Université de Montréal

Limited Sampling Strategies for Estimation of Cyclosporine Exposure in Pediatric Hematopoietic Stem Cell Transplant Recipients: Methodological Improvement and Introduction of Sampling Time Deviation Analysis

par

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Thèse présentée à la Faculté des études supérieures et postdoctorales

en vue de l'obtention du grade de docteur

Ph.D. en sciences pharmaceutiques

option technologie pharmaceutique

December, 2014

Université de Montréal

Faculté des études supérieures et postdoctorales

Cette thèse intitulée:

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Abstract

Therapeutic drug monitoring is recommended for dose adjustment of potent immunosuppressive agents. Accumulating evidence supports the usefulness of the area under the curve (AUC) for monitoring cyclosporine (CsA) in hematopoietic stem cell transplantation (HSCT). However, the use of AUC in clinical settings is restricted for practical reasons. Limited sampling strategies using regression (R-LSS) and Bayesian (B-LSS) approaches have been used to estimate AUC. However, for adequate application in clinical settings, these LSS approaches should be convenient in terms of the number of required concentration-time points as well as the duration of sampling. Furthermore, a particular attention should be given to ensure their adequate development and validation. Moreover, irregularity in the time of blood sample collection may have a non-negligible impact on the prediction performance of R-LSS; this impact has not yet been studied.

The investigation of these issues is the main focus of the current study in order to ultimately achieve convenient and reliable estimation of AUC using LSS. Pediatric HSCT patients receiving intravenous (IV) and oral (PO) CsA were investigated. Thorough regression and population-pharmacokinetic (Pop-PK) analyses were carried out in order to adequately develop and validate LSS. Several Pop-PK models were evaluated while bearing in mind their intended use for AUC estimation. The performance of B-LSS for targeting different versions of AUC was also investigated. Moreover, the impact of the deviations of actual sampling times from the planned nominal times on R-LSS prediction performance, in diverse sampling time deviation (STD) scenarios, was examined using a simulation approach.

These investigations led to the identification of LSS that have clinically acceptable predictive performance. These LSS are suitable for clinical application since they require 4 or fewer sampling points drawn within 4 hours post-dose. Besides, following the Pop-PK analysis, a two-compartment structural model with a lag time and a combined additive and proportional error was retained. However, the final covariate model did not improve B-LSS performance. It turned out that the structural models (without covariates) had a better performance. Moreover, B-LSS performed better for the estimation of the 'underlying' AUC derived from the Pop-PK simulated concentrations that exclude the residual errors, compared to their predictions of the observed AUC directly calculated using measured concentrations. Finally, our results showed that time deviation in blood sample collection have a significant impact on R-LSS prediction performance. This impact depends on the number of the involved samples, but more importantly on the duration of the sampling process. Furthermore, sampling errors at time points at which the concentration changes rapidly are critical for AUC prediction. Moreover, we have shown that R-LSS may have similar performance in terms of nominal times, but their tolerance to STD can be quite different. Hence, adequate consideration of the impact of STD can lead to a more reliable selection and use of R-LSS.

This thesis provided a thorough investigation of different issues related to LSS. Methodological improvement and proposition of new avenues have been realized while giving a careful consideration to ensure a convenient use in real-life clinical practice.

Keywords: Pharmacokinetics (PK), Population-pharmacokinetics (Pop-PK), Pharmacometrics, Therapeutic drug monitoring (TDM), Area under the curve (AUC), Limited sampling strategies (LSS), Weighted multiple regression, Bayesian approach, Sampling time deviation (STD), Cyclosporine (CsA), Pediatrics, Hematopoietic stem cell transplantation (HSCT).

Résumé

Le suivi thérapeutique est recommandé pour l'ajustement de la dose des agents immunosuppresseurs. La pertinence de l'utilisation de la surface sous la courbe (SSC) comme biomarqueur dans l'exercice du suivi thérapeutique de la cyclosporine (CsA) dans la transplantation des cellules souches hématopoïétiques est soutenue par un nombre croissant d'études. Cependant, pour des raisons intrinsèques à la méthode de calcul de la SSC, son utilisation en milieu clinique n'est pas pratique. Les stratégies d'échantillonnage limitées, basées sur des approches de régression (R-LSS) ou des approches Bayésiennes (B-LSS), représentent des alternatives pratiques pour une estimation satisfaisante de la SSC. Cependant, pour une application efficace de ces méthodologies, leur conception doit accommoder la réalité clinique, notamment en requérant un nombre minimal de concentrations échelonnées sur une courte durée d'échantillonnage. De plus, une attention particulière devrait être accordée à assurer leur développement et validation adéquates. Il est aussi important de mentionner que l'irrégularité dans le temps de la collecte des échantillons sanguins peut avoir un impact non-négligeable sur la performance prédictive des R-LSS. Or, à ce jour, cet impact n'a fait l'objet d'aucune étude.

Cette thèse de doctorat se penche sur ces problématiques afin de permettre une estimation précise et pratique de la SSC. Ces études ont été effectuées dans le cadre de l'utilisation de la CsA chez des patients pédiatriques ayant subi une greffe de cellules souches hématopoïétiques. D'abord, des approches de régression multiple ainsi que d'analyse pharmacocinétique de population (Pop-PK) ont été utilisées de façon constructive afin de développer et de valider adéquatement des LSS. Ensuite, plusieurs

modèles Pop-PK ont été évalués, tout en gardant à l'esprit leur utilisation prévue dans le contexte de l'estimation de la SSC. Aussi, la performance des B-LSS ciblant différentes versions de SSC a également été étudiée. Enfin, l'impact des écarts entre les temps d'échantillonnage sanguins réels et les temps nominaux planifiés, sur la performance de prédiction des R-LSS a été quantifié en utilisant une approche de simulation qui considère des scénarios diversifiés et réalistes représentant des erreurs potentielles dans la cédule des échantillons sanguins.

Ainsi, cette étude a d'abord conduit au développement de R-LSS et B-LSS ayant une performance clinique satisfaisante, et qui sont pratiques puisqu'elles impliquent 4 points d'échantillonnage ou moins obtenus dans les 4 heures post-dose. Une fois l'analyse Pop-PK effectuée, un modèle structural à deux compartiments avec un temps de délai a été retenu. Cependant, le modèle final - notamment avec covariables - n'a pas amélioré la performance des B-LSS comparativement aux modèles structuraux (sans covariables). En outre, nous avons démontré que les B-LSS exhibent une meilleure performance pour la SSC dérivée des concentrations simulées qui excluent les erreurs résiduelles, que nous avons nommée « underlying AUC », comparée à la SSC observée qui est directement calculée à partir des concentrations mesurées. Enfin, nos résultats ont prouvé que l'irrégularité des temps de la collecte des échantillons sanguins a un impact important sur la performance prédictive des R-LSS; cet impact est en fonction du nombre des échantillons requis, mais encore davantage en fonction de la durée du processus d'échantillonnage impliqué. Nous avons aussi mis en évidence que les erreurs d'échantillonnage commises aux moments où la concentration change rapidement sont celles qui affectent le plus le pouvoir prédictif des R-LSS. Plus intéressant, nous avons

mis en exergue que même si différentes R-LSS peuvent avoir des performances similaires lorsque basées sur des temps nominaux, leurs tolérances aux erreurs des temps d'échantillonnage peuvent largement différer. En fait, une considération adéquate de l'impact de ces erreurs peut conduire à une sélection et une utilisation plus fiables des R-LSS.

Par une investigation approfondie de différents aspects sous-jacents aux stratégies d'échantillonnages limités, cette thèse a pu fournir des améliorations méthodologiques notables, et proposer de nouvelles voies pour assurer leur utilisation de façon fiable et informée, tout en favorisant leur adéquation à la pratique clinique.

Mots-clés : Pharmacocinétique (PK), Pharmacocinétique de population (Pop-PK), Pharmacométrie, Suivi thérapeutique, Surface sous la courbe (SSC), Stratégies d'échantillonnage limité (LSS), Régression multiple pondérée, Approche Bayésienne, Écart de temps d'échantillonnage (STD), Cyclosporine (CsA), Pédiatrie, Transplantation des cellules souches hématopoïétiques.

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List of abbreviations

AUC	Area under the concentration-time curve
$AUC_{0\text{-}12h}$	AUC between drug administration and 12 hours post-dose
AUC_{full}*	Full AUC : AUC estimated using the trapezoidal method and the full set of avilaible concentrations
AUC_{obs}	Observed AUC: AUC_{full} estimated using observed concentrations (measured concentrations)
AUC_{IPRED}	'Underlying' AUC: AUC_{full} estimated using simulated concentrations (individual simulated concentrations without residual error)
AUC_{pred}*	Predicted AUC : AUC estimated using LSS method
$AUC_{nominal}$	Nominal AUC : AUC_{pred} using simulated concentrations at nominal times (without sampling time errors)
AUC_{actual}	Actual AUC : AUC_{pred} using simulated concentrations at actual times (with sampling time errors) in a given sampling time deviation scenario
AUMC	Area under the moment curve
B-LSS	Bayesian limited sampling strategy
CI	Confidence interval
C_{max}	Maximum concentration
CsA	Cyclosporine A
C_t	Concentration at time t in hours post-dose
E%	Relative prediction error
EI	Error indices

*In Chapter 4 (Article III) AUC_{full} and AUC_{pred} are calculated using simulated concentrations; however, they are generally calculated using measured concentrations.

ε_{ij}	Residual error for individual i at time j
FOCE	First order conditional estimation
GVHD	Graft-versus-host disease
HSCT	Hematopoietic stem cell transplantation
IV	Intravenous
LOOCV	Leave-one-out cross-validation
LSS	Limited sampling strategy(es)
ME	Mean prediction error
ME%	Mean relative prediction error
MRT	Mean residence time
η_i	Inter-individual variability for a given parameter of an individual i
NCA	Non-compartmental analyses
NONMEM	Nonlinear mixed effect model
OFV	Objective function value
PD	Pharmacodynamic
PK	Pharmacokinetic
PO	Oral
Pop-PK	Population pharmacokinetics
R^2	Correlation coefficient
R-LSS	Regression limited sampling strategy
RMSE	Root mean squared prediction error
RMSE%	Root mean squared relative prediction error
STD	Sampling time deviation
TDM	Therapeutic drug monitoring

t_{max}	Time spent to reach the maximum concentration
95^{th} PAE%	95^{th} percentile of absolute values of relative prediction errors

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À mes chers parents
À ma vie Walaa,
À mes espoirs Aya et Youssef et
À tous les êtres chers qui m'entourent
Qui font que la vie est si belle !

Acknowledgments

Words cannot express how grateful I am to my research director Dr. Fahima Nekka who provided to me, through her outstanding scientific and human qualities, a close follow-up, appropriate and clear instructions, excellent scientific and personal supports, and most importantly an exceptional attention and comprehension to accommodate and enhance my creative ideas and desire to learn and innovate.

I would like to express my sincere appreciation to my co-director Dr. Catherine Litalien for her significant and ongoing scientific and personal support and smart guidance; her excellent comments and contributions enhanced remarkably the quality and the impact of my work.

A special thanks to Dr. Jun Li who enriched my research experiences through his deep knowledge, great intellectual capacity, and constructive criticisms. Dr. Li helped me to turn limitations and obstacles to opportunity for innovation and accomplishment.

I want to show deep appreciation for CHU Sainte-Justine partners, namely the transplantation medical team, Dr. Yves Théorêt and Dr. Anne-Laure Lapeyraque for their vital collaboration.

I would also like to thank my committee members, Dr. Robert Bies, Dr. Grégoire Leclair, and Dr. Gaëlle Roullin for accepting to evaluate my Thesis; thanks for your attention, comments, and consideration. I also want to thank you for letting my defense be an enjoyable moment.

I would also like to express my gratitude to my parents, Mohammad and Sabah, my sister, Rawan, and my brothers Bashar and Mohannad, for the support, for the endless encouragement and for all the sacrifices that you've made on my behalf. Your prayer for me was what sustained me thus far.

I express my warm thanks to my colleagues and friends: Bassel, Fadi, Olivier, Guillaume, Liella, Morgan, Mario, Abbass, and Steven; thank you for your comfortable presence, for your smiles ... thank you for your help and for the extraordinary times I spent with you.

At the end, I would like to express my deepest appreciation to my beloved wife Walaa for her exceptional care and wonderful accompany; I will be forever grateful for your scarification and support in every moment of my course.

I want to thank everyone who supported me throughout the long and pleasurable course of my under and post-graduate studies.

Thank you,

Sarem

Chapter 1 Introduction

1 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) aims at improving patient care by individually adjusting the dose. As such, it is crucial in clinical practice in the presence of considerable inter- or intra-individual pharmacokinetic (PK) variability, unpredictable dose-response relationship, or narrow therapeutic window [1]. In fact, under these conditions an uniform dose can result in significant variations in drug concentration (up to 10-fold range) and hence may lead to under or overexposure with therapeutic failure or serious undesirable effects, respectively [2-4].

TDM involves the measurement of drug effects and concentrations, usually in plasma or blood, and the clinical interpretation of the results. It is used to individualize dosage to improve efficacy and avoid toxicity. TDM has been utilized in the clinical care of patients from the early 1960s [5]. Currently, this approach is routinely performed for dose adjustment of several medications in different therapeutic classes, such as digoxin (cardiovascular agents), phenytoin (antiepileptics), gentamicin (antibiotics), azathioprine (cancer chemotherapeutic agents), and cyclosporine (immunosuppressive agents). TDM goes beyond the individualization of therapy and can include detection of noncompliance to medical treatments, dose adjustment in patients with hepatic and renal dysfunction, as well as recognition of drug interactions.

The major limitation associated with TDM is the requirement of a well-established relationship between drug concentrations or derived PK markers and

therapeutic outcomes or toxicity. This pharmacokinetic/pharmacodynamic (PK/PD) relationship is necessary to establish therapeutic targets in order to guide dose adjustment. Therapeutic ranges are usually determined through retrospective studies that analyze available data to identify the values of drug concentrations or other surrogate markers (such as the area under the concentration-time curve (AUC)) associated with efficacy, treatment failure, and toxicity. These ranges are supposed to provide clinically acceptable efficacy with minimal risk of toxicity in most patients [6].

The lack of well-established PK/PD relationship may lead to use empirical ranges determined using the observed exposures in patients with normal clearance receiving standard doses. For example, Evans et al. used AUC values of the 50^{th} to 90^{th} percentile of patients treated with conventional doses of methotrexate to establish target systemic exposures of this drug, for acute lymphoblastic leukemia in pediatrics [7]. Nonetheless, further investigations are needed to prove the safety and efficacy of such 'observational' ranges.

Indeed, when possible the therapeutic range should be determined through prospective concentration-controlled studies (as an alternative to dose-controlled studies) [8]. In the former study design, patients are randomized to different arms; in each one the doses are adjusted through TDM approach to achieve a predefined level of drug exposure. The clinical outcomes are compared to determine the range associated with the best benefit/risk ratio. Such studies provide a high level of evidence and have a significant impact on clinical practice. As an example, one can refer to the randomized,

double-blind, multicenter concentration-controlled trial for determining a therapeutic range of mycophenolate mofetil by Van Gelder et al. [9].

In the context of TDM, the methods used for dose individualization can be classified into two main categories: 1) *a priori* methods, and 2) *a posteriori* methods [3, 10]. *A priori* methods use available patient characteristics (weight, body surface area, genotype, creatinine clearance, etc.) and associated technique such as PK models or dose calculation formulas to determine the required dose in a given patient in order to achieve the desired level of exposure. The *a priori* methods take into account some inter-individual variability by means of the patient characteristics integrated into PK models or calculation formulas and are easy to apply. However, their predictive performance is not expected to be as good as that of *a posterior* methods since they are not intended to consider intra-individual variability as well as unexplained inter-individual variability [10]. The *a posteriori* methods use the information obtained after drug administration (ex. drug concentrations) to calculate individual PK parameters and markers such as AUC and hence to estimate the adequate dose. These methods are particularly useful in the presence of large inter-individual variability as the dose adjustment will be based on actual drug exposure.

TDM has become a standard of care for individual dose optimization of immunosuppressant drugs. Several PK markers have been proposed to carry out this monitoring. The common approach in most transplantation centers is to use one blood concentration (C_0 for tacrolimus; C_0 or C_2 (C_t: concentration at time t in hours post-dose) for cyclosporine) [11-13] as the criterion to adjust the dosing regimen of these drugs.

However, treatment failure and toxicity still arise even when these monitoring parameters are within their target ranges. Thus, there is increasing interest in investigating other markers such as AUC which usually best reflects systemic drug exposure. In fact, AUC has been frequently studied as TDM marker for immunosuppressant drugs [2, 12, 14-16].

In addition to the individualization of therapy through therapeutic monitoring approach, AUC is also involved in other clinical and research settings, such as bioequivalence studies and *in vivo* evaluation of enzyme activities via probe substrates. Bioequivalence studies are legally required in several countries for the approval of generic products, product line extensions, and new dosage forms. AUC is one of the main bioequivalence metrics. For example, the Canadian bioequivalence guidance documents require that 90% confidence interval of the relative mean AUC of the test (generic) to reference (original) products should be within 80.0% to 125.0% inclusively [17]. Given the expected correlation between the drug effect and AUC, a more restrictive interval, 90.0% to 112.0% inclusively, is required for critical dose drugs which are associated with small therapeutic index. In addition, AUC of some drugs can be used as a marker to assess *in vivo* enzyme activities. For example, the systemic clearance of midazolam has been used as a marker to evaluate *in vivo* CYP3A activity [18]. The estimation of systemic clearance is, usually, based on AUC. Hence, there is increasing interest in developing practical and accurate methods for AUC estimation.

A dense concentration-time course sampling over a dosing interval has been used for an accurate estimation of the 'full' AUC (AUC_{full}) using the trapezoidal method. In clinical practice, AUC_{full} is calculated using the full set of the observed (measured)

concentrations and it is referred to as observed AUC (AUC_{obs}). However, this approach is associated with considerable disadvantages: prolongation of patient's hospital stay, expensive costs, patient discomfort, etc. Therefore, alternative estimation based on a limited sampling strategy (LSS) approach that allows a reliable estimation of AUC_{full}, using fewer samples drawn within a shorter interval post-dose, is needed. Particularly, the use of LSS is an appealing approach for AUC-based TDM in clinical practice. Nevertheless, well-established and validated LSS are required to ensure effective and safe AUC-guided dose adjustments.

2 Limited sampling strategies (LSS)

A LSS typically consists of a subset $\{C_{t_1},\ldots,C_{t_k}\}$† of the complete set of all available concentration-time points $\{C_{t_1},\ldots,C_{tn}\}$ used to estimate the AUC_{full}. For practical reasons, LSS consisting of a maximum of 4 concentration-time points are usually considered. According to the number of included points, LSS can be divided into 4 subgroups, namely one, two, three, and four concentration-time point subgroups.

The total number of possible LSS depends on the number of available concentration-time points. The general mathematical formula for combinations can be used to find the number of LSS of k points that can be chosen from a set of n points:

$$C_n^K = \frac{n!}{k!\,(n-k)!}$$

† C_{ti} refers to the concentration at time t_i in hours post-dose

So, for example, 9 available concentration-time points generate 9! / 1! (9-1)! = 9 LSS of one point, 36 LSS of 2 points, 84 LSS of 3 points, and 126 LSS of 4 points for a total of 255 possible LSS.

The current LSS development method often investigates a small group of LSS, pre-selected according to practical or PK considerations. Alternatively, this pre-selection is based on the coefficient of correlation (R^2) or it can be completely arbitrary at the investigator's discretion. This pre-selection procedure is intended to reduce the required computation workload. However, this justification is no longer relevant because of the increasing accessibility to helpful software and advanced calculation tools. Particularly, the use of the coefficient of correlation as the criterion of the pre-selection step may be misleading since this coefficient assesses correlation and cannot evaluate the prediction performance. Hence, in order to avoid missing a 'good' LSS through the unjustified but widely used pre-selection step, the performances of all possible LSS should be evaluated. Then, promising ones can be selected according to their predictive performance of AUC, along with other criteria designed to ensure their applicability in clinical settings. These criteria may include a restriction of sampling duration to a short period post-dose and the inclusion of a particular concentration-time point such as C_0.

LSS has been developed through two approaches, namely, the regression-based LSS (R-LSS) and Bayesian-based LSS (B-LSS). [19, 20].

2.1 Regression LSS

The regression LSS approach (R-LSS) is performed to define a linear relationship between one or more concentration-time points (as independent variables) and AUC (as

a dependent variable). Multiple linear regression can be used to model this relationship in the form of equation:

$$AUC_{pred} = F_0 + F_1 \times C_{t_1} + \ldots + F_k \times C_{t_k}$$

where $C_{t_1}, C_{t_2}, \ldots, C_{t_k}$ are the concentrations obtained at times $t_1, t_2, \ldots, t_k$; $F_0, F_1, \ldots, F_k$ are fitting coefficients estimated using multiple linear regression to reduce the deviation of predicted AUC (AUC_{pred}) from AUC_{full} by minimizing the objective function:

$$O = \sum_{i=1}^{n} \left(AUC_{pred}^{(i)} - AUC_{full}^{(i)} \right)^2$$

where i denotes the i^{th} profile; n is the number of studied profiles. Instead of the usually used ordinary multiple linear regression which only minimizes the squares of the prediction errors without taking into account their relative values when compared with AUC_{full}, one can use weighted MLR that includes a weight w in the objective function

$$O = \sum_{i=1}^{n} w^{(i)} \left(AUC_{pred}^{(i)} - AUC_{full}^{(i)} \right)^2$$

The weight w is chosen to take into account the relative value of the prediction errors and defined as $w^{(i)} = 1/AUC_{full}^{(i)\,2}$. This approach aims to prevent large relative errors in the prediction of small AUC values, which can occur with ordinary linear regression analysis.

2.2 Bayesian LSS

Bayesian estimation is generally applied using a nonlinear mixed effected population-pharmacokinetic (Pop-PK) model, so it can take into account the PK characteristics of the typical population, represented by the Pop-PK model and the typical values of its parameters. In addition, this approach accounts for the individual characteristics of patients by including co-variables such as body weight in the model. As *a posteriori* dose adjustment method, Bayesian estimation also considers the residual random effects by using measured drug concentrations. These concentrations help to account for the intra-individual variability, as well as the unexplained fraction of the inter-individual variability.

In Bayesian LSS (B-LSS), the estimated individual PK parameters are obtained through the empirical Bayesian approach. This estimation involves the use of the concentrations obtained in an individual patient according to the B-LSS to predict his PK parameters using a Pop-PK model. Then these parameters are used for the prediction of the patient's drug concentrations corresponding to a full PK profile (ex. 9 concentrations), as shown in Figure 1.1. Consequently, the AUC of a particular patient can be calculated. Hence, the Pop-PK model and the typical values of its parameters, considered as acquired prior knowledge of drug PK characteristics, helps to improve the estimation otherwise solely based on observed individual drug concentrations, considered as posterior knowledge measured after drug administration. More details about B-LSS approche are provided in Chapter 3.

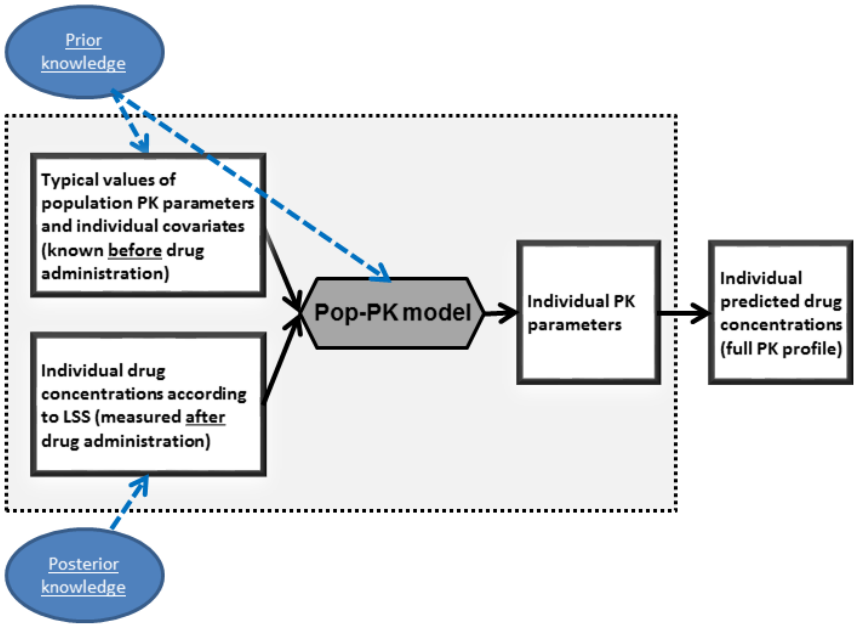

Figure 1.1: Bayesian LSS procedure.

2.3 Regression vs. Bayesian LSS

For its simplicity, the use of R-LSS is practical and widely spread as a bedside application. In fact, the development of R-LSS includes the definition of associated mathematical equations, which then can be simply incorporated in common computer programs (ex. Excel®). This implementation permits an effortless calculation of the predicted AUC. However, their use is highly restrictive since the involved blood samples are assumed to be collected at the planned nominal times according the LSS while excluding or ignoring any possible deviations.

Bayesian method has important advantages, including the possibility of checking of modeling efficiency, by comparing the measured concentrations and the predicted concentration-time curve. One outstanding advantage of the Bayesian approach is its flexibility in terms of sampling times since no condition is imposed by either the construction of the associated Pop-PK model or the prediction of individual PK parameters, and the real sampling times can be used in case of deviations from the nominal ones. Nevertheless, the B-LSS that utilizes the real times will have a different predictive performance than the planned one. In addition, the use of B-LSS can be hampered by the requirement of well-established Pop-PK models, trained professionals, and advanced software that are not, usually, accessible in daily practice.

	R-LSS	**B-LSS**
Approach	Linear regression	Empirical Bayesian approach
Application based on	Regression equation	Pop-PK model
Advantage	Simplicity of development and use	Flexibility for sampling time deviation
Inconvenience	Vulnerability to sampling time deviations	Requirement of trained professionals and access to specialized software

Table 1.1. Comparison between R-LSS and B-LSS.

3 Sampling time deviation (STD)

The remarkable technical advancement and the wide adaptation of good laboratory practice rules allow drug concentrations to be measured with considerable

accuracy and precision. However, unintentional errors in sample collection or processing still occur frequently in clinical practice. These errors can have a substantial impact on the accuracy of concentration measurements that will be inherited by surrogate markers such as AUC. Therefore, they can lead to wrong therapeutic decisions in the context of therapeutic drug monitoring. The effect of sample analytical processing errors is evaluated through analytical validations and quality control procedures. However, the impact of time errors in the sample collection is usually overlooked. It should be emphasized that, similarly to the noncompliance to medical therapy that may result in treatment failure in spite of using an effective drug [21-24], deviations in sample collection time may lead to significant errors in the estimation of PK markers in spite of using validated LSS (that would have acceptable prediction errors if samples were collected without deviation from planned nominal times). Consequently, a dose adaptation using these erroneous markers may lead to therapeutic failure or toxicity, In fact, considering sampling time deviation (STD) impact on the prediction performance of R-LSS is crucial to achieve a reliable AUS estimation for therapeutic drug monitoring. Figure 1.2 illustrates a simple example of STD.

Saint Marcoux et al. [25] investigated the impact of STD on the accuracy of measuring concentrations 2 hours post-dose (C_2) that were used as therapeutic drug monitoring marker. They found that a considerable difference, up to 30% from nominal C_2 values, can occur with STD of 15 minutes. However, the impact of STD on the estimation of AUC using R-LSS is a more complex issue since it generally involves multiple sampling points for which the effects of time deviations may build up, interact, and potentially amplify. Moreover, the impact of STD may be different according to

samples' positions on the concentration-time curve. Hence, a thorough investigation of this impact is necessary to determine the actual prediction errors of R-LSS in the presence of STD.

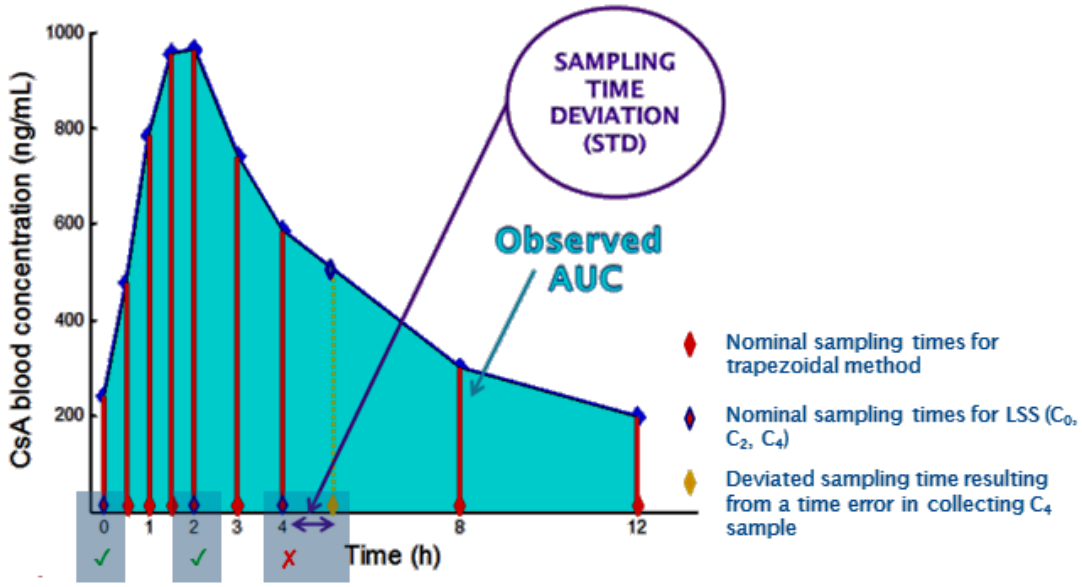

Figure 1.2: Example of a sample time deviation in one concentration-time point, the last sample of the LSS (that includes C_0, C_2, and C_4) being collected later than the planned nominal time of 4h post-dose; C_t is the concentration at time t in hours post-dose.

4 Management of STD in clinical settings

Appropriate management of STD in clinical settings is a critical subject. On one hand, overlooking STD can result in an inappropriate therapeutic drug monitoring decision. In fact, imprecisely estimated PK markers can cause failure to perceive or confirm the potential correlation between these markers and clinical outcomes. On the other hand, apart from sample collection performed in the strictly controlled conditions

such as phase I clinical trials and bioequivalence studies, sampling time errors are frequently observed. Hence, zero-tolerance approach cannot be suggested to avoid their undesirable effect on PK marker estimation. Such extreme approach can lead to ethical and sometimes clinical unacceptable frequent repeating of the sampling process that involves several samples in the case of LSS. However, procedures have to be implanted to reduce as possible its frequency and extent.

Nonetheless, one can suppose that STD are likely a part of usual human behavior and while they can be reduced they cannot be entirely eliminated. Thus, to ensure adequate therapeutic drug monitoring in the presence of STD, the clinical and technical staff should be aware of its impact. A vital assistance for an appropriate management of this issue consists of providing the medical team with instructions and materials that allow a timely assessment of the impact of STD. Thus, necessary interventions can be applied in order to ensure an accurate estimate of AUC in case of an unintentional sampling time error. These interventions may include the use of corrective methods or alternative LSS. The repetition of the whole sampling process may be advised as a last resort only in the situation where no other practical and reliable solutions exist.

5 Area under the concentration-time curve (AUC)

The simplest and most common approach for the estimation of AUC is a numerical approximation method according to the trapezoidal rule [26], Figure 1.3. This method is based on considering the AUC of each segment defined by a time interval between two consecutive samples as a trapezoid and calculates its area by multiplying the average concentration by the section width. The total AUC is finally calculated by

adding the segment areas together. It is, therefore, a linear combination of all the available concentrations.

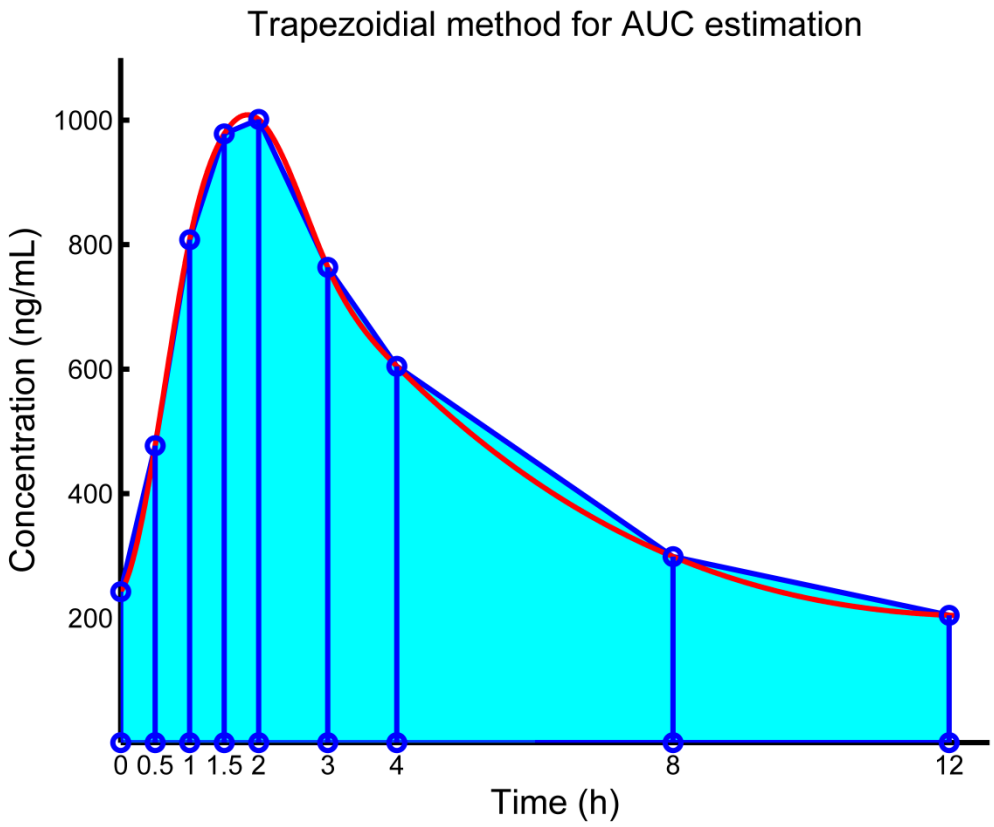

Figure 1.3: Trapezoidal estimation of AUC.

5.1 Full AUC

AUC_{full} is calculated from all available concentrations according to the trapezoidal rule. The predicted AUC using LSS is compared to AUC_{full} to evaluate the predictive performance of LSS. Two types of AUC_{full} can be defined according to the concentration type used for its calculation, namely observed AUC (derived from measured concentrations) and 'underlying' AUC (derived from simulated concentrations).

5.1.1 Observed AUC

Observed AUC (AUC_{obs}) is the AUC_{full} calculated from all available measured concentrations. However, these measured or observed concentrations are known to be affected by random errors originating from sample collection, analytical method, and data processing. These errors could be inherited by the AUC_{obs}; this attribute may raise the question of its reliability.

5.1.2 Underlying AUC

The real systemic exposure of a drug would be ideally estimated using a large number (if possible continuous) of concentrations over the whole dosing interval. In addition, these concentrations should be obtained using an exact quantitative measurement in samples collected accurately according to the planned protocol schedule. Such idealist concentration-time profile cannot be obtained in clinical settings for ethical and technical reasons. However, it can be simulated using the drug Pop-PK model and the observed concentrations. Hence, it would be interesting to consider alternatively the AUC_{full} calculated directly from the simulated concentrations that excludes the residual errors (measurement and data processing errors). These concentrations are represented by IPRED using the nomenclature of NONMEM® (the mostly used software in Pop-PK analyses). This AUC is referred to as 'underlying' AUC, and can be noted as AUC_{IPRED}. The difference between AUC_{obs} and 'underlying' AUC is illustrated in Figure 1.4.

Although the expected underlying concentrations cannot be directly measured in practice, they represent to a greater degree the inner property of a patient's PK as it is

not altered by random errors. Consequently, the 'underlying' AUC should better reflect drug effect than AUC_{obs} since its correlation with actual drug exposure is likely to be higher after eliminating the random noise associated with residual errors.

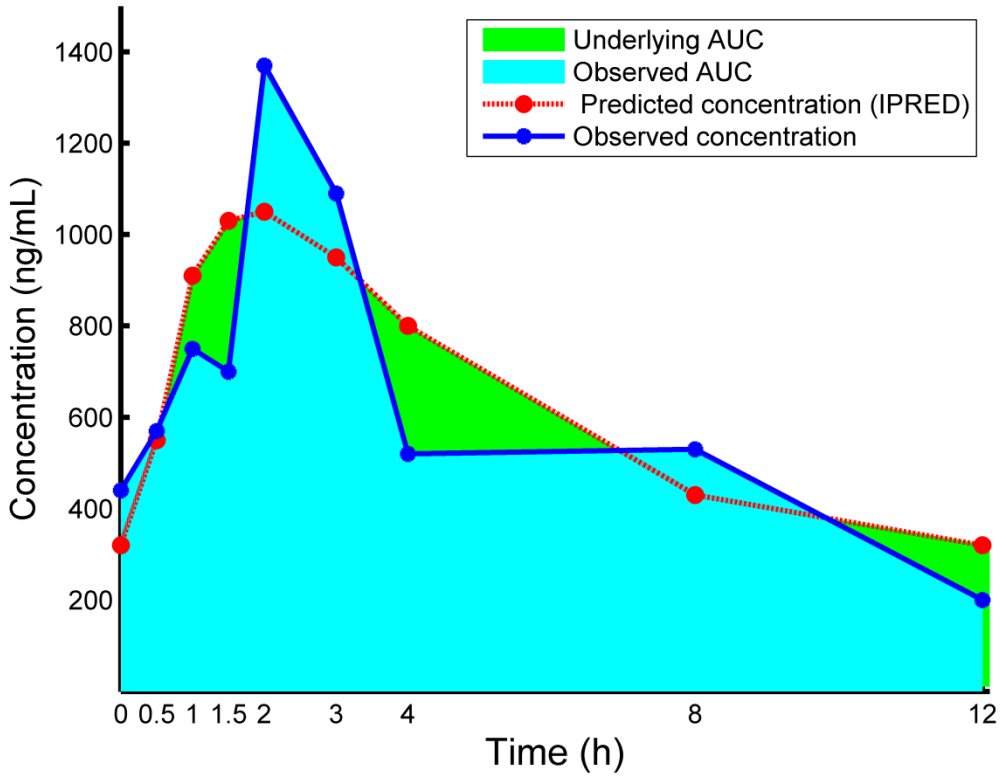

Figure 1.4: Typical example of 'underlying' AUC vs. observed AUC.

5.2 Predicted AUC using measured concentrations

Predicted AUC (AUC_{pred}) is the estimation of AUC using LSS. When measured concentrations are used the accuracy of estimation depends on their quality; time errors in sample collection can compromise quality of measurement and thus increase prediction errors particularly for R-LSS. As mentioned in a previous section, this

problem is usually overlooked and samples are assumed to be taken at nominal times while excluding or ignoring any possible deviations.

5.3 Predicted AUC using simulated concentration

In order to assess the impact of STD on the predictive performace of LSS, the difference between AUC predicted using concetrations obtained at nominal time ($AUC_{nominal}$) and that predicted using concetrations collected at actual times (AUC_{actual}) should be assessed and quantified. Adequate and comprehensive analyses of this problematic issue can only be realized using simulated concentrations to calculate these AUC. The use of simulated concentrations is intended to allow isolating the impact of STD from other sources of errors such as the analytical method.

5.3.1 Nominal AUC

$AUC_{nominal}$ is the predicted AUC using LSS if all required concentration-time points are collected correctly without any deviation from the nominal times of the LSS. However, we have to mention that even when sample collection is performed without error so that nominal time concentrations are available for the prediction of AUC using LSS, the prediction error will not be null since the AUC_{full} (used for the assessment of prediction error) is calculated by the trapezoidal method and not using LSS.

5.3.2 Actual AUC

AUC_{actual} is the predicted AUC using LSS if one or more required concentration-time points were collected with deviation from the nominal times of LSS. Different types (patterns) and level (extent) of deviation scenarios in sample collection can be

expected in clinical settings and thus should be taken into consideration to have adequate and comprehensive analyses of STD impact on LSS performance. Therefore, very intense sampling is needed to allow mimicking the diverse possible scenarios of time deviation in sample collection. The use of Pop-PK simulation is necessary to generate dense concentration-time profiles that allow investigation of such a complex topic.

6 Evaluation of the prediction performance

6.1 Cross-validation

The identification of the most appropriate LSS that provides accurate and reliable predictions is critical for their safe and confident application in clinical settings. The objective of cross-validation is to evaluate the predictive performance adequately. Notably, this technique allows checking whether a model (ex. LSS) is suffering of the self-fitting problem that is characterized by a substantial deterioration in the model's predictive performance when evaluated in independent data that were not used in model fitting. Hence, cross-validation gives an insight on how a model will generalize to a 'new' dataset which is independent from the studied population.

Typically, this process consists of two stages, namely the learning and validation steps (also referred to as training and testing steps). In the context of LSS investigations, the aim of the learning step is to define the LSS coefficients, which are the regression coefficients for R-LSS while they represent the typical values of Pop-PK parameters for B-LSS. Then, the LSS performances are verified in the validation step [27, 28].

Cross-validation methods can be classified as exhaustive and non-exhaustive techniques. The basic principle of the former is to split, in all possible ways, the available dataset into learning and validation subsets. For example, the leave-p-out-cross-validation (LPOCV) involves using p data elements (ex. PK profiles) as the validation subset and the remaining n-p data elements as the training subset (where n is the number of the studied data elements). This procedure is repeated according all possible ways to divide the original dataset into a validation subset of p data elements and a training subset including the remaining ones. LPOCV requires to learn and validate C_n^p times; so when n is large and p≠1, it becomes difficult to perform the statistical calculation. However, leave-one-out cross-validation (LOOCV), which is a particular case of leave-p-out cross-validation with p = 1, doesn't have the calculation problem of the general LPOCV because C_n^1 = n. The application of LOOCV method in LSS studies is described in a dedicated section below.

Non-exhaustive methods, such as K-fold cross-validation, are not intended to consider all possible splitting ways of the original dataset. In K-fold cross-validation technique, the investigated dataset is divided into K subsets, and the learning and validation steps are repeated K times. Each time, one of the K subsets is used for the validation, and the other K-1 subsets are put together to form the learning subset. One advantage of this method is that each data element is involved in the validation exactly once and in the training subset K-1 times, and it requires less calculation than exhaustive methods. However, this method shows bias and variance issues that decrease as K increases [29].

A variation of K-fold cross-validation is to divide the data randomly into training and validation sets for K different times as seen in Monte Carlo cross-validation. The avantage of this method (over k-fold cross-validation) is that the size of the training and validation subset is not dependent on the number of iterations (folds). However, some data elements may never be selected in the validation subset, whereas others may be selected more than once, and this may lead to biased results, particularly if K is small [29, 30]. The main disadvantage of the non-exhaustive methods is the non-constancy of the results if the analysis is repeated because of different randomization procedures used.

Two group cross-validation is a simplification of the K-fold cross-validation method, where k=2 and the learning and validation procedure are performed just once as described in the following section.

6.1.1 Two group cross-validation

This approach is widely used for its simplicity and it is also known as learning-testing split or holdout method. Hence, a part of the available dataset is used for the learning step (training subset) and the remaining data (validation subset) are used to evaluate the performance. The validation subset plays the role of 'new data', since they were not used in the estimating of LSS coefficients, Figure 1.5 outlines the application of the two group cross-validation in the context of LSS investigations.

However, for appropriate learning and validation of LSS, the available dataset should include enough PK profiles, thus it can be divided into two subsets of adequate size. The number of PK profiles in the learning subset has to be sufficient to represent adequately the features of the studied population, and also the number of PK profiles

included in the validation subset should be sufficient to evaluate adequately the predictive performance. Besides, this technique can have a high variance. Particularly, its outcomes may depend greatly on which data elements are parts of the training subset and which are included in the validation subset. Therefore, the results may differ significantly according to the size of each data subset and how the division is made [30].

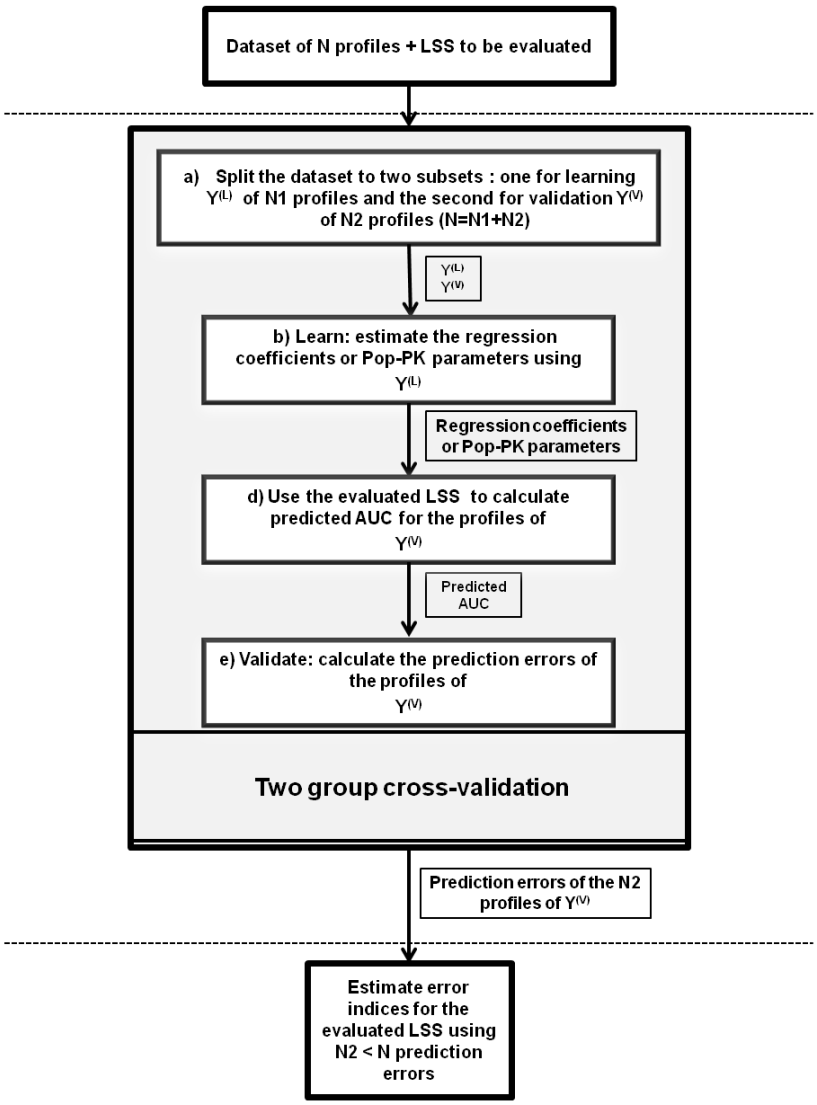

Figure 1.5: Outlines of two group cross-validation of LSS.

6.1.2 Leave-one-out cross-validation (LOOCV)

This approach allows adequate validation with a relatively small number of data elements. In this technique, the training and validation subsets are driven repeatedly from the available dataset so all data elements are exploited for the training and validation but in the same time the self-fitting phenomenon is avoided [27, 28].

In this approach, for every LSS, each PK profile is left out of the analysis in turn. This subset of profiles is noted by $Y^{(-i)}$ (i = 1, …, n), where $^{(-i)}$ expresses that all profiles are considered but the temporary excluded ith one. Then, $AUC_{pred}^{(i)}$ is calculated using the ith profile's concentrations along with the regression equation or Pop-PK parameters, developed using $Y^{(-i)}$, for R-LSS and B-LSS, respectively. Hence, the prediction of $AUC_{pred}^{(i)}$ does not involve the previous knowledge of the concentrations of the temporary excluded ith PK profile or its $AUC_{obs}^{(i)}$, in order to avoid the self-fitting phenomena. This operation is repeated until each one of the n available PK profiles has been left out of the analysis once. Using this approach, AUC_{pred} are estimated for all profiles, Figure 1.6.

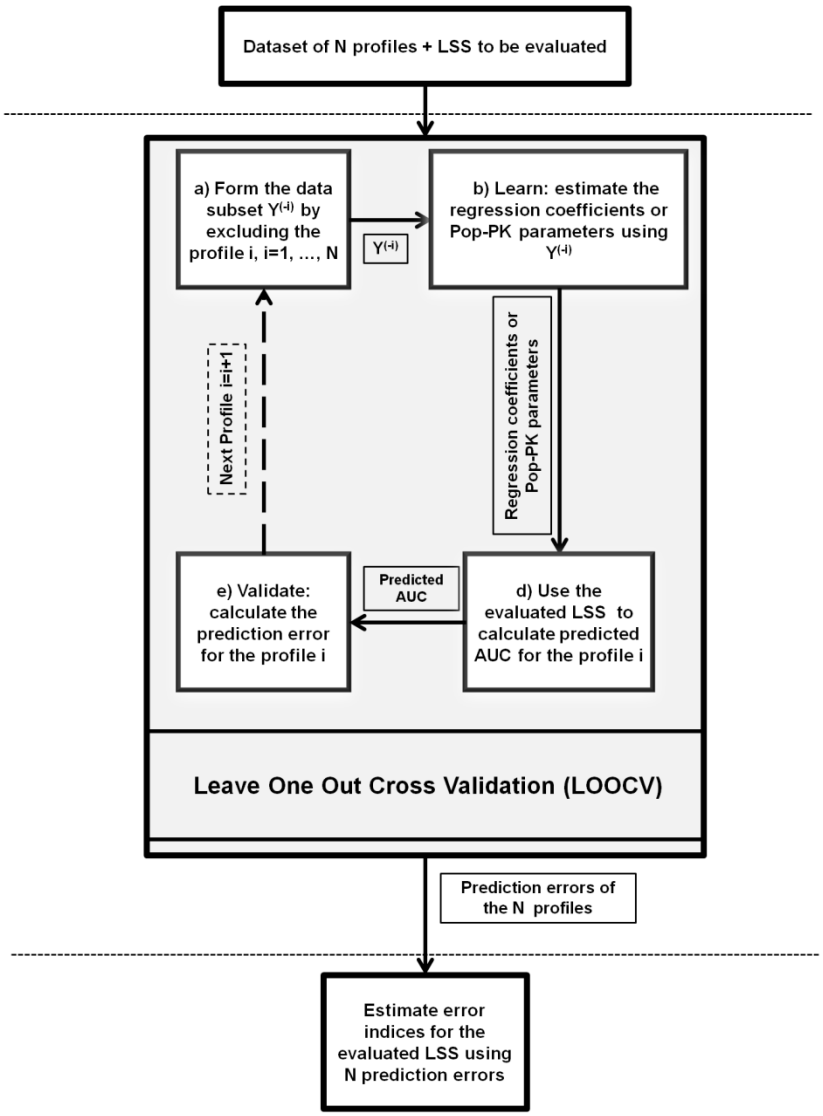

Figure 1.6: Outlines of leave-one-out cross-validation of LSS.

6.2 Error indices

The evaluation of the predictive performance of LSS can be accomplished statistically through error indices such as : relative prediction errors (E%), 95th percentile of the absolute values of relative prediction errors (95th PAE%), mean relative prediction error (ME%) and root mean squared relative prediction error (RMSE%); the last two

indices evaluate bias and precision, respectively [31]. Bias is the systematic error and represents the trend of constantly over- or under-estimating a variable. Precision is affected by the random error and reflects the extent of deviation in the prediction. These two error indices are negatively-oriented scores: lower values are better. Hence, a LSS will be less biased and more precise when the ME% and RMSE% are smaller. These indices are based on the following formulations:

$$E_i\% = \frac{AUC_{pred}^{(i)} - AUC_{full}^{(i)}}{AUC_{full}^{(i)}} \times 100 \quad (1)$$

95th PAE% = 95th percentile of the set $\left\{|E_i|\%\right\}_i^N$ in increasing order (2)

$$ME\% = \frac{1}{N}\sum_{i=1}^{N} \frac{AUC_{pred}^{(i)} - AUC_{full}^{(i)}}{AUC_{full}^{(i)}} \times 100 \quad (3)$$

$$RMSE\% = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{AUC_{pred}^{(i)} - AUC_{full}^{(i)}}{AUC_{full}^{(i)}}\right)^2} \times 100 \quad (4)$$

These formulations are used to estimate the errors indices in the following Chapters.

Sheiner and Beal [31] suggested guidelines for the evaluation of the predictive performance to ensure objective evaluation and comparability of results with other related studies. While these guidelines promote the measurement of the precision primarily by RMSE and alternatively using the median (not mean) absolute error (MAE), several authors continue to use the mean absolute errors instead. The comparison of these methods of precision measurements is not in the scope of this

thesis; nonetheless, one has to encourage using the former ones in favor of a more standard performance evaluation. Moreover, in the calculation of RMSE, the prediction errors are squared before they are averaged, so it gives a higher weight to large errors. This attribute indicates that RMSE is more useful when large errors are particularly undesirable which is the case in clinical settings.

Finally, graphical methods can also be used to evaluate the performance of LSS, such as Bland-Altman and Mountain plots [32, 33]. Bland-Altman plot is a manner of data plotting designed to assess the concordance between two different techniques, usually, a new technique (ex. LSS : AUC_{pred}) and a reference one (ex. trapezoidal method: AUC_{full}). In Bland-Altman plot, good LSS performance is characterized by narrow agreement limits (mean ± standard deviation range) as well as by homogeneous prediction errors for the small and the big AUC values, Figure 1.7.

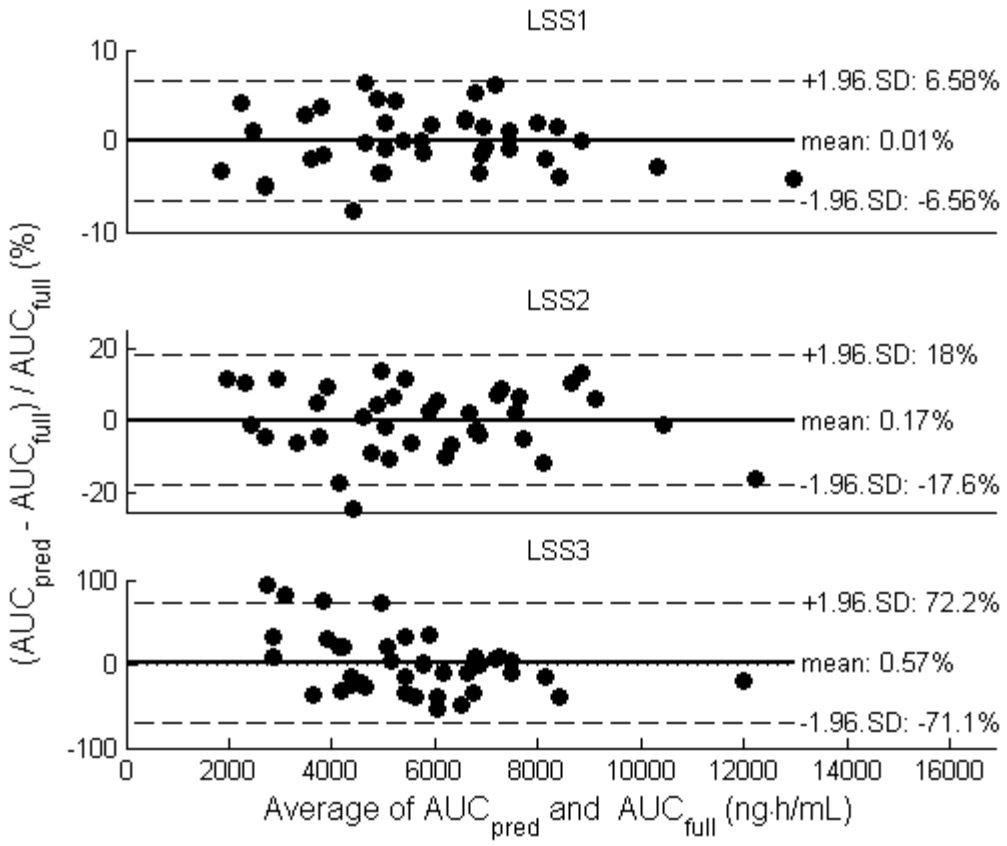

Figure 1.7: Bland-Altman analysis of agreement between the full AUC and predicted AUC using three LSS with different level of predictive performance. The solid line represents the mean of the relative difference between the full and predicted AUC; the dotted line represents ± 1.96 x standard deviation of the mean; AUC_{full}, full AUC; AUC_{pred}, predicted AUC.

LSS can also be closely compared to each other using the folder empirical cumulative distribution plot, more commonly known as the Mountain plot, which is performed by calculating a percentile for each ranked relative difference between the new and reference techniques (i.e. relative prediction errors). A folded plot can be obtained by the following alteration, for all percentiles over 50, percentile = 100 - percentile. Percentiles

are then plotted against the relative differences [33]. In Mountain plot, a good LSS performance is characterized by narrow, symmetric Mountain plot with centered peak, Figure 1.8.

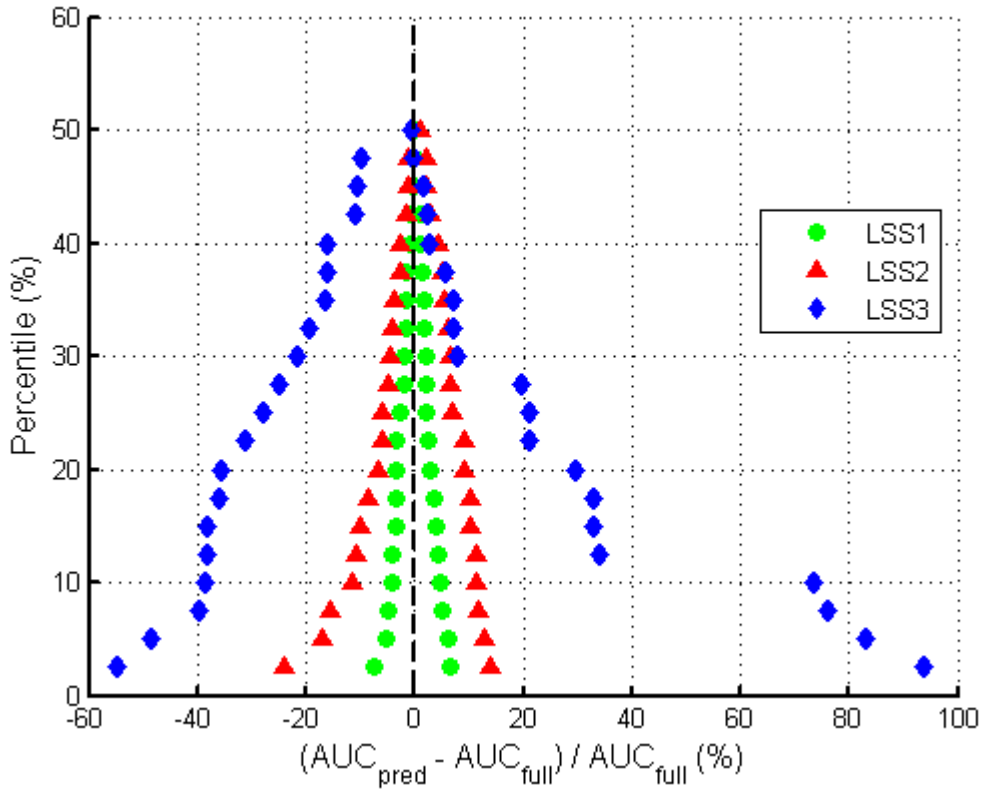

Figure 1.8: Mountain plots analyzing agreement between the full AUC and predicted AUC for the same three LSS evaluated in Figure 1.7.

7 Hematopoietic stem cell transplantation (HSCT)

Hematopoietic stem cell transplantation (HSCT) is usually performed after an intensive myeloablative treatment which leads to severe or complete depletion of bone marrow cells. This transplantation consists of an intravenous (IV) infusion of stem cells

to restore an appropriate hematopoietic function in patients with bone marrow or immune system disorders. HSCT can be autologous or allogenic, according to the source of the transferred stem cells [34, 35].

For patients who have no demonstrable malignancy in the blood or bone marrow (ex. patients with solid tumors), autologous transplantation is performed and the previously collected patient's stem cells are used as a rescue therapy after a medical intervention involving a high-dose myeloablative treatment.

Allogenic transplantation, which refers to the use of stem cells from a donor other than the treated patient, is used in many malignant and nonmalignant disorders in order to replace the defective hematopoietic system in the host with a normal one from the donor. The degree of HLA match between the host and the donor is a critical issue in determining the transplantation outcomes; well-matched transplants decrease the risk of graft rejection and medical complications.

There were more than 50 000 first HSCT reported from 1 327 centers in 71 countries in 2006, according to a global survey conducted by the Worldwide network for blood and marrow transplantation. These transplantations were mainly performed in America (36%) and in Europe (48%), Figure 1.9. Medical indications were lymphoma (54%), leukemia (34%), solid tumors (6%), nonmalignant disorder (5%) or, others conditions (1%) [34]. HSCT remains associated with significant morbidity and mortality and represents one example of high cost and highly specialized medicine.

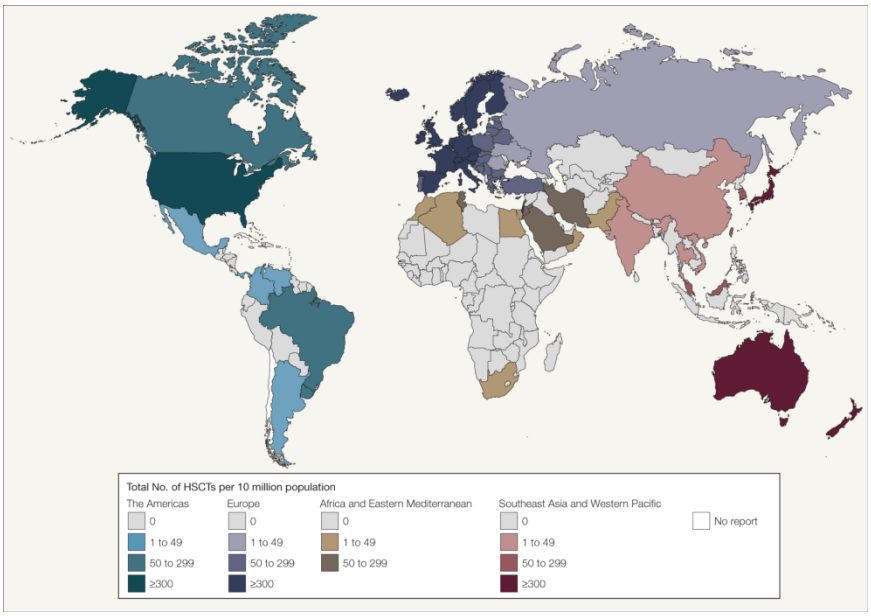

Figure 1.9: Global distribution of hematopoietic stem cell transplantations in 2006, adopted from Gratwohl et al. [34] with permission.

8 Graft-versus-host disease (GVHD)

Graft-versus-host disease (GVHD), which results from an indesirable immunological attack from the transferred immuno cells (particularly T-cells) on recipient organs or tissues, is the most important complication of allogeneic HSCT and is a major cause of morbidity and mortality in these patients [35]. Clinical manifestations involved the skin, the intestinal tract and the liver with GVHD classified into four grades (I-IV) depending on the degree of involvement of these three organs. The symptoms include dermatitis, cutaneous blisters, crampy abdominal pain with or without diarrhea, persistent nausea and vomiting, and hepatitis; other organ systems may be involved. GVHD is classified as acute when the symptoms manifest before 100 days after the transplantation and as chronic otherwise [36].

Acute GVHD (namely the grades II-IV) may occur in 35%–75% of allogeneic HSCT recipients [37]. While patients with grade II can have more than 80% probability of long-term survival, those with grade III and IV have a remarkably poor prognosis despite therapeutic interventions, with a long-term survival of 30% and 5%, respectively [37, 38].

Chronic GVHD may occur in 30–65% of allogeneic HSCT recipients. For survivors who were disease-free two years after transplantation, this complication was the main cause of death, accounting for 66% of deaths in those who received a transplant for aplastic anemia, and was the second main cause of death (after disease relapse), accounting for 23-36% of deaths in those who received a transplant for leukemia [36, 39].

GVHD can occur even when the donor is a 'perfectly' matched (HLA-identical) sibling, however, increasing levels of HLA-mismatching increase the probability and the severity of the disease. If the prophylaxis is not provided, serious acute GVHD affects almost every recipient. Steroids are the first line of treatment, but patients with steroid-refractory acute GVHD have long-term mortality rates that can reach 90% [36]. Hence, the GVHD prophylaxis is a standard of care for HSCT patients.

The prophylaxis is based on the peri- and post-transplant administration of immunosuppressive agents to protect the host from the donor's transferred immuno cells. Early studies showed the advantage of a combination prophylaxis using the calcineurin inhibitor, cyclosporine A (CsA), in combination with methotrexate over methotrexate alone [40]. Alternative combinations such as tacrulimus and methotrexate were investigated and a reduction in the incidence of acute GVHD was reported,

nonetheless there was no significant difference in the incidence of chronic GVHD as well as the overall and relapse-free survival rates [41-43]. In practice, the combination including CsA and methotrexate is the typical prevention regimen and is used routinely in most transplantation centers. Methotrexate is administrated intravenously on day 1, 3, 6, and 11 after HSCT, and CsA is administrated by continuous IV infusion from one day before transplantation until its oral (PO) administration can be tolerated. CsA dose is adjusted according to its blood concentrations as well as serum creatinine levels to ensure efficacy and avoid renal toxicity. CsA is continued PO for 6-8 weeks and then tapered gradually and stopped 6 months after HSCT in stable patients [40, 43].

9 Cyclosporine A (CsA)

CsA is mainly used as an immunosuppressant agent, and it is also prescribed as disease-modifying antirheumatic drug. Therapeutic drug monitoring is recommended for CsA dose adjustment because of its large PK variability and small therapeutic index [2, 12, 13]. CsA is a typical example of immunosuppressive agents for which LSS are widely developed to estimate AUC, particularly in solid organ transplantation [13]. Recent studies support using AUC for CsA monitoring in pediatric HSCT recipients [44, 45]. CsA served as a drug model in the LSS investigations carried out in this thesis.

This drug is a cyclic polypeptide of 11 amino acids that is poorly soluble in water; the chemical structure of CsA is illustrated in Figure 1.10.

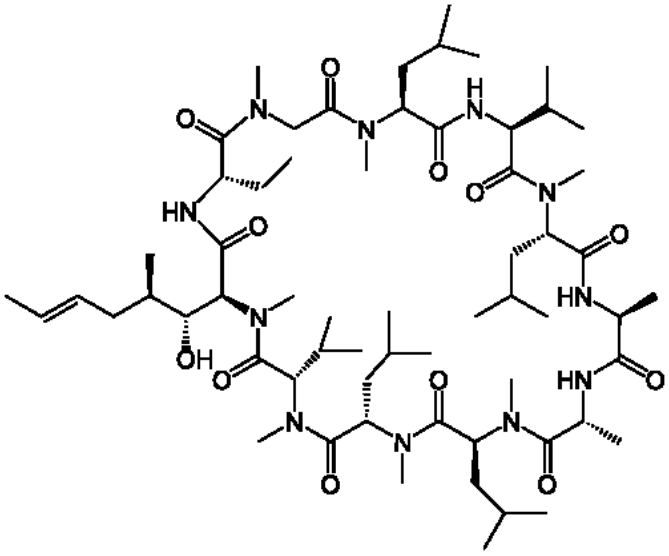

Figure 1.10: The chemical structure of cyclosporine.

9.1 Development

CsA was discovered in Sandoz Laboratory in Basel, Switzerland in 1972. The other immunosuppressive agents, which were available at that time such as methotrexate, had significant toxicities, particularly due to their remarkable cytotoxic effects. Therefore, CsA, for its ability to suppress the immuno system without disturbing other cells significantly, was a revolution in organ transplantation medications and has become a cornerstone in immunosuppressant therapy. CsA is efficient in both suppressing the humoral- and cell-mediated immunity and does not cause bone marrow suppression. Hence, the elimination of anemia, leukopenia, and thrombocytopenia as adverse effects made CsA an interesting alternative immunosuppressive drug, particularly in HSCT recipients.

CsA was originally brought to market under the brand-name Sandimmune®, which is available as soft gelatin capsules, oral solutions, and formulations for IV

administration. However, CsA has a very poor solubility in water, so Sandimmune® formulations are non-aqueous and exhibit variable absorption. Neoral® is a newer microemulsified form of CsA that is better absorbed [46]. Conventional (nonmodified) Sandimmune® and modified formulations (Neoral®) are not bioequivalent and should not be used interchangeably without close medical supervision. Neoral® is available as a solution and as soft gelatin capsules for PO administration (Figure 1.11).

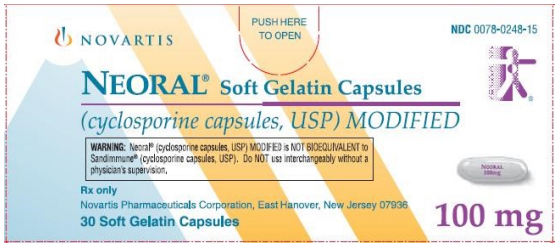

Figure 1.11: Package label for 100 mg Neoral® capsules.

9.2 Mechanism of action

The biochemical effect of calcineurin inhibitors, such as CsA, is ultimately the inhibition of T-cell activation [16, 47]. Calcineurin is a calcium/calmodulin-dependent protein phosphatase. Binding of foreign antigens to receptors on the T-cell surface leads to several signaling pathways, which in turn increase intracellular calcium concentration [Ca^{2+}]. Increased [Ca^{2+}] activates calmodulin and calcineurin B, which then activate the catalytic subunit of calcineurin called calcineurin A. This calcineurin dephosphorylates the nuclear factor of activated T-cell (NFAT) and permits this transcription factor to enter the nucleus (Figure 1.12). Then, it can influence synthesis and release of lymphokines particularly the interleukin-2 (IL-2), which is a regulator of T-cell proliferation and differentiation. The principal antagonistic action of CsA is binding to

the catalytic subunit of the calcineurin complex, leading to decreased dephosphorylating of NFAT, and so decreasing T-cell proliferation and growth [16, 47].

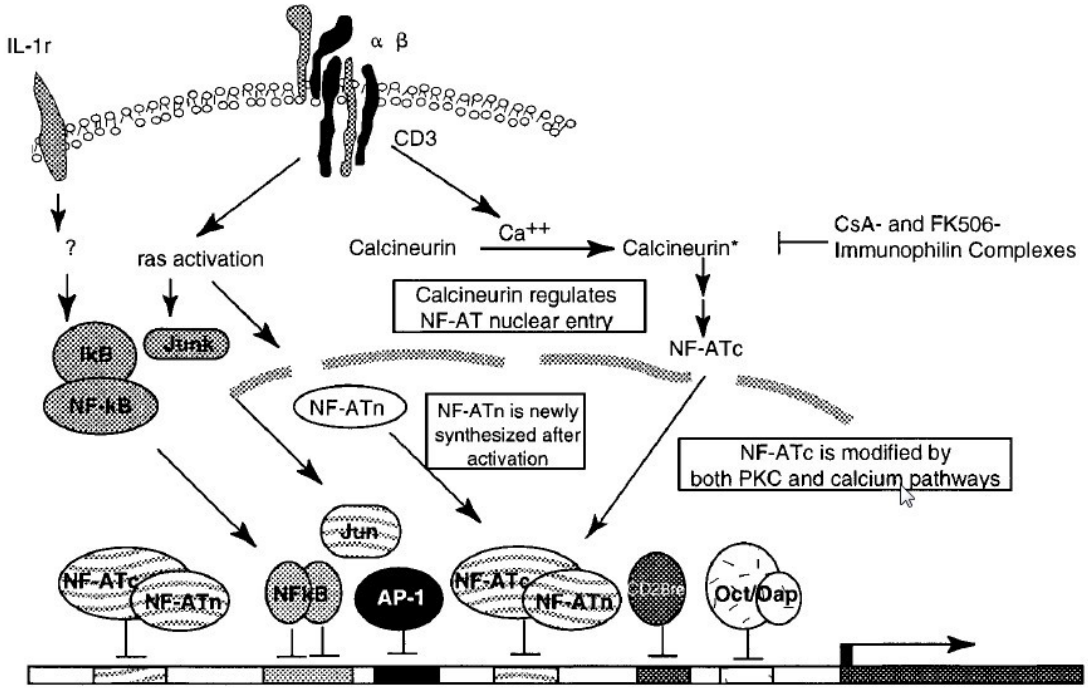

Figure 1.12: Schematic representation of a signaling pathway in which an increase in intracellular calcium leads to triggering the phosphatase activity of calcineurin, adopted from Ho et al. [47] with permission.

9.3 Clinical use

CsA is a cornerstone agent for the prophylaxis of graft-versus-host disease in HSCT patients. It is also indicated for the prophylaxis of organ rejection in kidney, liver, and heart allogeneic transplants. In addition, it can be prescribed alone or in combination for rheumatoid arthritis and psoriasis when first-line therapies have not adequately provided satisfactory outcomes [16].

9.4 Pharmacokinetics

9.4.1 Absorption

Following PO administration CsA absorption is variable, and it undergoes extensive first-pass metabolism. Modified oral formulations (Neoral®) have greater bioavailability than the conventional oral formulations (Sandimmune®) [46, 48-50]. Neoral® capsules are bioequivalent to Neoral® solution [51]. Generally, food affects CsA absorption and causes a small (< 20%) but consistent decrease in bioavailability [46]. however, the Neoral® solution can be mixed with fruit juice without affecting the rate or extent of CsA absorption [51, 52]. In HSCT recipients, graft-versus-host disease can result in diffuse inflammation that affects intestinal integrity which may cause reduction and delay in CsA absorption [53, 54].

9.4.2 Distribution

CsA is widely distributed into body fluids and tissues; most of the drug is distributed outside the blood volume and accumulates in fat-rich organs, including adipose tissue and liver [55, 56]. In the blood, it is extensively distributed in erythrocytes in a saturable manner that depends on hematocrit, temperature, and the concentration of plasma proteins. Whole blood concentration was roughly twice the serum concentration when blood was separated at 37 degrees Celsius [57]. In the plasma, it binds to proteins, mainly to lipoproteins and, to a lesser extent, to albumin. The unbound fraction of CsA in plasma is approximately 2% [55, 56]. Schematic overview of CsA distribution and fate is shown in Figure 1.13.

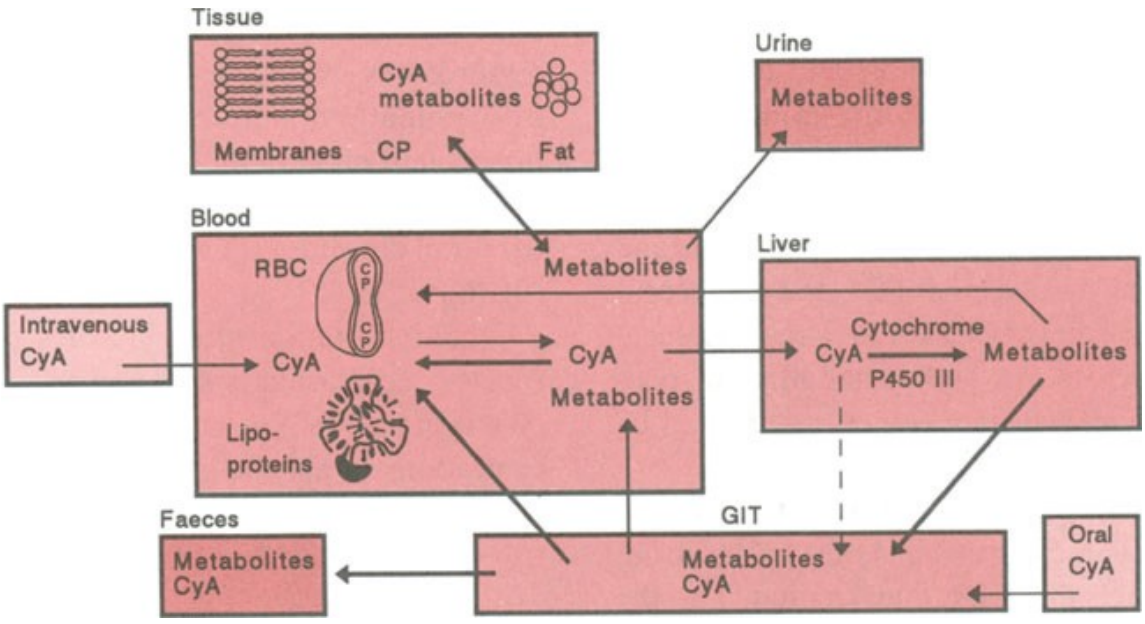

Figure 1.13: Schematic overview of the distribution and the fate of cyclosporine and its metabolites in the body. RBC refers to red blood cells; CP, cyclophilin; CyA, cyclosporine; GIT, gastrointestinal tract; adopted from Faht et al [56] with permission.

9.4.3 Metabolism

CsA is mostly metabolized in the liver, principally by CYP3A, primarily CYP3A4, and to a less extent in the GI tract and the kidney [58, 59]. Hence, CsA has a high possibility of significant and multiple drug interactions, particularly with other drugs metabolized though CYP3A4 [56].

9.4.4 Elimination

CsA is excreted almost entirely as metabolites, with less than 1% appearing unchanged in the urine or feces. Elimination is primarily via bile with less than 5% excreted in the urine. The terminal half-life averages 8–27 hours (range: 4–50 hours). In

HSCT recipients, the clearance is higher in comparison to solid organ transplantation [53, 54, 60].

9.5 AUC monitoring and clinical outcomes

In HSCT patients, high exposure of CsA is associated with leukemia relapse. On the other hand, low exposure increases the risk of graft-versus-host disease and the related mortality [61-63]. AUC describes better the bioavailable dose than single concentration-time point such as C_0 and should correlate to a greater degree with the above-mentioned clinical outcomes.

In pediatric HSCT, accumulating evidence supports performing CsA monitoring using AUC [44, 45]. A recent study has shown that steady state AUC of CsA was the PK index that correlated best with an anti- graft-versus-host disease effect; in addition, AUC values were significantly different in patients with diverse graft-versus-host disease grades [44]. In another study performed in pediatric HSCT patients diagnosed with acute myeloid leukemia, although no correlation was observed between CsA AUC and the incidence of acute graft-versus-host disease, AUC values below 3000 ng × h/mL were significantly associated with better survival rates and a lower risk of relapse while C_0 was not linked with these outcomes [45].

Moreover, in the setting of solid organ transplantations in several studies, AUC has been shown to be a good predictor of clinical outcomes such as acute and chronic rejection, graft survival, and nephrotoxicity [11, 15, 64-72].

Grant et al. studied the relationship between C_0, the maximum concentration (C_{max}), and AUC and graft rejection rates during the first month after transplantation, in *de novo* liver transplant recipients. On postoperative days 5 and 10, for Neoral® formulation, they found a significant inverse relationship between graft rejection and C_{max} and AUC. No such relationship was shown for C_0 [64].

Mahalati et al. found that AUC values were significantly lower in patients with acute renal rejection and significantly higher in patients with nephrotoxicity; nevertheless, 44% and 53% of patients with acute rejection and with nephrotoxicity, respectively, had C_0 within the therapeutic range [65].

Citterio et al. defined a cut-off concentration point at steady state (AUC/dose interval) = 450 ng/mL to discriminate patients who may be at risk for chronic renal rejection; nonetheless, those patients had similar C_0 values; additionally, 18% of the patients had an average concentration less than the cut-off point [66].

Selen et al. examined several PK parameters as predictors of patient, graft, and rejection-free survival rates in early renal transplants; they found patients with an initial steady state (AUC/dose interval) ≥ 550 ng/mL had higher 1-year (88 %) and 6-year (66 %) graft survival rates than patients with steady state < 550 ng/mL, who had 1- and 6-year graft survival rates of 80 % and 59 %, respectively. Moreover, patients with steady state < 550 ng/mL had more severe rejection episodes than patients with steady state ≥ 550 ng/mL (grades II and III; 71 % vs. 50 %; P = 0.036). In contrast, the C_0 and C_{max} values did not correlate with patient, graft, or rejection-free survival rates [67].

Bowles et al. investigated whether CsA AUC provided more useful information than single CsA concentration-time points regarding renal allograft rejection. Their results demonstrated that the mean AUC was lower at the time of rejection (3821 ng.h/mL) than that of a matched group of non-rejecting patients (5479 ng.h/mL, $P \leq 0.02$). More interestingly, an AUC above 6400 h.ng/mL significantly differentiates rejection from non-rejection groups, whereas C_0 and C_{max} did not have such cut-off value [68].

Kaplan et al. showed that recipients of simultaneous pancreas/kidney transplants without repeated rejection had significantly higher AUC than did those with recurrent rejection: 4663 vs. 2454 ng.h/mL, $P < 0.05$ [69].

Grevel et al. confirmed that CsA concentration at steady state (AUC/dose interval) was significantly lower in patients suffering from renal rejection (with rejection mean = 127 ng/mL, without rejection mean = 163 ng/mL, $P = 0.027$). In addition, they demonstrated through a logistic regression analysis that the probability of rejection can be decreased by up to 40% if steady state concentrations never drop below 250 ng/mL [71].

Kasiske et al. found that renal transplant recipients with acute rejection within 2 or 4 weeks after study have 13-19% lower AUC ($P < 0.05$) compared to those who were rejection-free [72].

Kelles et al. pointed out that, in pediatric renal transplant recipients, AUC of patients with biopsy-proven CsA toxicity were significantly higher than those of patients without toxicity ($P < 0.05$) despite similar C_0 [73].

Even though several studies demonstrated a correlation between CsA AUC and clinical outcomes, further prospective trials are still needed to confirm whether AUC-based monitoring can further increase efficacy and avoid toxicity. However, evaluating the value of AUC as a marker for therapeutic drug monitoring of CsA is outside the scope of this thesis.

10 Pharmacokinetic modeling

Pharmacokinetics describes how the body affects a particular drug after administration through the mechanisms of absorption, distribution, metabolism, and elimination. Modeling generally involves the use of mathematical formulations and statistical approaches to describe and quantify observed data. PK models are used to describe the fate of a drug from its administration up to the moment at which it is completely eliminated from the body. In addition, PK model enables the simulation of new conditions such as changes in dose and posology, changes in absorption and elimination rate or extent, and the impact of drug interactions. Furthermore, applied with pharmacodynamic (PD) modeling, PK/PD analysis can be used to determine effective concentrations and to establish dosing regimens [74, 75].

10.1 Non-compartmental analyses

Non-compartmental analyses (NCA) do not involve the assumption of drug distribution in specified physiologic or virtual compartment(s). NCA is generally the preferred methodology when the main purpose of investigation is to estimate the drug's associated PK parameters, such as the maximum concentration (C_{max}), the time spent to reach it (t_{max}), elimination half-life, etc., since it requires fewer assumptions than compartment approaches.

Non-compartmental approach is based on the theory of statistical moments which is a mathematical concept explaining the distribution of data. Statistical moment approach was applied to PK analyses by the late 1970s [76, 77]. In PK modeling, statistical moments calculated from a set of concentration-time points correspond to an estimate of the true moments; much like the mean of a sample represents an estimate of the true mean of the entire population.

The first three statistical moments represent AUC (calculated from moment zero), mean residence time (first moment), and variance of residence time (second moment). Typically, only the first two moments are used in PK studies. The zero moment defines the AUC from time zero to infinity (AUC_{inf}) and relates the exposure of the drug to the concentrations as defined by the Equation 1.

$$AUC = \int_0^\infty C\,(t)dt \tag{1}$$

where C is the drug concentration and t is the time

AUC is typically calculated using the trapezoidal method that is performed by adding multiple small trapezoidal areas. Several approaches exist such as the linear and logarithmic trapezoidal rules that use the equations 2 and 3, respectively, to calculate AUC [78, 79].

$$AUC_{0-t_n} = \sum_{i=1}^{n-1} \frac{1}{2}(t_{i+1} - t_i)\ (C_{i+1} + C_i) \quad (2)$$

$$AUC_{0-t_n} = \sum_{i=1}^{n-1} \frac{(t_{i+1} - t_i)\ (C_i - C_{i+1})}{log(C_i / C_{i+1})} \quad (3)$$

AUC_{inf} is the sum of AUC from time 0 to the last measurable concentration (AUC_{0-t_n}) and the extrapolated AUC beyond the last measurable concentration to infinity ($AUC_{t_n\text{-}inf}$). The latter part is calculated as the last measurable concentration (C_{Last}) divided by the apparent terminal elimination rate constant (K_{el}).

$$AUC_{inf} = AUC_{0-t_n} + (C_{Last}/K_{el}) \quad (4)$$

For a drug with first-order elimination, K_{el} is calculated from the slope of the logarithm of the concentration-time curve during the apparent terminal phase of the PK profile.

The first statistical moment is involved in the measurement of the mean residence time (MRT) that shows how long drug molecules may spend in the body. MRT calculation is based on the area under the moment curve (AUMC) [80]. For a bolus IV administration, MRT is estimated by Equation 6.

$$AUMC = \int_0^{\infty} t\, C\,(t) dt \tag{5}$$

$$MRT = AUC / AUMC \tag{6}$$

AUMC has no physiological value and is simply a mathematical variable used to determine other PK parameters. In fact, the concentration-time curve, particularly its statistic moments AUC and MRT, can be used to obtain other useful PK parameters. For example, AUC is used to calculate the bioavailability (F) and the clearance (CL) as shown in the equations 7 and 8. These PK parameters, F and CL, measure the fraction of the drug administered dose that reaches the systemic circulation and the volume of blood or plasma from which the drug is removed per unit of time, respectively.

$$F = Dose_{IV} \times AUC_{PO} / Dose_{PO} \times AUC_{IV} \tag{7}$$

$$CL = Dose_{po} \times F / AUC_{po} \tag{8}$$

Another important PK parameter is the total volume of distribution at the study state (V_{ss}) that represent apparent volume into which the bioavailable part of the administered dose would have to distribute to achieve the measured concentration. This PK parameter is, usually, defined in compartmental analyses. However, V_{ss} can be calculated using MRT without the assumption of a particular compartment model [81].

$$V_{ss} = CL \times MRT \tag{9}$$

Furthermore, other PK parameter such as C_{max} and t_{max}, which correspond to the rate and extent of absorption, are usually drawn directly from the concentration-time curve without any interpolation of the data.

10.2 Compartmental approach

Compartmental PK models are rather simple mathematical schemes that represent complex physiologic spaces or processes. Compartmental models consist of hypothetical compartments representing the body or different components of the body and are used to explain how the drug is absorbed, distributed, and eliminated.

In empirical PK analysis, each compartment represents a virtual volume in which a drug has homogeneous distribution. Multiple compartments with rational scheme and connections can be proposed to explain complexes PK features. Movement between compartments is described by rate constants that are, usually, labeled as K_{ij} (where i and j are the connected compartments), Figure 1.14.

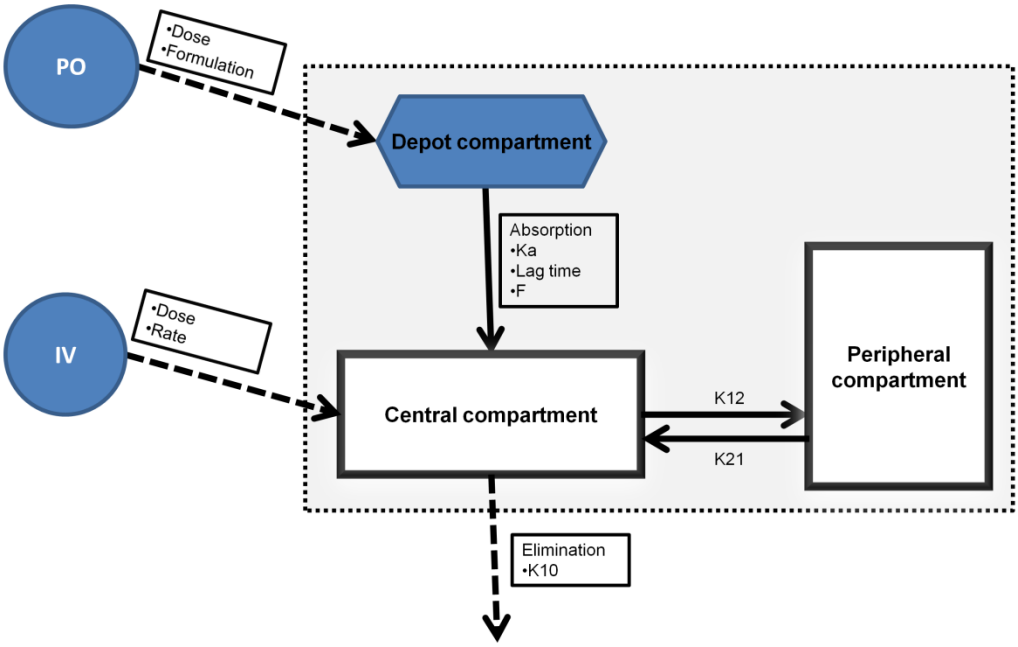

Figure 1.14: Typical scheme of two compartment PK model.

Highly infused tissues such as the liver and kidneys are generally considered as one compartment (central compartment) in equilibrium with the systemic circulation. More poorly attained tissue as adipose tissue and skin may be considered to form a peripheral compartment. Central compartment can act as the depot and elimination one or it can be connected to other compartments where those processes take place. Transfers between these compartments can be of one or two directions with rate of order 0 (active transport with constant speed), order 1 (passive diffusion), or nonlinear. To select the best fitting for PK data, several models are tested, and the choice of the best model is based on visual and statistical criteria.

In general, the PK model to estimate the PK parameters of an individual 'i' can be written as:

$$Y_{ij} = f(\theta_i, X_{ij}) + \varepsilon_{ij}$$

where Y_{ij} is the predicted value Y (ex. plasma concentraion) for the individual i at time j using the function f, which is a nonlinear function linking individual parameters θi (ex. CL and V), constants x_{ij} (ex. dose, sampling time), and Y_{ij}. The residual error ε_{ij}, difference between prediction and observation for the individual i at time j, represents measurement errors, model errors, etc. In individual approach, the estimation of PK parameters is based on individual PK profiles that are examined one by one, independently from each other.

10.3 Population pharmacokinetics (Pop-PK)

Population analyses of PK data including multiple PK profiles can be performed using two main approaches: conventional two-stage approach and mixed effect modeling approach. In the two-stage approach, in the first stage, the parameters are determined in each individual separately using individual concentration–time profiles. In the second stage, the descriptive statistics of these individual parameters, notably their mean and variance, are estimated [82]. The main difference between this approach and mixed effect modeling approach is the method of estimating PK parameters. While two-stage analysis adopts an independent individual approach and requires a relatively intense and regular sampling for each profile, the later approach seeks to extract the PK parameters collectively from spare data without a requirement of regular or frequent individual sampling, Figure 1.15. As it is possible to achieve PK analysis from limited and irregular data collected in a large population, the mixed effect modeling approach is mainly useful during phases II and III clinical trials. It also allows performing PK

investigation in special populations as pediatric populations for which an intensive sampling is ethically restricted [74].

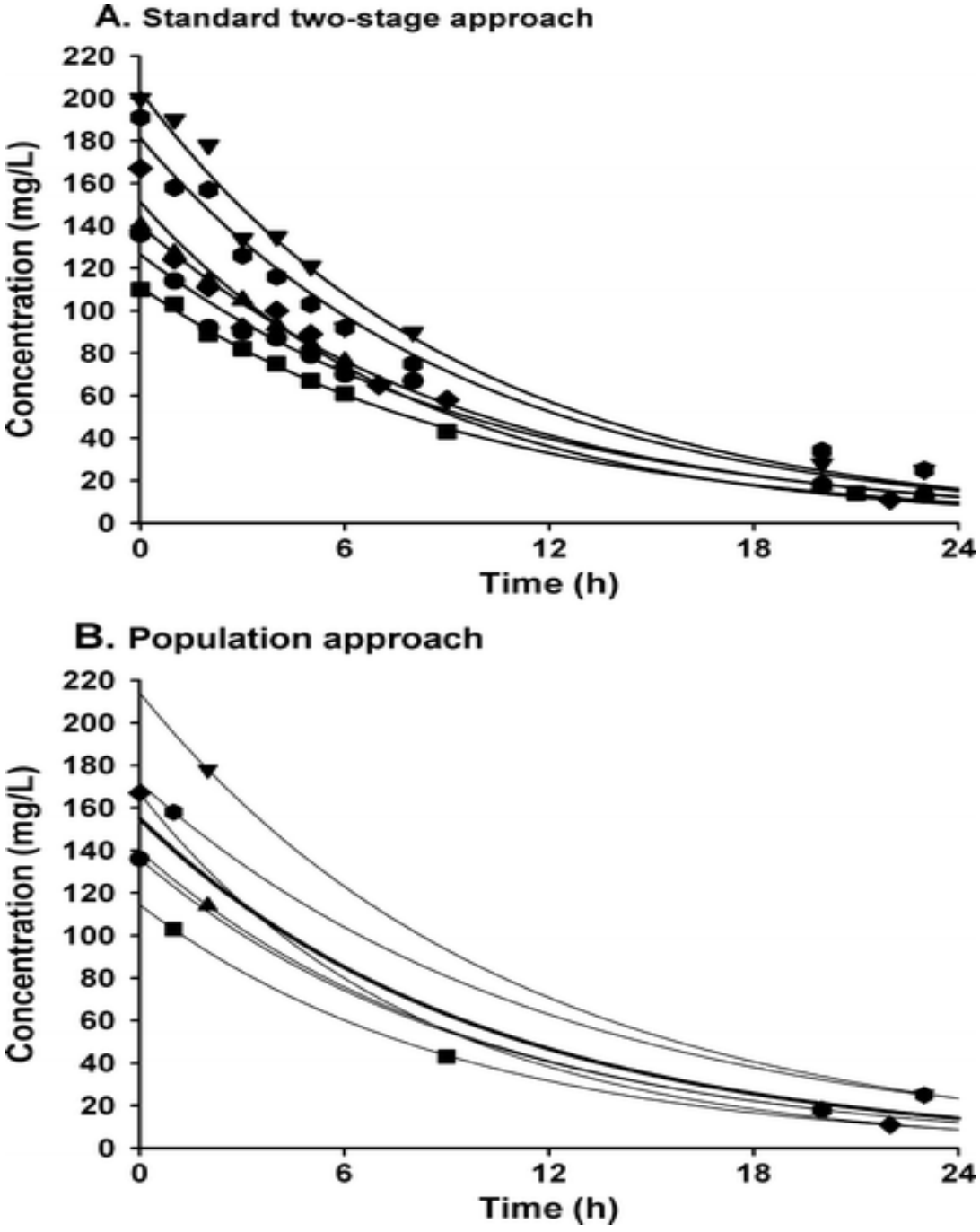

Figure 1.15: Concentration–time profiles of the same study using two different approaches. In (a) the standard two-stage approach is applied to a rich dataset. (b) shows

the population approach with mixed effect modeling applied to the same dataset using only two data points for each individual so that a sparse dataset is created. In (a), in each of the six individuals 10 samples are available. The different symbols correspond to different individuals. Each black line corresponds to a separate fit to the 10 data points of each individual. In (b), which uses the mixed effect modeling approach, two samples of the 10 per subject in (a) are used. The different symbols correspond to the six different individuals. The black line illustrates the concentration–time plot based on the population mean values of the PK parameters (PRED). The grey lines show the plots of the individual patients, which are based on the population mean values together with the measured concentrations of the specific individual (IPRED), adopted from De Cock et al. [74] with permission.

Pop-PK analyses are typically performed using nonlinear mixed effect models (NONMEM) proposed by Sheiner and Beal since 1972 [83, 84]. In this approach, PK parameters may be considered as random variables whose typical value and distribution (variability) are estimated. Hence, the PK profiles of a drug in a given population are modeled using fixed effect parameters that define the typical values and random effect parameters that describe the variability of the PK parameters in the population. These parameters are included in the structural and statistical (error) parts, respectively, of the mixed effect model. However, some PK parameters may not have variability (or are supposed without variability) because the available data do not allow to estimate its variability. For example, the variability in absorption lag time cannot be determined if the data do not include early samples to detect individual distinctions in this parameter. The structural part of the model also includes independent variables (ex. dose, time,

infusion rate) and additional variables or covariates (ex. weight, creatinine clearance) that belong to the fixed effect category. The covariates are added into the model to explain a fraction of the variability of PK parameters.

The main feature of the Pop-PK approach is that it quantifies various levels of PK variability while seeking to describe their sources. This variability may be inter-individual, intra-individual, and residual [74]. The source of variability may be related to demographic factors (age, weight, sex, race, morbidity, etc.), environmental (smoking, diet, compliance, co-medication, etc.), genetic (genetic polymorphism of enzymes and receptors, etc.), physiological (circadian cycle, activity, etc.), or other.

Inter-individual variability (η_i) corresponds to the difference between the typical parameter of a population and that of an individual: $\eta_i \sim N(0, \omega^2)$, ω^2 quantifies the inter-individual variability. It is also possible to consider an intra-individual variability that corresponds to the variance over time of the parameter value in an individual 'i', Figure 1.16.

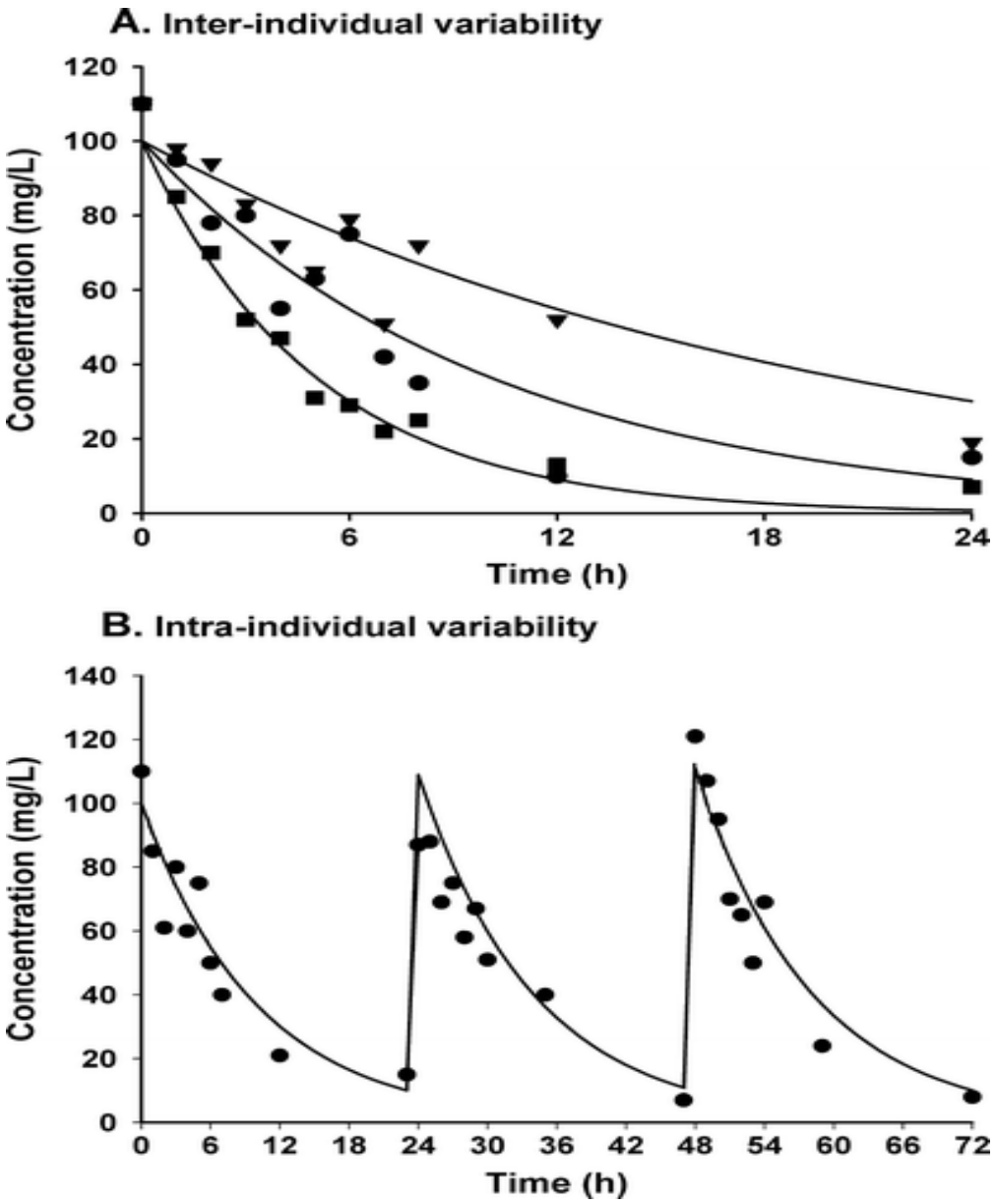

Figure 1.16: In (a), the inter-individual variability among three individuals who received the same dose is shown. (b) presents the intra-individual or residual variability by showing the concentration–time profile after repeated administration. Both these random variables are assumed to be normally distributed with a mean of zero and a variance of ω^2 or σ^2 respectively, adopted from De cock et al. [74] with permission.

The residual variability (ε) refers to the unexplained variability in the observed data after considering other sources of variability. It is associated, for example, with measurement errors, model errors, etc. It quantifies the difference between the value predicted by the model and the observed value: $\varepsilon \sim N(0, \sigma^2)$. In general, the equation of a Pop-PK nonlinear mixed effect model is:

$$C_{ij} = f(\theta, n_i, X_{ij}, Z_i, \varepsilon_{ij})$$

where C_{ij} is the predicted concentration in subject i at time j, f is compartmental model, θ vector of typical population parameters, η_i vector of inter-individual variability associated with θ, X_{ij} vector of independent fixed effect parameters including dose, sampling time, etc., Z_i vector of selected covariates, and ε_{ij} residual variability. Often the residual variability includes intra-individual variability.

10.3.1 Model optimization and selection criteria

The parameters of Pop-PK models are estimated so that the model predictions are most likely similar to available observed data. This similarity is, usually, assessed using maximum likelihood approaches. In this context, NONMEM® software uses quasi-Newton algorithm and an extended least square method to minimize a model performance evaluation criterion called objective function value (OFV) which is considered as the cornerstone criterion for model construction, development, and comparison [85].

Even though, other graphical and statistical methods are commonly used during modeling procedure, they are used often as complementary and secondary decisive

factors [86]. For example, in covariate analysis, forward inclusion backward exclusion method is communally used [85, 87]. In this method, OFV is lonely used as a criterion in the statistical tests performed to evaluate if the enhancement (or deterioration) of a model, observed after adding (or removing) a covariate, is statistically significant. However, the application of this method is habitually preceded by covariate-parameter plots and clinical evaluations as a complementary procedure to ensure a more selective and rational inclusion of plausible covariates and consequently ending up with a clinically meaningful and useful Pop-PK model.

11 Primary hypothesis

LSS can be used as a convenient approach to accurately estimate AUC in clinical settings. However, identifying reliable LSS requires the use of a well-established methodology that addresses the crucial development and application issues of LSS.

12 Objectives

12.1 General objective

This research aims to establish a comprehensive methodology for the development and selection of LSS for convenient and accurate estimation of AUC. Furthermore, critical questions regarding LSS development and use are studied and discussed. These questions include among other: the choice of the best Pop-PK model for B-LSS application and the effect of sampling errors on R-LSS performance.

12.2 Specific objectives

1. Identify and validate practical and reliable R-LSS and B-LSS for the prediction of AUC following IV and PO CsA in pediatric HSCT patients;
2. Develop Pop-PK models for CsA in the pediatric HSCT population;
3. Select the best Pop-PK model for B-LSS application by considering its intended use for AUC prediction;
4. Evaluate the performance of B-LSS when targeting the prediction of different versions of AUC;
5. Investigate STD impact on the predictive performance of R-LSS in diverse STD scenarios.

These issues are investigated in the following chapters.

13 Presentation of involved articles

This thesis includes three articles that have been underwent peer-reviews and published in acknowledged scientific journals.

These articles address the objectives of this thesis and are presented in the following chapters. Namely, **Chapter 2 (Article I)** establishes a comprehensive methodology for the development and selection of practical and accurate R- LSS. In **Chapter 3 (Article II),** the outlines of this methodology are adopted for investigating B-LSS, as a frequently used Pop-PK approach for AUC estimation. The 'final' covariate model, developed using the standard diagnostic criteria, is usually implemented for B-LSS application without rational justification. Thus, the AUC prediction errors of several Pop-PK models, in addition to the 'final' one, are compared in order to identify the most adequate model for B-LSS application. Besides, the performance of B-LSS when targeting the prediction of different versions of AUC is evaluated. Finally, in **Chapter 4**

(Article III), in order to evaluate the reliability of R-LSS, the impact of STD on the estimation of AUC by R-LSS, as a major concern that delays R-LSS adoption in clinical practice, is thoroughly investigated. In addition, the integration of STD analysis. as an essential element in R-LSS development procedure to guide their selection and use in clinical setting, is proposed and discussed.

For the three articles, Sarem participated in the design of the study, conducted the analysis, interpreted and discussed the results, and drafted the manuscript. The agreements of all the coauthors, to include these articles in the present thesis, have been received. The title, authors, authors' participations, and publication journal for each article are listed below:

Article 1: Limited Sampling Strategies for Estimating Intravenous and Oral Cyclosporine Area Under the Curve in Pediatric Hematopoietic Stem Cell Transplantation

S Sarem, MSc, F Nekka, PhD, O Barrière, PhD, H Bittencourt, MD, M Duval, MD, P Teira, MD, E Haddad, MD, PhD, Y Théorêt, PhD ,A-L Lapeyraque, MD and C Litalien, MD

SS participated in study design, performed data analysis and interpretation, and drafted the manuscript. JL, FN, and CL participated in study design and coordination, data analysis and interpretation, and helped to draft the manuscript. OB helped to perform data analysis. YT and AL conceived the clinical study and helped to draft the manuscript and for acquisition of data. HB, MD, PT, and EH helped to review the manuscript and for acquisition of data.

Therapeutic Drug Monitoring (Published).

Article 2: Bayesian Approach Application for Cyclosporine Area Under the Curve Estimation Using Limited Sampling Strategies in Pediatric Hematopoietic Stem Cell Transplantation

S Sarem, MSc, J Li PhD, O Barrière PhD, C Litalien, MD; Y Théorêt, PhD, A-L Lapeyraque, MD, F Nekka, PhD

SS participated in study design, performed data analysis and interpretation, and drafted the manuscript. JL, FN, participated in study design and coordination, data analysis and interpretation, and helped to draft the manuscript. OB helped to perform data analysis. CL, YT and AL conceived the clinical study and helped for acquisition of data.

Theoretical Biology and Medical Modelling (Published).

Article 3: Impact of Sampling Time Deviations on the Prediction of Area under Curve Using Limited Sampling Strategies

S Sarem, MSc, F Nekka, PhD, I.S. Ahmed PhD, C Litalien, MD, J Li PhD

SS participated in study design, performed data analysis and interpretation, and drafted the manuscript. JL, FN, participated in study design and coordination, data analysis and interpretation, and helped to draft the manuscript. CL and IA participated in study design and data interpretation and helped to draft the manuscript.

Biopharmaceutics & Drug Disposition (Published).

Chapter 2 (Article I/ Therapeutic Drug Monitoring journal): Limited Sampling Strategies for Estimating Intravenous and Oral Cyclosporine Area Under the Curve in Pediatric Hematopoietic Stem Cell Transplantation

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Abstract

Background: The optimal monitoring strategy for cyclosporine (CsA) in pediatric hematopoietic stem cell transplantation (HSCT) patients remains unclear. Although there is a growing interest in the use of the area under the concentration-time curve (AUC), measurement of AUC in clinical settings is often impractical. The objective of this study was to identify and validate limited sampling strategies (LSS) for the prediction of CsA AUC following intravenous (IV) and oral (PO) administration in this population.

Methods: Sixty-eight pediatric patients who underwent HSCT and received CsA were investigated. Twelve hour pharmacokinetic profiles (n=138) performed per standard of care were collected. Weighted multiple linear regression was used to investigate all possible LSS consisting of four or fewer concentration-time points. Their predictive performance was evaluated by leave-one-out cross-validation and external validation by measuring the root mean squared relative error (RMSE%) and the 95th percentile of the absolute relative error (95th PAE%). Values less than 20% were considered clinically acceptable.

Results: Nine LSS (4 IV and 5 PO) convenient for clinical application proved to have clinically acceptable performance. Notably, LSS based on C_0, C_2, C_4 was found to be accurate for estimation of CsA exposure following both IV and PO administration with the 95th PAE% of 19.7% and 17.5%, respectively.

Conclusions: LSS using 3 or 4 concentration-time points obtained within 4 hours post-dose provide a convenient and reliable method to estimate CsA exposure in this population. These LSS may facilitate future research aiming at better defining the relationship between AUC and clinical outcomes.

Keywords: Pediatric hematopoietic stem cell transplantation (HSCT), Cyclosporine (CsA), Therapeutic drug monitoring (TDM), Area under the curve (AUC), Limited sampling strategy (LSS), Weighted multiple linear regression (MLR).

Introduction

Cyclosporine (CsA), a potent immunosuppressive agent, is widely used in hematopoietic stem cell transplantation (HSCT) for the prevention of graft-versus-host disease, which continues to be a major complication following allogeneic stem cell transplantation. Due to its narrow therapeutic index and large inter- and intra-individual variability, therapeutic drug monitoring has become a standard of care for CsA dosing optimization in order to prevent graft-versus-host disease while minimizing the occurrence of adverse effects and disease relapse (graft-versus-leukemia effect) [1, 2], although the optimal monitoring strategy remains unclear.

The most widely used approach for CsA monitoring employed in HSCT patients is to determine whole blood trough concentration (C_0) to adjust CsA dosing regimen. However, there is accumulating evidence indicating that C_0 is not a good marker to assess CsA exposure in HSCT, with growing interest in the area under the concentration-time curve (AUC) because it is generally considered to be the best indicator of drug exposure [3-7]. Moreover, in the setting of solid organ transplantations, AUC has been shown to be a better predictor of acute and chronic rejection, graft survival, and nephrotoxicity than C_0 [1, 8-15]. In pediatric HSCT recipients, one study has shown that CsA steady-state AUC was the pharmacokinetic (PK) index that correlated best with an anti- graft-versus-host disease effect; in addition, AUC values were significantly different in patients with diverse graft-versus-host disease grades [3]. In another study performed in pediatric HSCT patients diagnosed with acute myeloid leukemia, although no correlation was observed between CsA AUC and the incidence of acute graft-versus-host disease, $AUC_{0\text{-}12h}$ values below 3000 ng × h/mL were significantly

associated with better survival rates and a lower risk of relapse while C_0 was not linked with these outcomes [4].

Even though the above findings support the use of AUC-based CsA therapeutic drug monitoring, measurement of full AUC in clinical settings is often impractical, particularly in children, as it necessitates obtaining multiple blood samples over the entire dosing interval. Alternatively, limited sampling strategies (LSS) allow reliable AUC estimation using a restricted number of samples drawn within a short period after drug administration. Several LSS have been reported for CsA AUC prediction in pediatric solid organ transplant recipients [2, 16]. However, they cannot be used in HSCT patients since they have not been validated in this transplant population. Moreover, HSCT recipients have different pharmacokinetics compared to solid organ transplant recipients; absorption is diminished and delayed [17], and clearance is higher [18]. In addition, intestinal integrity of HSCT recipients is often disrupted by the development of graft-versus-host disease and mucositis of the intestinal tract, resulting in diffuse inflammation which can affect intestinal absorption of CsA [17].

Recently, several groups have developed LSS to predict CsA AUC in adult HSCT [19-22]. Nevertheless, LSS remain to be specifically developed and validated for pediatric HSCT patients. The pharmacokinetics of CsA differ between adult and pediatric HSCT populations; children exhibit faster systemic clearance (normalized by body weight) of the drug and require higher CsA doses to achieve similar target blood concentrations [17, 18, 23, 24]. To date, a few studies have developed LSS for the prediction of CsA AUC in pediatric HSCT recipients [7, 25, 26]. Of these studies, two investigated pediatric patients who received CsA by intravenous (IV) infusion [7, 25]. Although these investigations reported regression LSS (R-LSS) that performed well, they required samples to be drawn within eight hours post-dose,

which may impede their use outside of a research environment. Only one study developed LSS based on a Bayesian approach (B-LSS) for orally administrated (PO) or IV administered CsA [26], but this investigation failed to perform proper validation with an independent set of data.

Therefore, the objective of this study was to identify and adequately validate practical R-LSS for AUC estimation of CsA following IV or PO administration in a pediatric HSCT population. The study aimed to establish the most comprehensive methodology developed to date, based on three main approaches: 1) an exhaustive LSS evaluation; 2) the use of a validation method to address the self-fitting issue; 3) the use of rational criteria for the selection of the best LSS.

Materials and Methods

Study population and design

Pediatric patients (< 19 years) considered for inclusion in this retrospective study received IV (2-hour infusion) or PO CsA twice daily for graft-versus-host disease prophylaxis after undergoing HSCT from a sibling or unrelated donor/cord blood at the CHU Sainte-Justine, and had 12h PK profiles performed as per standard of care. This study was approved by the Institutional Research Ethics Committee of the CHU Sainte-Justine. Pharmacokinetic, demographic, and other clinical data were collected from patient medical records. The study population comprised two cohorts of patients, a development cohort (Cohort A) and an external validation cohort (cohort B). The development cohort included 25 pediatric patients (15 males and 10 females) over a 12-month period from August 2009 to August 2010 (Table

2.1). The external validation cohort included 47 pediatric patients (31 males and 16 females) over a 24-month period from September 2010 to September 2012. The median age of Cohorts A and B patients at transplantation was 10.2 years (range 0.5 – 18.2) and 9.7 years (range 0.2-18.5), respectively. Four patients were included in both cohorts; their PK profiles performed between August 2009 and August 2010 were included in Cohort A and the following ones in Cohort B.

Table 2.1. Summary of patient characteristics for development cohort (Cohort A).

Parameter	**Number or median (range)**	
	IV	PO
Patients (n)	19	20
Sex: male/female (n)	10/9	12/8
Age at transplantation (yr)	10.5 (1-18)	11.1(0.5-18.2)
Transplantation type: Sibling/Unrelated (n)	10/9	13/7
Qualified PK profiles (n)	23	39
Formulation (n)	23 (IV)	19 (Susp) /20 (Cap)
Time post transplantation (mth)	0.13 (0.1 – 1.7)	1.28 (0.7-9.1)
Age at PK profile (yr)	10.4 (1 – 17.9)	11.9 (1.2-18.3)
Weight (kg)	33 (10 – 81)	38 (8-83)
Cyclosporine dose (mg/kg/d)	2.5 (1 – 3.2)	4.2 (1-8.3)
Concomitant medication (n)		
Corticosteroid	13	26† †
Calcium channel blocker	12	26† †
Azole antifungal	18	18† †
Albumin (g/L)	32 (19 – 48)	32 (22-41) ‡
Creatinine (μmol/L)	33 (12 – 358)	50 (13-117) ‡
Bilirubin (μmol/L)	11 (5 – 64)	10 (3-596) ‡
AST (U/L)	20 (9-42)	24 (13-125) ‡
ALT (U/L)	24 (9 – 85)	31 (19-69) ‡
GGT (U/L)	35 (8-94)	32 (9-217) ‡
AP (U/L)	87 (1.9-203)	110 (53-302) ‡
Hb (g/dL)	93 (64-143)	87 (64-122) ‡
Hct (%)	25 (18-44)	26 (19-36) ‡

ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; Cap, capsule; Hb, hemoglobin; Hct, hematocrit; IV, intravenous; GGT, γ- glutamyltranspeptidase; n: number; PK, pharmacokinetic; PO, oral administration; Susp, suspension

††Data available for 37 profiles.

‡ Data available for 38 profiles.

Cyclosporine dose adjustment

Since 2010, the medical team at the CHU Sainte-Justine caring for HSCT patients moved from C_0- to AUC-based monitoring in light of controversy regarding the usefulness of dose adjustments based on CsA C_0 [6]. CsA dose adjustments were made by the treating physician in accordance with institutional target $AUC_{0\text{-}12h}$ values, which were defined based on published data from renal transplantation studies [27, 28] and one adult HSCT study [29]. These were adapted by the team according to the patient's underlying disease (three target ranges: $AUC_{0\text{-}12h}$ from 3000–5000 ng × h/mL for patients with malignant disease in whom a graft-versus-leukemia effect is desirable, $AUC_{0\text{-}12h}$ from 5000–7000 ng × h/mL for patients with sickle cell anemia who are at increased risk of seizures and in whom limitation of CsA neurotoxicity is desirable, and $AUC_{0\text{-}12h}$ from 7000–9000 ng × h/mL for patients with nonmalignant disease (e.g.: immunodeficiency syndromes, bone marrow failure syndromes) to optimize graft-versus-host disease prophylaxis). The correlation between these institutional targets and clinical outcomes has not yet been clearly established.

Sample collection and analytical methods

Serial blood samples were drawn before and at 2, 3, 4, 6, 8, 10, and 12 h after starting IV infusion (2-h infusion) and at 0.5, 1, 1.5, 2, 3, 4, 8 and 12 h after PO CsA administration. Concentrations were measured using ARCHITECTi2000SR® (Abbott Laboratories, Abbott Park, Illinois, USA). The lower and upper limits of detection were 30 and 1500 ng/mL, respectively. Blood samples with CsA concentrations > 1500 ng/mL were diluted with blank blood. The between-run coefficients of variation were 9.95% at 87 ng/mL, 8.64% at 340

ng/mL, and 9.25% at 850 ng/mL. All available steady-state PK profiles containing at least seven concentration-time points were incorporated in this study.

Cohort A included 23 IV and 39 PO profiles. Fourteen patients had both IV and PO profiles (18 IV and 26 PO profiles), 5 patients had only IV profiles (5 profiles), and 6 patients underwent only PO profiles (13 profiles). Cohort B included 40 IV and 36 PO profiles. Twenty-three patients had both IV and PO profiles (25 IV and 25 PO profiles), 13 patients had only IV profiles (15 profiles), and 11 patients underwent only PO profiles (11 profiles). The associated observed $AUC_{0\text{-}12h}$ (AUC_{obs}) were calculated according to the trapezoidal method. Individual CsA concentration-time profiles are shown in Figure 2.1 and Figure 2.2.

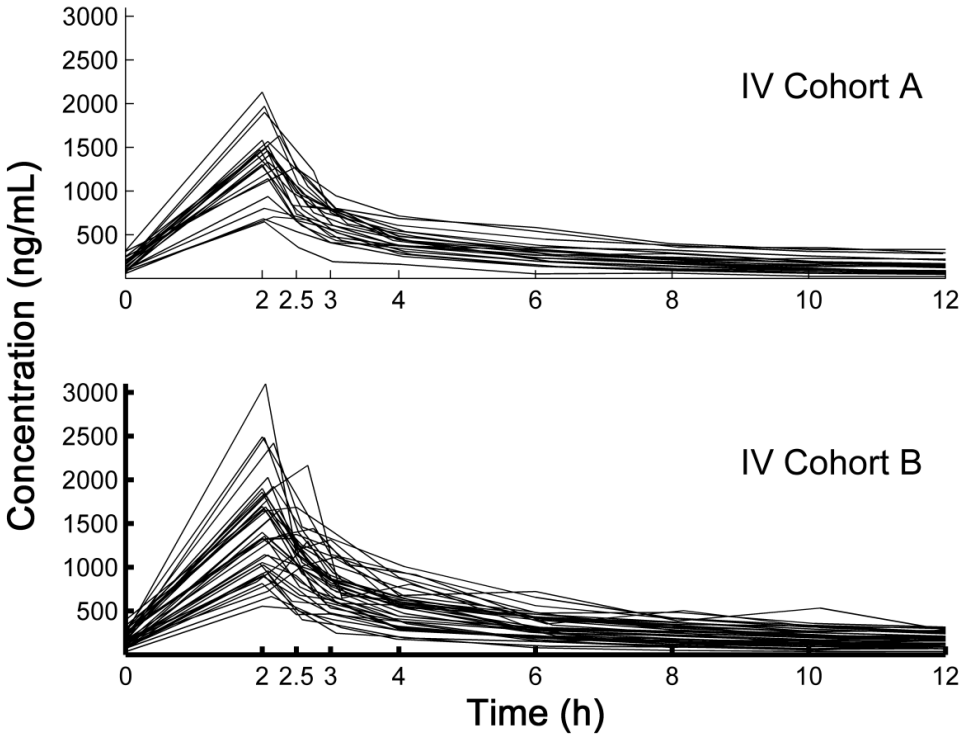

Figure 2.1: Concentration-time course for the available IV profiles of Cohort A (development cohort) and Cohort B (external validation cohort).

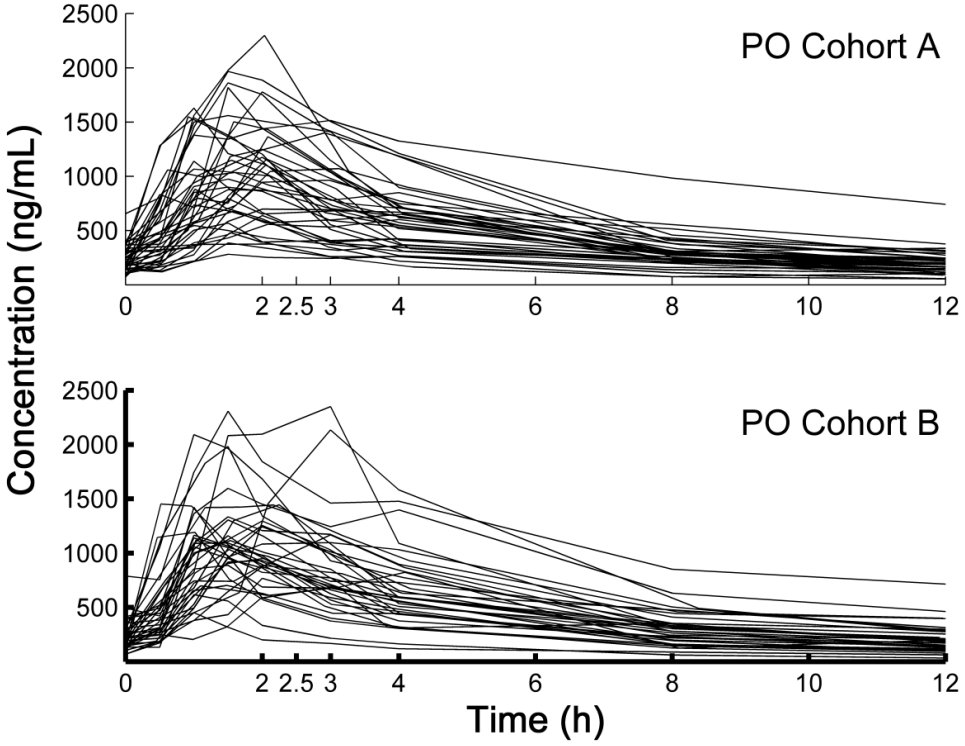

Figure 2.2: Concentration-time course for the available PO profiles of Cohort A (development cohort) and Cohort B (external validation cohort).

For PO administration, CsA concentration 2 hours after drug intake (C_2) was greater or equal to C_4 for 95 % of the profiles with a median C_4/C_2 ratio of 0.62 (0.29 – 1.35).

LSS development and validation

LSS development and validation were performed independently for IV and PO profiles through five steps: LSS definition, leave-one-out cross-validation (LOOCV), performance evaluation, selection, and external validation, as illustrated in Figure 2.3.

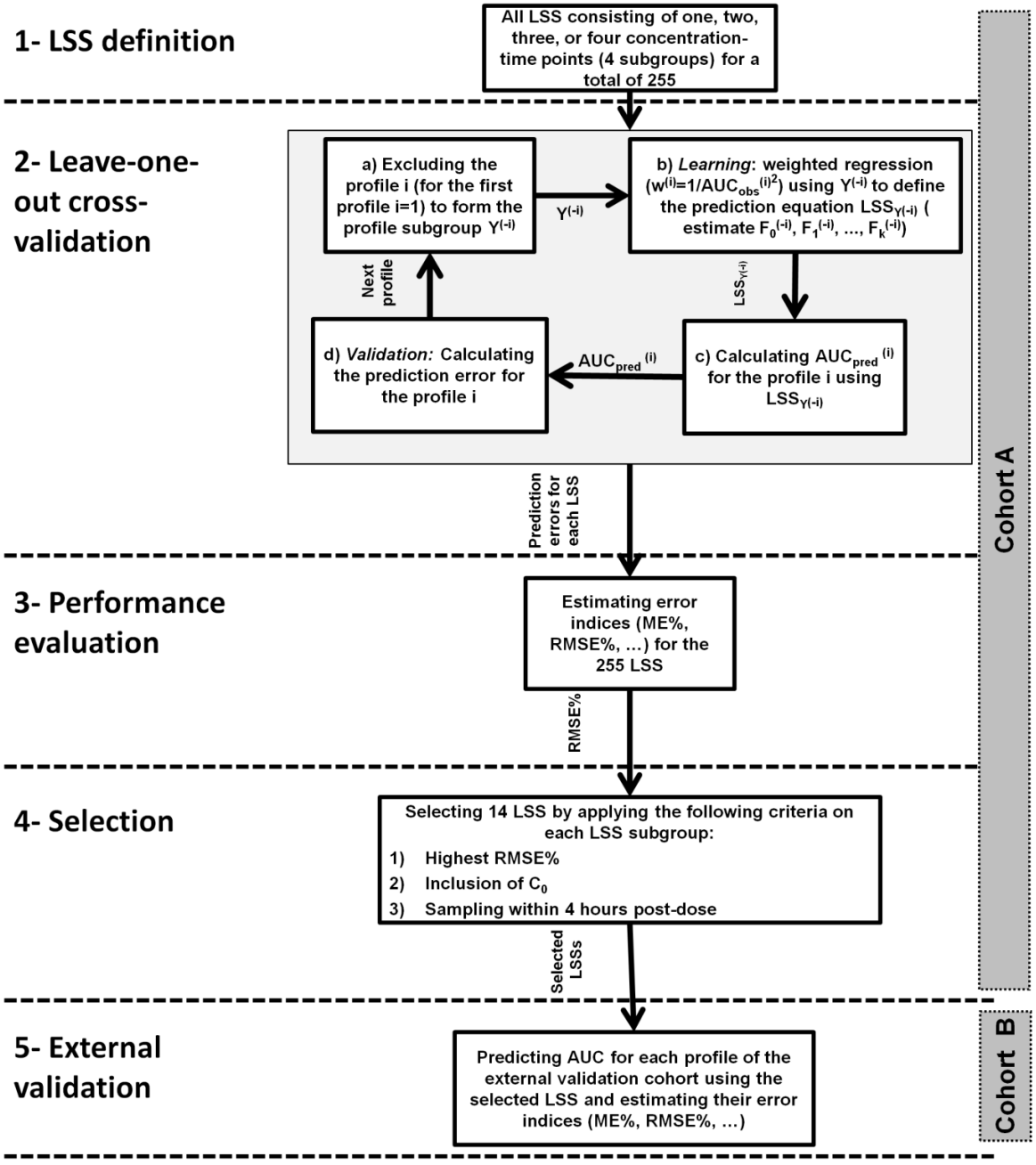

Figure 2.3: LSS development procedure; AUC_{obs}, observed AUC; AUC_{pred}, predicted AUC; F, fitting coefficients; $^{(i)}$ denotes the i^{th} profile; $(^{-i})$ denotes all profiles apart from the temporary excluded i^{th} one; ME%, relative mean prediction error; RMSE%, relative root mean squared prediction error; Y, subgroup of profiles.

Step 1: LSS definition

A 'limited Sampling Strategy' consists of a subset of all available concentration-time points used to predict the full AUC. For practical reasons, it was decided *a priori* to consider LSS consisting of a maximum of four concentration-time points. Thus, all LSS consisting of one, two, three, or four concentration-time points were considered (255 in total). The LSS were divided into four subgroups according to the number of concentration-time points. Weighted multiple linear regression (MLR) was used to model the linear relationship between LSS concentration-time points (independent variables) and AUC (dependent variable) in the form of:

$$AUC_{pred} = F_0 + F_1 \times C_{t_1} + \ldots + F_k \times C_{t_k}$$

where $C_{t_1}, C_{t_2}, \ldots, C_{t_k}$ are the concentrations obtained at times $t_1, t_2, \ldots, t_k$; $F_0, F_1, \ldots, F_k$ are fitting coefficients estimated using weighted MLR to reduce the deviation of predicted AUC (AUC_{pred}) from AUC_{obs} by minimizing the objective function:

$$O = \sum_{i=1}^{n} w^{(i)} \left(AUC_{pred}^{(i)} - AUC_{obs}^{(i)} \right)^2$$

where i denotes the i^{th} profile; n is the number of profiles; 'w' is the weight defined as $w^{(i)} = 1/AUC_{obs}^{(i)\,2}$. Thus, weighted MLR includes a weight w in the objective function which was chosen to take into account the relative value of the error. This prevents large relative errors in the prediction of small AUC values, which can occur with ordinary MLR.

Step 2: Leave-one-out cross-validation

Leave-one-out cross-validation was used as it allows validation (predictive performance evaluation) based on a relatively small dataset [30]. In this approach, for every

LSS, each profile is left out of the analysis in turn. This subgroup of profiles is symbolized as $Y^{(-i)}$ (I = 1,…, n), where $^{(-i)}$ denotes all profiles apart from the temporary excluded ith one. Then, the fitting coefficients $F_0^{(-i)}, F_1^{(-i)}, \ldots, F_k^{(-i)}$ are defined by performing weighted MLR on $C_{t_1}^{(-i)}, \ldots, C_{t_k}^{(-i)}$ and $AUC_{obs}^{(-i)}$. Consequently, $AUC_{pred}^{(i)}$ is calculated using the following equation $AUC_{pred}^{(i)} = F_0^{(-i)} + F_1^{(-i)} \times C_{t_1}^{(i)} + \ldots + F_k^{(-i)} \times C_{t_k}^{(i)}$. Hence, the prediction of $AUC_{pred}^{(i)}$ does not involve the knowledge of $AUC_{obs}^{(i)}$, to avoid self-fitting phenomena. This is repeated until each one of the n available profiles has been left out of the analysis on one occasion. Using this approach, AUC_{pred} was estimated for all profiles.

Step 3: Performance evaluation

Evaluation of the predictive performance of the 255 LSS was accomplished statistically through error indices and graphically with appropriate plots. For each LSS, the relative prediction error (E%) and 95th percentile of its absolute values (95th PAE%), mean relative prediction error (ME%) and root mean squared relative prediction error (RMSE%) were calculated; the last two indices evaluate bias and precision, respectively [31]. The following equations were used:

$$E\% = \frac{AUC_{pred}^{(i)} - AUC_{obs}^{(i)}}{AUC_{obs}^{(i)}} \times 100$$

$$ME\% = \frac{1}{N} \sum_{i}^{N} \frac{AUC_{pred}^{(i)} - AUC_{obs}^{(i)}}{AUC_{obs}^{(i)}} \times 100$$

$$RMSE\% = \sqrt{\frac{1}{N}\sum_{i}^{N}\left(\frac{AUC_{pred}^{(i)} - AUC_{obs}^{(i)}}{AUC_{obs}^{(i)}}\right)^2} \times 100$$

Step 4: Selection

Three LSS were chosen from each LSS subgroup based on the presence of the following three criteria: 1) highest predictive performance according to ME% and RMSE%; 2) inclusion of C_0 as it is a reference point well recognized by practitioners that allows compliance to be checked and aids the identification of patients with high CsA clearance, and 3) concentration-time points within four hours of CsA administration. Moreover, in each subgroup, the LSS with the highest predictive performance was also selected regardless of whether it met the last two practical criteria or not.

Step 5: External validation

The predictive performance of the selected LSS was further evaluated in an external dataset (Cohort B) using the errors indices described in step 3.

LSS considered suitable for clinical application were defined as those with RMSE% and 95th PAE% of less than 20% after both leave-one-out cross-validation and external validation. Modeling and computations were performed using MATLAB® (version 2008b, The MathWorks Inc, Natick, Massachusetts, U.S.A.).

Results

A total of 255 LSS derived from the development cohort (Cohort A) were considered in this investigation. As all had negligible bias following leave-one-out cross-validation (ME% less than 1.5% and 95% CI including 0), LSS selection was performed using RMSE% as the performance criterion along with the other two predefined criteria. Consequently, 28 LSS (14

for IV CsA and 14 for PO CsA) were selected, as shown in Table 2.2. The predictive performance of selected LSS, as determined by leave-one-out cross-validation, is presented in Table 2.3 and Table 2.4. The single concentration-time point LSS with the best performance involved C_4. LSS based solely on C_0 had a poor predictive ability with 95th PAE% of 67.11 and 80.11% for IV and PO CsA, respectively. More frequent and/or longer sampling resulted in better prediction. The best overall predictive performances were obtained with LSS that consisted of C_2, C_3, C_6, and C_8 and $C_{0.5}$, C_2, C_4, and C_8 with 95th PAE% values of 5.2 and 6.3% for IV and PO CsA, respectively.

Table 2.2. Selected LSS for cyclosporine $AUC_{0\text{-}12}$ prediction following IV and PO administration.

Concentration-time points	**LSS equation: AUC_{pred} (ng × h/mL)**
IV	
C_2, C_3, C_6, C_8	107.47 + 1.25 C_2 + 1.66 C_3 + 3.64 C_6 + 4.83 C_8
C_0, C_2, $C_{2.5}$, C_4	-509.54 + 4.01 C_0 + 1.23 C_2 + 0.83 $C_{2.5}$ + 5.51 C_4
C_0,C_2, C_3, C_4	-457.51 + 5.23 C_0 + 1.27 C_2 + 1.25 C_3 + 4.70 C_4
C_0, C_2, $C_{2.5}$, C_3	-529.35 + 8.55 C_0 + 1.19 C_2 + 0.71 C2.5 + 2.94 C_3
C_2, C_4, C_{10}	0.33 + 1.38 C_2 + 4.64 C_4 + 6.07 C_{10}
C_0, C_2, C_4	**-424.37 + 3.77 C_0 + 1.38 C_2 + 6.62 C_4**
C_0, C_2, C_3	-473.01 + 9.06 C_0 + 1.27 C_2 + 3.55 C_3
C_0, $C_{2.5}$, C_4	-311.49 + 2.79 C_0 + 1.82 $C_{2.5}$ + 7.29 C_4
C_2, C_6	297.00 + 1.67 C_2 + 8.22 C_6
C_0, C_3	-223.32 + 9.91 C_0 + 5.62 C_3
C_0, C_4	-32.99 + 1.80 C_0 + 10.67 C_4
C_0, $C_{2.5}$	-250.28 + 8.05 C_0 + 4.38 $C_{2.5}$
C_4	-50.65 + 11.35 C_4
C_0	1853.15 + 17.97 C_0
PO	
$C_{0.5}$, C_2, C_4, C_8	131.49 + 1.00 $C_{0.5}$ + 1.74 C_2 + 3.04 C_4 + 5.52 C_8
C_0, C_1, C_2, C_4	-45.58 + 4.78 C_0 + 0.99 C_1 + 1.40 C_2 + 4.16 C_4
C_0,$C_{0.5}$, C_2, C_4	77.53 + 3.85 C_0 + 1.05 $C_{0.5}$ + 1.81 C_2 + 4.13 C_4
C_0, C_1, C_3, C_4	57.53 + 3.77 C_0 + 1.45 C_1 + 2.18 C_3 + 3.33 C_4
$C_{1.5}$, C_4, C_8	75.66 + 1.87 $C_{1.5}$ + 3.46 C_4 + 6.12 C_8
C_0, $C_{1.5}$, C_4	-46.41 + 4.84 C_0 + 2.00 $C_{1.5}$ + 4.51 C_4
C_0, C_2, C_4	**141.82 + 5.20 C_0 + 2.16 C_2 + 3.71 C_4**
C_0, C_1, C_4	62.23 + 3.75 C_0 + 1.67 C_1 + 5.77 C_4
C_2, C_8	286.02 + 2.70 C_2 + 9.42 C_8
C_0, C_2	108.19 + 9.11 C_0 + 3.43 C_2
C_0, C_3	641.37 + 4.74 C_0 + 5.17 C_3
C_0, C_4	723.23 + 3.35 C_0 + 6.93 C_4
C_4	1007.22 + 7.78 C_4
C_0	1897.22 + 14.12 C_0

AUC_{pred}, predicted area under the concentration-time curve.

Table 2.3. Predictive performance of the selected LSS to estimate cyclosporine AUC following IV administration.

Concentration-time points	Leave-One-Out Cross-Validation (Cohort A)			External Validation (Cohort B)		
	ME% (95% CI)	RMSE% (95% CI)	95th PAE%	ME% (95% CI)	RMSE% (95% CI)	95th PAE%
C_2, C_3, C_6, C_8	-0.12(-1.23, 0.99)	2.51(1.56, 3.18)	5.19	-1.51 (-3.09, 0.06)	5.08 (3.46, 6.30)	11.58
C_0, C_2, $C_{2.5}$, C_4	0.13(-1.41, 1.68)	3.49(2.73, 4.11)	5.81	2.25 (-0.77, 5.28)	9.6 (6.55, 11.90)	21.65
C_0,C_2, C_3, C_4	0.18(-1.57, 1.92)	3.95(2.57, 4.96)	8.13	1.77 (-0.82, 4.36)	8.20 (4.36, 10.75)	15.47
C_0, C_2, $C_{2.5}$, C_3	0.11(-2.27, 2.49)	5.38(3.50, 6.76)	11.30	1.03 (-1.28, 3.34)	7.20 (5.20, 8.76)	15.60
C_2, C_4, C_{10}	-0.04(-1.58, 1.51)	3.50(2.39, 4.33)	6.63	0.08 (-1.62, 1.80)	5.29 (3.78, 6.46)	11.80
C_0, C_2, C_4	**0.11(-1.87, 2.10)**	**4.49(3.10, 5.54)**	**8.91**	**1.69 (-1.46, 4.84)**	**9.88 (6.18, 12.53)**	**19.73**
C_0, C_2, C_3	0.10(-2.35, 2.55)	5.54(3.49, 7.01)	12.09	0.61 (-2.20, 3.42)	8.70 (5.76, 10.87)	19.89
C_0, $C_{2.5}$, C_4	-0.01(-3.81, 3.78)	8.59(5.61, 10.77)	18.51	6.3 (0.51, 12.08)	18.94 (10.64, 24.58)	48.76
C_2, C_6	0.06(-2.21, 2.34)	5.15(3.28, 6.50)	10.36	-4.31 (-7.06, -1.55)	9.53 (6.58, 11.77)	21.99
C_0, C_3	0.12(-4.13, 4.37)	9.60(5.61, 12.37)	18.60	3.77 (-1.10, 8.64)	15.51 (9.69, 19.67)	34.44
C_0, C_4	0.41(-3.88, 4.70)	9.71(6.71, 11.98)	18.32	5.87 (-0.77, 12.52)	21.34 (9.16, 19.43)	51.95
C_0, $C_{2.5}$	0.64(-5.26, 6.53)	13.35(9.30, 16.43)	23.89	4.09 (-0.98, 9.17)	16.20 (11.07, 20.06)	38.26
C_4	0.24(-4.01, 4.50)	9.64(6.74, 11.84)	16.99	6.42 (-0.70, 13.155)	22.93 (9.39, 31.04)	57.15
C_0	0.98(-12.8, 14.75)	31.16(9.92, 45.17)	67.11	-6.64 (-12.47, -0.80)	19.20 (15.84, 22.06)	33.33

CI: confidence interval; C_{tj}: concentration at time t_j in hours post-dose; ME%: mean relative prediction error; RMSE%: root mean squared relative prediction error; 95th PAE%: 95th percentile of absolute values of relative prediction errors.

Table 2.4: Predictive performance of the selected LSS to estimate cyclosporine AUC following PO administration.

Concentration-time points	Leave-One-Out Cross-Validation (Cohort A)			External Validation (Cohort B)		
	ME% (95% CI)	RMSE% (95% CI)	95th PAE%	ME% (95% CI)	RMSE% (95% CI)	95th PAE%
$C_{0.5}$, C_2, C_4, C_8	0.01(-1.08, 1.10)	3.31(2.59, 3.90)	6.30	-2.43(-3.83, -1.03)	4.75 (3.18, 5.91)	9.54
C_0, C_1, C_2, C_4	0.06(-2.06, 2.19)	6.48(5.15, 7.57)	12.17	-0.13(-2.12, 1.86)	5.8 (3.56, 7.40)	11.83
C_0¸$C_{0.5}$, C_2, C_4	0.11(-2.15, 2.36)	6.87(5.12, 8.25)	13.70	-2.20(-4.40, 0.01)	6.78 (4.74, 8.33)	14.58
C_0, C_1, C_3, C_4	0.09(-2.31, 2.48)	7.30(5.79, 8.54)	13.44	3.42 (0.96, 5.88)	7.94 (5.07, 10.02)	19.7
$C_{1.5}$, C_4, C_8	0.05(-1.80, 1.90)	5.63(4.22, 6.74)	11.82	0.43(-2.20, 1.33)	5.16(3.77, 6.25)	10.25
C_0, $C_{1.5}$, C_4	0.15(-2.62, 2.92)	8.43(5.97, 10.32)	16.71	-0.33(-3.07, 2.41)	7.99(5.81, 9.68)	16.18
C_0, C_2, C_4	**0.17(-2.78, 3.11)**	**8.97(6.75, 10.73)**	**16.48**	**-3.15 (-5.96, -0.34)**	**8.78 (6.39, 10.65)**	**17.55**
C_0, C_1, C_4	0.16(-2.95, 3.27)	9.48(6.81, 11.55)	15.77	2.38 (-1.12, 5.88)	10.46 (5.78, 13.62)	24.03
C_2, C_8	0.15(-2.65, 2.94)	8.52(6.20, 10.34)	16.04	-4.59 (-8.17, -1.00)	11.42 (6.27, 14.88)	21.88
C_0, C_2	0.16(-4.41, 4.74)	13.94(10.94, 16.39)	26.74	-5.95 (-10.10, -1.80)	13.47 (9.47, 16.53)	25.62
C_0, C_3	0.21(-4.95, 5.37)	15.71(11.57, 18.97)	34.85	1.01 (-3.95, 5.98)	14.51 (10.15, 17.84)	26.94
C_0, C_4	0.32(-5.21, 5.86)	16.86(13.12, 19.91)	32.70	-1.98 (-7.06, 3.10)	14.94 (10.59, 18.28)	28.53
C_4	0.36(-5.34, 6.05)	17.35(13.64, 20.39)	28.37	-0.18 (-5.98, 5.62)	16.90 (12.31, 20.50)	30.78
C_0	0.57(-11.29, 12.42)	36.10(25.39, 44.29)	80.11	-10.06 (-18.52, -1.62)	26.61 (17.6, 33.27)	48.90

CI: confidence interval; C_{tj}: concentration at time t_j in hours post-dose; ME%: mean relative prediction error; RMSE%: root mean squared relative prediction error; 95th PAE%: 95th percentile of absolute values of relative prediction errors.

The results of the external validation (Cohort B) are also shown in Table 2.3 and Table 2.4. The prediction error indices were highly correlated with those of leave-one-out cross-validation for PO CsA; LSS ranks in each subgroup (based on RMSE%) were always preserved. However these error indices were higher than those of leave-one-out cross-validation for IV CsA. Nonetheless, 13 of the selected LSS (6 for IV and 7 for PO CsA) had predictive performances suitable for clinical application, with RMSE% and 95th PAE% values of less than 20% in both leave-one-out cross-validation and external validation. Among these LSS, nine met all predefined selection criteria, four for IV CsA ((C_0, C_2, C_4), (C_0, C_2, C_3), (C_0; C_2, C_3, C_4), (C_0; C_2, $C_{2.5}$, C_3)), and five for PO CsA ((C_0, $C_{1.5}$, C_4), (C_0 ; C_2, C_4), (C_0; C_1, C_2, C_4), (C_0; $C_{0.5}$, C_2, C_4) (C_0; C_1, C_3, C_4)).

The predictive performances of three of the selected LSS, namely, the best ((C_2, C_3, C_6, C_8) for IV CsA and ($C_{0.5}$, C_2, C_4, C_8) for PO CsA), the worst (C_0), as well as a clinically practical LSS (C_0, C_2, C_4), are shown graphically; Bland-Altman analysis showed limits of agreement of less than 20% for all but the C_0 LSS (Figure 2.4).

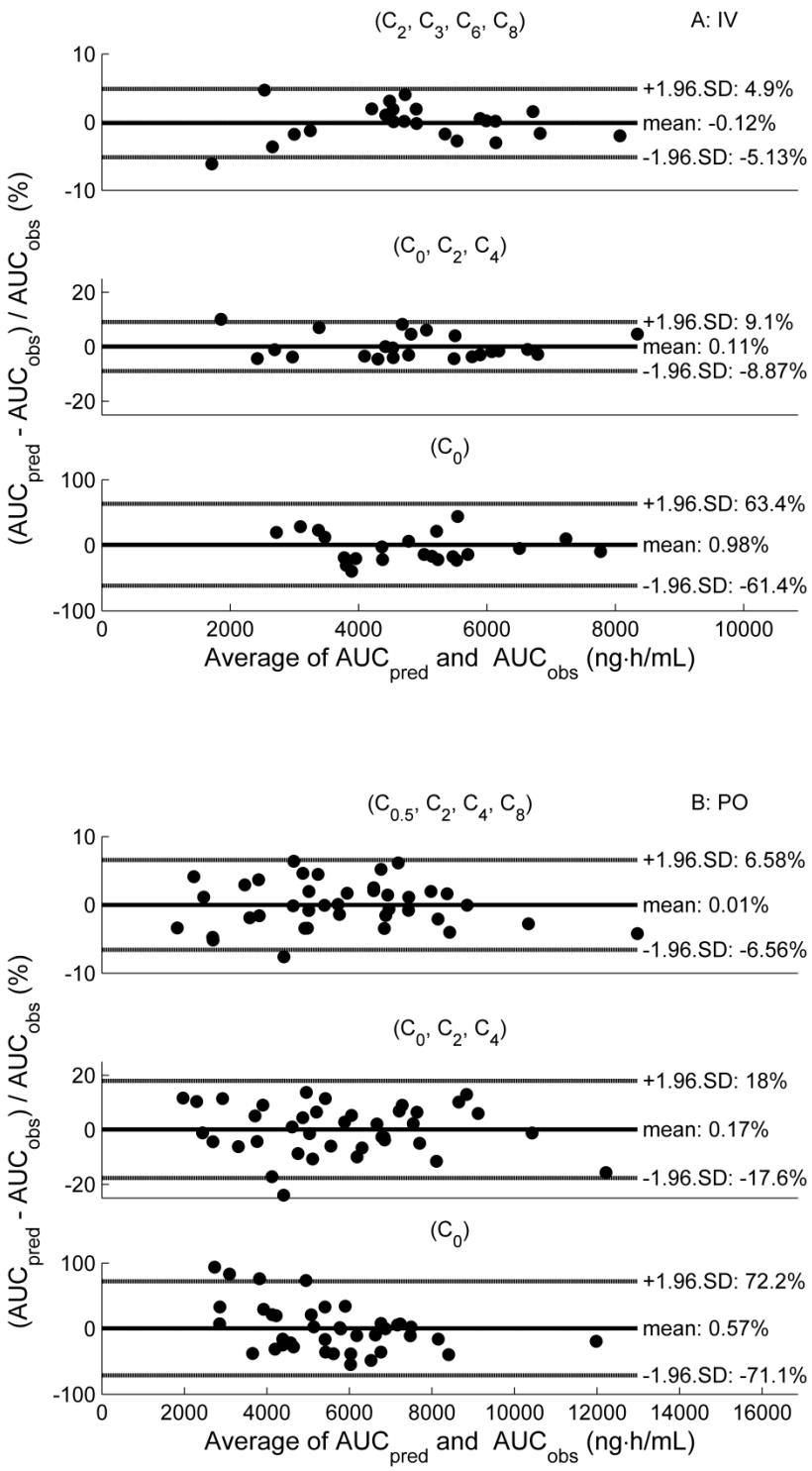

Figure 2.4: Bland-Altman analysis of agreement between observed AUC and predicted AUC using leave-one-out cross-validation. A) Prediction of cyclosporine AUC following IV

administration using three LSS: (C_2, C_3, C_6, C_8), (C_0, C_2, C_4), and (C_0); B) Prediction of cyclosporine AUC following PO administration using three LSS: ($C_{0.5}$, C_2, C_4, C_8), (C_0, C_2, C_4), and (C_0). The solid line represents the mean of the relative difference between observed and predicted AUC; the dotted line represents ± 1.96 x standard deviation of the mean; AUC_{obs}, observed AUC; AUC_{pred}, predicted AUC.

Discussion

This study consists of the largest pediatric cohort (68 patients and 138 PK profiles) investigated to date to develop and validate LSS for estimating IV and PO CsA AUC in children undergoing HSCT. Furthermore, the present study reports for the first time validated R-LSS for CsA administered orally in this population. Nine LSS (four for IV and five for PO CsA) convenient for application in clinical settings (four or fewer concentration-time points within 4 hours of drug administration) proved to have clinically acceptable performance. These LSS had RMSE% and 95th PAE% values of less than 20% in both leave-one-out cross-validation and external validation. Notably, LSS based on sampling at three concentration-time points (C_0, C_2, C_4) was found to be accurate for CsA estimation following both IV and PO administration. Thus this LSS may represent a favorable method for clinical application, because use of the same sampling protocol following both IV and PO administration may limit the risks of procedural errors. LSS based solely on C_0 had a poor predictive ability, which is in agreement with previous reports indicating poor correlation between C_0 and CsA exposure [6, 7]. LSS with highest predictive performance in each subgroup were also identified whether or not they met the predefined practical criteria in order to assess the value of these criteria in terms of performance. As shown in Table 2.3 and Table 2.4, improved prediction performance could be

achieved if the requirement of including C_0 was omitted and the sampling period was prolonged beyond four hours. However, considering the more clinically convenient LSS developed in this study that offer acceptable precision for clinical use, this gain in performance needs to be balanced against inconveniences associated with late sampling times in daily practice.

In this study, leave-one-out cross-validation was initially performed in the development cohort (Cohort A) to estimate error indices of all possible one, two, three, or four concentration-time point LSS. This allowed selection of the best LSS according to their predictive performance along with practical criteria. Then, external validation was carried out to evaluate the performance of the selected LSS in an independent dataset (Cohort B) to avoid overestimation of the predictive performance. Prediction error indices estimated in the external validation for selected IV LSS were found to be higher than those obtained by leave-one-out cross-validation. These differences in predictive capacity may be secondary to the fact that IV PK profiles used for external validation (Cohort B) were more heterogeneous compared to those of Cohort A (Figure 2.1). For the selected PO LSS, the results of external validation were in line with those of leave-one-out cross-validation. Interestingly, PO PK profiles of both development and external validation cohorts displayed considerable variability (Figure 2.2). These observations reinforce the need for external validation and suggest that the presence of heterogeneity in the development cohort, although potentially associated with higher error indices estimated by leave-one-out cross-validation, increase robustness of LSS to accurately estimate CsA exposure in real-life settings. The coefficient of determination (R^2) only estimates association and provides no information regarding predictive performance [31]; hence it is not reported in this paper.

The performances as well as the sampling times of the LSS proposed in the current study are comparable with those reported in adult HSCT population. For PO CsA, Hadjibabaie et al. found that the R-LSS including C_0, C_2, and C_4 had the best performance among 50 tested LSS with a median absolute relative error of 4.8% [20]. Eljebari et al. performed pre-selection steps and validated 24 R-LSS. The LSS that consisted of $C_{0.5}$, C_2, and C_4 had the best performance with RMSE% of 9.6% [21]. In another study, Eljebari et al. developed a B-LSS consisting of $C_{0.5}$, C_2 and C_4, with RMSE% of 12.07% [21]. Wilhelm et al. suggested C_0, C_2, and C_3 as the best LSS for Bayesian estimating of IV (3-hour infusion) and PO CsA AUC with mean absolute relative errors of 3.7% [22].

In the pediatric HSCT population, Willemze et al. reported B-LSS that involved sampling within 4 hours post-dose and had smaller error indices; the LSS consisting of C_0, C_2, and C_4 had a median absolute relative errors of 3.3% [26]. Nonetheless, their error indices were estimated using a small number of PK profiles (9 and 7 profiles for IV and PO CsA, respectively) and concentration-time points (6 per profile). Moreover, they did not carry out an external validation and the error indices were not reported separately for PO and IV LSS. The suggested R-LSS by Dupuis et al. had good performances but required samples to be drawn within eight hours and were developed and validated for IV CsA only [25]. In this paper, we developed the first validated R-LSS for PO CsA in pediatric HSCT, in addition to a more practical IV LSS for use in the clinical setting; these approaches do not require well trained professionals nor any sophisticated software such as is required to conduct B-LSS.

Even though the LSS developed in this study allow accurate and precise CsA AUC estimation, to obtain the full clinical benefit, well-established AUC targets are required. There is at least one ongoing pharmacokinetic/pharmacodynamic trial that is aiming to better define

the target CsA AUC in pediatric HSCT recipients (ClinicalTrials.gov Identifier: NCT02175615). Furthermore, the predictive performances reported in this paper are only applicable for patients whose conditions and characteristics are comparable to those of patients considered in this investigation's development and validation of LSS. For example, caution should be exercised if the proposed LSS are used in patients with C_4/C_2 ratios outside the range reported in this study (0.29 – 1.35). In addition, CsA concentrations should be measured with the specific immunoassay used in this study since immunoassays can also measure some of the drug's metabolites and consequently overestimate the actual concentrations present [32]. Finally, as R-LSS rely on timed concentrations for AUC estimation, accurate sampling time is required in order to insure reliable prediction.

In conclusion, the R-LSS developed in this study can predict CsA AUC in pediatric HSCT following both IV and PO administration with clinically acceptable prediction errors. They require four or fewer concentration-time points drawn within four hours after drug administration and, hence, are suitable for clinical use. Improved prediction could be achieved by increasing the sampling frequency and/or duration. These LSS may facilitate future research aiming at better defining the relationship between systemic exposure (AUC) and clinical outcomes such as prevention of graft-versus-host disease, disease relapse, CsA toxicities and overall survival. Subsequently, these LSS may also be employed in prospective trials to determine whether AUC-based monitoring of CsA is superior to C_0-based monitoring, for short- and long-term outcomes in this population.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the transplantation medical team at CHU Sainte-Justine for their collaboration. This work has been supported by FQRNT and FRSQ. MITACS and the Centre de Recherches Mathématiques of Université de Montréal are acknowledged for their support.

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Chapter 3 (Article II/Theoretical Biology and Medical Modelling journal): Bayesian Approach for the Estimation of Cyclosporine Area Under the Curve Using Limited Sampling Strategies in Pediatric Hematopoietic Stem Cell Transplantation

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Abstract

Background: The optimal marker for cyclosporine (CsA) monitoring in transplantation patients remains controversial. However, there is a growing interest in the use of the area under the concentration-time curve (AUC), particularly for CsA dose adjustment in pediatric hematopoietic stem cell transplantation (HSCT). In this paper, we develop Bayesian limited sampling strategies (B-LSS) for CsA AUC estimation using population pharmacokinetic (Pop-PK) models and investigate related issues, with the aim to improve B-LSS prediction performance.

Methods: Twenty five pediatric HSCT patients receiving intravenous and oral CsA were investigated. Pop-PK analyses were carried out and the predictive performance of B-LSS was evaluated using the final Pop-PK model and several related ones. The performance of B-LSS when targeting different versions of AUC was also discussed.

Results: A two-compartment structure model with a lag time and a combined additive and proportional error is retained. The final covariate model does not improve the B-LSS prediction performance. The best performing models for intravenous and oral CsA are the structure ones with combined and additive error, respectively. Twelve B-LSS, consisting of 4 or fewer sampling points obtained within 4 hours post-dose, predict AUC with 95^{th} percentile of the absolute values of relative prediction errors of 20% or less. Moreover, B-LSS perform better for the prediction of the 'underlying' AUC derived from the Pop-PK model estimated concentrations that exclude the residual errors, in comparison to their prediction of the observed AUC directly calculated using measured concentrations.

Conclusions: B-LSS can adequately estimate CsA AUC. However, B-LSS performance is not perfectly in line with the standard Pop-PK model selection criteria; hence the final

model might not be ideal for AUC prediction purpose. Therefore, for B-LSS application, Pop-PK model diagnostic criteria should additionally account for AUC prediction errors.

Keywords

Bayesian approach, Population pharmacokinetics (Pop-PK), Cyclosporine (CsA), Area under the curve (AUC), Limited sampling strategy (LSS).

Background

Therapeutic drug monitoring is a common practice for the use of immunosuppressant drugs, which generally exhibit considerable inter- or intra-pharmacokinetic (PK) variability and narrow therapeutic window [1]. A non-monitored dosing can increase the risk for therapeutic failure or induce serious undesirable effects. Currently, therapeutic drug monitoring approach, which involves the measurement of drug concentrations and their interpretation, has become a standard of care in immunosuppressant therapy for dose optimization, with the aim of maximizing therapeutic benefits and minimizing adverse effects [1, 2]. In clinical practice, the pre-dose concentration (C_0) is widely used as a PK marker for the therapeutic drug monitoring due to its accessibility. Nonetheless, treatment failure, adverse effects, and toxicity can still arise even in situations where C_0 is within the recognized therapeutic range [3,4]. These risks call for the implication of other PK based surrogates, such as the area under the concentration-time curve (AUC) which is generally known as the best indicator of drug systemic exposure. While its use as an optimal marker for immunosuppressant agents monitoring remains controversial its correlation with clinical outcomes is increasingly being investigated [5-7].

When estimating AUC, we generally refer to the observed AUC, usually denoted $AUC_{obs,}$ which is obtained using the trapezoidal method. This method can be cumbersome for patients and their care providers since it requires a frequent sampling over a time interval long enough to fully represent the drug disposition. As an alternative, limited sampling strategies (LSS) have been proposed to predict AUC with an adequate precision, using a reduced number of sampling points drawn within a short time interval. LSS have

been applied with two main approaches, namely, the multiple linear regression-based LSS (R-LSS) and Bayesian-based LSS (B-LSS).

The regression approach aims to establish a linear relationship between one or more concentration-time points (independent variables) and AUC (dependent variable) in the form of the following equation:

$$AUC_{pred} = F_0 + F_1 \times C_{t_1} + \ldots + F_k \times C_{t_k}$$

where C_{t1}, C_{t2}, …, C_{tk} are the concentrations sampled at times t_1, t_2, …, t_k, respectively; and F_0 , F_1, …, F_k are regression coefficients. For its simplicity, the use of regression LSS is widely spread as a bedside application. However, its use is highly restrictive since samples are assumed to be taken on nominal sampling times, excluding thus any possible deviation.

The B-LSS approach requires the use of several drug concentrations in addition to a well-established population pharmacokinetic (Pop-PK) model for the estimation of AUC. This model, considered as the acquired *prior* knowledge of drug characteristics, helps to improve the estimation, otherwise solely based on the observed drug concentrations. With the B-LSS method, the estimated individual PK parameters are obtained using the empirical Bayesian approach; these parameters are then used for the prediction of drug concentrations and, consequently, the estimation of AUC.

One advantage of the Bayesian approach over the regression LSS is its flexibility in terms of sampling time deviations which are readily considered when building the associated Pop-PK model and predicting the individual PK parameters; since the real sampling times can be used in case of sampling deviations from the nominal times.

Nevertheless, the use of B-LSS can be hampered by the need for trained professionals and specialized software. This situation is however changing progressively since many PK software packages with user-friendly interfaces are now made available.

In both LSS approaches, the estimation of AUC aims to approximate the real AUC that could be reachable in ideal conditions of frequent blood samplings associated with perfect measurements that reflect precisely drug concentrations. However, only few samples are usually available. In addition, these samples are generally affected by different sources of errors, emanating from sample collection, measurement method, and data processing. These limitations can potentially be inherited by the observed AUC, and consequently raising the question of its reliability.

It would be thus interesting to alternatively consider the AUC calculated directly from the estimated individual concentrations using the Pop-PK model, assuming the exclusion of the residual errors. These estimated concentrations are denoted IPRED in the usual notation of NONMEM®, the mostly used software in Pop-PK analyses. We refer to this AUC as 'underlying' AUC. The difference between observed AUC and 'underlying' AUC is illustrated in Figure 3.1.

Although 'underlying' AUC cannot be directly measured in practice, we believe that it represents the intrinsic property of a patient's PK profile as it is not altered by residual errors and hence can be a better predictor for drug effects, compared to the observed AUC where the residual errors are always present.

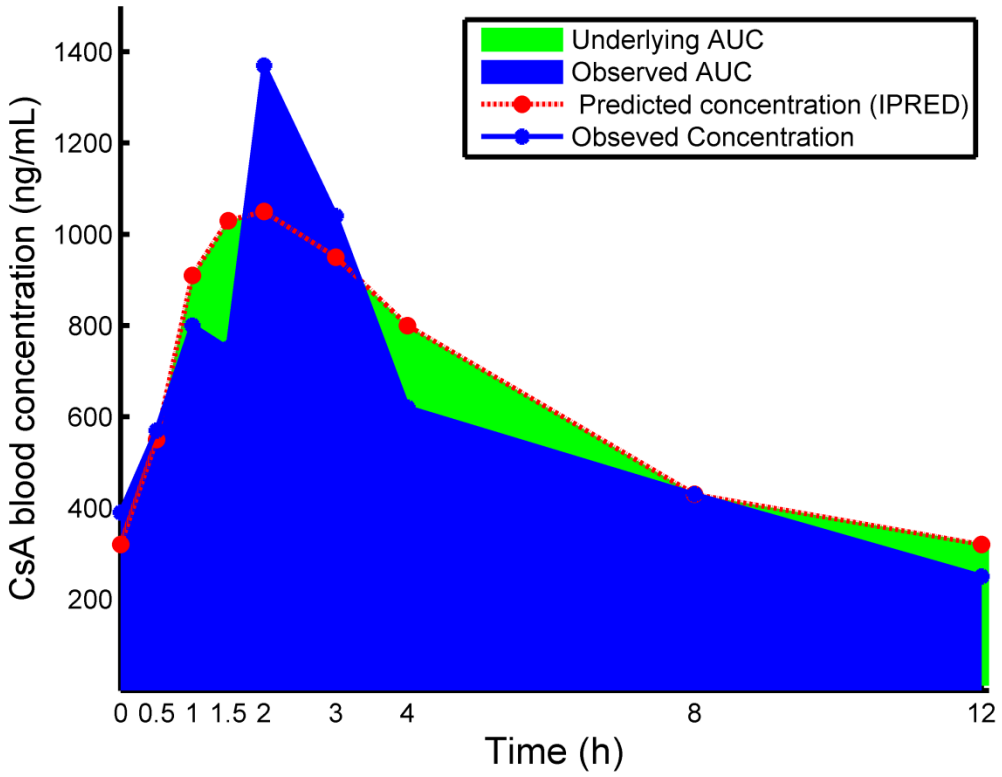

Figure 3.1: Underlying AUC (7448 ng.h/mL) Vs. Observed AUC (7017 ng.h/mL).

Cyclosporine (CsA) is a typical example of immunosuppressive agents where LSS are widely used. Therapeutic drug monitoring is recommended for CsA dose adjustment because of its large PK variability and small therapeutic index [2, 8]. CsA is used mainly in hematopoietic stem cell transplantation (HSCT) for the prophylaxis of graft-versus-host disease. In this context, there is a growing interest in the use of AUC as a therapeutic drug monitoring marker [6, 7]. However, prospective trials are still needed to evaluate the efficacy of AUC guided dose adjustment.

In HSCT, graft-versus-host disease can result in diffuse inflammation that affects intestinal integrity, thus causing reduction and delay in CsA absorption, while the clearance is reported higher in comparison to solid organ transplantation [9, 10]. Recently, LSS have been applied by several research groups to predict AUC for HSCT in adults [11-

14]. However, their results cannot be directly transferred to pediatric patients who generally require higher doses, as they have faster systemic clearance and lower CsA exposure [9, 10, 15, 16]. Therefore, particular B-LSS in pediatric patients need to be developed and validated.

To our knowledge, only three LSS studies for the prediction of CsA AUC in pediatric HSCT have been published. Willemze et al. found a good performance using B-LSS for intravenous (IV) and oral (PO) CsA; however, they were tested on a small number of combinations of sampling points, with no validation reported [17]. Based on a population of 24 pediatric patients receiving 2 hours BID infusion, Sibbald et al. reported R-LSS [18]. These LSS are developed only for PK profiles obtained after the first CsA IV dose and their application is restricted to this particular condition. Recently, Dupuis et al. validated these LSS and reported new R-LSS for the prediction of AUC at the steady state [19]. The latter LSS showed a good performance but required samples to be drawn within 8 hours post-dose and were developed and validated only for IV CsA.

In this paper, we will develop practical B-LSS for the prediction of AUC in pediatric HSCT patients after IV and PO CsA administrations. In this context and based on available PK data of our pediatric population, we developed Pop-PK models of CsA following the general Pop-PK modeling steps, but with a particular care for its intended use in AUC prediction by B-LSS. Furthermore, to insure the LSS applicability in clinical settings, the number of concentration-time points and sampling duration were restricted to 4 points or fewer drawn within 4 hours post-dose. Performance of these LSS is evaluated using well-established error indices.

Materials and methods

Patients

Pediatric patients receiving IV (2 hours infusion) or PO CsA twice daily for graft-versus-host disease prophylaxis after undergoing HSCT from a sibling or unrelated donor, at the Centre Hospitalier Universitaire Sainte-Justine, were considered for inclusion in this retrospective study. Patients who were 19 years old or more were excluded. The study was approved by the institutional research ethics committee at the Centre Hospitalier Universitaire Sainte-Justine. Twenty-five pediatric patients were eligible for inclusion in this study over a period from August 2009 to August 2010. Eighteen of these patients have IV and PO pharmacokinetic profiles. Patients' characteristics are summarized in Table 3.1.

Cyclosporine dose adjustment

Since 2010, the medical team at the Centre Hospitalier Universitaire Sainte-Justine caring for HSCT patients moved from C_0- to AUC-based monitoring in light of controversy regarding the usefulness of dose adjustments based on CsA C_0 [20]. Hence, CsA dose adjustments were made by the treating physician in accordance with institutional target $AUC_{0\text{-}12h}$ values, which were defined based on published data from renal transplantation studies [21, 22] and one adult HSCT study [23]. These were adapted by the team according to the patient's underlying diseases.

Table 3.1: Patients' information summary.

Parameter (unit)	Number or median (range)	
	IV	PO
Patients	19	20
Sex: male/female	10/9	12/8
Age at transplantation (year)	10.5 (1–18)	11.1(0.5-18.2)
Transplantation type: Sibling/Unrelated	10/9	13/7
Included PK profiles	23	39
Formulation	23 (IV)	19 (Susp) /20 (Cap)
Time post transplantation (month)	0.13 (0.1 - 1.7)	1.28 (0.7 - 9.1)
Age at PK profile (year)	10.4 (1–17.9)	11.9 (1.2 - 18.3)
Weight (kg)	33 (10–81)	38 (8–83)
Cyclosporine dose (mg/kg/day)	2.5 (1–3.2)	4.2 (1–8.3)
Concomitant corticosteroid	13	26†
Albumin (g/L)	32 (19–48)	32 (22–41)‡
Creatinine (μmol/L)	33 (12–358)	50 (13–117)‡
Bilirubin (μmol/L)	11 (5–64)	10 (3–596)‡
AST (U/L)	20 (9–42)	24 (13–125)‡
ALT (U/L)	24 (9–85)	31 (19–69)‡
GGT (U/L)	35 (8–94)	32 (9–217)‡
AP (U/L)	87 (1.9 - 203)	110 (53–302)‡
Hb (g/dL)	93 (64–143)	87 (64–122)‡
Hct (%)	25 (18–44)	26 (19–36)‡

ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; Hb: hemoglobin; Hct: hematocrit; IV: intravenous administration; GGT: γ- glutamyltranspeptidase; PK: pharmacokinetic; PO: oral administration; Susp: suspension; Cap: capsule

† Data available for 37 profiles.

‡ Data available for 38 profiles.

PK data

All available steady state PK profiles that contained at least 7 concentration-time points were incorporated in this study for a total of 23 IV and 39 PO profiles. Blood samples were drawn before and at 2, 3, 4, 6, 8, 10, and 12 hours after CsA administration

for IV profiles and at 0.5, 1, 1.5, 2, 3, 4, 8 and 12 hours after CsA administration for PO profiles. Concentrations were measured using ARCHITECTi2000SR® (Abbott Laboratories, Abbott Park, Illinois, USA). The lower and upper limits of detection were 30 and 1500 ng/mL, respectively. The between-run coefficients of variation were 9.95% at 87 ng/mL, 8.64% at 340 ng/mL, and 9.25% at 850 ng/mL. Blood samples with CsA concentrations > 1500 ng/mL were diluted with blank blood. The associated observed AUC were calculated using the trapezoidal method. Individual CsA concentration-time profiles are reported in Figure 3.2.

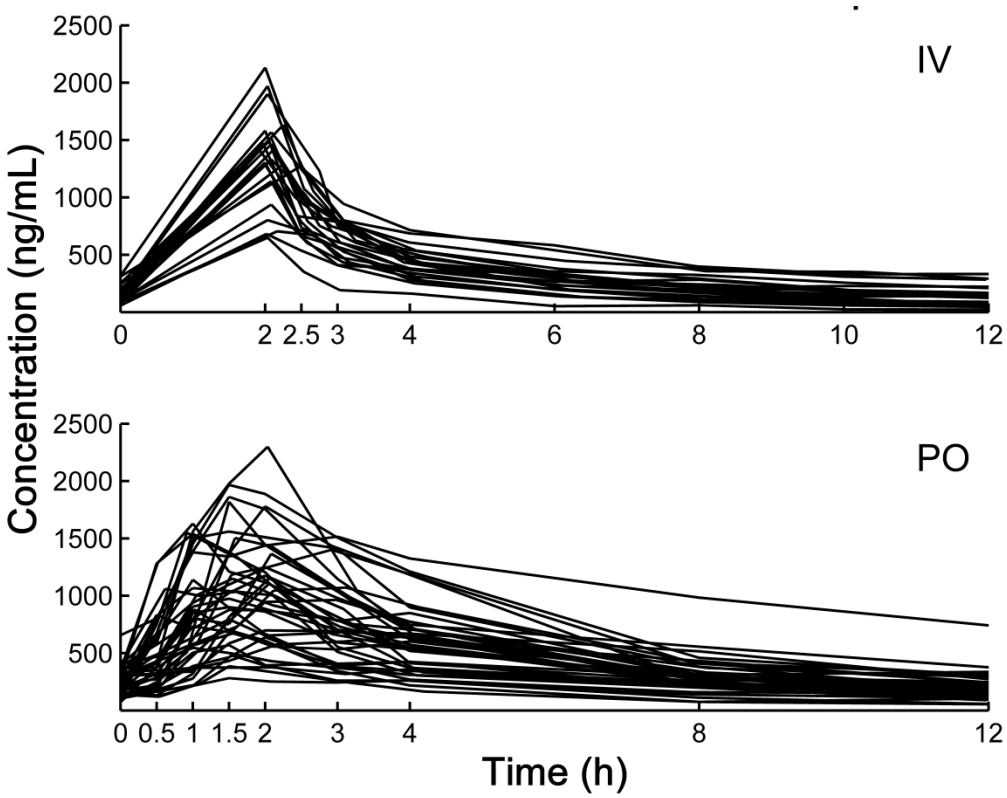

Figure 3.2: Concentration-time courses for the available full profiles.

Development of Pop-PK model

Population PK analyses were performed using the nonlinear mixed effect approach as implemented in NONMEM® software (Version VII). The first order conditional estimation with interaction (FOCE-I) method was used to determine PK parameters and the associated variability. To define the structural model, one, two and three compartment models with first-order absorption and elimination were used to analyze available CsA data. The lag time in absorption was also tested for each model. The exponential model was used to describe inter-individual variability for PK parameters as expressed in Eq.1:

$$\theta_{ij} = \theta_j \times \mathrm{EXP}(\eta_{ij}) \quad (1)$$

where θ_{ij} is the j^{th} PK parameter for the i^{th} individual, θ_j is the typical value of the population parameter; $\eta_{i,ij}$ is a random variable characterizing the between subject variability. A combined version of additive and proportional models was used to test for residual variability (Eq.2):

$$C_{obs} = C_{pred} \times (1+\varepsilon_1) + \varepsilon_2 \quad (2)$$

where C_{obs} and C_{pred} are the observed and predicted CsA blood concentrations, respectively; ε_1 and ε_2 are random variables describing the unexplained residual variability.

The structural model was developed based on statistical significance in the reduction of the objective function value (OFV) using likelihood ratio test (LRT), as well as other standard indicators such as the model stability and the improvement in model

fitting. As usually done in pediatric Pop-PK modeling, weight had been initially integrated as an allometric scaling factor for the clearance and the volume of distribution [24]. The covariate model was then established by the forward inclusion backward elimination strategy, using Perl speaks NONMEM (PsN) script [25], in which a change of OFV greater than 6.63 and 7.87, associated with a p-value of 0.01 and 0.005, was used as selection criteria for statistical significance, respectively. A total number of 19 covariates were included in the plan. With a careful checking of graphical relationship and consideration of their clinical meaning, potentially meaningful covariates were tested (see model development details in Appendix of this Chapter).

B-LSS development and validation

Using the nine available sampling points of each PK profile included in this study, we evaluated the performance of all possible combinations that contain one, two, three, or four concentration-time points, which gives rise to a total number of 255 LSS to be tested. These LSS were divided into four subgroups according to the number of concentration-time points included in the LSS plan. To allow validation despite the small number of available data, we used the leave-one-out cross-validation approach [26].

We briefly recall that, when using the leave-one-out cross-validation approach, each PK profile is left out in turn from the analysis, which gives rise to a partial dataset noted as $Y^{(-i)}$, i = 1, …, N, where i stands for the temporary excluded i^{th} PK profile. Using the available Pop-PK model of CsA, we estimate the PK parameters associated with the partial dataset $Y^{(-i)}$.Then to estimate PK parameters of the excluded profile, the standard empirical Bayesian approach, as implemented in NONMEM®, is performed using, as initial values, the Pop-PK parameters previously obtained for $Y^{(-i)}$. This estimation

involves the LSS associated concentrations of the excluded profile. These PK parameters obtained for the i^{th} profile are then used to predict its full concentration-time course that includes the 9 concentration-time points of the sampling protocol. Finally, the predicted AUC for the i^{th} profile is calculated using the trapezoidal method.

Performance of the 255 LSS is evaluated using error indices [27]. For each LSS, relative error (E%), the 95th percentile of the absolute values of relative prediction errors (95th PAE%), mean relative prediction error (ME%) and root mean squared relative prediction error (RMSE%) were calculated. These estimates were based on the following formulations:

$$E_i\% = \frac{AUC_{pred}^{(i)} - AUC_{obs}^{(i)}}{AUC_{obs}^{(i)}} \times 100 \quad (3)$$

$$95^{th}\ \text{PAE\%} = 95^{th}\ \text{percentile of the set } \left\{|E_i|\%\right\}_i^N \text{ in increasing order} \quad (4)$$

$$ME\% = \frac{1}{N}\sum_i^N \frac{AUC_{pred}^{(i)} - AUC_{obs}^{(i)}}{AUC_{obs}^{(i)}} \times 100 \quad (5)$$

$$RMSE\% = \sqrt{\frac{1}{N}\sum_i^N \left(\frac{AUC_{pred}^{(i)} - AUC_{obs}^{(i)}}{AUC_{obs}^{(i)}}\right)^2} \times 100 \quad (6)$$

Moreover, since relative errors can induce bias when applied to highly asymmetrical data, the symmetry of the distribution and the range of estimated relative errors were also verified [28].

For each LSS subgroup defined above, four representative B-LSS were chosen to represent the overall performance. In each subgroup, the first chosen B-LSS corresponds to the one that has the highest predictive performance according to 95th PAE%. In addition to

this criterion, the following three B-LSS were selected according to two clinically oriented restrictions, namely the inclusion of C_0 and the limitation of sampling to an interval of 4 hours post-dose. As reported in the Results Section, 28 B-LSS (14 for IV and 14 for PO CsA) are obtained for each evaluated Pop-PK model.

Figure 3.3 depicts the above procedure of B-LSS development and validation.

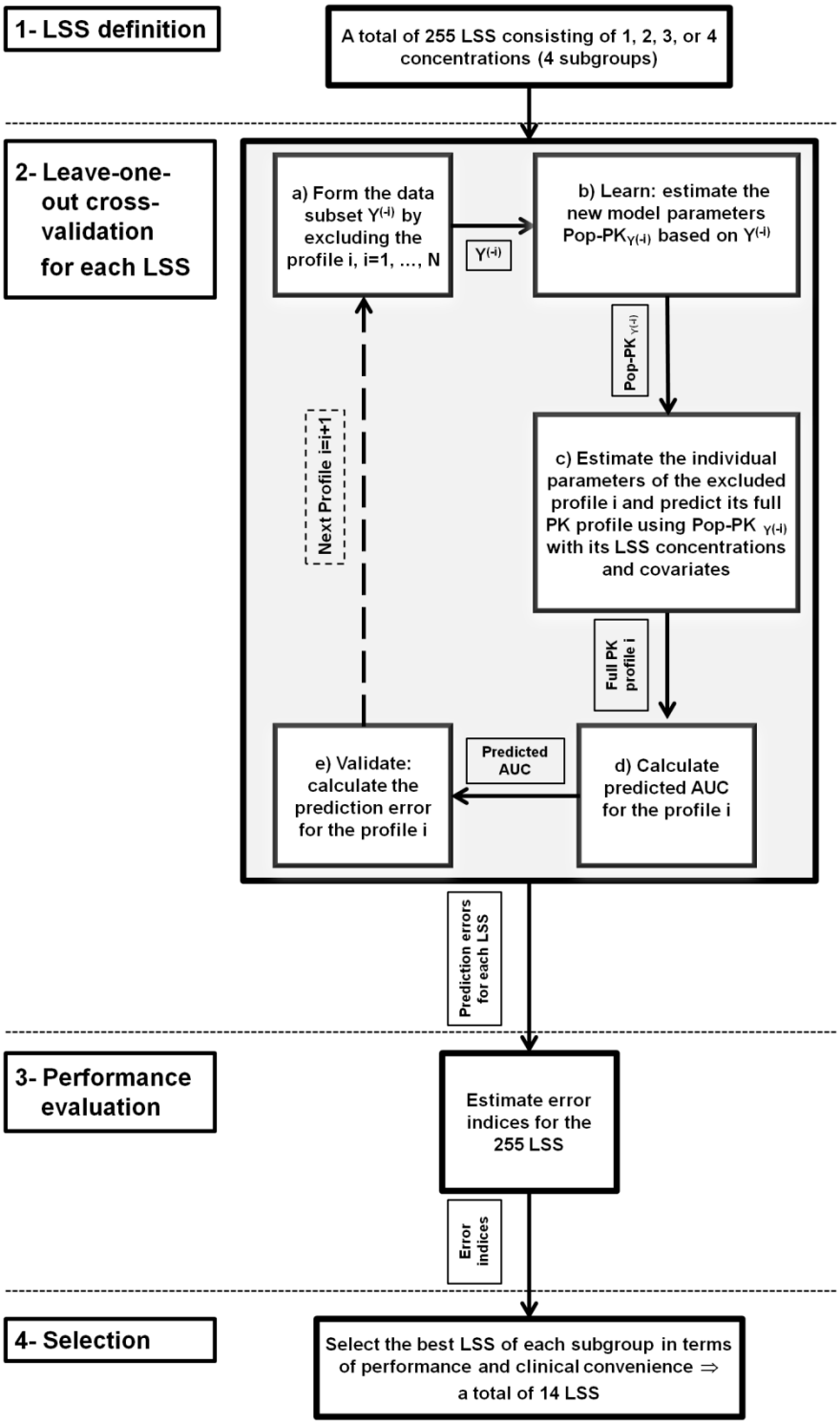

Figure 3.3: B-LSS development procedure; Y is the group of all profiles; $Y^{(-i)}$ is the subgroup of all profiles except the i^{th} one, where i = 1,2…,N.

Analysis of B-LSS performance

When investigating B-LSS performance using the structural model as well as the final model developed following the standard Pop-PK procedure, we noticed that the final model was not associated with the best performance. This non anticipated result raised the concern about the appropriateness of the final Pop-PK model, with regard to B-LSS application. Indeed, the decision for the final model is mainly determined through objective function value (OFV), a criterion which may not be adequate to optimize a model for B-LSS application. Hence, we decided to investigate the B-LSS performance using intermediate Pop-PK models that differ from the final one in terms of error models and included covariates. To report their B-LSS performance, we chose to use the 95^{th} PAE% for its simplicity and clinical relevance [8]. The results for other performance indices, not reported here for space restriction, were consistent with those of the 95^{th} PAE%.

As mentioned above, we also estimated the 'underlying' AUC and used it as a reference for AUC predicted through B-LSS. Then the performance of B-LSS in this context is compared to their performance for the prediction of observed AUC.

The commercial software package MATLAB® (2008b, The Math Works Inc, Natick, Massachusetts, U.S.A.) and NONMEM® (version VII, Icon Development Solutions, Ellicott City, MD) were used for modeling implementation and computations.

Results

Final Pop-PK model

The initial model analyses for the description of CsA PK data suggested a two-compartment structure with a combined additive and proportional error model. This structural model was parameterized in terms of: clearance (CL), apparent volume of distribution of the central compartment (Vc), apparent volume of distribution of the peripheral compartment (Vp), inter-compartmental transfer rate (Q), absorption rate (KA), lag time in oral absorption (ALAG), and oral bioavailability (F). Inter-individual variability was estimated for CL, Vc, Q, KA, and F.

Moreover, as usually suggested in pediatric literature, clearance and volume were scaled by weight with powers of ¾ and 1, respectively [24]. With this addition to the structural model, we have performed a standard covariate analysis which led to the final model that included weight (WT), age at profile date (AG), time post transplantation (TPT), alkaline phosphatase (AP), and dosage form (FORM). The details of model construction and estimated parameters can be found in the Appendix.

Pop-PK model selection based on associated B-LSS performance

The structural model with combined errors (Model 4 in Table 3.2) and the structural model with additive errors (Model 6 in Table 3.2) were selected as the best models for performing AUC prediction using B-LSS, for IV and PO profiles, respectively. Their selection was based on their performance in terms of 95th PAE%. Associated to these two models, 16 LSS (11 for IV and 5 for PO) had 95th PAE% of 20% or less.

The performance of B-LSS was evaluated using the structural, final, and several related Pop-PK models that differ from the final one in terms of error models as well as the covariates included. For each model, 28 LSS (14 for IV and 14 for PO) were selected using the above performance criteria. The results of the evaluated models are shown in Table 3.2. It is worth emphasizing that the final model did not give the best prediction for AUC though it has the least OFV. Associated to this model, 10 LSS (9 for IV and 1 for PO) had 95^{th} PAE% of 20% or less.

Table 3.2. Performance of B-LSS for cyclosporine AUC prediction using selected Pop-PK models.

	Model 1	95th PAE%	Model 2	95th PAE%	Model 3	95th PAE%	Model 4	95th PAE%	Model 5	95th PAE%	Model 6	95th PAE%
OFV	**−1900**		−1895		−1613		**−1790**		−1773		**−1447**	
IV	**C2, C2.5, C6, C10**	***10***	C2, C3, C6, C8	*11*	C2, C2.5, C6, C10	23	**C2, C2.5, C8, C10**	*7*	C2, C2.5, C8, C10	*8*	C2, C2.5, C4, C6	*15*
	C0, C2, C3, C4	***18***	C0, C2, C3, C4	*18*	C0, C2, C2.5, C4	27	**C0, C2, C3, C4**	***14***	C0, C2, C3, C4	*14*	C0, C2.5, C3, C4	22
	C0, C2, C2.5, C4	***19***	C0, C2, C2.5, C4	*19*	C0, C2, C3, C4	27	**C0, C2, C2.5, C3**	***15***	C0, C2, C2.5, C3	*15*	C0, C2, C2.5, C4	24
	C0, C2, C2.5, C3	***19***	C0, C2, C2.5, C3	*19*	C0, C2, C2.5, C3	27	**C0, C2, C2.5, C4**	***16***	C0, C2, C2.5, C4	*16*	C0, C2, C2.5, C3	24
	C2, C2.5, C10	***12***	C2, C3, C8	*13*	C2, C2.5, C6	24	**C2, C2.5, C8**	***10***	C2, C2.5, C8	*10*	C2.5, C8, C10	*16*
	C0, C2, C3	***20***	C0, C2.5, C3	*20*	C0, C2, C3	28	**C0, C2, C3**	***16***	C0, C2, C3	*16*	C0, C2.5, C4	*20*
	C0, C3, C4	***20***	C0, C2, C3	21	C0, C2, C4	28	**C0, C2.5, C3**	***18***	C0, C2.5, C4	*19*	C0, C2, C4	22
	C0, C2.5, C3	***20***	C0, C3, C4	21	C0, C2.5, C3	28	**C0, C2.5, C4**	***19***	C0, C2.5, C3	*19*	C0, C2.5, C3	24
	C2.5, C6	***17***	C2.5, C6	*17*	C2.5, C6	27	**C2.5, C8**	***15***	C2.5, C8	*15*	C2.5, C8	*20*
	C0, C3	**21**	C0, C3	22	C0, C3	31	**C0, C2.5**	***20***	C0, C2.5	21	C0, C2.5	23
	C0, C4	**23**	C0, C4	24	C0, C2.5	32	**C0, C3**	***20***	C0, C3	21	C0, C2	27
	C0, C2.5	**24**	C0, C2.5	25	C0, C4	37	**C0, C4**	**25**	C0, C4	24	C0, C3	30
	C6	**24**	C6	23	C3	33	**C4**	**23**	C4	22	C2.5	28
	C0	**37**	C0	37	C0	45	**C0**	**40**	C0	38	C0	51
PO	**C1, C3, C4, C12**	***14***	C1, C3, C4, C8	*15*	C1, C3, C4, C8	*16*	C1.5, C3, C4, C12	*13*	C1.5, C3, C4, C12	*13*	**C1.5, C3, C4, C8**	***14***
	C0, C1, C3, C4	**27**	C0, C1, C3, C4	32	C0, C1, C3, C4	*20*	C0, C1, C2, C4	23	C0, C1.5, C3, C4	25	**C0, C1.5, C2, C4**	***16***
	C0, C1, C2, C4	**28**	C0, C1, C2, C4	33	C0, C1, C2, C4	22	C0, C1.5, C3, C4	24	C0, C1, C2, C4	25	**C0, C1.5, C3, C4**	***16***
	C0, C0.5, C3, C4	**32**	C0, C0.5, C3, C4	35	C0, C1.5, C2, C4	24	C0, C1, C3, C4	25	C0, C1, C3, C4	26	**C0, C1, C3, C4**	***18***
	C1, C3, C4	**21**	C1, C3, C4	22	C1, C3, C4	20	C2, C4, C12	*16*	C1, C3, C8	*17*	**C0.5, C3, C4**	***18***
	C0, C1, C4	**35**	C0, C1, C4	39	C0, C1, C4	30	C0, C1, C4	30	C0, C1, C4	30	**C0, C2, C4**	**24**
	C0, C1.5, C4	**38**	C0, C0.5, C4	41	C0, C1.5, C4	33	C0, C2, C4	30	C0, C2, C4	33	**C0, C1, C4**	**24**
	C0, C2, C4	**40**	C0, C1.5, C4	42	C0, C1, C3	35	C0, C2, C3	35	C0, C1.5, C4	36	**C0, C1, C3**	**28**
	C1, C4	**27**	C1.5, C4	31	C1, C4	29	C1, C4	24	C1, C4	26	**C1, C4**	**25**
	C0, C2	**61**	C0, C2	64	C0, C4	50	C0, C3	45	C0, C3	46	**C0, C3**	**41**
	C0, C1	**65**	C0, C4	68	C0, C3	51	C0, C1	47	C0, C1	49	**C0, C4**	**43**
	C0, C4	**66**	C0, C1	69	C0, C1.5	61	C0, C2	54	C0, C2	55	**C0, C2**	**44**
	C3	45	C3	49	C4	41	C3	40	C3	40	**C3**	**33**
	C0	99	C0	99	C0	100	C0	86	C0	85	**C0**	**86**

Model 1: significant covariates included, combined error model (final model)

Model 2: significant covariates included, proportional error model

Model 3: significant covariates included, additive error model

Model 4: no covariates, combined error model (selected structural model): best model for B-LSS application regarding IV profiles

Model 5: no covariates, proportional error model

Model 6: no covariates, additive error model: best model for B-LSS application regarding PO profiles

C_t: concentration at time t in hours post-dose, OFV: objective function value, 95^{th} APE%: 95^{th} percentile of absolute values of relative errors

Bayesian LSS performance

Twelve B-LSS (8 for IV and 4 for PO, using models no. 4 and 6, respectively) that required 4 or fewer concentration-time points obtained within 4 hours post-dose, estimate AUC with 95th PAE% of 20% or less, Table 3.2. Among these LSS, (C_0, C_2, C_3, C_4) for IV and (C_0, $C_{1.5}$, C_2, C_4) for PO CsA, had the best performance, with 95th PAE% of 14% and 16%, respectively. However, it is possible to reduce the prediction error if a prolonged sampling period beyond 4 hours post-dose is allowed. For example, the LSS (C_2, $C_{2.5}$, C_8, C_{10}) had a reduced PAE% of 7% for IV CsA.

Furthermore, the prediction of the 'underlying' AUC revealed that the selected B-LSS often had a better performance when the 'underlying' AUC was estimated rather than the observed AUC. Indeed, under the same conditions of 4 or fewer sampling points within 4 hours post-dose, we identified 15 B-LSS(instead of 12), that have 95th PAE% of 20% or less, Table 3.3.

Table 3.3. Performance of B-LSS for the prediction of observed and 'underlying' AUC using selected Pop-PK models.

	LSS #	Concentration-time points	Error indices (for observed AUC)						Error index for 'underlying' AUC
			RMSE% (Confidence interval)	ME% (Confidence interval)	E%			95th PAE%:	95th PAE%
					<−20%	[−20%, 20%]	>20%		
IV	1	C2, C2.5, C8, C10	4.39(3.26, 5.28)	1.19(−0.67, 3.06)	0	23	0	*7*	*14*
	2	C0, C2, C3, C4	7.30(4.37, 9.36)	1.93(−1.18, 5.04)	0	23	0	*14*	*17*
	3	C0, C2, C2.5, C3	7.71(4.32, 10.01)	1.75(−1.57, 5.07)	0	22	1	*15*	*17*
	4	C0, C2, C2.5, C4	7.61(2.33, 10.51)	2.83(−0.30, 5.95)	0	22	1	*16*	*19*
	5	C2, C2.5, C8	5.70(4.30, 6.81)	3.24(1.17, 5.31)	0	23	0	*10*	*17*
	6	C0, C2, C3	8.14(4.63, 10.54)	2.40(−1.04, 5.84)	0	22	1	*16*	*19*
	7	C0, C2.5, C3	9.85(7.16, 11.95)	−0.61(−4.96, 3.73)	0	23	0	*18*	*14*
	8	C0, C2.5, C4	9.53(6.42, 11.85)	0.89(−3.30, 5.09)	0	23	0	*19*	*16*
	9	C2.5, C8	8.64(6.49, 10.36)	0.58(−3.24, 4.39)	0	23	0	*15*	*14*
	10	C0, C2.5	11.33(8.25, 13.74)	1.05(−3.93, 6.04)	1	22	0	*20*	*19*
	11	C0, C3	11.42(8.55, 13.69)	−0.94(−5.97, 4.09)	1	22	0	*20*	*15*
	12	C0, C4	12.11(8.19, 15.04)	2.65(−2.58, 7.87)	1	21	1	25	*19*
	13	C4	13.88(10.30, 16.71)	4.36(−1.46, 10.19)	1	18	4	23	25
	14	C0	23.32(16.81, 28.37)	0.55(−9.75, 10.86)	6	10	7	40	38
PO	15	C1.5, C3, C4, C8	6.98(5.29, 8.33)	−3.20(−5.24,−1.17)	0	39	0	*14*	*16*
	16	C0, C1.5, C2, C4	7.50(4.97, 9.36)	0.70(−1.76, 3.15)	0	38	1	*16*	*11*
	17	C0, C1.5, C3, C4	7.60(5.48, 9.25)	−0.35(−2.85, 2.14)	0	39	0	*16*	*14*
	18	C0, C1, C3, C4	9.34(6.62, 11.42)	0.43(−2.63, 3.50)	1	38	0	*18*	*16*
	19	C0.5, C3, C4	11.73(7.27, 14.91)	0.08(−3.77, 3.93)	1	38	0	*18*	*15*
	20	C0, C2, C4	11.02(8.00, 13.37)	3.40(−0.04, 6.84)	1	36	2	24	*19*
	21	C0, C1, C4	10.26(6.11, 13.15)	0.48(−2.89, 3.84)	1	37	1	24	*20*
	22	C0, C1, C3	13.08(9.20, 16.04)	2.07(−2.17, 6.31)	2	34	3	28	21
	23	C1, C4	11.96(8.36, 14.70)	−2.86(−6.68, 0.95)	4	34	1	25	25
	24	C0, C3	18.65(11.05,23.94)	2.16(−3.92, 8.24)	4	31	4	41	34
	25	C0, C4	21.37(16.26,25.47)	2.09(−4.89, 9.07)	6	23	10	43	38
	26	C0, C2	21.19(12.55,27.21)	8.61(2.25, 14.97)	2	29	8	44	36
	27	C3	17.46(12.77,21.14)	−3.17(−8.81, 2.47)	7	29	3	33	30
	28	C0	43.26(31.81,52.26)	15.62(2.37, 28.87)	7	14	18	86	87

The selected Pop-PK models are the structural model with combined errors (Model 4 in Table 3.2) and the one with additive errors (Model 6 in Table 3.2) for IV and PO CsA, respectively.

C_t: concentration at time t in hours post-dose, ME%: relative mean prediction error, RMSE%: relative root mean squared prediction error, 95th APE%: 95th percentile of absolute values of relative prediction errors.

Discussion

Limited sampling strategies are gaining ground over extensive sampling in the drug development process and clinical practice, particularly in pediatric therapies. With the increasing use of Pop-PK modeling and Bayesian philosophy in drug R&D, we can notice the recent transition from classical R-LSS approach towards B-LSS. The current work investigates the use of B-LSS in the estimation of AUC, for CsA administered through IV or PO routes in pediatric HSCT. Taking into account clinical considerations, our approach uses the empirical Bayesian method as implemented in NONMEM® for the selection of the smallest set of sampling points (i.e. LSS) that allow accurate estimation of individual AUC.

Through LSS development process, we have been led to question the appropriateness, for B-LSS application, of the final Pop-PK model, particularly when its development is mainly driven by the objective function value OFV. For this, we have tested several related Pop-PK models and found that the usually referred as the final one does not necessarily provide the best B-LSS performance for AUC prediction. This is in fact not counterintuitive since this final model is chosen under curve fitting criteria. The PK parameters found through this goodness of fit criteria might not give the best estimation of AUC, which is indeed a summary of the information carried by the concentration curve. It would be interesting in the future to directly integrate an additional constraint that minimizes prediction errors in AUC, within the model optimization process, in order to account for both curve fitting and AUC estimation.

The prediction error of B-LSS depends on the Pop-PK-model used to predict drug concentrations. Several Pop-PK model components such as covariate and error models can significantly influence the performance of B-LSS. In a standard Pop-PK model development, reduction in model objective function value OFV is the main criterion to judge the quality of the model. In this approach, Pop-PK parameters are estimated and optimized via a restricted *maximum likelihood* method implemented in NONMEM®. However, for B-LSS application to estimate AUC, a more efficient selection of Pop-PK models can be achieved by the additional consideration of the impact of Pop-PK model components on AUC prediction rather than only considering their impact on PK parameters estimation. In our case, for example, even though the structural model with combined error shows a better overall fit for PO profiles, it underestimates C_{max} of individual profiles. The structural model with additive error allowed a better estimation of C_{max} that has the main contribution to AUC value calculated by trapezoidal method and therefore this model is associated with a better performance of B-LSS.

To develop B-LSS for CsA in pediatric HSCT and investigate its performance, we developed a Pop-PK model following the standard procedure, from the structural model to the final covariate model, while carefully keeping the intermediate tested models for comparison. In order to identify the model that best predicts AUC, the performance of the final model was compared with intermediate ones that differ in one or more model components. For each model, all possible one, two, three, or four concentration-time point LSS were investigated and their predictive performance

evaluated. Moreover, we studied the situation when B-LSS are targeting the 'underlying' AUC rather than the observed AUC and found that the B-LSS prediction performance is improved. Indeed, we have used the two models (no. 4 and 6 in Table 3.2 for IV and PO CsA, respectively), which have the smallest prediction errors for the observed AUC, and obtained a better performance when the 'underlying' AUC was estimated.

The studied population covers a wide range of demographic and clinical characteristics that enables large applicability of the developed LSS. In addition, to avoid the overestimation of the predictive performance, the dataset used for validation has to be different from the one used for learning. However, the small number of initially available PK profiles, a common issue in pediatric research, led us to use leave-one-out cross-validation approach. This method is generally used as an alternative to compensate for small datasets. When evaluating the LSS performance, relative errors indices, namely E%, ME% and RMSE%, were computed. However, we are aware that the use of relative errors might induce the bias when applied to highly asymmetrical data, thus their distribution was considered [28]. The 95^{th} PAE% was used to initially compare B-LSS performance for the Pop-PK models since it is more frequently used in clinical setting for the evaluation of errors. Other error indices were calculated as well for all considered models and the detailed results are reported in Table 3.3 for the best performing ones, namely, for models no. 4 and 6 of Table 3.2 Particularly, the 12 proposed B-LSS (8 IV and 4 for PO CsA) were verified for the absence of bias and their ME% were not significantly different from 0. Even though the LSS developed in this study allow accurate and precise CsA AUC estimation, we have to mention that further

prospective trials are still needed to determine whether AUC-based monitoring can increase efficacy and avoid toxicity. However, evaluating the value of AUC as a marker for therapeutic drug monitoring is outside the scope of this paper.

In the current study, using standard model diagnostic criteria, we have constructed a two compartment Pop-PK model with lag time and combined error to characterize CsA PK data, which is in agreement with previous HSCT studies [11, 13, 17, 20, 29]. In our structural model, CL was estimated to be 14.8 L/h with an inter-individual variability of 31% (14.8, 31%), which are similar to the reported ones for pediatric patients (11.3, 36%) [17] and (15.3, 17%) [20]. However, higher values of CL were reported for adult populations (22.3, 27.7%) [11], (25.4, 38.7%) [13], and (52, 42%) [29]. The covariate influence on CL was described in two studies. Willemze et al. [7] has shown power and linear relationships between CL and WT as well as CL and time post transplantation (TPT), respectively. Kim et al. [29] reported linear relationships between CL and sex as well as hematocrit. In our study, WT was included as an allometric covariate for both CL and V_C, which is in agreement with the findings of Willemze et al. [7] and generally adopted in pediatric PK modeling [24]. In addition, we found relationships between CL and alkaline phosphatase (AP) as well as age at profile date (AG), the former relationship is compatible with the fact that CsA has hepatic metabolism and that its elimination depends on liver function. HSCT complication includes chronic and acute liver graft-versus-host disease for which alkaline phosphatase is a clinical marker [30,31]. In addition our investigation confirmed the inverse correlation between age and two PK parameters, namely, CL and Vc [15,

16]. Moreover, our results regarding the central and peripheral volume of distribution were within the range of the reported studies [11, 13, 17, 20, 29], where values largely vary (Vc: 12.9-52, Vp: 59.9-496). Furthermore, a lag time for CsA absorption was previously reported in three studies [11, 13, 17]. The present investigation showed the influence of two clinically relevant covariates on lag time, namely, time post transplantation and FORM. In HSCT patients, time post transplantation is related to the intestinal integrity that can affect CsA absorption [9] and, as expected, capsules need additional time to be available for absorption when compared to suspension. KA value was higher than that reported in adults [11, 13, 29] and close to estimates of Willemze et al. in pediatrics [17]. The CsA bioavailability in our study was estimated to be 59% with an IIV of 30%, which compares well with reported values [11, 17].

Conclusion

B-LSS requiring 4 or fewer concentration-time points obtained within 4 hours post-dose can estimate CsA AUC in pediatric HSCT with acceptable prediction errors. However, the Pop-PK model developed using the standard model diagnostic criteria, does not always lead to the best model for B-LSS application. As we have seen in this paper, even the final covariate model gives a better fitting for concentration data in the sense of objective function value (OFV) than the structural model, the latter has a better AUC prediction than the former. Thus, for improved B-LSS application, more considerations with focus on the error in AUC prediction have to be taken into account in the development of Pop-PK models. Moreover, in the case where the prediction of the

'underlying' AUC is preferred compared to the observed AUC, as the residual error is excluded in the former, B-LSS can have a better performance.

Abbreviations

95^{th} PAE%, 95^{th} percentile of absolute values of relative prediction errors; AUC, Area under the concentration-time curve; Observed AUC, AUC estimated using the trapezoidal method and observed concentrations; Predicted AUC, AUC estimated using LSS method and observed concentrations; Underlying AUC, AUC estimated using the trapezoidal method and individual predicted concentrations without residual error; B-LSS, Bayesian limited sampling strategy; CsA, Cyclosporine; C_t, Concentration at time t in hours post-dose; E%, Relative error; IV, Intravenous administration; LSS, Limited sampling strategy; ME%, Mean relative prediction error; OFV, Objective function value; PO, Oral administration; Pop-PK, Population pharmacokinetics; RMSE%, Root mean squared relative prediction error

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SS participated in study design, performed data analysis and interpretation, and drafted the manuscript. JL, FN, participated in study design and coordination, data analysis and interpretation, and helped to draft the manuscript. OB helped to perform

data analysis. CL, YT and AL conceived the clinical study, and participated in its design and coordination, and helped for acquisition of data. All authors read and approved the final manuscript.

Acknowledgements

This work has been supported by FRSQ scholarship, FQRNT Projet d'équipe, NSERC Discovery grants and NSERC-Industrial Chair in Pharmacometrics in partnership with Pfizer, Novartis and inVentiv Health Clinics. The authors would like to thank the transplantation medical team at CHU Sainte-Justine for their collaboration.

Appendix

Pop-PK model development

Population PK analyses were performed using the nonlinear mixed effect model approach as implemented in NONMEM® software (Version VII). The first order conditional estimation with interaction (FOCE-I) method was used to determine PK parameters.

To define the structural model, one, two and three compartment models with first-order absorption and elimination were used to analyze available CsA data. The lag time in absorption was also tested for each model. The exponential model was used to describe inter-individual variability for PK parameters as expressed in Eq.1:

$$\theta_{ij} = \theta_j \times \mathrm{EXP}(\eta_{ij}) \quad (1)$$

where θ_{ij} is the j^{th} PK parameter for the i^{th} individual, θ_j is the typical value of the population parameter; $\eta_{i,ij}$ is a random variable characterizing the between subject variability. A combined version of additive and proportional models was used to test for the residual variability (Eq.2):

$$C_{obs} = C_{pred} \times (1+\varepsilon_1) + \varepsilon_2 \quad (2)$$

where C_{obs} and C_{pred} are the observed and predicted CsA blood concentrations, respectively; ε_1 and ε_2 are random variables describing the unexplained residual variability.

The structural model was developed based on statistical significance in the reduction of the objective function value (OFV) using likelihood ratio test (LRT), as well as other criteria such as the model stability and the improvement in model fitting. As usually done in pediatric Pop-PK modeling, weight was initially integrated as an allometric scaling factor for the clearance and volume of distribution. The covariate model was established using the forward inclusion backward elimination method. This approach was accomplished through three steps. In the first step, we set up the basic model by including weight as an allometric scaling factor for the clearance and volume of distribution into the structural model. Scatter plots of model parameters against each covariate helped to evaluate the potential covariate impact and the relation patterns. In the second step, each candidate covariate was screened in turn by incorporating it into the basic model to develop the intermediate models toward a full one. The difference in OFV obtained for a model with n + 1 covariates and the nested one with n covariates approximates the χ^2 distribution with one degree of freedom, and a change of OFV greater than 6.63, associated with a p-value of 0.01, was considered for statistical significance. The following covariate were considered: weight (WT), age at profile date (AG), time post transplantation (TPT), sex, dosage form (FORM), co-administration of corticosteroid, calcium channel blocker and azole antifungal, blood urea nitrogen, albumin, total protein, total bilirubin, aspartate aminotransferase (ALT), alanine

aminotransferase (AST), gamma glutamyl transpeptidase (GGP), alkaline phosphatase (AP), hemoglobin, hematocrit, and red blood cell count. Only potentially clinically meaningful relationships were considered. Hence, we have tested the influence on CL of WT, AG, sex, FORM, co-administration of corticosteroid, calcium channel blocker and azole antifungal, blood urea nitrogen, albumin, total protein, total bilirubin, ALT, AST, GGP, AP, hemoglobin, hematocrit and red blood cell count; we have tested the influence on Vc, Q, and Vp of WT, AG, and sex; and finally tested the influence on KA and ALAG of AG, sex, TPT, and FORM. Sex, FORM, and co-medications were included in the model as categorical covariates in a linear mode. Other covariates were included as continuous ones in linear, exponential, and power modes; these covariates are centered to their median values. In the backward step, each covariate was independently removed from the full model to confirm its importance. An increase in OFV of more than 7.87 (p-value, 0.005) was required to confirm that the covariate was significant. The final Pop-PK model included all significant covariates. The Perl speaks NONMEM (PsN) toolkit was used for stepwise covariate model building [25].

Pop-PK results

The initial analyses without covariates showed that a two-compartment model with lag time and combined error model described the CsA PK profile better than the other tested models. Thus, this is chosen as the structural model in the current study. The estimated PK parameters were CL, Vc, Vp, Q, KA, ALAG, and F. Inter-individual variability OMEGA can be estimated for CL, Vc, Q, KA and F; inter-individual

variability of Vc is highly correlated with that of CL and was estimated as a linear function of it. Parameter estimates for the structural model with combined and additive error model are shown in Table 3.4.

Table 3.4. Parameter estimates for the two structural Pop-PK models selected for B-LSS application.

PK parameters		Structural Pop-PK Model with Combined Residual Error				Structural Pop-PK Model with Additive Residual Error			
		NONMEM fixed effect parameters		Inter-individual variability%		NONMEM fixed effect parameters		Inter-individual variability%	
		estimate	RSE%	estimate	RSE%	estimate	RSE%	estimate	RSE%
CL (L/h)		14.82	7	31	14	14.49	8	32	15
Vc (L)		31.8	9	†		24.91	10	†	
Q (L/h)		13.49	13	80	11	13.14	15	100	14
Vp (L)		104.6	10	-	-	86.15	8	-	-
KA (1/h)		0.71	16	83	11	0.58	13	75	10
ALAG (h)		0.39	6	-	-	0.39	6	-	-
F		0.61	10	32	24	0.61	11	29	24
θ8		0.86	10	-	-	1.02	15	-	-
Cov (CL, Q)		-	-	44	27	-	-	48	22
Residual error	Prop.	17.5				-			
	Add.	15 ng/mL				100 ng/mL			

The selected Pop-PK models are the structural model with combined errors (Model 4 in Table 3.2) and the one with additive errors (Model 6 in Table 3.2) for IV and PO CsA, respectively.

† Inter-individual variability (Vc) = Inter-individual variability (CL) × θ8

CL: clearance, Vc: apparent volume of distribution of the central compartment, Vp: apparent volume of distribution of the peripheral compartment, Q: inter-compartmental transfer rate, KA absorption rate, ALAG: lag time in oral absorption, F: oral bioavailability, RSE%: relative standard errors.

The final model comprises the following covariates: WT, AG, TPT, and AP, and FORM. The estimated parameters are reported in Table 3.5. The relative standard errors (% RSE) of the parameters were acceptable, with a range from 0.05 to 0.30. Figure 3.4 shows the relationship between the observed and the predicted CsA concentrations based on the final parameter estimates (PRED). Figure 3.5 shows the relationship between the observed and the individual predicted concentrations (IPRED). Both plots show good correlation, suggesting that the final model explains well the observed data, although

peak concentrations in several individuals were slightly underestimated by PRED. The values and distribution of weighted residual (WRES) were unsatisfactory confirming the adequate use of FOCE-I instead of FO as an estimation method. The conditional weighted residuals (CWRES) for model-predicted concentrations shown in the narrow rectangular distribution in function of observed concentration and time, Figure 3.6, are also acceptable.

Table 3.5. Final Pop-PK model parameter estimates.

PK parameters		NONMEM fixed effect parameters		Inter-individual variability%	
		Estimate	RSE%	Estimate	RSE%
CL (L/h)		θ1 = 15.66	5	17	11
		θ10 = −0.32	19		
		θ11 = 0.0017	29		
Vc (L)		θ2 = 36.68	9	2	7
		θ13 = −0.39	16		
Q (L/h)		θ3 = 14.71	9	55	15
		θ12 = 0.023	18		
Vp (L)		θ4 = 105	10	-	-
KA (1/h)		θ5 = 0.8	15	72	10
ALAG (h)		θ6 = 0.46	3	-	-
		θ8 = 0.005	5		
		θ9 = −0.39 for suspension	19		
		θ9 = −0.022 for capsule	18		
		θ14 = −0.014	30		
F		θ7 = 0.59	8	30	15
Residual error	Prop.	16%			
	Add.	19 ng/mL			

CL: clearance, Vc: apparent volume of distribution of the central compartment, Vp: apparent volume of distribution of the peripheral compartment, Q: inter-compartmental transfer rate, KA absorption rate, ALAG: lag time in oral absorption, F: oral bioavailability, WT: weight, AG: age at profile date, TPT: time post transplantation, AP: alkaline phosphatase, FORM: dosage form (suspension or capsule), RSE%: relative standard errors.

$$CL = THETA(1) \times ((WT/36.1) ** 0.75) \times ((AG/11.82)**THETA(10)) \times (1+THETA(11) \times (AP - 99)) \times EXP(ETA(1))$$

$$Vc = THETA(2) \times (WT/36.1) \times ((AG/11.82)**THETA(13)) \times EXP(ETA(2))$$

$$Q = THETA(3) \times (1 + THETA(12) \times (WT - 36.1)) \times EXP(ETA(3))$$

$$Vp = THETA(4)$$

$$KA = THETA(5) \times EXP(ETA(4))$$

$$ALAG = THETA(6) \times EXP(THETA(8) \times (TPT - 3.71)) \times (1 + THETA(9)) \times (1 + THETA(14) \times (AG - 11.82))$$

$$F = THETA(7) \times EXP(ETA(5))$$

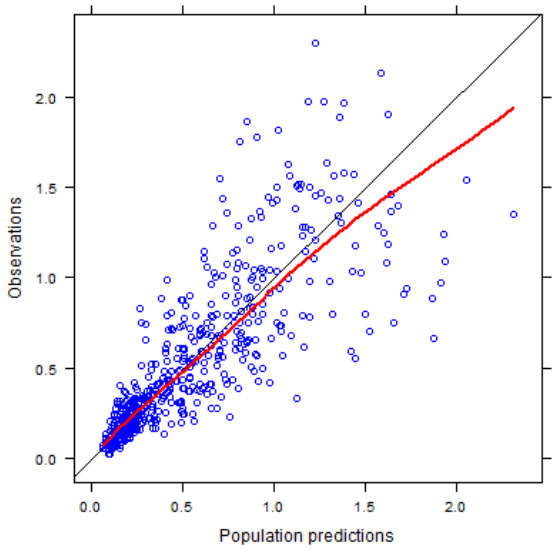

Figure 3.4: Relationship between the observed and the predicted cyclosporine concentrations based on the final parameter estimates (PRED).

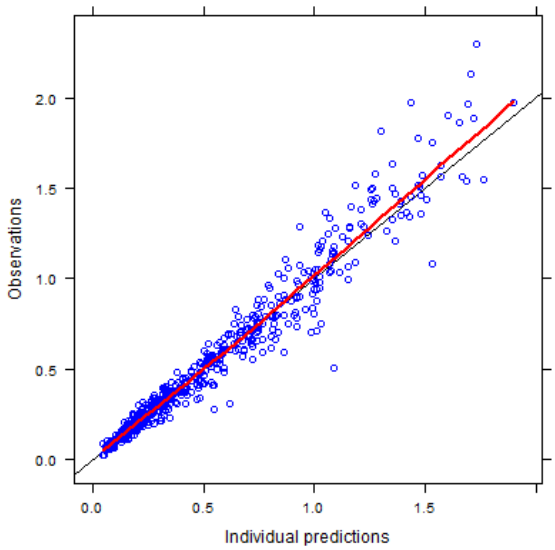

Figure 3.5 Relationship between the observed and the individual predicted concentrations (IPRED), black line: line of identity, red line, loess predictions.

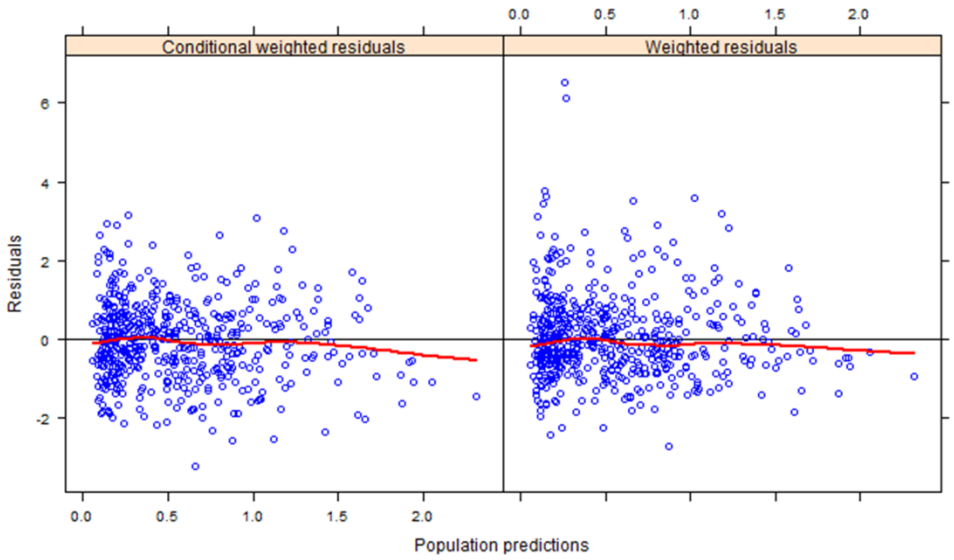

Figure 3.6 Distribution of weighted residual (WRES) and conditional weighted residuals (CWRES), black line: line of identity, red line, loess predictions.

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Chapter 4 (Article III/Biopharmaceutics & Drug Disposition journal): Impact of Sampling Time Deviations on the Prediction of the Area Under the Curve Using Regression Limited Sampling Strategies

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Abstract

The regression limited sampling strategy approach (R-LSS), which is based on a small number of blood samples drawn at selected time points, has been used as an alternative method for the estimation of the area under the concentration-time curve (AUC). However, deviations from planned sampling times may affect the performance of R-LSS, and thus influence the related therapeutic decisions and outcomes. The aim of this study was to investigate the impact of different sampling time deviation (STD) scenarios on the estimation of AUC by R-LSS using a simulation approach. Three types of scenarios were considered going from the simplest case of fixed deviations, to random deviations and then to a more realistic case where deviations of mixed nature can occur. In addition, the sensitivity of R-LSS to STD in each involved sampling point was evaluated. A significant impact of STD on the performance of R-LSS was demonstrated. The tolerance of R-LSS to STD was found to depend not only on the number of sampling points but more importantly on the duration of the sampling process. Sensitivity analysis showed that sampling points at which rapid concentration changes occur were relatively more critical for AUC prediction by R-LSS. As a practical approach, nomograms were proposed, where the expected predictive performance of R-LSS was provided as a function of STD information. The investigation of STD impact on the predictive performance of R-LSS is a critical element of R-LSS studies and should be routinely performed to guide their selection and use.

Keywords: Sampling time deviation (STD), Limited sampling strategy (LSS), Area under the curve (AUC), Cyclosporine (CsA), Therapeutic drug monitoring (TDM)

Introduction

Therapeutic drug monitoring is a standard of care for dosing optimization of drugs that exhibit a narrow therapeutic index and large inter- and intra-individual variability in order to minimize the risk of treatment failure and dose-related serious side effects. In clinical practice, the trough concentration (C_0) is often used as a dosing individualization marker; although in some instances its use has been disputed over and other therapeutic drug monitoring strategies have been suggested [1-5]. This is the case for cyclosporine (CsA), a widely used immunosuppressive agent, for which several published works have shown that pharmacokinetic (PK) markers other than C_0, such as the area under the concentration-time curve (AUC), are more related to clinical outcomes [6-12]. The large intra-individual variability of CsA can affect the value of using PK parameters (such as AUC) to guide dose adjustment. This being said, two recent studies for CsA in pediatric hematopoietic stem cell transplantation patients showed relationships between AUC of CsA and clinical outcomes [11,12].

In order to estimate AUC accurately, multiple blood samples over the dosing interval are generally used. The cost of this rich sampling approach and its inconvenience to patients prevent its widespread application in clinical settings. As an alternative, limited sampling strategies (LSS), relying on the smallest possible number of blood samples drawn at selected time points (often with 4 or fewer samples), have become an appealing tool for the estimation of AUC. Two main LSS approaches have been used, namely, the multiple linear regression-based (R-LSS) and the Bayesian-based (B-LSS) methods [13, 14].

Since B-LSS resorts to advanced population pharmacokinetic (Pop-PK) models, which often require additional modeling and computational skills, the R-LSS, involving only linear equations, is commonly used. However, despite its simplicity, R-LSS application is restrictive since the real times at which sampling occcurs (termed actual times) are assumed to correspond exactly to the selected sampling times (termed nominal times), without accounting for possible sampling time deviations (STD) that can occur in a real-life setting. Hence, one advantage of B-LSS over R-LSS is its flexibility in terms of sampling times since no related condition is imposed by either the construction of the associated Pop-PK model or the prediction of individual PK parameters, and the actual sampling times can be used in case of deviations from the nominal ones.Indeed, while analytical tools and techniques have greatly progressed in analyzing drug samples in the last decades, effective therapeutic drug monitoring is still hindered by human factors, whether in sample collection or processing. Putting aside systematic errors from the laboratory work, time errors in sample collection are believed to be a crucial problem. Irregularity in the time of collecting blood samples may have a non-negligible impact on the estimation of therapeutic markers and thus influence clinical decisions and outcomes [14].

Few studies have been devoted to this concern. In one study [15] where C_2 (Ct is the concentration at time t in hours post-dose) was used as the therapeutic marker for CsA dosing adjustment, the authors investigated concentration errors as a function of STD. They found that, when increasing the STD, the relative concentration error with respect to nominal C_2 could increase significantly. A considerable difference (up to 30%) from nominal C_2 values (which are themselves subjected to analytical inaccuracy

and imprecision) could result from a STD of 15 minutes. However, the estimation of AUC using R-LSS generally involves more than one sampling point for which the impact of STD may interact, accumulate, and even amplify. Moreover, this impact can differ from one sampling point to the other. All these issues call for a thorough investigation of the predictive performance of R-LSS in the presence of STD in order to set up guidelines for their selection and use.

Therefore the aim of this study was to investigate the impact of different STD scenarios on the estimation of AUC by R-LSS. To address this issue, a simulation approach was adopted and CsA chosen as a drug model.

Materials and Methods

Regression limited sampling strategy

AUC is generally estimated using the linear trapezoidal method which requires a dense sampling of 6 to 10 time points over a dosing interval. This approach limits the use of AUC as a dose adjustment criterion in daily practice. As an alternative, AUC can be estimated using limited sampling methods which only need a small number of samples collected at a priori selected time points. A commonly used approach is the multiple linear regression-based LSS (R-LSS). Mathematically, the estimation of AUC using R-LSS can be expressed as:

$$AUC_{pred} = F_0 + F_1 \times C_{t_1} + \ldots + F_k \times C_{t_k}$$

where $C_{t_1}, C_{t_2}, \ldots, C_{t_k}$ are the observed concentrations measured at a chosen set of time points $t_1, t_2, \ldots, t_k$ and $F_0, F_1, \ldots, F_k$ are the regression coefficients.

In the current work, various scenarios of STD from nominal times (i.e. STD from selected time points) were generated in order to evaluate their impact on the precision of the estimation of AUC using R-LSS. To perform this evaluation, CsA, for which we have previously reported R-LSS [16] and B-LSS [17], was used as a drug model. Namely, the R-LSS developed for oral (PO) CsA in pediatric hematopoietic stem cell transplantation (HSCT) were investigated, Table 4.1. Among these R-LSS, the nine LSS that have had clinically acceptable prediction error indices were retained to be reevaluated in terms of their performance in the presence of STD.

Table 4.1. Investigated R-LSS.

LSS#	Concentration-time points	LSS equation: AUC_{pred} (ng × h/mL)	Error indices		
			ME% (95% CI)	RMSE% (95% CI)	95th PAE%
LSS1	$C_{0.5}$, C_2, C_4, C_8	131.49 + 1.00 $C_{0.5}$ + 1.74 C_2 + 3.04 C_4 + 5.52 C_8	0.01 (-1.08, 1.10)	3.31 (2.59, 3.90)	6.30
LSS2	C_0, C_1, C_2, C_4	-45.58 + 4.78 C_0 + 0.99 C_1 + 1.40 C_2 + 4.16 C_4	0.06 (-2.06, 2.19)	6.48 (5.15, 7.57)	12.17
LSS3	C_0,$C_{0.5}$, C_2, C_4	77.53 + 3.85 C_0 + 1.05 $C_{0.5}$ + 1.81 C_2 + 4.13 C_4	0.11 (-2.15, 2.36)	6.87 (5.12, 8.25)	13.70
LSS4	C_0, C_1, C_3, C_4	57.53 + 3.77 C_0 + 1.45 C_1 + 2.18 C_3 + 3.33 C_4	0.09 (-2.31, 2.48)	7.30 (5.79, 8.54)	13.44
LSS5	$C_{1.5}$, C_4, C_8	75.66 + 1.87 $C_{1.5}$ + 3.46 C_4 + 6.12 C_8	0.05 (-1.80, 1.90)	5.63 (4.22, 6.74)	11.82
LSS6	C_0, $C_{1.5}$, C_4	-46.41 + 4.84 C_0 + 2.00 $C_{1.5}$ + 4.51 C_4	0.15 (-2.62, 2.92)	8.43 (5.97, 10.32)	16.71
LSS7	C_0, C_2, C_4	141.82 + 5.20 C_0 + 2.16 C_2 + 3.71 C_4	0.17 (-2.78, 3.11)	8.97 (6.75, 10.73)	16.48
LSS8	C_0, C_1, C_4	62.23 + 3.75 C_0 + 1.67 C_1 + 5.77 C_4	0.16 (-2.95, 3.27)	9.48 (6.81, 11.55)	15.77
LSS9	C_2, C_8	286.02 + 2.70 C_2 + 9.42 C_8	0.15 (-2.65, 2.94)	8.52 (6.20, 10.34)	16.04

CI, confidence interval; C_t, concentration at time t in hours post-dose.

Sampling time deviation scenarios

Three types of scenarios were considered going from the simplest case of fixed errors, to random errors represented by the most probable (generally normal) distributions and then to a more realistic case where errors of mixed nature can occur.

The first type involved fixed time lengths that were uniformly added to or deduced from all nominal times simultaneously in order to represent an actual delay or advance in sampling times, respectively. Deviations up to 20 minutes were considered (±1, 2, 3, 4,…,20 minutes). The second type involved random deviations, which were generated by assuming a normal distribution centered on nominal times with standard deviations (σ) ranging from 1 to 20 minutes, up to a maximum STD of 30 minutes. Actual times were obtained by the addition of these random deviations to nominal times. For each standard deviation value, 1000 cases of STD were generated for each R-LSS. The third type involved the combination of fixed and random deviations together. To produce this type of scenarios, STD were generated by assuming a normal distribution centered on fixed deviations between -15 and +15 minutes from nominal times (±1, 2, 3, 4,…,15 minutes).

Additionally, in order to identify those time points that are more vulnerable to STD in each R-LSS, a sensitivity analysis was performed for each time point separately, through the same procedure used to simulate sampling error scenarios for fixed and random STD.

Concentration time profiles

For ethical and practical reasons, the above sampling scenarios were tested through a PK simulation approach, in the population in whom the investigated R-LSS were developed [16]. This reference population consisted of 25 pediatric patients (median of 10.2 years (range, 0.5 -18.2)) receiving CsA twice daily for graft-versus-host disease prophylaxis after HSCT. They underwent 39 steady-state PK profiles, with nominal time points at 0, 0.5, 1, 1.5, 2, 3, 4, 8 and 12 h after PO CsA administration. In the current investigation, all the concentrations at different time points (actual and nominal time points) were simulated using a Pop-PK model previously developed for the reference population [17] (Figure 4.1). Of a two-compartment nature with a lag time in absorption, the Pop-PK model identified a large inter-individual variability in the main PK parameters represented by lognormal distributions, and an unexplained variability represented by a combined additive and proportional error model. As suggested in the pediatric literature [18], allometric scaling was applied to the base model using the power law of 3/4 and 1 for the scaling of clearance and volume of distribution by weight, respectively. Moreover, several covariates were identified, including weight, age, time post transplantation, alkaline phosphatase, and formulation (capsule or suspension).

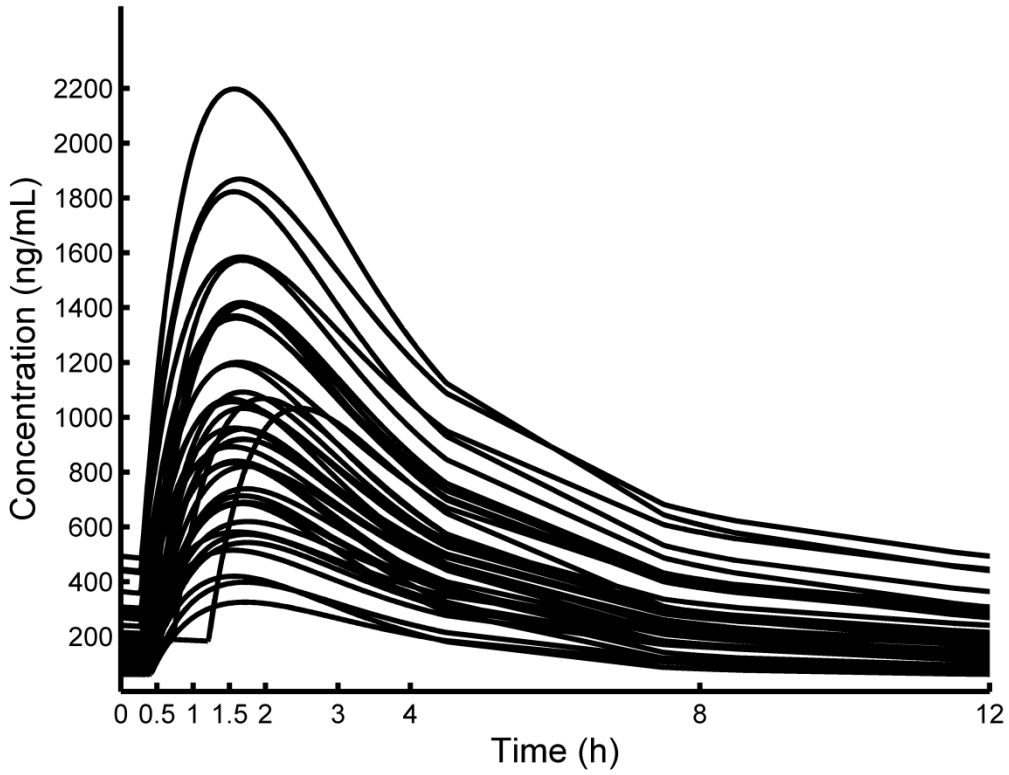

Figure 4.1: Concentration-time course of simulated PK profiles for PO cyclosporine in pediatric hematopoietic stem cell transplantation recipients.

Estimation of AUC

For a given R-LSS, the predicted AUC (AUC_{pred}) was calculated using simulated concentrations at nominal times ($AUC_{nominal}$) and simulated concentrations at actual times (AUC_{actual}). The simulated PK profiles included the concentrations at every minute within the range of the studied sampling deviations, so that the concentrations associated with any scenario of STD are accessible for the calculation of the associated AUC_{actual}. As mentioned in the Introduction, for AUC calculation using R-LSS, the simulated

concentrations at actual times are assumed to correspond exactly to the planned nominal times.

In addition, $AUC_{full,}$ which refers to the AUC obtained using simulated concentrations at all nominal times, was calculated using the trapezoidal method. The precision of R-LSS estimation of AUC was evaluated using prediction error indices that compare AUC_{full} with AUC_{pred} ($AUC_{nominal}$ and AUC_{actual}). The use of simulated data to calculate AUC_{full} and AUC_{pred} allowed isolating the impact of STD from other error sources such as the analytical method or data transcription.

Error indices

The predictive performance of LSS was evaluated using the following error indices (EI): 1) the relative prediction error (E%), 2) the 95th percentile of the absolute values of relative prediction errors (95th PAE%), 3) the mean relative prediction error (ME%) and 4) the root mean squared relative prediction error (RMSE%). These estimates were based on the following formulations:

$$E_i\% = \frac{AUC_{pred}^{(i)} - AUC_{full}^{(i)}}{AUC_{full}^{(i)}} \times 100 \quad (1)$$

95th PAE% = 95th percentile of the increasingly ordered set $\left\{\left|E_i\right|\%\right\}_{i=1}^{N}$ (2)

$$ME\% = \frac{1}{N}\sum_{i=1}^{N}\frac{AUC_{pred}^{(i)} - AUC_{full}^{(i)}}{AUC_{full}^{(i)}} \times 100 \quad (3)$$

$$RMSE\% = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(\frac{AUC_{pred}^{(i)} - AUC_{full}^{(i)}}{AUC_{full}^{(i)}}\right)^2} \times 100 \quad (4)$$

where i denotes the ith profile and AUC_{pred} refers to the predicted AUC using R-LSS, which can be $AUC_{nominal}$ or AUC_{actual}. Consequently, one has nominal and actual error indices, $EI_{nominal}$ and EI_{actual}, which refer to the prediction error indices due to the LSS

approximation of AUC_{full} when sampling occurs at nominal and actual times, respectively.

For each STD scenario, the ratios EI_{actual} /$EI_{nominal}$ (ex. 95^{th} $PAE\%_{actual}$/95^{th} $PAE\%_{nominal}$) were calculated, representing the effect of STD on AUC estimation for a given R-LSS. Then, the expected error indices in the presence of STD (EI_{STD}) were calculated by using these ratios to scale EI of the investigated R-LSS estimated using real data ($EI_{reference}$) [16].This was expressed as:

$$EI_{STD} = \frac{EI_{actual}}{EI_{nominal}} \times EI_{reference}$$

where EI_{STD} refers to 95^{th} $PAE\%_{STD}$, $ME\%_{STD}$ or $RMSE\%_{STD}$ and are reported as such in the Results section.

In order to provide a graphical visualization of the impact of STD, nomograms were used to illustrate LSS performance within a plane of sampling error attributes, which consists of fixed and random STD, as the x and y axes, respectively. These nomograms provide an insight of LSS tolerance to STD and classify their performance for various combinations of fixed and random STD pair, as stable or not, according to a given threshold of predictive error (ex. 95^{th} $PAE\%_{STD} \leq 20\%$).

Software

The commercial software package MATLAB® (2008b, The Math Works Inc, Natick, Massachusetts, U.S.A.) and NONMEM® (version VII, Icon Development Solutions, Ellicott City, MD) were used for calculations and simulations.

Results

Diverse scenarios involving fixed, random, and mixed STD were investigated for their impact on the estimation of AUC using R-LSS. Nine R-LSS for CsA AUC estimation in pediatrics, reported in Table 4.1, were studied. They were divided into 3 groups according to the number of required concentrations. Four from these R-LSS, namely LSS1 (that requires the following concentrations: $C_{0.5}$, C_2, C_4, C_8), LSS6 (C_0, $C_{1.5}$, C_4), LSS7 (C_0, C_2, C_4) and LSS8 (C_0, C_1, C_4) were chosen to highlight the impact of STD. The choice of these LSS was guided by their stability (LSS1, best), susceptibility (LSS8, worst) as well as comparability (LSS6 and LSS7, similar performance for nominal times but likely to be discriminated through STD analysis).

For fixed STD, the ratio between $E\%_{actual}$ and $E\%_{nominal}$ was correlated with the absolute value of the fixed sampling error with symmetric (the impact of STD depends mainly on its absolute values, LSS8) and slightly irregular (the impact of STD depends on its value but also on its type, delayed or in advance, LSS1, LSS6 and LSS7) shapes, Figure 4.2. The absence of symmetry indicates a different impact of STD whether the samplings are performed ahead or after nominal times. Every LSS has a different pattern of stability against STD dictated by the overall impact of time deviations of its sampling points. As shown, LSS1 was less sensitive to STD and had the best stability while LSS8 had the worst. Moreover, even though LSS6 and LSS7 had comparable performance in terms of AUC estimation using real data (Table 4.1), the latter was less affected by STD.

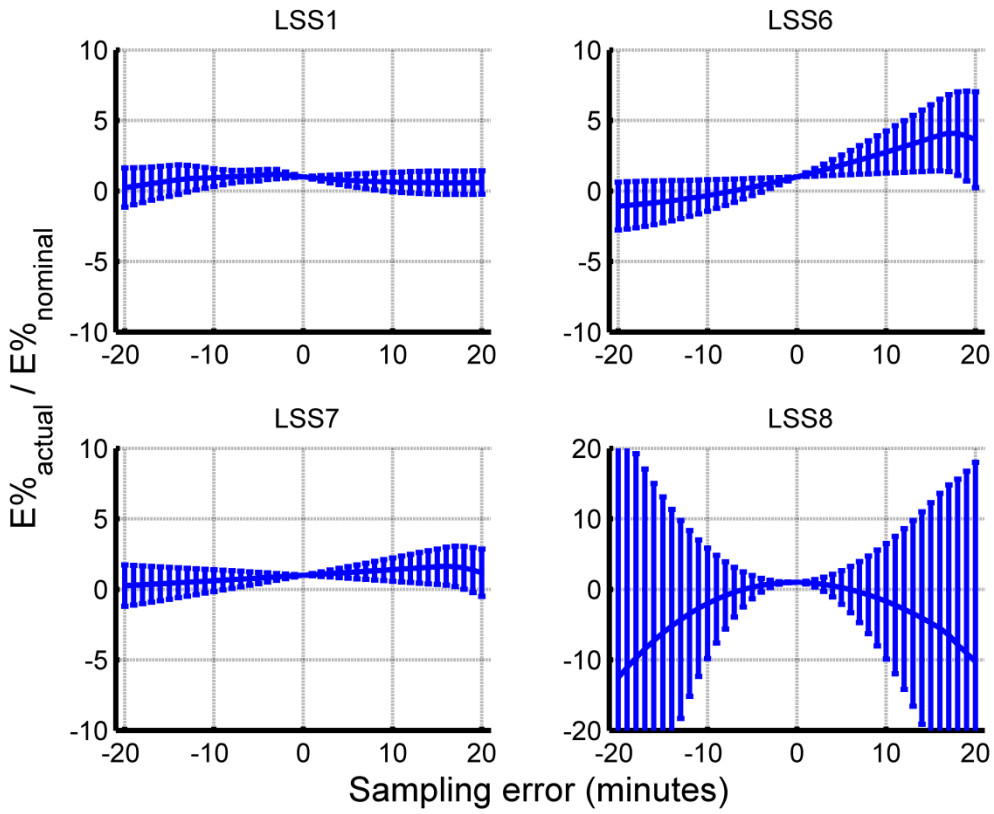

Figure 4.2: Fixed STD impact: the ratio $E\%_{actual}/E\%_{nominal}$ for four LSS (LSS1, LSS6, LSS7, and LSS8) used for cyclosporine AUC prediction (mean ± SD), $E\%_{actual}$ is the relative prediction error using actual times, $E\%_{nominal}$ is the relative prediction error using nominal times.

For random STD, eight of the nine studied LSS (all except LSS8) were stable against sampling errors when considering STD with a standard deviation up to 5 minutes (Table 4.2 and Figure 4.3). However, only four LSS (LSS1, LSS4, LSS5, and LSS9) still had 95^{th} $PAE\%_{STD}$ less than 20% when standard deviation increases to 10 minutes. In fact, an extended sampling to 8 hours, beyond the restriction of 4-hour post-dose, increased the stability as observed in the cases of LSS1, LSS5, and LSS9. Similarly, 4-point LSS were less affected by STD than 3-point LSS. Particularly, the prediction error

of 3-point LSS can increase dramatically in the presence of STD as seen with LSS6 and LSS8. For all LSS, the prediction performance had an apparent inverse regression relationship with STD and only one LSS, namely LSS1, requiring 4 sampling points and an extended sampling duration of 8 hours, could predict AUC with 95^{th} $PAE\%_{STD}$ less than 20% in spite of standard deviation up to 20 minutes.

Table 4.2. Error indices' ratios (EI_{actual} /$EI_{nominal}$) for random STD (described by the standard deviations of sampling errors in minutes).

LSS#	**EI_{actual} /$EI_{nominal}$**					
	$RMSE\%_{actual}$ / $RMSE\%_{nominal}$			**95^{th} $PAE\%_{actual}$/95^{th} $PAE\%_{nominal}$**		
	5 (min)	**10 (min)**	**15 (min)**	**5 (min)**	**10 (min)**	**15 (min)**
LSS1	1.31	1.75	2.06	1.50	2.02	2.39
LSS2	1.18	1.69	2.24	1.17	1.76	2.34
LSS3	1.14	1.44	1.62	1.44	1.91	2.20
LSS4	1.19	1.67	2.14	1.06	1.41	1.78
LSS5	1.16	1.58	1.90	1.17	1.57	1.86
LSS6	1.16	1.67	2.11	1.15	1.64	2.11
LSS7	1.05	1.18	1.33	1.09	1.26	1.44
LSS8	1.81	3.30	4.40	1.66	3.05	4.04
LSS9	1.02	1.07	1.13	1.06	1.17	1.26

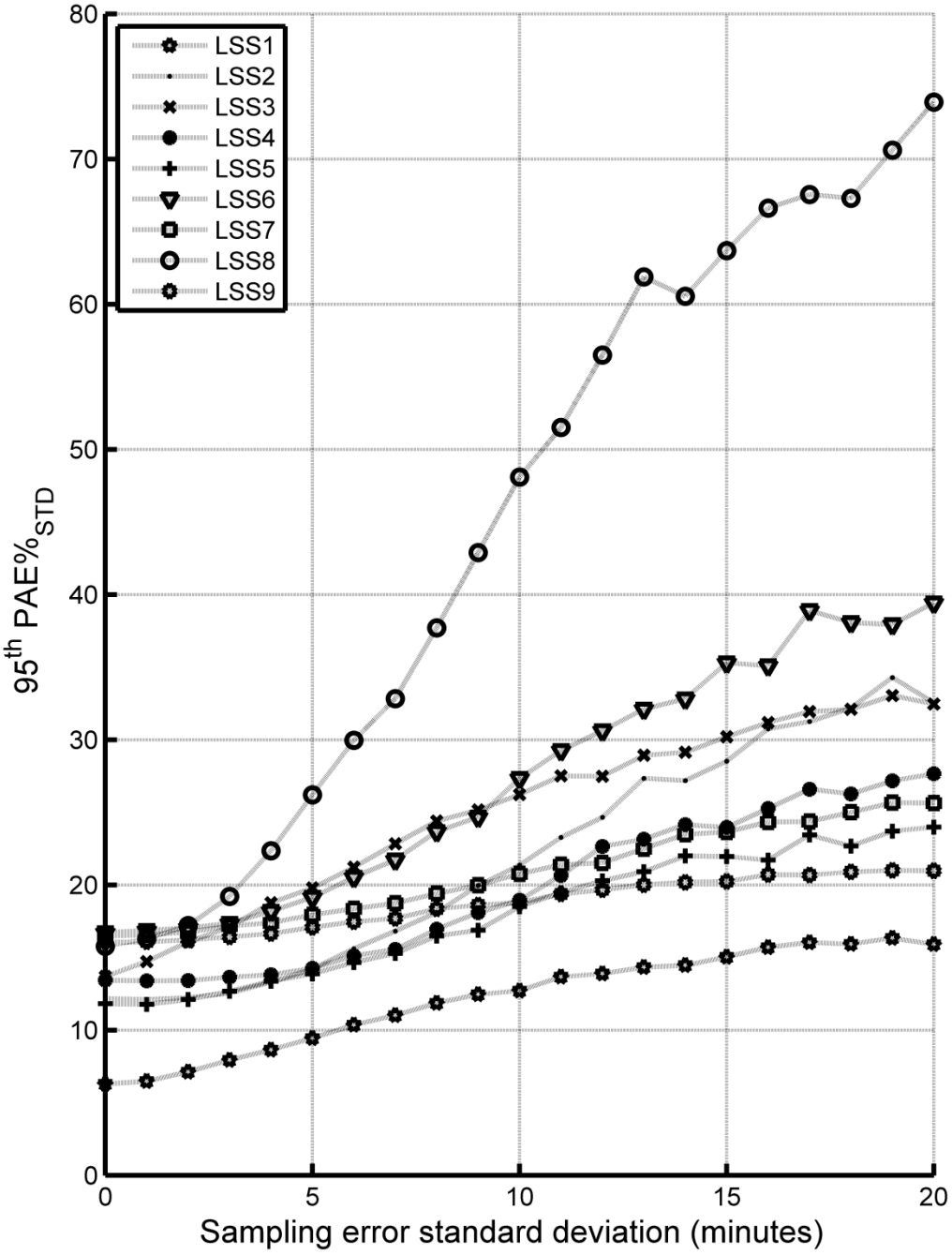

Figure 4.3: Random STD impact: 95th PAE%$_{STD}$ for the nine LSS used for cyclosporine AUC prediction. Random STD is described by the standard deviation of the sampling errors, 95th PAE%$_{STD}$ is the 95th percentile of absolute values of relative prediction errors in the presense of STD**.**

When considering mixed STD, Figure 4.4, LSS1 and LSS8 did not show a further decline in their predictive performance compared with random STD alone. In contrast, fixed deviation combined with random deviation further reduced the prediction performance of LSS6 and LSS7.

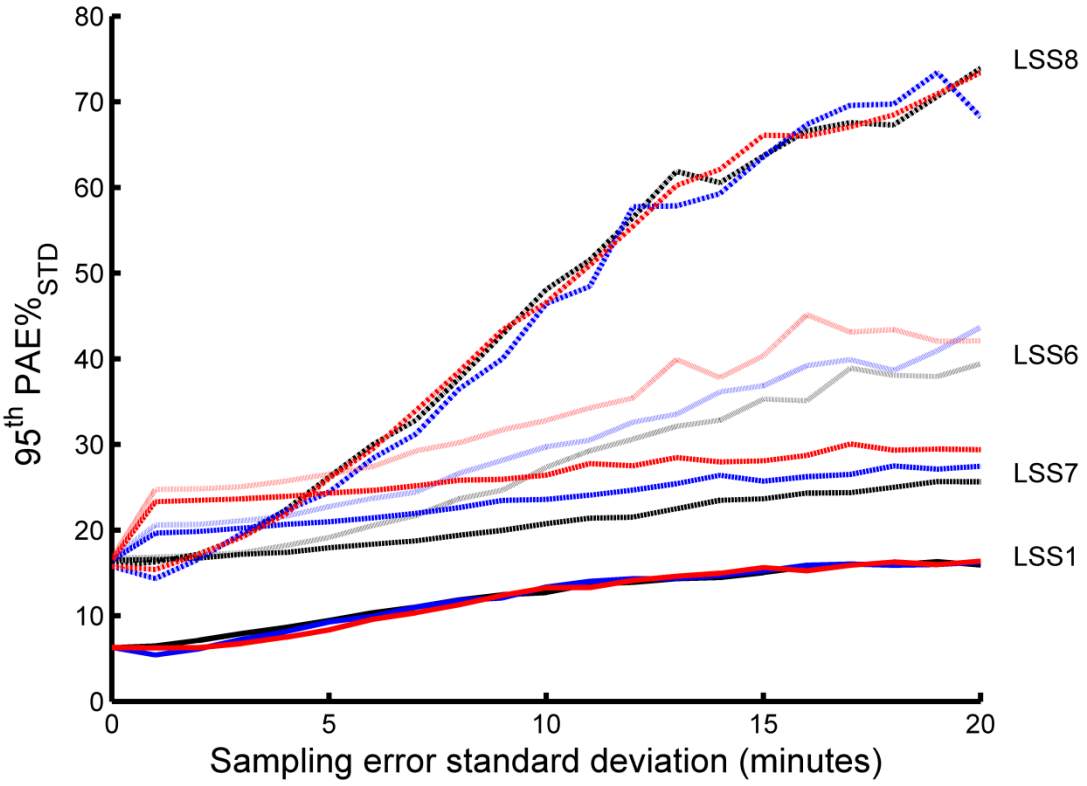

Figure 4.4: Mixed STD Impact (fixed deviations of 5 (in blue) and 15 (in red) minutes accompanied with random STD as well as random STD alone (in black)): 95^{th} $PAE\%_{STD}$ for four LSS (LSS1, LSS6, LSS7, and LSS8) used for cyclosporine AUC prediction. Random STD is described by the standard deviation of the sampling errors, 95^{th} $PAE\%_{STD}$ is the 95^{th} percentile of absolute values of relative prediction errors in the presense of STD.

Figure 4.5 displays different zones of 95th PAE%$_{STD}$ values for four LSS. LSS1 had a large clinically acceptable zone of 95th PAE%$_{STD}$ ≤ 20%, while LSS8 had the smallest one. These findings are in agreement with the above results. Moreover, LSS7 had a larger acceptable zone of 95th PAE%$_{STD}$ ≤ 20%, compared to LSS6. Although, both LSS had similar predictive error indices when STD was not considered (Table 4.1), the nomograms revealed a better robustness of LSS7 in the presence of STD. Thus, the proposed nomograms provide an additional objective way for the comparison of LSS ability to estimate AUC, particularly in the clinical setting where STD are expected. It is also worth noticing that nomogram zones were not always centered on zero values of the fixed STD axes. This can be explained by the fact that the LSS do not necessarily have their best performance when nominal time concentrations (in the absence of all types of STD) are used; and some STD may have a positive impact by decreasing the prediction error. However, such observation does not indicate a sustained association between the improvement of LSS performance and a given STD, and should not lead to the recommendation of an advance or late sampling.

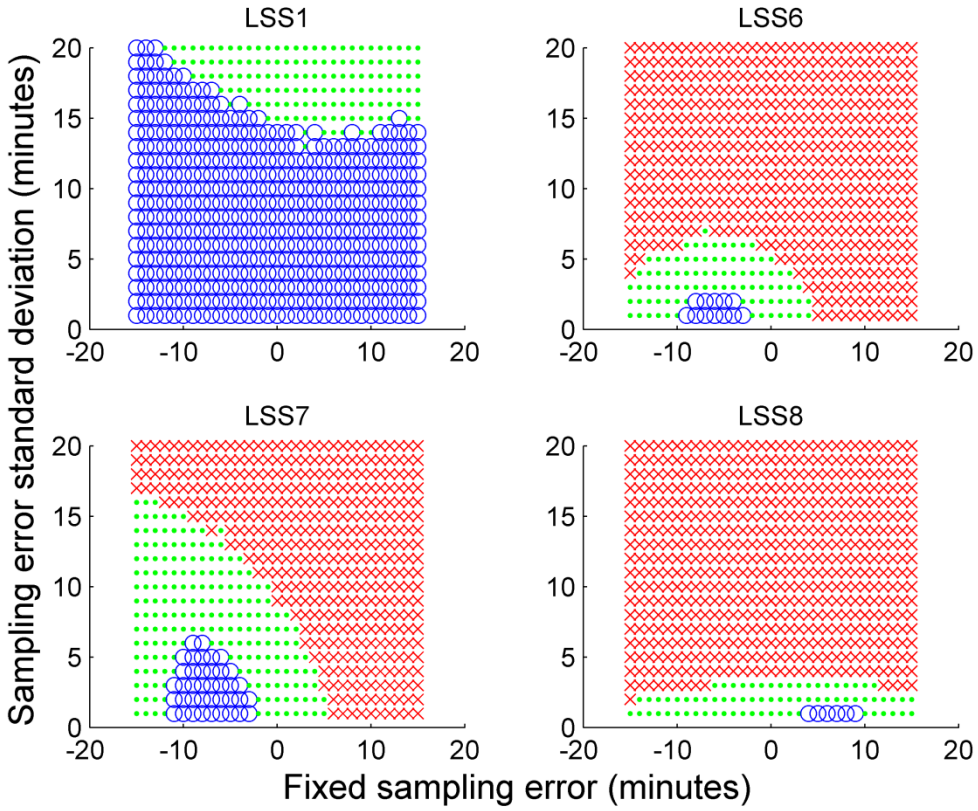

Figure 4.5: Nomograms for various combinations of random and fixed STD for four LSS (LSS1, LSS6, LSS7, and LSS8) used for cyclosporine AUC prediction. Red ×, for 95th PAE%$_{STD}$ more than 20%; green ●, equal or less than 20%; and blue ○ equal or less than 15%. Random STD is described by the standard deviation of the sampling errors, 95th PAE%$_{STD}$ is the 95th percentile of absolute values of relative prediction errors in the presense of STD**.**

Sensitivity analysis for each sampling point in the presence of fixed and random STD highlighted that certain sampling points are more crucial for the prediction of AUC (Figure 4.6 and Figure 4.7, respectively). Figure 4.6 illustrates the impact of fixed STD on R-LSS performance when a time error occurs only on one sampling point, while

keeping a full compliance at other sampling times. It shows that C_8 was the least sensitive point in LSS1 since its curve had an almost flat shape while $C_{0.5}$ was the most sensitive to STD with a relatively abrupt and strong decrease in the predictive performance. The sensitivity of C_2 and C_4 was between these two extreme cases. For other LSS, C_0 was always the least sensitive point, likely because less variation of concentration occurs around this sampling time, particularly in the presence of lag time in absorption, while C_1, and C_4 were the sampling point most affected by STD. For example, in the case of LSS7, a sampling delay of 10 minutes can be tolerated for C_0 or C_2 but not for C_4, considering the 95th $PAE\%_{STD}$ threshold of 20%. Thus, LSS performance was more sensitive to STD for those time points for which the concentration shows a rapid change, in other words, where the concentration-time curve has a large slope.

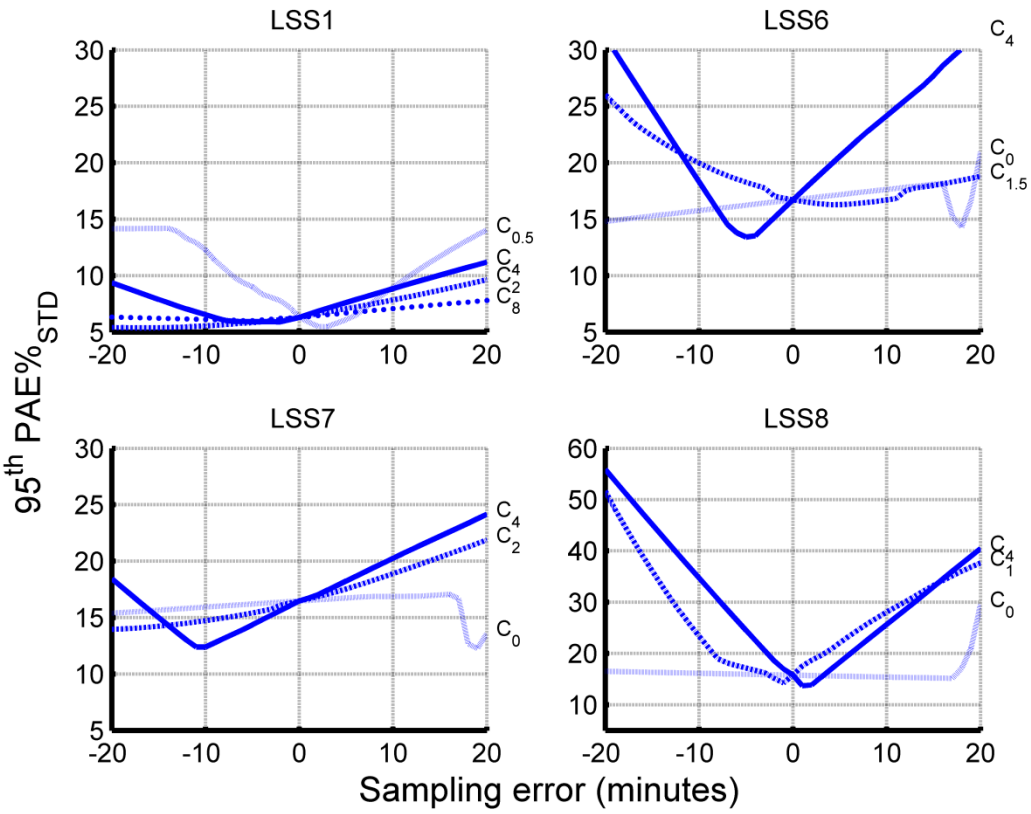

Figure 4.6: Sensitivity analysis for each sampling point in the presence of fixed STD for four LSS (LSS1, LSS6, LSS7, and LSS8) used for cyclosporine AUC prediction, 95^{th} $PAE\%_{STD}$ is the 95^{th} percentile of absolute values of relative prediction errors in the presense of STD, C_t is the concentration at time t in hours post-dose.

These sensitivity results are confirmed in Figure 4.7, where analysis was performed using random STD with the additional advantage of better delineating sampling points in terms of R-LSS sensitivity, where a sampling time point of a larger impact corresponds to a sensitivity curve with a greater slope. As an example, for LSS8, STD impact can be ordered as $C_0 < C_4 < C_1$.

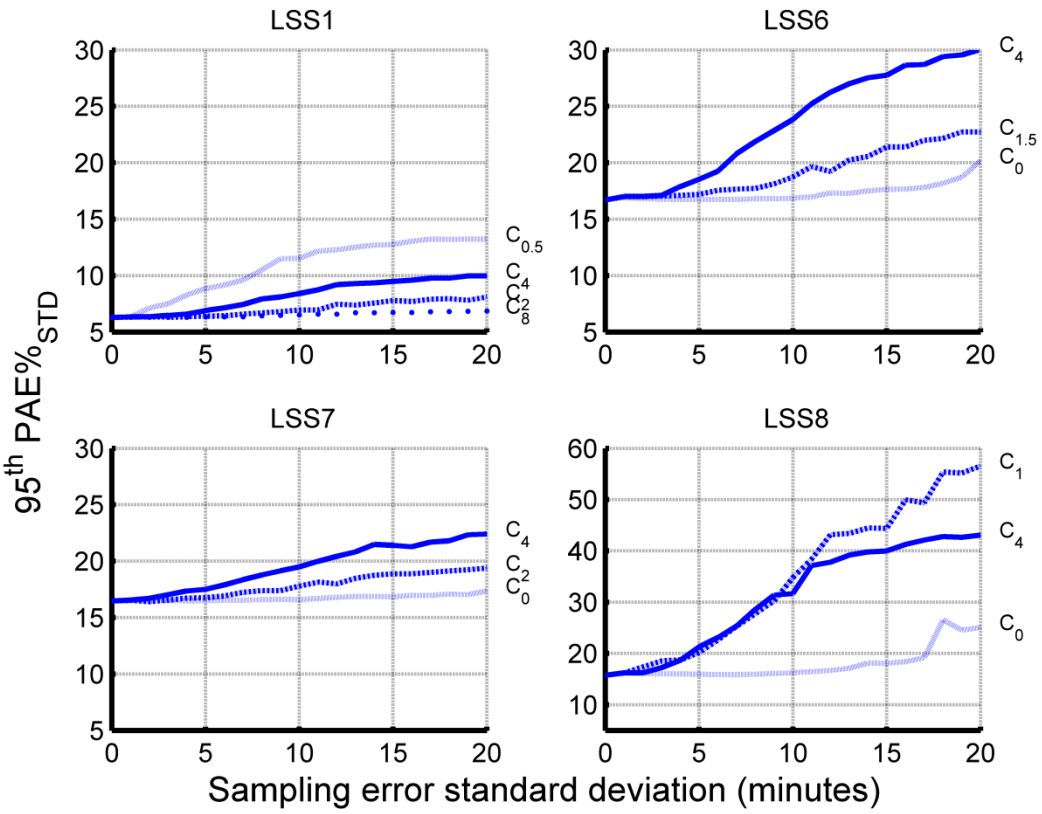

Figure 4.7: Sensitivity analysis for each sampling point in the presence of random STD for four LSS (LSS1, LSS6, LSS7, LSS8) used for cyclosporine AUC prediction. Random STD is described by the standard deviation of the sampling errors, 95^{th} $PAE\%_{STD}$ is 95^{th} percentile of absolute values of relative prediction errors in the presense of STD, C_t is the concentration at time t in hours post-dose.

Discussion

A regression limited sampling approach has the advantage of being a simple and efficient way to estimate AUC, using a small number of sampling points obtained within a short time interval. However, its performance can be hampered by errors in sampling times, which is recognized as an unavoidable human factor that should be accounted for.

Similar to the issue of noncompliance in drug intake, known for its consequences on drug fate [19-22], the deviation from nominal sampling times of R-LSS may have a non-negligible impact on AUC estimation and thus on clinical decision and therapeutic outcomes. Using CsA as a drug model, this study showed a significant impact of these deviations on the performance of R-LSS in predicting AUC. Therefore, a more reliable use of R-LSS should account for this aspect.

The tolerance of R-LSS to STD does not only depend on the number of sampling points but more importantly on the whole duration of the sampling process. In fact, adding a late sampling point to a R-LSS does not only decrease its 'nominal' prediction error, but also increases its stability in case of STD. Another important finding is that, some R-LSS may have similar performance in terms of nominal times, but their tolerance to STD can be quite different, offering an additional criterion in the selection of R-LSS, with a preference for those which are more stable (tolerant to STD). In fact, the investigation of the impact of STD on the estimation of AUC using R-LSS is a vital element that should be routinely performed to guide their selection and use.

To our knowledge, STD issue has not been previously investigated in the context of AUC estimation. This lack of investigation could be due to the difficulty (for ethical and practical reasons) of performing clinical studies using an irregular sampling protocol or intensive concentration-time samplings to mimic diverse sampling scenarios. This calls for a simulation-based approach. Hence, in the current paper, a Pop-PK model was used for simulating dense concentration-time profiles. Although an empirical polynomial regression model can also be used for such simulations, we adopted the Pop-PK model as more mechanistic information can be taken into account, such as the drug

distribution between compartments and the absorption lag time. The use of these simulated data enabled us to thoroughly investigate the impact of a diversity of sampling scenarios on R-LSS performance. The current study allowed reevaluating R-LSS performance from a new angle of view.

In order to have a clear idea about the relationship between STD and R-LSS performance, nomograms using different thresholds of error indices were proposed. The conception of these nomograms is in line with the nomographic charts that are usually used to assist clinical practice. Particularly, the nomograms can be useful in establishing sampling protocols that account for STD. As shown in Figure 4.5, for given values of 95^{th} $PAE\%_{STD}$, different zones of tolerance to sampling errors can be clearly delineated in the STD plane composed of the average of sampling errors (fixed STD, x axis) and their standard deviations (random STD, y axis). In fact, for each R-LSS, a nomogram can be developed to indicate the robustness of R-LSS to STD; hence R-LSS that have low tolerance to STD can be readily recognized to draw more attention in clinical practice in order to avoid errors during sampling procedure. Furthermore, during R-LSS development and selections process, these nomograms could also provide valuable information regarding the stability of R-LSS performance against STD.

In case a sampling error occurs, it is crucial to recognize its impact and to decide whether additional or alternative procedures are necessary. As a bedside application, sensitivity analysis can be used to assess the impact of a sampling error on a single time point. If this sampling error is expected to result in an unacceptable AUC prediction error for the used R-LSS, an alternative R-LSS with complementary or substitute time points can be proposed, instead of restarting the whole sampling procedure. This

alternative R-LSS usually includes additional late sampling point that can provide an accurate prediction of AUC in spite of the encountered sampling error. For example, in LSS7 (C_0, C_2, C_4), a sampling delay of 15 minutes in C_4 can result in a prediction of AUC with 95th PAE%$_{STD}$ > 20%, Figure 4.6. In this case, C_8 can be added in order to use the R-LSS composed of C_0, C_2, C_4, C_8, (AUC=162.03 + 1.65 C_0+ 2.09 C_2 + 2.57 C_4 + 5.43 C_8) as an alternative. The new formula can predict AUC with 95th PAE%$_{STD}$ of 14.9% in spite of 15 minutes error in C_4. Another alternative is to rule out C_4 and use R-LSS composed of C_0, C_2, C_8, (AUC=152.24 + 2.27 C_0 + 2.77 C_2 + 7.81 C_8), but in this case with a 95th PAE% of 17.8% [16]. A comprehensive strategy to substitute R-LSS by alternative ones would be interesting to establish. This is however beyond the scope of the current paper and could be pursued in a future work.

Conclusion

Sampling time errors can considerably affect the predictive performance of R-LSS. These limited sampling strategies may behave differently in the presence of distinctive scenarios of STD and exhibit various tolerability patterns. The impact of STD should be considered as an additional R-LSS selection criterion with a preference for those which are more stable and can also be used to point out restrictions and caveats for sampling errors that significantly affect the performance of R-LSS. Therefore, the investigation of STD impact on the prediction performance is an important element of R-LSS studies and should be routinely performed to guide their selection and use.

Acknowledgements

This work was supported by FRSQ Student Scholarship, held by S. Sarem, NSERC Discovery Grant, NSERC-Industrial Chair in partnership with Novartis, Pfizer and inVentiv Health Clinical as well as FRQNT Projet d'équipe, hold by F. Nekka.

Conflict of Interest

The authors have no conflict of interest.

List of abbreviations

AUC	Area under the concentration-time curve
AUC_{full}	Full AUC : AUC estimated using the trapezoidal method and the full set of nominal simulated concentrations
AUC_{pred}	Predicted AUC : AUC estimated using LSS method and simulated concentrations
$AUC_{nominal}$	Nominal AUC : AUC estimated using LSS method and nominal simulated concentrations
AUC_{actual}	Actual AUC : AUC estimated using LSS method and actual simulated concentrations in diverse sampling time deviation scenarios
CsA	Cyclosporine
C_t	Concentration at time t in hours post-dose
E%	Relative error
EI	Error indices
ME%	Mean relative prediction error
Pop-PK	Population pharmacokinetics
R-LSS	Regression limited sampling strategy
RMSE%	Root mean squared relative prediction error
STD	Sampling time deviation
95^{th} PAE%	95^{th} percentile of absolute values of relative prediction errors

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Chapter 5 Discussion

The main goal of this thesis was to establish a comprehensive methodology for the development and selection of practical LSS for an accurate estimation of AUC. To achieve this objective, CsA was used as a drug model for which LSS were developed to predict its AUC in pediatric HSCT.

The conventional approaches usually used in LSS investigations were carefully reevaluated and improved. Specifically, the weighted linear regression, which includes a weight in its regression objective function in order to account for the relative value of the prediction error, was used to build up the R-LSS. The addition of this weight aimed to prevent large relative prediction errors in the estimation of small AUC that can occur when using the ordinary linear regression instead. Moreover, **a more sound choice of Pop-PK models, rather than the routine use of the 'final' model, was proposed to improve the predictive performance of B-LSS.** In addition, the performance of B-LSS for targeting different versions of AUC was also investigated by comparing their predictions of the 'underlying' AUC which excludes the residual errors with those of the observed AUC directly calculated using the measured concentrations. As a final point, **STD, which is recognized to occur frequently in clinical practice, was studied and its impact on R-LSS analyzed in order to improve their selection process and support their application in clinical settings.**

For a thorough LSS investigation, **all possible combinations of concentration-time points were evaluated, with no preliminary pre-selection phase in order to avoid exclusion of potentially suitable candidates.** Then, LSS were selected according

to their predictive performance while paying careful attention to their applicability in clinical settings. As illustrated in Figure 5.1, in each LSS subgroup defined according to the number of included concentration-time points, representative LSS were chosen. First, **LSS were selected according to their predictive performance in addition to two criteria that were considered to favor more practical LSS:** 1) inclusion of C_0 since it is a reference point well recognized by the health professionals, in addtion it allows compliance to be checked and helps identifying patients with high clearance; 2) restriction to a sampling period of 4 hours post-dose for all included concentrations. Screening LSS using these practical criteria, which aim to select LSS that are more appealing for clinicians, may lead to overtake those associated with the best predictive performance. So, in each subgroup, the LSS which has the highest predictive performance was also selected whether it meets the last two practical criteria or not. In this way, the cost, in terms of performance, of considering practical criteria could also be assessed. In addition, the division into subgroups defined by the number of concentration-time points, when performing the selection process of LSS, allowed to evaluate the correlation between the number of these points and the predictive performance.

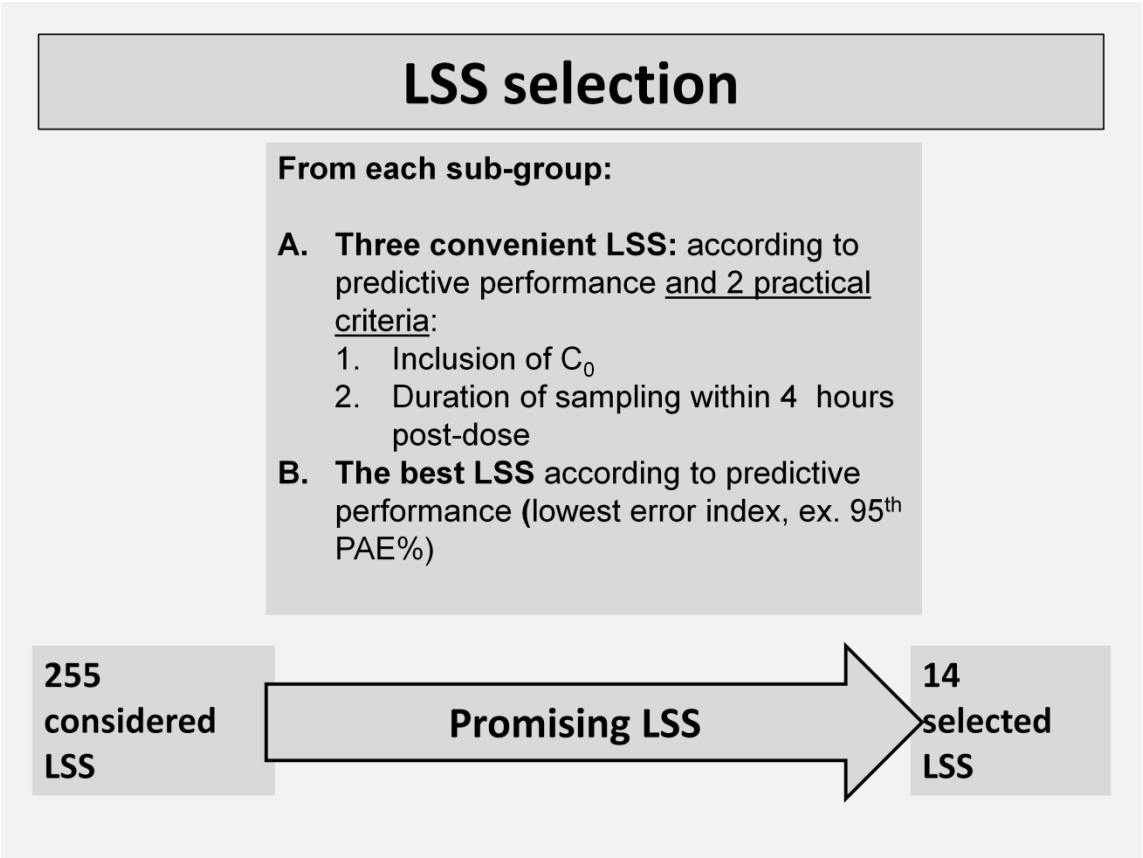


Figure 5.1: LSS selection scheme; all possible LSS of 4 or fewer points were derived from available profiles; these LSS were screened using practical criteria and predictive error indices to select the most convenient and accurate LSS, 95th PAE% is 95th percentile of absolute values of relative prediction errors.

The optimal marker for CsA monitoring in transplantation patients remains controversial, nonetheless, **there is a growing interest in the use of AUC, particularly for CsA dose adjustment in pediatric HSCT.** The issue of the value of AUC as a marker for therapeutic drug monitoring is outside the scope of this thesis, and eventually prospective trials are needed to confirm the advantage of AUC-guided dose adjustment in termes of clinical outcomes. However, our study plays a key role in order to achieve,

in the end, adequate therapeutic drug monitoring for CsA. To reach this objective, one has indeed to begin with developing practical monitoring tools such as LSS for the estimation of AUC; then clinical or PK/PD studies can be conducted to establish AUC-based therapeutic window and dosing guidelines. Finally, prospective trials can be carried out to evaluate the efficacy and safety of AUC-guided dosing regimens and to decide whether or not AUC-based monitoring is superior to that based on C_0 for short- and long-term outcomes. The present study addresses the first goal by providing practical LSS, which accurately estimate AUC, and making them more accessible in clinical settings. Particularly, **the reported LSS are desired to overcome the practical and ethical difficulties, mainly in the pediatric population, that may delay performing the needed trials to adequately evaluate AUC as a therapeutic drug monitoring marker.**

The error indices were selected carefully to adequately charectrize the performance of the evaluated LSS. The coefficient of determination (R^2) only estimates the association between observed and predicted values and provides no information regarding the predictive performance; hence, it was not used for LSS evaluation. Absolute error indices, such as ME and RMSE, which are based on absolute (not relative) prediction errors, provide precious means for estimating the performance of LSS. However, they do not take into account the relative value of prediction errors. The relative error indices, such as ME% and RMSE%, provide more easily interpretable evaluation of the predictive performance, compared to absolute ones. However, their use has to be surrounded by cautions to ensure that their values are not affected by outliers or data asymmetry [88].

In addition, although RMSE% represents the deviation of the estimate (ex. AUC_{pred}) from the target of the prediction (ex. AUC_{full}) its utility to guide LSS use in clinical practice is limited since its value cannot be straightforwardly interpreted in terms of an intelligible prediction error interval. For example, RMSE% does not involve that all or even 95% of the prediction errors are smaller than its value while 95th PAE% does. Hence, **the 95th PAE% as well as the number of prediction errors within selected intervals can be reported to evaluate the LSS performance in a clinically oriented way.** Nonetheless ME% as well as RMSE% should continue to be routinely reported for a complete predictive performance evaluation of LSS. ME% is necessary to identify the biased LSS, while RMSE% is usually used to compare the precision of prediction.

The dataset used for the validation of LSS has to be different from that used in their learning process in order to avoid the overestimation of their predictive performance. However, the small size of available data, which is a common issue in pediatric research, may lead to use the leave-one-out cross-validation, a recognized validation technique in cases of small data. For the development of R-LSS in the Article I (Chapter 2) of this thesis, the initial design of the study included one cohort, namely Cohort A (PK profiles performed from August 2009 to August 2010), with a leave-one-out cross-validation planned. **Several developed R-LSS were shown to accurately estimate AUC according to this validation. Though, we decided to further challenge these R-LSS by testing their reliability in an external cohort**, specifically Cohort B as it became available through collecting additional PK profiles (from September 2010 to September 2012). **This external validation did not delay the progress of the**

planned R-LSS investigations since the additional PK profiles were collected while processing and analyzing the initially available data (Cohort A).

The estimated performance of LSS and their reliability in real-life settings depends on the heterogeneity of the development cohort and hence its ability to accurately represent the studied population. The prediction error indices calculated in the external validation for the selected R-LSS for IV CsA were higher than those obtained by the leave-one-out cross-validation. These differences in predictive capacity may be due to the fact that the set of available IV PK profiles used for the leave-one-out cross-validation (Cohort A) was relatively small and more homogeneous compared to that used for the exteral validation (Cohort B), as shown in Figure 2.1 in Article I. These observations support the value of the external validation and indicate that **when the available data are limited, and hence cannot exhibit the expected variability of the studied population, the validation results using leave-one-out cross-validation or other validation methods should be considered with caution** since in these conditions over-estimation of the predictive performance ought to be anticipated.

As a general rule, the estimated predictive performances of LSS are only applicable for patients whose conditions and characteristics are comparable to those considered in the LSS's development and validation. For example, Patients receiving CsA three times a day (TID) were excluded from our LSS studies since they have different PK profiles compared to the investigated ones who received CsA twice a day (BID); therefore, the developed LSS are not intended for use in patients receiving CsA TID.

The performance of the reported R-LSS has been evaluated in the presence of large inter and intra-individual variability (several patients had multiple PK profile) and, as mentioned above, they are expected to maintain their predictive performance when applied in a similar population. The next step after AUC estimation is to decide if a dose adjustment is necessary; **the large intra-individual variability of CsA can affect the value of the use of PK parameters (such as AUC) to guide dose adjustment [52, 89].** This being said, two recent studies for CsA in pediatric hematopoietic stem cell transplantation showed relationships between AUC of CsA and clinical outcomes [44, 45]. Nonetheless, as mentioned above further studies are still needed to directly confirm the effectiveness and safety of AUC-guided dosing regimens.

Patients' characteristics were not accounted for in the equations of R-LSS as independent variables since no correlation was found in the studied population between LSS performance (their relative prediction errors) and these characteristics when evaluated using scatter plots. The Appendix I shows the scatter plots for patient's characteristics (covariates) that were retained in the final CsA Pop-PK model. As an example, refer to the figures below showing relative prediction errors vs. patient age and concomitant azole antifungal drugs (Figure 5.2) and concomitant calcium-channel blocker (Figure 5.3) for the estimation of AUC by the R-LSS (C_0, C_2, C_4). [52]However, **since concomitant medications and other patients' characteristics can increase or decrease systemic drug exposure (AUC), reliable LSS have to predict accurately AUC over a wide range of values, and this is the case of the reported LSS,** as already shown in Bland-Altman Plots.

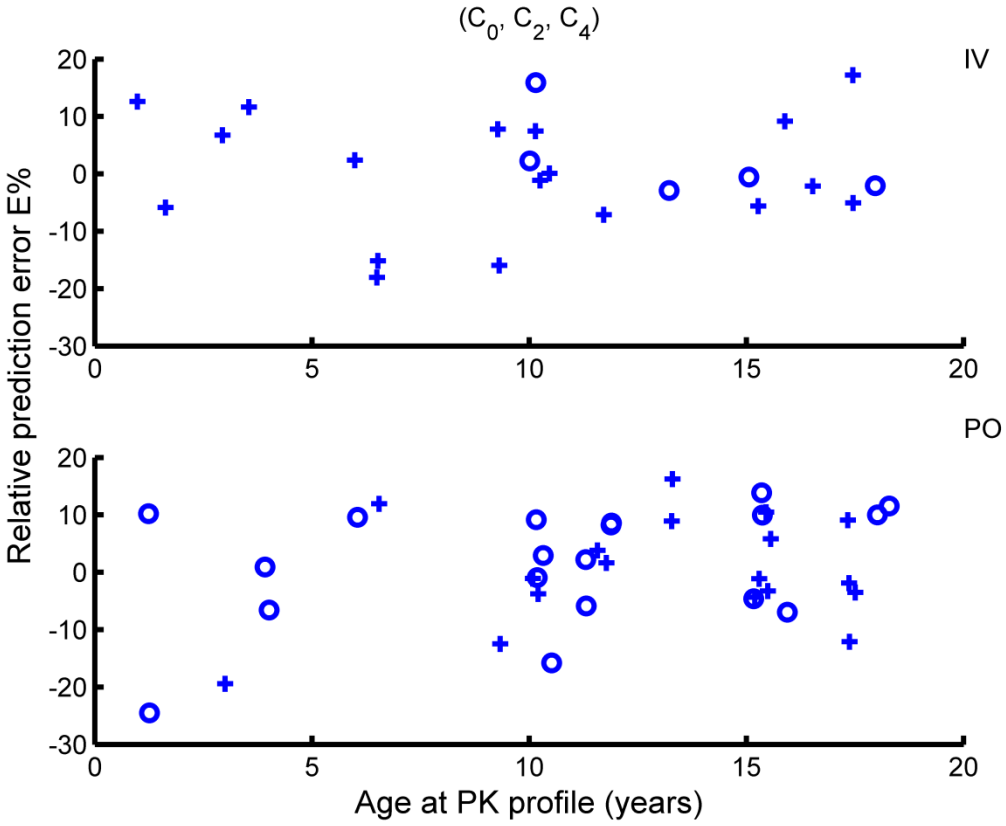


Figure 5.2: Prediction errors vs. concomitant azole antifungal drugs (+ for yes, ○ no) and patient age for the R-LSS (C_0, C_2, C_4) used for estimating AUC of cyclosporine administrated A) IV and B) PO

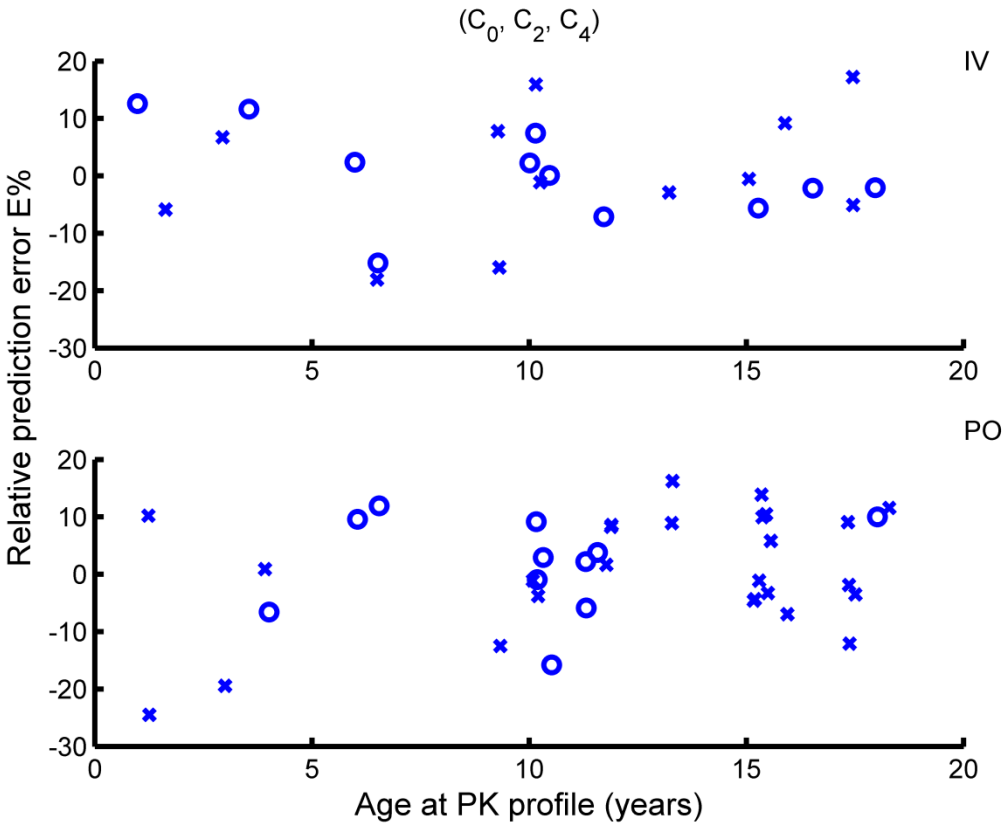


Figure 5.3: Prediction errors vs. concomitant calcium-channel blockers (× for yes, ○ no) and patient age for the R-LSS (C0, C2, C4) used for estimating AUC of cyclosporine administrated A) IV and B) PO.

In addition to the limited number of available profiles, accessible data may have other limitations, usually due to ethical and practical considerations, such as the limited number of samples collected for each PK profile. The limitations of collected PK data can impede performing deep and comprehensive studies. **Particularly, in order to carry out investigations requiring a rich sampling approach, the use of Pop-PK simulation is an indispensable approach.** Besides, the use of simulated data offers other interesting opportunities such as excluding or controlling the random noise

associated with residual errors (ex. estimating 'underlying' AUC), expanding the characteristics (ex. age) of the studied population, and considering a variety of PK features (ex. simulating patients with a renal dysfunction (low clearance)). Simulation approaches are increasingly used to enhance the cost-effectiveness in drug development, particularly in pediatrics [90]. In the Articles II and III included in this thesis,the simulated concentrations using a Pop-PK model that excludes the residual errors, i.e. IPRED in NONMEM® nomenclature, were used to allow broad investigations. In Article II, the 'underlying' AUC was estimated since its correlation with actual drug exposure is likely to be higher than AUC_{obs} once the random noise associated with residual errors is eliminated.

In Article III, **the use of simulated data for the calculation of the full and predicted AUC allows to take apart sampling time error from the other errors, such as analytical method errors and data registration mistakes, that usually contribute to the inaccuracy in AUC prediction.** However, we were aware that excluding the other sources of errors in AUC estimation can lead to nominal error indices ($EI_{nominal}$ calculated using simulated concentration at nominal time without sampling times errors) that are likely smaller than those calculated using real data, as shown in Table 5.1. Likewise, the actual error indices (EI_{actual} calculated using simulated concentration at actual times with sampling time errors) cannot be compared directly with those calculated using real data for estimating the impact of STD on R-LSS predictive performance. As shown in Figure 5.4, even though the STD can increase nominal 95th PAE% by almost 5 folds for LSS8 its actual 95th PAE% remains beyond the 20% threshold. Therefore, referring to actual 95th PAE% to estimate the impact of

STD can be misleading since this estimation does not consider the other sources of errors that are expected in real-life settings.

However, the EI_{actual} can be compared directly with the $EI_{nominal}$ which have been calculated in the same simulated conditions, and their ratios ($EI_{actual}/EI_{nominal}$) represent the impact of STD on LSS performance. Therefore more realistic error indices to be considered in clinical practice in case of STD can be estimated by linearly scaling these ratios using reference error indices ($EI_{reference}$) calculated using real data as in the following formula:

$$EI_{STD} = \left(\frac{EI_{actual}}{EI_{nominal}}\right) \times EI_{reference}$$

where EI_{STD} is the estimated error indices in the evaluated STD scenario.

Table 5.1. Reference and nominal 95th PAE% of the R-LSS studied for investigating STD impact.

LSS#	Concentration-time points	LSS equation: AUC_{pred} (ng × h/mL)	**95th PAE%**	
			Reference	**Nominal**
LSS1	$C_{0.5}$, C_2, C_4, C_8	131.49 + 1.00 $C_{0.5}$ + 1.74 C_2 + 3.04 C_4 + 5.52 C_8	**6.30**	**3.7**
LSS2	C_0, C_1, C_2, C_4	-45.58 + 4.78 C_0 + 0.99 C_1 + 1.40 C_2 + 4.16 C_4	**12.17**	**4.0**
LSS3	C_0,$C_{0.5}$, C_2, C_4	77.53 + 3.85 C_0 + 1.05 $C_{0.5}$ + 1.81 C_2 + 4.13 C_4	**13.70**	**4.9**
LSS4	C_0, C_1, C_3, C_4	57.53 + 3.77 C_0 + 1.45 C_1 + 2.18 C_3 + 3.33 C_4	**13.44**	**5.8**
LSS5	$C_{1.5}$, C_4, C_8	75.66 + 1.87 $C_{1.5}$ + 3.46 C_4 + 6.12 C_8	**11.82**	**3.4**
LSS6	C_0, $C_{1.5}$, C_4	-46.41 + 4.84 C_0 + 2.00 $C_{1.5}$ + 4.51 C_4	**16.71**	**4.1**
LSS7	C_0, C_2, C_4	141.82 + 5.20 C_0 + 2.16 C_2 + 3.71 C_4	**16.48**	**6.3**
LSS8	C_0, C_1, C_4	62.23 + 3.75 C_0 + 1.67 C_1 + 5.77 C_4	**15.77**	**3.0**
LSS9	C_2, C_8	286.02 + 2.70 C_2 + 9.42 C_8	**16.04**	**6.5**

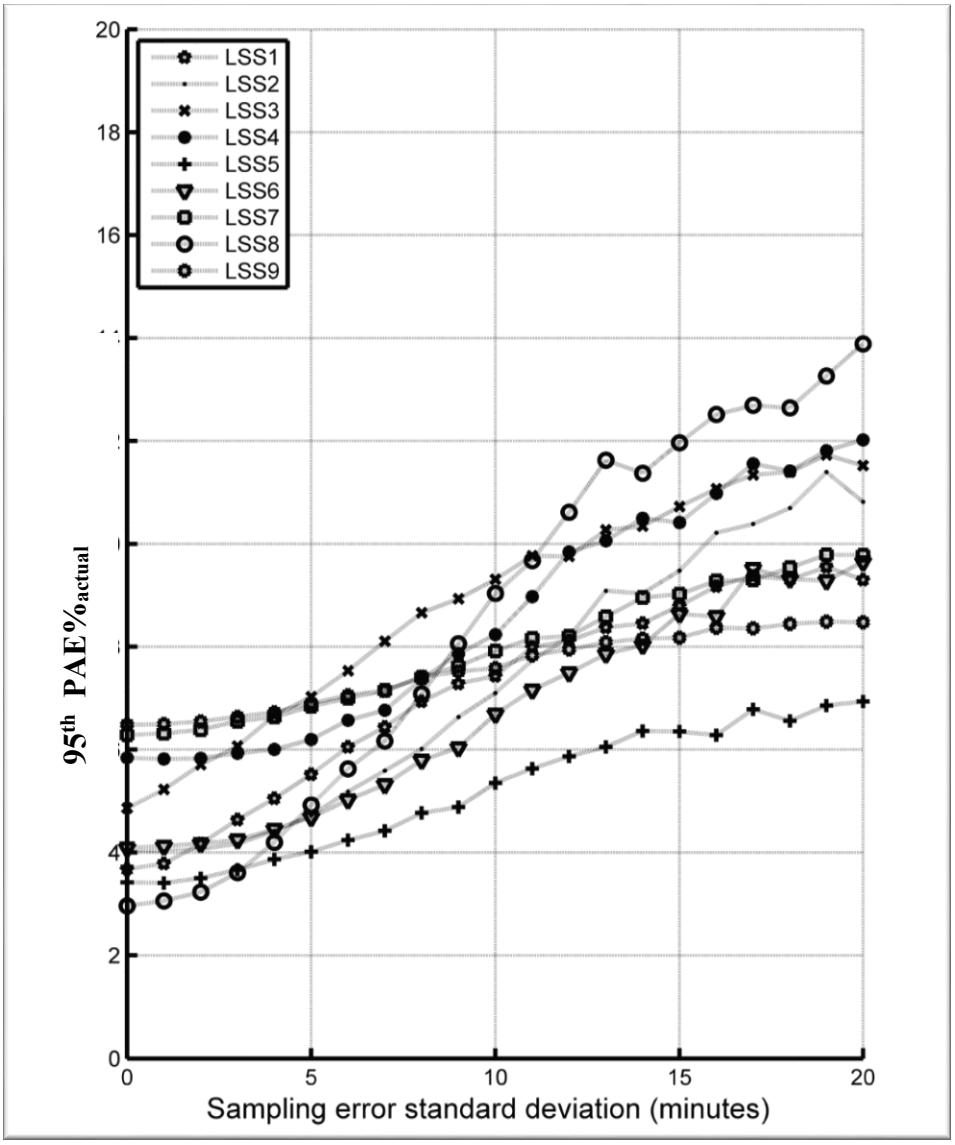


Figure 5.4: Random STD influence represented by 95th PAE%$_{actual}$ (instead of 95th PAE%$_{STD}$) for nine investigated R-LSS (shown in Table 5.1) used for cyclosporine AUC prediction. Random STD is described by the standard deviation of the sampling errors, 95th PAE%$_{STD}$ is the 95th percentile of absolute values of relative prediction errors in the presense of STD**,** 95th PAE%$_{actual}$ is 95th percentile of absolute values of prediction errors initially estimated using simulated concentrations at actual sampling times.

Assessment of the impact of STD can be used as an additional LSS evaluation criterion to discriminate R-LSS that have similar prediction errors in terms of 'nominal' (without STD) predictive performance. In Article I, the R-LSS composed of C_0, C_2, and C_4, which had clinically acceptable prediction errors, was considered the most practically convenient. Other LSS such as the one involving the concentrations C_0, C_1, and C_4 had similar characteristics (it satisfied the practical selection criteria and even it had a slightly better prediction error). However, even though these two R-LSS had comparable 'nominal' predictive performance (16.48% and 15.77% 95^{th} PAE%, respectively) their tolerance to STD is quite different. The later LSS degrade dramatically while the former is more stable in the presence of STD, Figure 5.5.

In addition, STD investigation revealed that the tolerance of R-LSS for STD depends on the number of chosen samplings and more importantly on the whole duration of the sampling process. In fact, adding a supplementary or a late sample point to R-LSS, will not only decrease its 'nominal' prediction errors, but may also increase its stability in case of STD as seen with the LSS ($C_{0.5}$, C_2, C_4, and C_8). Since a reliable prediction, in real-life settings where STD are frequent, is mandatory for safe implementation of LSS in clinical practice, those LSS which are more stable against STD are preferable. In this sense, **the investigation of STD should be routinely performed as an essential element in the development procedure of R-LSS (i.e. R-LSS development procedure should routinely include STD investigations in addtion to the basic learning and validation steps) to guide their selection and use.**

LSS#	Concentrations-time points				95th PAE%
LSS1	$C_{0.5}$	C_2	C_4	C_8	6.30
LSS6	C_0	$C_{1.5}$	C_4		16.71
LSS7	C_0	C_2	C_4		16.48
LSS8	C_0	C_1	C_4		15.77

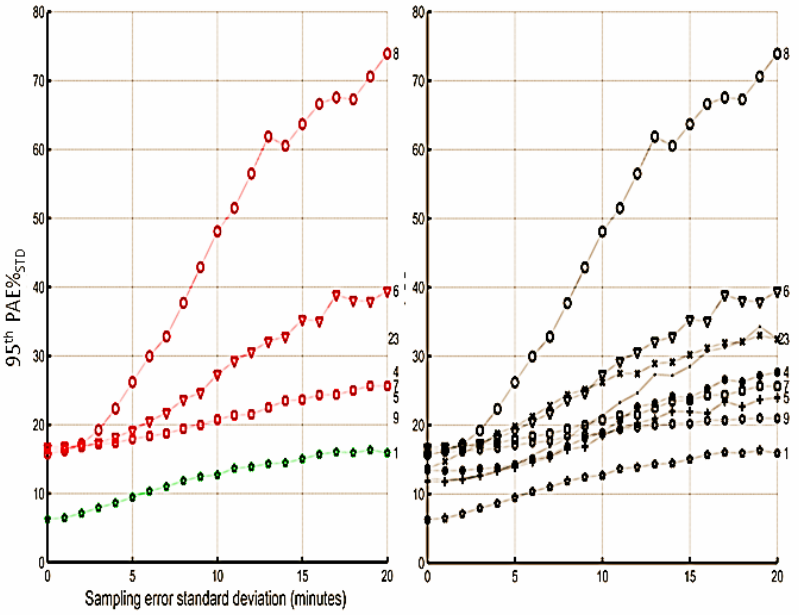


Figure 5.5: Random STD impact: 95th PAE%STD for the nine R-LSS studied for investigating STD impact (right plots) and four selected ones (center plots: LSS1, LSS6, LSS7, LSS8) used for cyclosporine AUC prediction, LSS1 had the best tolerability to STD, LSS7 had a better tolerability than LSS6 and LSS8 even though these three LSS share similar performance regarding nominal times. Random STD is described by the standard deviation of the sampling errors, 95th PAE%STD is 95th percentile of absolute values of relative prediction errors in the presense of STD.

In fact, **the final R-LSS choice[3] for application in clinical practice should be personalized and guided by a rational judgment that carefully evaluates the**

[3] An initial choice should be made between R-LSS and B-LSS based on 1) their predictive performances (which are comparable in the case of CsA in pediatric HSCT), 2) feasibility of implantation and use of B-LSS (currently a major limitation for B-LSS adoption in clinical practice), 3) tolerance of R-LSS against STD (according to STD analysis), 4) the target AUC (Observed vs. Underling).

performance-cost balance of each LSS. This evaluation has to consider the required predictive performance according to patient's clinical situation, anticipated extent and frequency of STD as well as the implied cost (frequency and duration of sampling and financial cost). For example, the R-LSS (C_0, C_2, C_4) can be selected for a stable patient consulting in an external clinic at a hospital for whom a prediction error of 20% can be acceptable and a sampling duration of 4 hours is perferred. Then, during LSS application, STD assessment still has an important role since it allows identifying critical (sensible to STD) sampling points of each LSS in order to reduce sampling time errors at these points. For the R-LSS (C_0, C_2, C_4), one should pay particular attention to accuratly collect the sample of C_4, Figure 5.6.

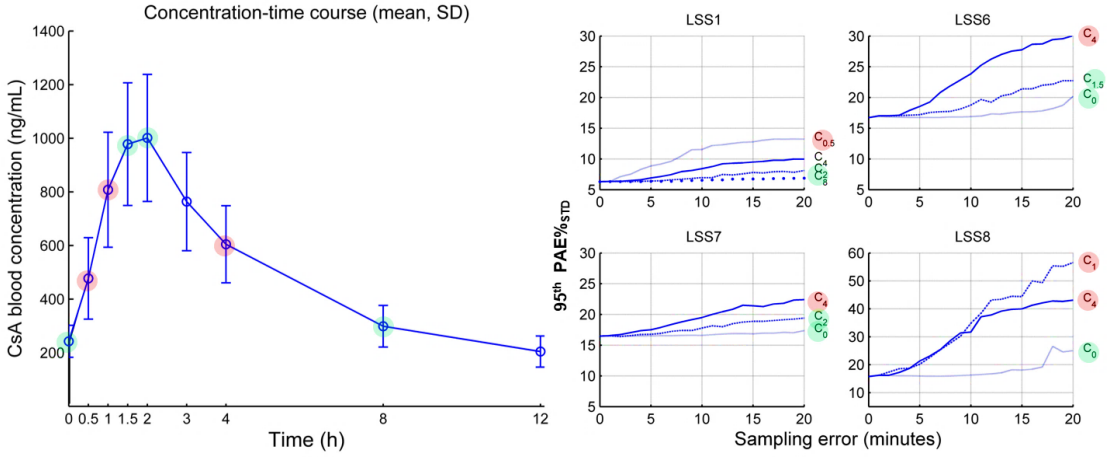


Figure 5.6: Sensitivity analysis, for each sampling point in the presence of random STD for four R-LSS (LSS1, LSS6, LSS7, LSS8) used for cyclosporine AUC prediction, showing two categories of sampling points A) with high tolerance for STD highlighted in green and B) with low tolerance for STD highlighted in red. Random STD is

described by the standard deviation of the sampling errors, 95^{th} $PAE\%_{STD}$ is 95^{th} percentile of absolute values of relative prediction errors in the presence of STD, Ct is the concentration at time t in hours post-dose.

However, **during sample collection, in case of encountering a sampling time error, its impact should be evaluated and a decision can be taken promptly to use an alternative R-LSS if this sampling error may lead to estimate the AUC with unacceptable prediction error, as shown in Figure 5.7.** The alternative R-LSS can use all or a subset of the samples already performed and usually includes an additional late sampling point in order to provide an accurate prediction of AUC. Even in cases where the impact of STD does not lead to a clinically significant prediction error, the medical team should be informed that a sampling error has been encountered, and an updated expected prediction error should be reported. Table 5.2 presents four R-LSS that can be used as alternative LSS in case of significant sampling errors since they are relatively stable against STD. Appendix II reports the STD analysis for these LSS. User-friendly software can be designed to assist the implantation of such interactive approach.

Finally, the previous R-LSS approaches that predetermine some R-LSS to be used in all patients, without systematic consideration of STD, may lead to erroneous clinical decision and hamper therapeutic outcomes. Thus, this thesis proposes a more 'personalized' and interactive approach that provides the best LSS for each patient and suggests alternatives when a significant sampling error is involved.

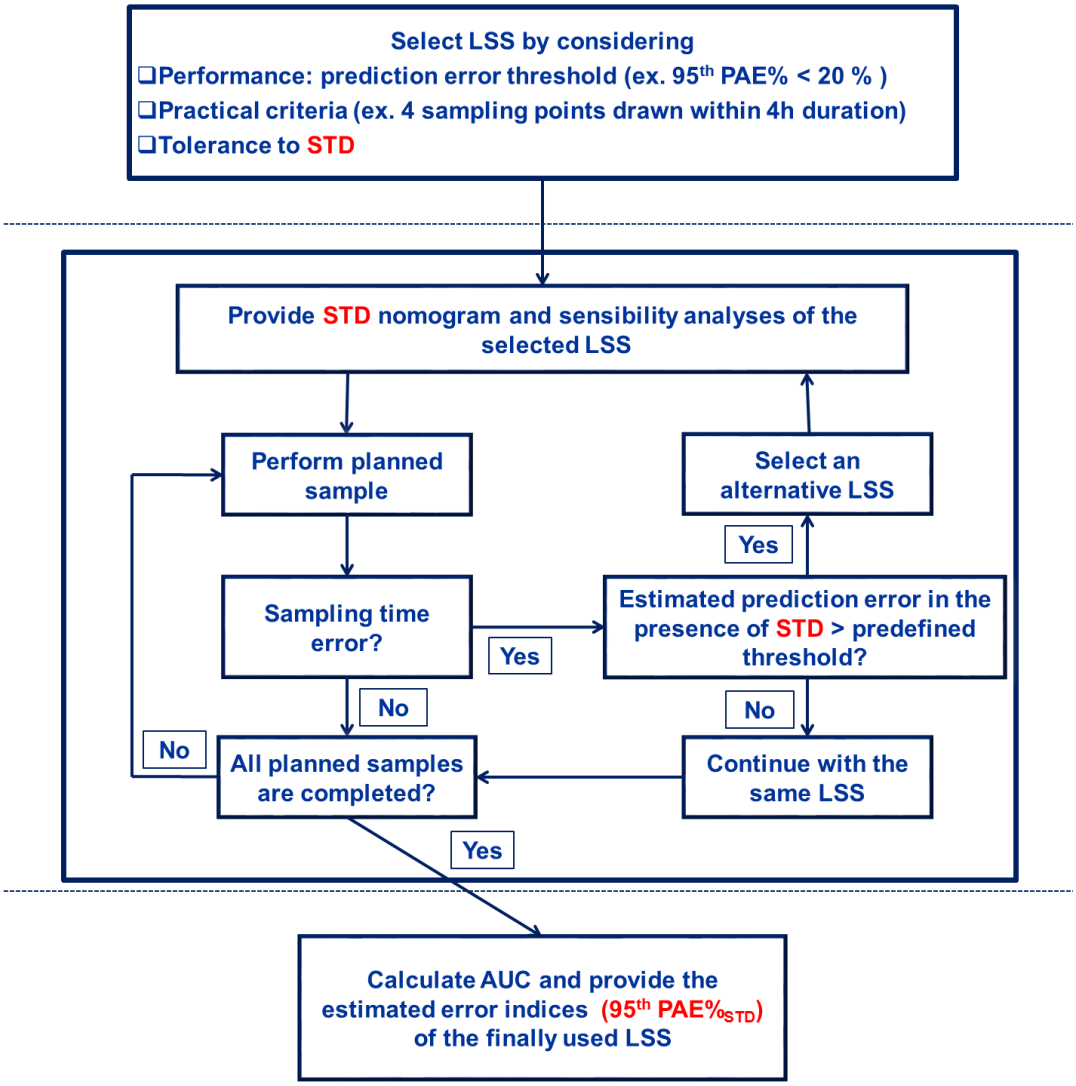


Figure 5.7: Proposed approach for considering STD in the application of R-LSS, this approach involves the selection of the best LSS to be used for each patient individually and the use of alternative LSS in case of a significant sampling error, 95^{th} $PAE\%_{STD}$ is 95^{th} percentile of absolute values of relative prediction errors in the presence of STD.

Table 5.2: Predictive performance of four additional (alternative) R-LSS to estimate cyclosporine $AUC_{0\text{-}12h}$ following PO administration.

Concentration-time points	Equations AUC_{pred} (ng × h/mL)=	ME% (95% CI)	RMSE% (95% CI)	95th PAE%
C_0, $C_{1.5}$, C_4, C_8	-13.22 + 1.46 C_0 + 1.92 $C_{1.5}$ + 3.42 C_4 + 5.16 C_8	0.03(-1.78, 1.85)	5.52(3.80, 6.82)	10.29
C_0, C_2, C_4, C_8	162.03 + 1.65 C_0 + 2.09 C_2 + 2.57 C_4 + 5.43 C_8	0.05(-1.88, 1.98)	5.87(4.62, 6.90)	11.35
C_0, C_1, C_4, C_8	115.86 + 0.98 C_0 + 1.53 C_1 + 4.88 C_4 + 4.26 C8	0.09(-2.63, 2.80)	8.26(6.31, 9.83)	15.20
C_0, C_2, C_8	152.24 + 2.27 C_0 + 2.77 C_2 + 7.81 C_8	0.11(-2.63, 2.86)	8.36(6.10, 10.14)	17.89

CI: confidence interval; C_{tj}: concentration at time t_j in hours post-dose; ME%: mean relative prediction error; RMSE%: root mean squared relative prediction error; 95th PAE%: 95th percentile of absolute values of relative prediction errors.

Chapter 6 Conclusion

Comprehensive methodology and advanced STD analysis have been presented in order to achieve improved development and selection of LSS for AUC estimation.

R-LSS and B-LSS, issued from this thesis, can predict CsA AUC in pediatric HSCT with clinically acceptable prediction errors. These LSS require four or fewer concentration-time points drawn within 4 hours post-dose and, hence, are convenient for clinical setting. Enhanced prediction could be achieved by increasing the sampling frequency or duration.

For B-LSS, the conventional development procedure should be reviewed and improved. Notably, Pop-PK model construction, using the standard diagnostic criteria, does not always lead to the best model for B-LSS application. Indeed, the final covariate model gives a better fitting for concentration-time data in the sense of OFV than the structural model that does not include covariates; though the latter can better predict AUC. Pop-PK models specifically optimized for AUC estimation using B-LSS are needed. In order to develop appropriate and specific Pop-PK models for B-LSS, additional considerations, with a focus on their intended use for AUC prediction, have to be taken into account.

In addition, in the case where the prediction of the 'underlying' AUC is preferred rather than the observed AUC, as the residual error is excluded in the former, B-LSS can have a better performance.

For R-LSS, STD can have a significant impact on their predictive performance and should not be ignored; overlooking STD can lead to considerable errors in the estimation of AUC and hence can result in inappropriate AUC-based dose adjustment.

Since R-LSS behave differently in the presence of various scenarios of STD and show different tolerability patterns, the investigation of the effect of STD on the prediction performance has to be integrated as an essential element in R-LSS development procedure to guide their selection and use.

Chapter 7 Perspectives

This thesis addressed major issues related to LSS development and clinical use; however there is still room for further investigations. Notably, it would be interesting to consider the impact of inter-occasion and inter-individual variability on the robustness of LSS. Indeed, similarly to the different sensitivities of LSS to STD, they can also differ in their sensitivity to inter-occasion and inter-individual variability.

The STD analysis provided a thorough view of sampling error impact on R-LSS performance. Nonetheless, an effective integration of STD considerations in clinical practice requires the 'translation' of the reported comprehensive statistical indices and graphics, that represent the impact of STD, to clear and practical rules which can be easily understood and applied by the medical and technical staff (as example of such rules: for accurate estimation using LSS (C0, C2, C4), the sampling time error should not exceed 10 minutes for C4 and 15 minutes for C0 or C2 in case of a single error, and should not exceed 5 minutes for any sample in case of multiple sampling errors). Moreover, by analogy to identifying those time errors that cannot be tolerated for each R-LSS, it may be useful to set other rational rules in order to avoid the use of LSS when the PK profile seems to be problematic and hence large prediction error can be expected. These rules may be based on PK profile features or the relations between measured concentration values (ex. for accurate estimation using LSS (C0, C2, C8), the ratio C_2 /C_8 should be greater than 2).

The technical progress may make the development and use of R-LSS and B-LSS more accessible in the near future. In fact, in spite of the availability of a well-established methodology and adequate PK data, medical research teams may defer the

development of R-LSS for other drugs and populations. Indeed, this task is difficult and time consuming when it should be carried out using non specialized software such as Excel® with manual repetition of the whole procedure for each evaluated LSS. Hence, R-LSS approach may be reinforced by integrating the established methodology in an interactive software with a user friendly interface that allows the automatization of R-LSS development and selection. Moreover, this software may include advanced utilities and features such as STD analysis, and it can allow in-depth statistical investigations (Jackknife and Bootstrap confidence intervals for error indices, ranking and grouping of LSS according to multiple criteria, etc.). Besides, the use of interactive sample collection protocol, that accounts for actual sampling times and can promptly propose alternative R-LSS, may be facilitated if the data of LSS predictive performance and STD impact are integrated in an interactive smart phone application.

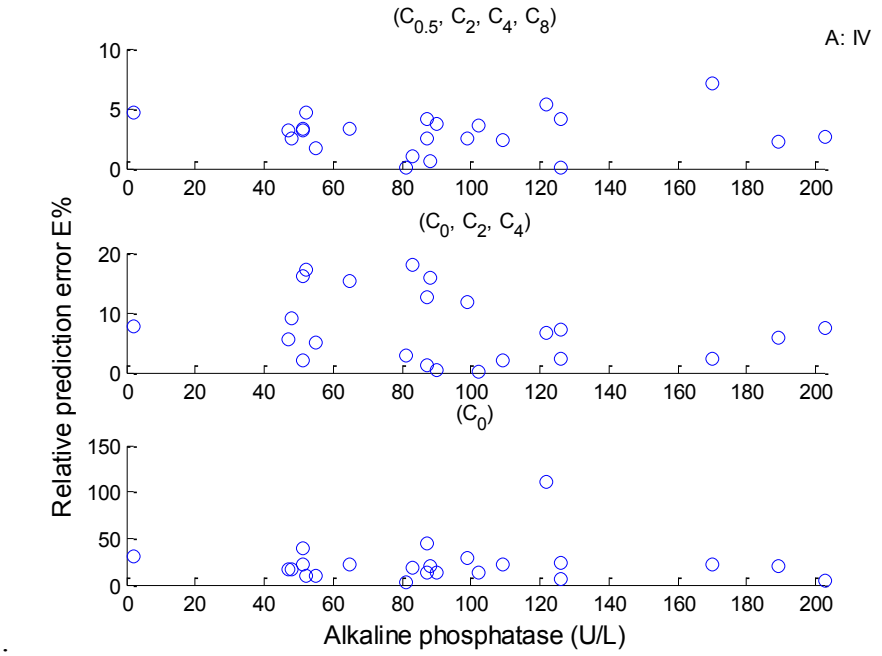
For B-LSS, in order to be more adapted for clinical practice, they have to terminate their association with the requirement of trained professionals and access to specialized software. Namely, the implementation of cloud-based application or software, to facilitate B-LSS development and use, will be very helpful. Furthermore, an advanced model optimization and selection criteria should be investigated (ex. modified OFV that account for AUC prediction errors), rather than the routine use of conventional standards such as the usual OFV, for developing Pop-PK models that are optimized to provide the best prediction using B-LSS.

In this thesis the prediction of CsA AUC has been studied as a typical case, however the developed methodology and STD analysis can be applied to investigate and improve the estimation of AUC using LSS for other drugs and populations.

Appendices

Appendix I

Relative prediction errors vs. patients' covariates, for estimating cyclosporine AUC by R-LSS after PO and IV administration.



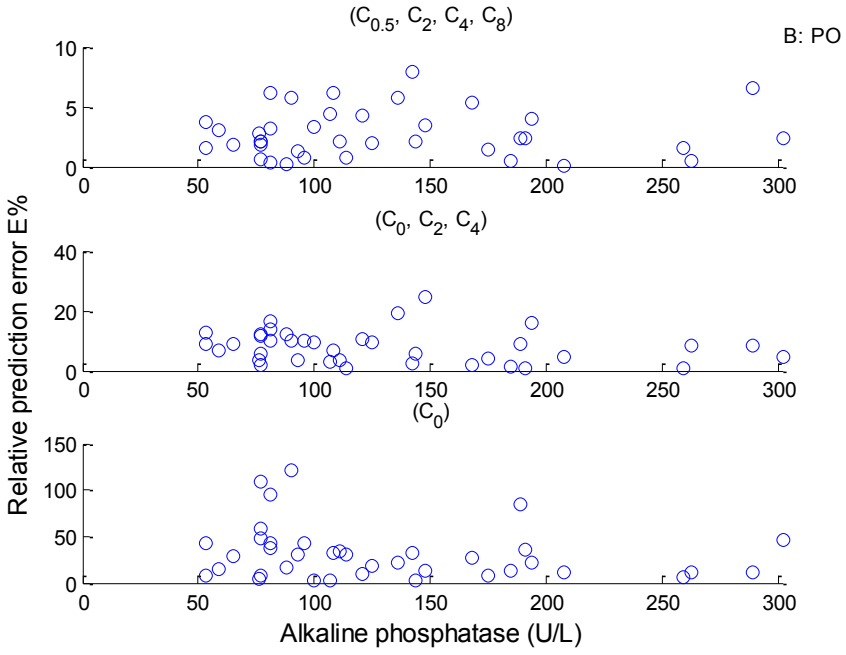


Figure I.1: Relative prediction errors vs. alkaline phosphatase, for estimating cyclosporine AUC by three R-LSS.

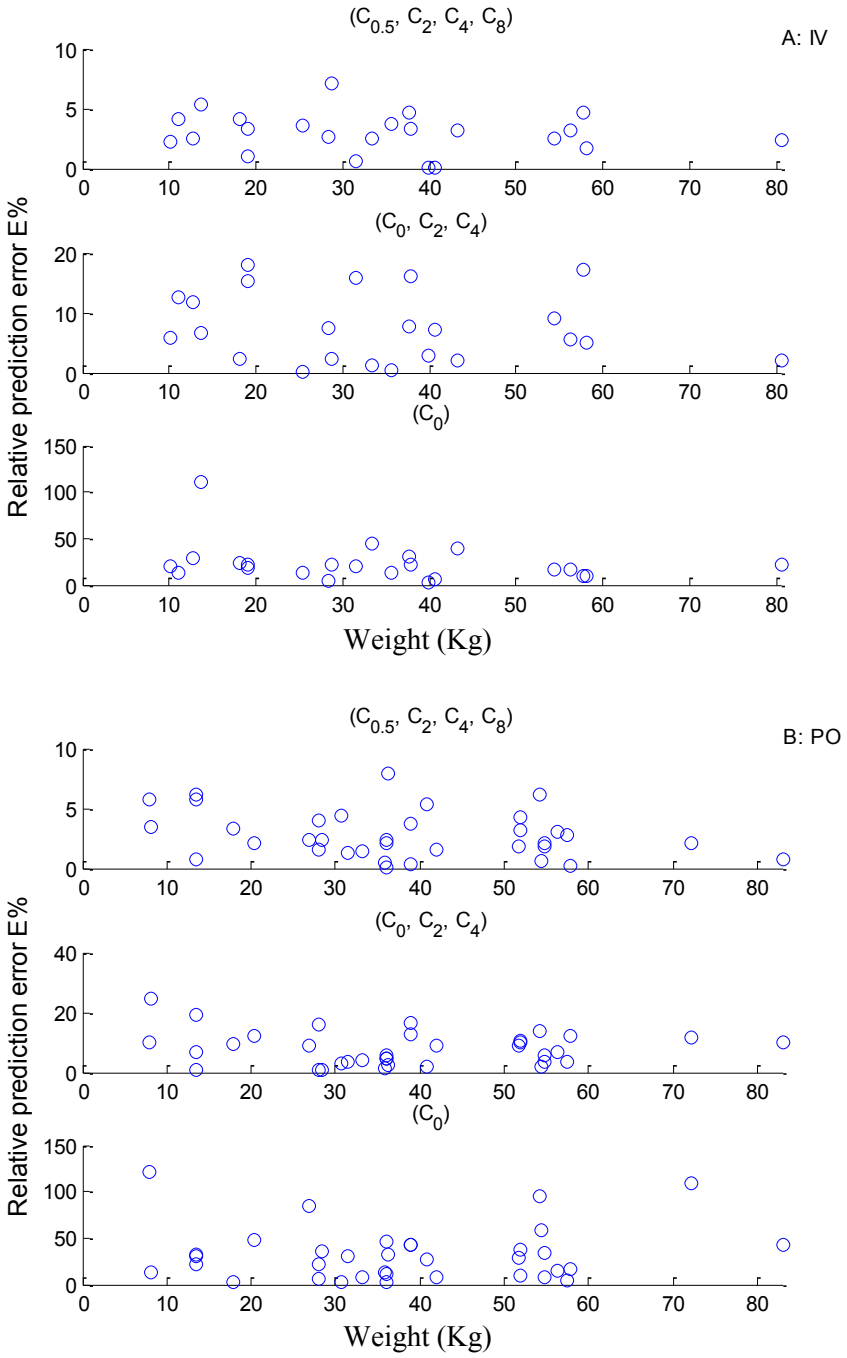


Figure I.2: Relative prediction errors vs. weight, for estimating cyclosporine AUC by three R-LSS.

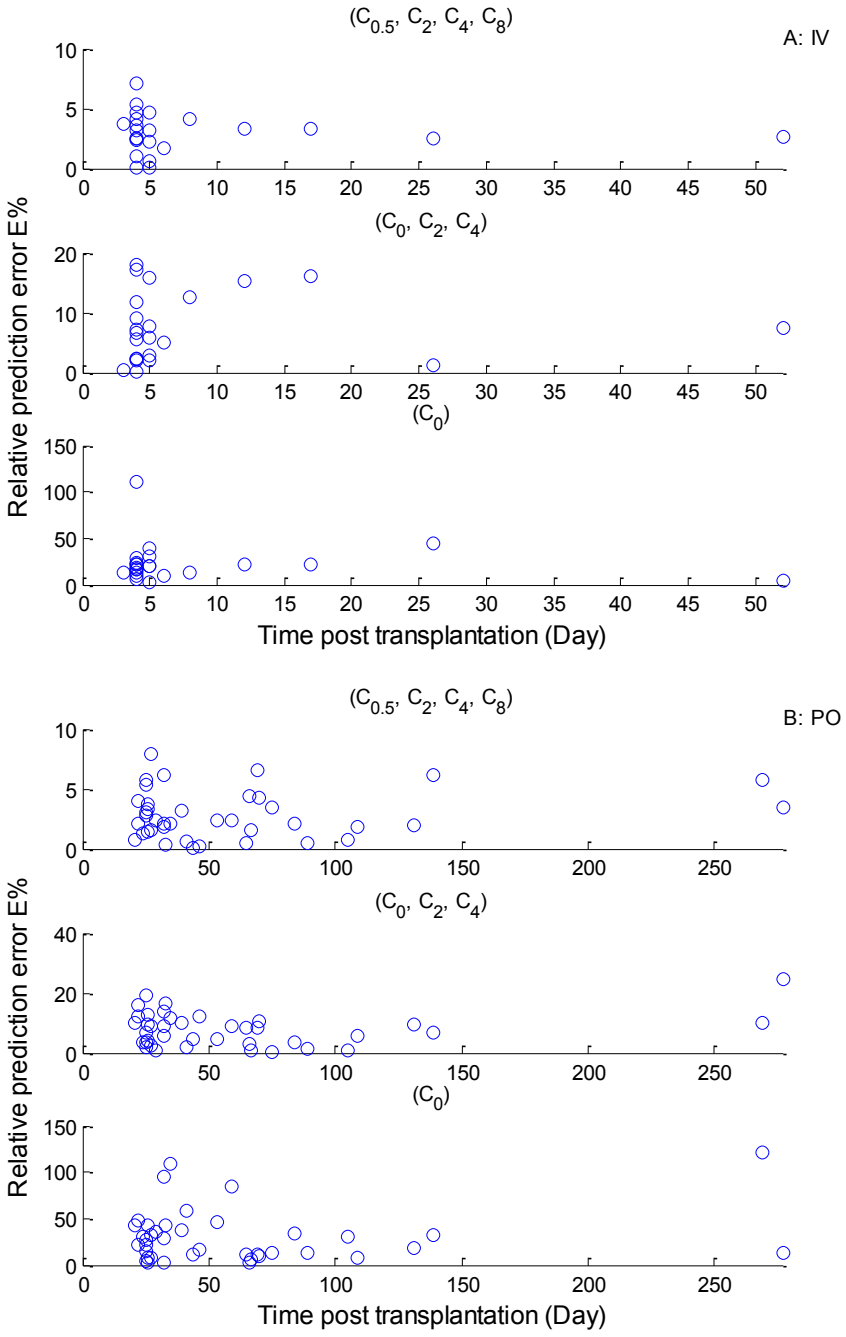


Figure I.3: Relative prediction errors vs. time post transplantation, for estimating cyclosporine AUC by three R-LSS.

Appendix II

Analyses of sampling time deviation impact for the four additional R-LSS reported in Table 5.2.

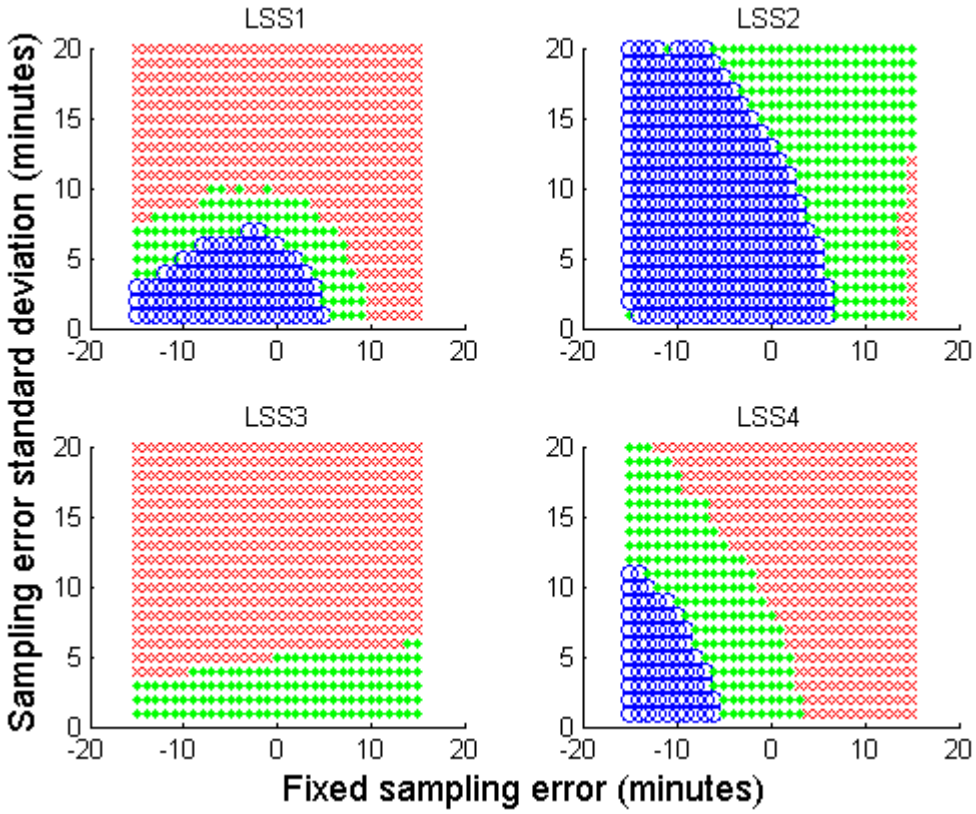


Figure II.1: Nomograms for various combinations of random and fixed STD for four additional R-LSS (LSS1: C_0, $C_{1.5}$, C_4, C_8; LSS2: C_0, C_2, C_4, C_8; LSS3: C_0, C_1, C_4, C_8; and LSS4: C_0, C_2, C_8) used for cyclosporine AUC prediction; red ×, for 95th PAE%$_{STD}$ more than 20%; green ●, equal or less than 20%; and blue ○ equal or less than 15%. Random STD is described by the standard deviation of the sampling errors, 95th PAE%$_{STD}$ is the 95th percentile of absolute values of relative prediction errors in the presense of STD.

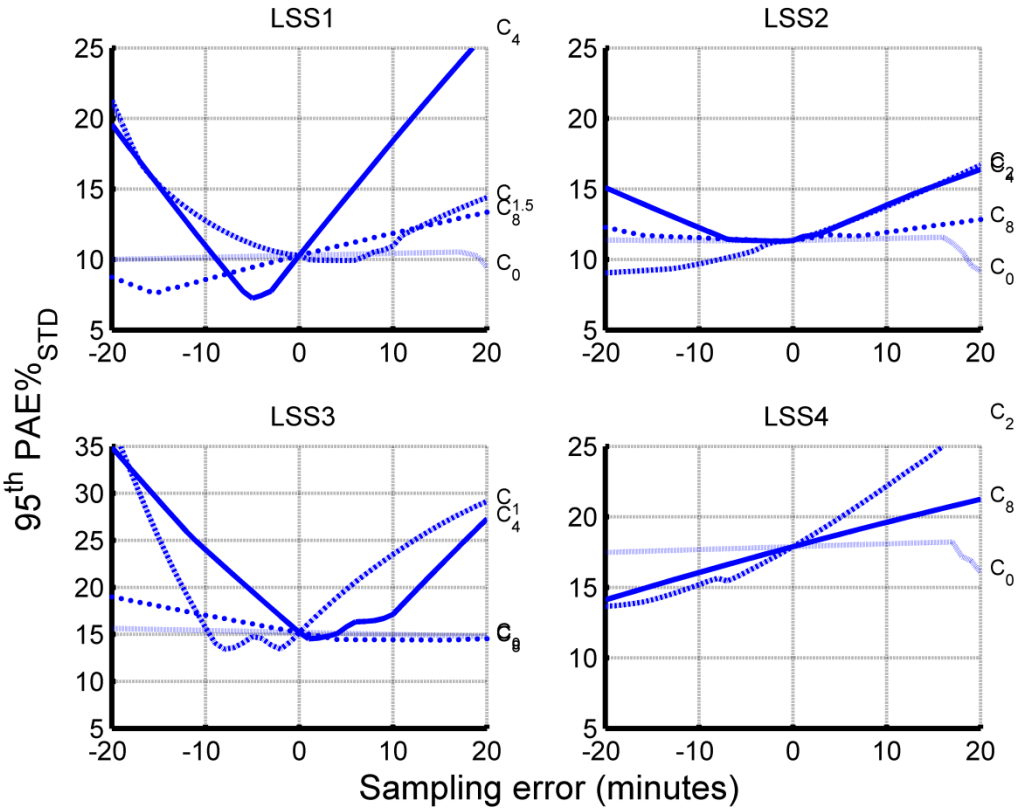


Figure II.2: Sensitivity analysis for each sampling point in the presence of fixed STD for four additional R-LSS (LSS1: C_0, $C_{1.5}$, C_4, C_8; LSS2: C_0, C_2, C_4, C_8; LSS3: C_0, C_1, C_4, C_8; and LSS4: C_0, C_2, C_8) used for cyclosporine AUC prediction, 95^{th} $PAE\%_{STD}$ is the 95^{th} percentile of absolute values of relative prediction errors in the presense of STD, C_t is the concentration at time t in hours post-dose.

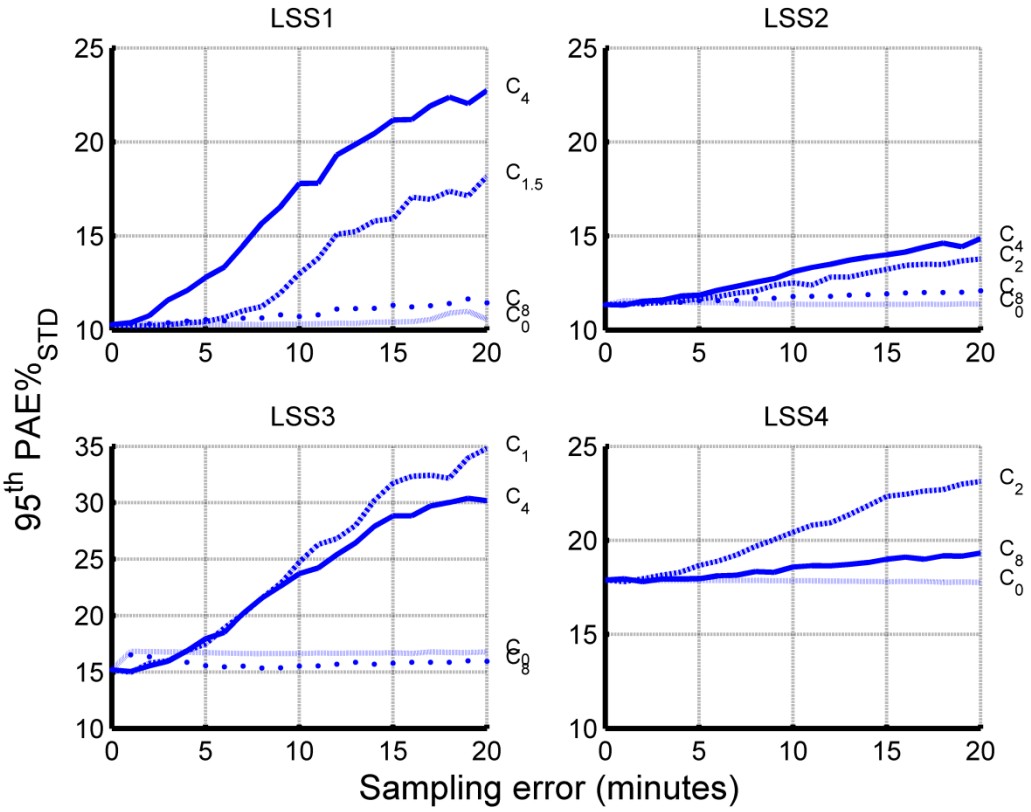


Figure II.3: Sensitivity analysis for each sampling point in the presence of random STD for four LSS additional R-LSS (LSS1: C_0, $C_{1.5}$, C_4, C_8; LSS2: C_0, C_2, C_4, C_8; LSS3: C_0, C_1, C_4, C_8; and LSS4: C_0, C_2, C_8) used for cyclosporine AUC prediction. Random STD is described by the standard deviation of the sampling errors, 95^{th} $PAE\%_{STD}$ is 95^{th} percentile of absolute values of relative prediction errors in the presense of STD, C_t is the concentration at time t in hours post-dose.

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