Université de Montréal

Population Pharmacokinetic Analysis of Cyclosporine A Using Standard Two-Stage (STS) and Nonlinear Mixed-Effects Modeling (NONMEM) Methods

Par

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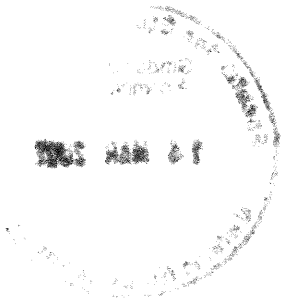

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Ce mémoire intitulé:

Population Pharmacokinetic Analysis of Cyclosporine A Using Standard Two-Stage (STS) and Nonlinear Mixed-Effects Modeling (NONMEM) Methods

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Abstract

More than two decades of extensive research and clinical experience with cyclosporine A (CsA) immunosuppression has significantly contributed to improve transplant survival rates and overall clinical outcome. Wide CsA inter- and intra-individual biopharmaceutic and pharmacokinetic variability has complicated the relationship between dose, blood levels, and outcome, making it difficult to achieve the desired therapeutic response. Several approaches can be used in order to overcome these potential complicating factors, one of which is to optimize the CsA dosage regimen to reach a target blood CsA concentration. In doing so, it is crucial to choose the best kinetic model, which provides a good description of the pharmacokinetic behavior of CsA. In fact, a good description of the kinetic process is essential for proper account of the variability. Hence, it has to be decided which compartmental model is best suited to minimize the discrepancy between the data and our mathematical predictions. This model can then reliably describe the time-concentration profile yielding accurate PK parameters to optimize the CsA dosage regimen, as CsA pharmacokinetics have been documented as one- [Anderson JE et al., 1994], two- [Wu G et al, 1996], and three-compartment models [Karlsson MO, Lindberg-Freijs A, 1990 and Anderson JE et al., 1994] and even a physiological pharmacokinetic model including 14 tissue and two blood compartments [Bernareggi B, Rowland M, 1991].

The objective of this study is to compare three models of Cyclosporine A (CsA) population pharmacokinetics using two methods of analysis to elucidate which model describes CsA pharmacokinetics most accurately and which method is the most suitable for this purpose. Clinical data of 52 bone marrow transplant recipients were gathered for estimation of CsA pharmacokinetics. The blood CsA concentration-time profile in each of 52 adult bone marrow transplant patients taking the first course of CsA treatment was fitted by one-, two-, and three-compartment open models to obtain relevant pharmacokinetic parameter estimates. Population pharmacokinetic parameters were

estimated using the standard two-stage (STS) and nonlinear mixed-effects modeling (NONMEM) methods. The predictions of blood CsA concentrations by one-, two-, and three-compartment open models using the maximum likelihood estimation (**MLE**) method were evaluated employing STS and NONMEM methods.

In the STS method, for each patient, the residual sum of squares was determined for each one of the three investigated pharmacokinetic models. Afterwards, the method of minimum Akaike information criterion estimation (**AIC**) was used to determine the model that most adequately describe the pharmacokinetic data, based on the number of blood levels, the residual sum of squares of the specific pharmacokinetic model, and the number of parameters in the same model. While the Akaike's information criterion (AIC) favored the two-compartment model to describe CsA concentration-time profiles, the predictive performance analyses (bias and precision) showed that both two- and three-compartment models were better than the one-compartment for prediction. Furthermore, the three-compartment model was slightly superior to the two-compartment model in terms of prediction of CsA blood levels, however, the test of redundancy rejected its use. The same procedure was carried out for the NONMEM method, which confirmed the use of a two-compartment open model in describing the kinetic profile of CsA after considering the diagnostic test of redundancy and model selection criterion (likelihood ratio).

In conclusion, based upon AIC values, bias and precision, a two-compartment model best describes CsA population pharmacokinetics, as built by NONMEM and STS, and it is able to predict CsA levels equally well in our population of bone marrow transplant patients. Furthermore, the NONMEM and the STS methods appeared to be suitable methods of population pharmacokinetic analysis for the rich data and for this purpose they can equally offer reliable and accurate results.

Keywords : Population pharmacokinetics, Cyclosporine A, Sandimune, Immunosuppression, Bone Marrow Transplantation, Compartmental Modeling, Standard Two-Stage (STS), Nonlinear mixed-Effects Modeling, NONMEM, Model Comparison.

Résumé

Après plus de deux décennies de recherches étendues et d'expériences cliniques sur la cyclosporine A (CsA), l'utilisation de cet immunosuppresseur a considérablement amélioré le taux de survie des greffés. Toutefois, l'énorme variabilité inter- et intra-individuelle de la biopharmaceutique et pharmacocinétique de la CsA complique la relation entre la dose, les concentrations sanguines, et les prévisions de la réponse thérapeutique ciblée. Plusieurs approches sont utiles afin de surmonter ces complications, par exemple l'optimisation du schéma posologique de la CsA pour atteindre une concentration sanguine ciblée. Le choix du modèle cinétique est alors crucial pour fournir une prédiction précise du comportement pharmacocinétique du médicament.

Le modèle compartimental est souvent utilisé en PK pour son adéquation entre les données observées et ses prévisions mathématiques. Ce modèle peut alors décrire le profil de temps-concentration en donnant des paramètres précis de PK pour optimiser le schéma posologique de la CsA. La pharmacocinétique de la CsA a été modélisée par une approche à un- [Anderson JE et autres, 1994], deux- [Wu G et autres, 1996], et trois-compartiments [Karlsson MO, Lindberg-Freijs A, 1990 et Anderson JE et autres, 1994] et même par un modèle pharmacocinétique physiologique comprenant 14 compartiments tissulaires et sanguins [Bernareggi B, Rowland M, 1991].

L'objectif de cette étude de population est de comparer deux approches pour déterminer le modèle compartimental qui décrit le mieux la pharmacocinétique de la CsA administrée en perfusion chez des patients avant une greffe de moelle osseuse. Nous avons modélisé les profils temps-concentrations sanguines de la CsA chez 52 patients par des modèles ouverts à un-, deux-, et trois-compartiments. Les paramètres pharmacocinétiques de population ont été estimés en utilisant la méthode standard à deux étapes (STS) et la méthode à effets mixtes nonlinéaires (NONMEM). Les

performances des 3 modèles testés dans cette étude sont comparées par le critère d'Akaike (AIC), l'erreur résiduelle, et la redondance des paramètres.

Les deux méthodes montrent que le modèle à deux-compartiments décrit le mieux la pharmacocinétique de la CsA. Ce modèle peut prévoir avec précision les concentrations sanguines de la CsA dans une population de patients avant transplantation. La méthode STS se révèle être aussi une méthode appropriée d'analyse de pharmacocinétique de population pour les conditions de données riches présentes dans cette étude. Les deux méthodes déterminent qu'elles peuvent également offrir des résultats fiables et précis.

Mots clés : Pharmacocinétiques de population, cyclosporine A, Sandimune, greffe de moelle osseuse, modèle compartimental, méthode standard à deux étapes (STS), méthode à effets mixtes nonlinéaires, NONMEM, comparaison de modèle.

Introduction

Cyclosporine A (Sandimune®) is a member of a family of drugs that possess immunosuppressive activity. Since the introduction of cyclosporine A (CsA) into clinical practice in the early 1980's, it has been shown that this drug is widely used to prevent the prophylaxis of organ transplantation and to treat some autoimmune diseases, increasing the survival rate in patients. Unfortunately, the pharmacokinetic profile of CsA is characterized by great variations in blood concentration levels after oral or intravenous administration [Ptachcinski RJ, 1986], and it differs from patient to patient. It is then essential to permanently maintain the CsA residual blood concentrations in a narrow therapeutic window in order to preserve the optimal relationship between tolerance and effectiveness of the drug. Serious clinical consequences are associated with CsA narrow therapeutic range and its high inter- and intra-individual variability. Low blood concentrations (underexposure) of CsA may contribute to an increased incident of acute rejection and subsequent graft loss, whereas high blood concentrations (overexposure) can result in nephrotoxicity, hepatotoxicity, and other malignancies [Meyer MM, 1993; Dantal J, 1998]. Therefore, it has been recommended that dosing schedules should be guided by routine pharmacokinetic monitoring for individualized therapy [Kahan BD, 1990].

One of the clinical applications of CsA is to prevent graft rejection following bone marrow transplantation (BMT) and also in prevention or treatment of graft-versus-host disease (GVHD). Initially used by Powles [Powles, 1978], CsA has been used as the main immunosuppressive treatment to prevent GVHD after BMT for decades in order to improve survival rates. Despite long experience with CsA in a clinical setting for decades and many publications regarding its use, the application of CsA in the treatment of BMT patients is not optimal. This is due to the lack of proper definition of the variability of this important drug.

Several approaches can be used in order to overcome these potential complicating factors, one of which is to optimize the CsA dosage regimen to reach a target blood CsA concentration through population approach. In doing so, it is crucial to choose the **best kinetic model** which best describes the pharmacokinetic behavior of CsA and accounts for the variability. Indeed, selecting a model that well describes CsA kinetic process and estimates the population characteristics is the major challenge of achieving optimal immunosuppression. This can improve the accuracy of CsA dosing guidelines.

The objectives of the current study were to:

- estimate cyclosporine population pharmacokinetic parameters, using standard two-stage (STS) and nonlinear mixed-effects modeling (NONMEM), and quantify the interindividual variability found with such experimental (rich) data;
- compare mean PK parameter estimates and interindividual variability obtained by the STS and NONMEM methods, and to determine which method is more appropriate for this data rich situation.

Experimental data of 52 adult candidates of bone marrow transplantation were gathered for estimation of CsA pharmacokinetics. The blood CsA concentration-time profile in each of these pretransplant candidates, taking the first course of CsA treatment through infusion, was fitted by one-, two-, and three-compartment open models to obtain relevant pharmacokinetic parameter estimates. From the best model selected, population parameters (mean, interindividual variability) were estimated using the STS and NONMEM methods maximizing the likelihood function.

In the first chapter, a background of pharmacokinetics and pharmacokinetic modeling in an individual and a group of individuals (population) is presented. We also distinguish between experimental and routine (clinical) pharmacokinetic data as requiring different approaches for data analysis. In the second chapter, *Methodology*, the method of maximum likelihood (**ML**) estimation is presented. Emphasis is placed

on population methods, by which mean population parameters and pharmacokinetic variability can be measured. Finally in the third chapter, *Application*, we will focus on the estimates of the mean population parameters and the relative interindividual variability of CsA in a group of pretransplant candidates of BMT, in the context of regression models relating pharmacokinetic parameters to the measured observations (blood levels). Comparison of the estimation methods are then considered to show the specificity of the estimation methods from the point of view of data analysis for this essential but highly variable drug.

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"I am because we are."

I am grateful to so many for so much. My sincerest thanks are due to all those that have assisted me to get this far. I would like to express my deepest gratitude to both my supervisors:

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To my wife

Contents

List of Tables

List of Figures

Notation

Normal lower case	scalar.
Bold lower case	column vector.
Bold upper case	matrix.
Embossed upper case	unity.
$\mathbf{v}_i$	i^{th} component of vector **v**.
det (**M**)	determinant of matrix **M**.
$\mathbf{M}^{-1}$	inverse of the matrix **M**.
$\mathbf{M}^{T}$	transpose of the matrix **M**.

LIST OF **SYMBOLS** AND **ABBREVIATIONS** OF GENERAL OCCURRENCE

$a \times b$	vector cross product.
Δ	forward difference.
$\int$	integration symbol.
$\int_b^a$	definite integral.
∂	partial differentiation (derivative).
$\sim$	asymptotic relation.
$\times$	operation for multiplication group.
Π	product symbol.
Σ	summation symbol.
Θ	asymptotic function.
A_i, α_i	coefficient and exponent of the ith exponential term of a polyexponential disposition equation, [**macroconstants**].

AIC	Akaike Information Criterion.
arg max j(**p**)	value of **p** that maximizes j(.).
arg	argument.
C_{max}	maximum (peak) drug concentration after single dose administration.
CL	clearance.
cmt	compartment.
CV	coefficient of variation.
D	dose.
E (.)	expectation.
e, e	error.
e, e_j	noise for j^{th} measurement.
F (p)	Fisher information matrix.
$\mathbf{I_n}$	n x n identity matrix.
I (.)	quantity of information.
i.i.d.	independent and identically distributed.
i.v.	intravenous.
j_{ML} (.)	maximum likelihood criterion.
k_0	zero-order input or infusion rate constant.
k_e	elimination rate constant.
k_{ij}, k_{ji}	apparent first-order intercompartmental transfer rate constant, where i = 1, 2, …, j = 1, 2, …, $i \neq j$.
L_y (**p**)	likelihood of **p** given the observations **y**.
ln	logarithmic function.
$\mathbf{M}^i$	matrix of weight and normalization of the data.
ML	maximum likelihood.
MLE	maximum likelihood estimator.
N (m, V)	normal (Guassian) random variable distribution, with mean **m** and covariance **V**.

n_c	number of covariates.
n_i	number of individuals.
n_p	number of parameters.
n_q	number of covariates.
n_t	number of time-observations.
N	sample size.
$\mathbf{p}, \mathbf{p}^i$	vector of the kinetic parameters, for individual **i**.
$\mathbf{p}^*$	true value of the parameter for the individual **i**.
$\mathbf{p}_0$	vector of the population mean parameters.
$\mathbf{P}^i_{ML}$	precision matrix of the parameters for the individual **i**.
$\mathbf{p}^i_{ML}$	estimated value of the parameter by the maximum likelihood criterion for the individual **i**.
pdf	probability distribution function.
PD	pharmacodynamics.
PK	pharmacokinetics.
$\mathbf{Q}^i$	precision matrix of the covariates.
Q	intercompartmental clearance.
$\mathbf{q}, \mathbf{q}^i$	vector of the covariates, possibly for the individual **i**.
$\mathbf{R}_0$	zero-order input or infusion rate constant.
RMS	root mean square.
S	matrix of normalization.
SD, σ	standard deviation.
SE	standard error of means.
T	duration of constant-rate infusion.
t	time of the j^{th} measurement.
$\mathbf{t}, \mathbf{t}_j$	time variable or post-infusion time.
u (t)	system input, route and quantity of administered dose.
V, V_1	volume of distribution at central compartment.
Var (.)	variance.

V_{ss}	volume of distribution at steady state..
$\mathbf{X}^i$	weighting matrix for individual **i**.
$\mathbf{x}, \mathbf{x}^i$	vector value for all n variables, for individual **i**.
y_j, y_j^i	j^{th} observation, possibly for individual **i**.
$y_m(t_j, \mathbf{p})$	model output.
$\mathbf{y}, \mathbf{y}^i$	vector of kinetic observations, possibly for individual **i**.
z	observations for the covariate **q**.
γ	smoothing parameter.
δ	Delta function.
μ	mean population parameters.
ε	residual error encompassing intraindividual variability and measurement error.
σ, σ_j^i	variance of measurement error, possibly for individual **i** and the measurement **j**.
σ^2, σ_{ii}	variance.
σ_{ij}, ϖ_{ij}	covariance.
ρ	correlation coefficient.
$\mathbf{\Psi}^i$	covariance matrix of observations for individual **i**.
Ω	a priori covariance of the parameters.
θ, θ_0	regression parameter.
η, η_i	independent and identically distributed random variable with zero mean, interindividual variability.
$\pi(.)$	probability density.
$\pi_b(b)$	pdf of the measurement error.
$\pi(\mathbf{p})$	a priori pdf of the parameters.
$\pi_{p,q}(\mathbf{p},\mathbf{q})$	joint pdf of the parameters and the covariates.
$\pi_y(\mathbf{y})$	marginal pdf of the observations **y**.

$\pi_p(\mathbf{p}/\mathbf{y})$	a posteriori conditional pdf of the parameters **p** given the observations **y**.
$\pi_y(\mathbf{y}/\mathbf{p})$	conditional pdf of **y**, if giving the parameter **p** generating probabilities or likelihood of **y**.
$\frac{\partial j}{\partial \mathbf{p}}$	gradient of the criterion compared to the parameters.
$\frac{\partial^2 j}{\partial \mathbf{p}\partial \mathbf{p}^T}$, $\mathbf{H_i}$	Hessian of the criterion.

Preface

The use of pharmacokinetic principles in clinical practice has partly evolved as a result of advances in clinical pharmacology and biopharmaceutics. Clinical pharmacology, which is the study of the selective biological activity of drugs on living organisms, offers the unique opportunity to carefully investigate pharmacotherapy in human subjects. When relevant dosage regimens are administered, a drug with potential biological activities causes a response in the living organism. However, this response varies from one individual to another; and as in all biological phenomena, the pharmacotherapy of a group of individuals results in considerable differences in response to the same stimulus. By implementing pharmacokinetic and pharmacological studies, one can demonstrate this large interpatient variability. This variability is expressed at the same time in an experimental context (in healthy volunteers) [Sheiner, 1984; Fhüler et al., 1984] and in a clinical context (in patients) [Grasela and Sheiner, 1991; Steimer et al., 1994]. Nature, amplitude and duration of the effect of a drug with standardized dosage regimen vary in different individuals. To analyze this variability of response, one can distinguish three levels of interactions in the relationship between the organism and the drug. These relationships are tightly interconnected and can be expressed as:

- *Biopharmaceutics*, encompassing the study of the relationship between the nature and intensity of biological effects and the various formulation factors such as the rate of drug delivery. These effects are generally proportional to the total amount of drug made available to the body (drug input).
- *Pharmacokinetics*, the action of the organism on drugs, i.e. the time course of drug absorption, distribution, metabolism, and excretion.

- *Pharmacodynamis*, the effect of drugs (therapeutic effects or side-effects) on the organism.

Many factors: morphological, physiological, genetic, pathological and environmental, specific for each individual interact with all these stages and generate a variability of response in the total population. In extreme cases, a standardized amount of a drug could induce a wide variability in response in a group of individuals, from inefficacy to toxic effects [Follath et al., 1983]. This situation is frequently seen with anti-cancer agents. To compensate for this therapeutic variability, our main activity is focused on the input of the system, i.e. the choice of the route of administration, the galenic form and the amount of dose. For example, the adjustment of dosage regimen in a group of individuals where anti-cancer agents are used, one seeks to provide the optimal dosage regimen for each individual in order to reach the target concentration and decrease the adverse effects. When the route and the amount of administration are selected, it is the quantitative knowledge of the variability in pharmacokinetic (and in some cases pharmacodynamic) processes within a population which allows an improvement toward individual treatment.

Feasibly, population pharmacokinetic studies [Steimer et al., 1986] are thus necessary to quantify this variability. Today, therefore, such studies are applied at different phases of (new) drug development (usually phase II and III). This information, provided by a group of subjects, is analyzed by statistical methods. It provides a quantification of the variability, which makes it possible for example to detect subpopulations at the risk starting from the knowledge of demographic variables (age, weight, sex, etc...). Mathematical modeling is employed to copy the functional properties of a real system (the organism) from an artificial mechanism (the mathematical model). It, *in vivo*, allows a simulation of new protocols of administration without experimentation. To found these developments, we present in this chapter a recall on the modeling of the individual kinetics, which is the basis of all pharmacokinetic studies. The principal population approaches will be later exposed in the following sections.

Background 1

Pharmacokinetics

Pharmacokinetics, which is the study of time course of physiological transport processes (i.e. absorption, distribution, elimination, and excretion) and metabolism of drugs and chemicals (*ADEM*), has traditionally been used to describe the concentration-time profile of drugs (and/or metabolites) in living organisms. It also concerns the relationship of these processes to the intensity and time course of pharmacologic (therapeutic and toxicologic) effects of drugs and chemicals. To achieve this goal, competence in mathematics at least through calculus and pharmacokinetic modeling are essential to describe, predict and, in some cases, understand the fate of drugs in the body. Used to describe the kinetic processes, mathematical models are identified through observational data and they supply estimates of the PK parameters in a given mathematical structure. After a short outline of types of pharmacokinetic approaches, we will recall the population methods used for the present study.

Types of Pharmacokinetic Studies

Pharmacokinetic modeling is implemented based on two different approaches: *Compartmental* and *Noncompartmental*.

1. Compartmental Pharmacokinetics

The most commonly employed approach to the pharmacokinetic characterization of a drug or a metabolite is to represent the body as a homogeneous

well-stirred system of compartments into which the drug is distributed uniformly, and to assume that the rate of transfer between and from compartments follow first-order or linear kinetics [Jacquez JA, 1996]. It means that the rate of change of drug concentration in the compartment(s) reflects quantitatively the change in drug concentrations throughout the body, even though it has no physiologic or anatomic reality [Jacquez JA, 1985]. In fact, the main purpose of using compartmental approach is to define the entire system of a living organism throughout pharmacokinetic parameters based on curve fitting of individual(s) data.

2. Noncompartmental Pharmacokinetics

On the other hand, noncompartmental methods do not require the assumption of a specific compartmental model for either drug or metabolite. In fact, these methods can be applied to virtually any compartmental model, provided that we can assume linear pharmacokinetics. Noncompartmental methods are used to estimate certain pharmacokinetic parameters without specifically referring to them as such. Usually based on the estimation of the area under the concentration-time curve, these methods are mostly used to estimate bioavailability, clearance, apparent volume of distribution, and the fraction of a dose of a drug that is converted to a specific metabolite. These methods are also used to predict the average steady-state concentration of a drug and its metabolites after a single dose administration, and the time required to reach that point when a fixed dose of a drug is given at regular intervals [Gibaldi M, Perrier D, 1982].

Pharmacokinetic Modeling

Mathematical modeling has been used as a tool to understand various kinetic processes for centuries. Over the last few decades great strides have been made in our understanding of these kinetic processes particularly those governing the fate of drugs in man and animals, ***pharmacokinetics***. This growth has come with the advent of

analytical techniques capable of specific measurement of minute quantities of drugs and their metabolites in biological fluids, and in computational techniques required to analyse the resultant data. In fact, the use of computer technology in pharmacokinetic modeling has recently allowed researchers to investigate problems that were once described as impossible and frivolous tasks. Pharmacokinetics has thus graduated from being an essentially theoretical subject to one of vital practical interest to all those involved in the design, evaluation and administration of drugs. The ability to study pharmacokinetics with elegance, speed, and accuracy has been a great benefit for the advancement of scientific research in the field of clinical pharmacology.

In pharmacokinetics, the data are analyzed using a mathematical representation of a part or the whole of an organism. Broadly then, the purposes of pharmacokinetic modeling are, on one hand, to reduce and simplify data to a number of meaningful parameter values and on the other hand, to use the reduced data to predict either the results of future experiments or the results of a host of studies which would be too costly and time-consuming to complete (Wagner, 1968 and 1975). While mathematics is undoubtedly a powerful tool, its power must be meticulously handled when used in pharmacokinetic modeling. To use models properly, it requires not only an understanding of mathematics, but also a fundamental knowledge of the kinetic process under study, which reflects the physiological reality. Once a pharmacokinetic model is developed for a particular drug or a kinetic system, this does not constitute the end of the problem. In fact, modeling can be thought of as an iterative process. The accumulated knowledge in a field should provide some justification for the use of a pharmacokinetic model and the structure, and parameters of the model should have meaning in terms of known processes and the structure of the real system. Otherwise a model description may be of little relevance to the real data.

In the majority of the PK studies, the observations are defined by plasma (or blood) concentrations measured after drug administrations. After considering the judicious choice of model assumptions, it is the series of observations y and the knowledge of drug administration $u(t)$ that enable one to model the real process. The

optimal mechanistic model will provide the greatest simplifications while providing an adequately accurate representation of the processes affecting phenomena of interest. Fig.1.1 presents the functional diagram of modeling through stages of the data collection, the mathematical model, the choice of estimation method, and the optimization criterion. The following paragraphs summarize these four stages by specifying some methods available for this purpose. We limit our presentation to the models most studied in pharmacokinetics.

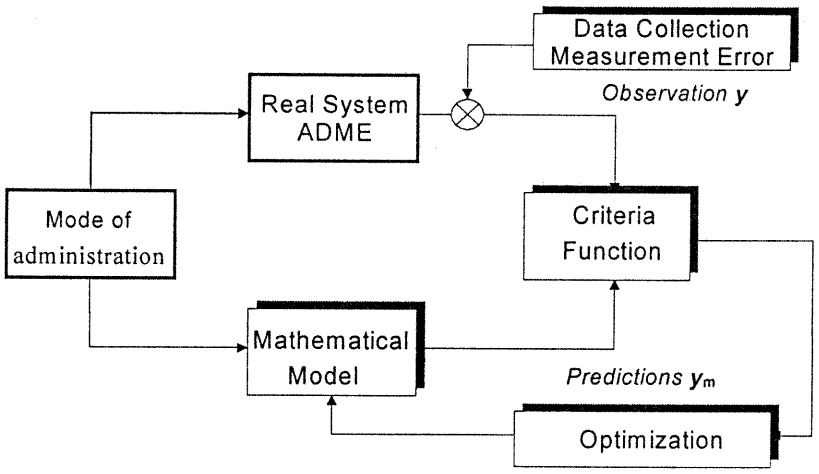


Figure1.1 – Four stages of modeling.

Data Collection

From a modeling perspective it is extremely important that the actual dosing and sampling times are recorded and used in the analysis. An integral part of the data collection process is the measurement of the dependent variable. This might involve a careful determination of a pharmacological effect or the accurate analysis of drug (or metabolite) concentrations in a biological sample (usually plasma or blood), in order to acquire the time course of drug concentration or effect. Basically, the plasma (or blood) concentration of a drug decreases until total elimination is reached. Fig.1.2 represents the kinetics of a drug intravenously administered.

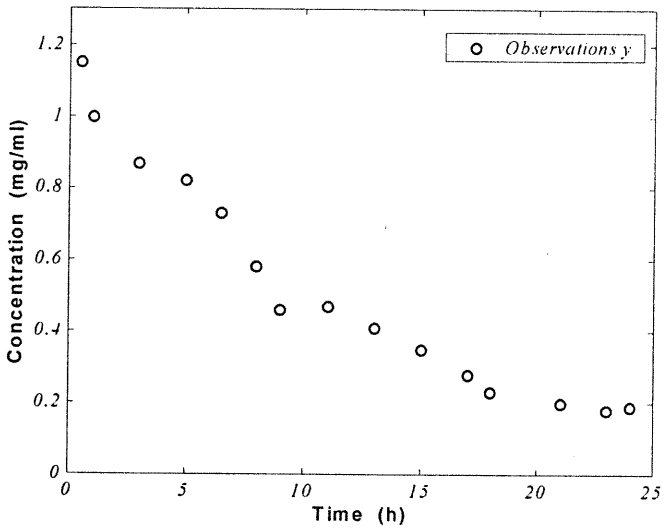


Figure 1.2 – The time course of a hypothetical drug concentrations.

Not only do we need to collect these data as accurately as possible, but we should also keep in mind the precision with which these data are measured. The information about the precision of the data should be used in developing an appropriate weighting scheme to use during the modeling.

Sampling

To observe the pharmacological phenomenon, one should collect the drug effects or concentrations through a definite time series, since it continuously evolves in time. Once the data have been collected, one can begin considering which models may be most useful. Based on the data collection and models used, one may suggest that improvements should be made in the data collection scheme. Additional samples or a different type of sampling schedule may be needed for a better representation of the drug kinetics. Moreover, it is necessary to take into account the high cost and ethical issues involved in patient care, which limits the establishment of a sampling protocol.

The Measurement

Samples are then analyzed for determination of the drug concentrations and/or its metabolites based on a (more or less) reliable technique. It is thus necessary to associate each measurement an uncertainty which results from an accumulation of small experimental errors. One can thus apply the theorem of the central limit and assume that the total error is distributed according to a Gaussian law (Fig.1.3). According to this law, the observations y_j at times t_j are assumed to be independently distributed following Gaussian (Normal distribution) errors ε_j, with mean zero and variance σ_j^2. In order to propose a model to calculate σ_j^2, one should study the reproducibility of measurements of *central limit theorem* [Efron B, 1982]. In the majority of cases, this study establishes a proportionality (relative error) between the standard deviation and the measured concentration (Fig.1.4) of the form

$$\sigma_j = a \cdot y_j + c$$

where **a** and **c** are constant. When the range of measured concentrations is close to the limit of quantification, the linearity becomes less evident and a polynomial regression model must be applied.

$$\sigma_j = a.y_j^{\alpha} + c$$

Even though, significant effort may be needed to incorporate uncertainties into the modeling process, this could potentially result in providing useful information that can aid in decision-making. Uncertainty analysis provides insights into the level of confidence and credibility of measurements, and model estimates as well. Further, it can lead to the identification of the key sources of uncertainty which merit further research, as well as the sources of uncertainty that are not important with respect to a given response.

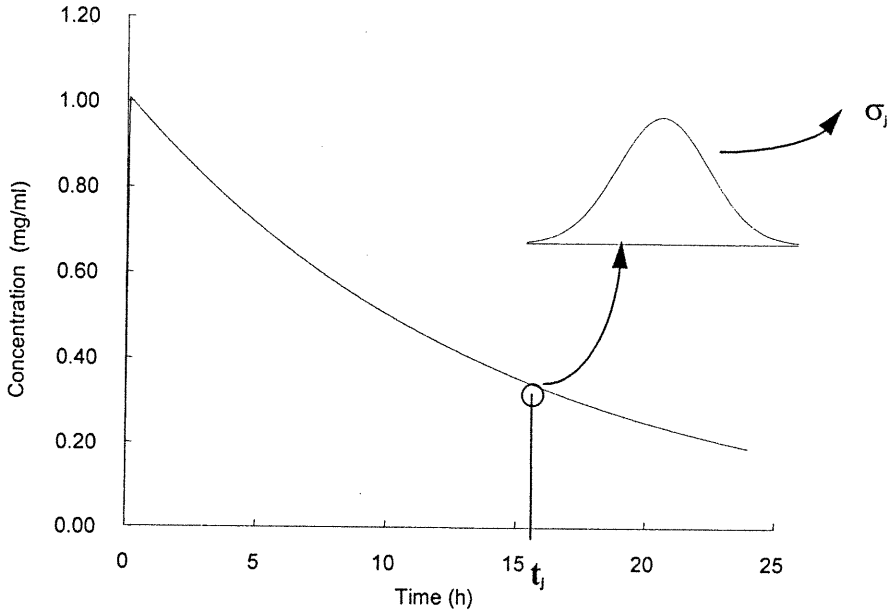


Figure 1.3 – Nuisance of Gaussian measurement.

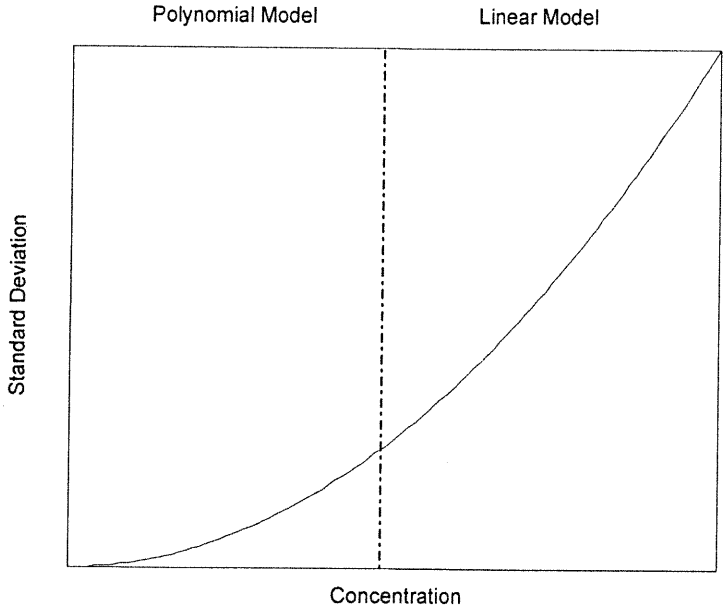


Figure1.4 – Relation between the standard deviation σ_j and the measured concentration y_j.

Mathematical model

Mathematical modeling are necessarily simplified descriptions of certain aspects of reality by mathematical means $y_m(.)$, thereby allowing one to concentrate on the factors believed to be important in the description of the observed data.

Modeling strategies consist of four basic steps [Edwards D & Hamson M, 1990]:

- clearly stating the problem or purpose of modeling,
- clearly stating the assumptions in model building,
- providing the rationale for model selection, and
- validating the model using internal or external data.

In the case of PK modeling, the biological processes involved in the elaboration of the observed drug concentration are regarded with the overall purpose of allowing a quantitative description of the real system, and even more important, a prediction beyond the existing data. Therefore, the choice of the model is a key stage as a whole, which can describe a biological phenomenon. For instance, the kinetic process of two individuals receiving the same drug can be described by means of a unique model structure $y_m(.)$. However, they differ in numerical values of their *parameters* **p**.

Before continuing with modeling techniques, we should briefly review a type of models which can well define the kinetic processes studied in pharmacokinetics. In general, **Compartmental Modeling** can well describe distribution and elimination of the molecules (Fig.1.5) [Rowland and Tucker, 1986]. This general class of models is a system which is made up of a finite number of interconnected subsystems, called compartments. The number of compartments defining the model structure rarely exceeds three compartments, often limited by the technique of drug detection in the samples. All compartments are considered as *homogeneous* and *well-mixed*, and the compartments interact by exchanging materials. Because the interactions between compartments are transfers of material, some type of mass conversion condition holds for all transfers between compartments and, to and from the environment [Jacquez JA,

1985]. These may be inputs from the environment into one or more of the compartments and there may be outputs (excretion) from one or more compartments into the environment. Represented by arrows in Fig.1.5, these rate constants are called *microconstants*. If there are no exchanges with the environment the system is said to be *closed*, otherwise it is an *open system*. The rate of change of concentration within the compartment is a function of the concentration in that compartment and in compartments to which it is connected. Thus it could be said that the forces driving the dynamics are *local*.

Another characteristic of compartmental models, most often used in pharmacokinetics and in their standard form, is that the differential equations are *linear* and follow *first-order kinetics*, thus ensuring that an *analytical solution* can be obtained. These are the main characteristics of compartmental models and the mathematical theory of the behavior of such systems is called compartmental analysis or the theory of compartmental systems. The simplest compartmental model in pharmacokinetics is a model comprising only a *single compartment*, which includes the systemic blood circulation and frequently called *central compartment*. Into this central compartment the drug enters from the site of administration, which can be from different influx pathways (i.e. bolus administration, infusion, oral route, etc...). In most PK investigations, a two-compartment model can adequately describe the kinetic processes.

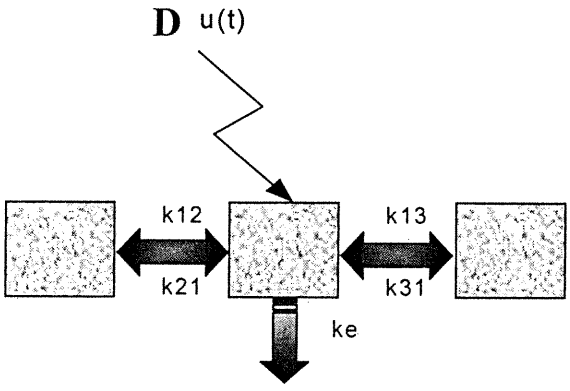


Figure 1.5 – Compartmental Structure: a 3-compartment model, **V** and **k** represent the volume of distribution and constant rate of transfer, respectively .

There are two major topological families (i.e., mathematical groups involving properties unaltered under a homeomorphism) of compartment models, *the catenary* and *the mammillary* type of model [Holz M & Fahr A, 2001]. A catenary model consists of a chain of interconnected compartments while a mammillary model comprises a central compartment interacting with a number of peripheral compartments around it. If all rate constants are ***first order***, the time course of the concentrations in the compartments will always follow a sum of exponentials. The solutions for any $y_m(t)$ function are (almost) easily calculated by a mathematical procedure called "*inverse Laplace transformation*", which is briefly explained in the following section.

Solution of The Kinetic Equations

To obtain the concentration versus time profiles of a compartment, the differential equations for the compartment system must be solved. The most promising way to solve such systems is to apply Laplace transformation which transforms the set of differential equations into a set of simple algebraic equations. Solutions of a set of algebraic equations can be easily found with the help of computer software using matrix inversion. The final step is the back-transformation from the Laplace domain to the natural time domain. Only for systems including *first order rates* this back-transformation is straightforward since it does always lead to a sum of exponentials [Rowland and Tucker, 1986], often generalized by the equation

$$y_m(t) = \sum_{k=1}^{n_c} A_k . e^{-\alpha_k t}$$

In the most traditional case, $y_m(t)$ represents the blood concentration at time *t*. The number of exponential terms is equal to the number of compartments in the structure. Thus, for any compartment, the coefficient-exponent couples (A_k, α_k) are the parameters of the model called *macroconstants* of the system. They are a function of the microconstants previously defined. In a more physiological context, one prefers to use the clearance, the volume of distribution and the elimination half-life to describe

kinetics. All these parametrizations are used to characterize individuals' kinetics, and some simple rules controlling their inter-relationship [Rowland, 1984].

For instance, for an instantaneous intravenous administration and a single compartment disposition, the model becomes

$$y_m(t) = \left(\frac{Dose}{Volume}\right)\exp\left(-\frac{Clearance}{Volume}t\right)$$

where Dose represents the input $\mathbf{u}(t)$, and Clearance and Volume are parameters that can be interpreted physiologically. Note that $y_m(t)$ is proportional to the dose administered (above equation).

Having specified the structure of the model, it is necessary to determine the numerical values of A_k and α_k (or clearance and volume) where the predictions $y_m(t)$ are close enough to the observations y. In the following section, we will describe the method used for this estimation.

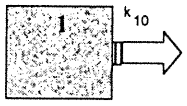
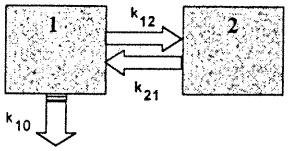
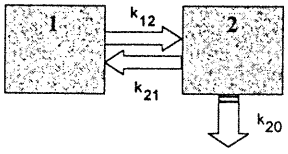
For more complicated systems the back-transformation to closed time functions might be impossible and numerical integration algorithms must be applied. A great advantage of the Laplace transformation technique is that the compartment system may always be split into an input and a disposition part. The input system represents the drug delivery system and the disposition system describes what happens to the drug once it has reached the input compartment. The input might be an exponentially decaying flux (e.g. first order absorption from the gut) and the disposition system might be a one- or two-compartment system with elimination representing the central compartment. By choosing the appropriate input function and disposition model, one can simply calculate the time courses of drug concentrations in any of the compartments and then fit these curves to any given data according to the least-squares principle. Table 1a shows some basic inputs and table 1b some disposition functions for the central compartment.

Table 1.1 – Holz M , Fahr A. *Compartment Modeling.*

(Advanced Drug Delivery Reviews. 2001; 48: 249– 264, p.260.)

Table 1.1

Basic compartment model composition. (a) Input functions. (b) System disposition functions.

Input type	**Laplace transform**	**Time function (input rate)**
(a)		
i.v. bolus dose	D	Dirac's δ-pulse with area = D
$K = D/T$ → Constant Rate	$\frac{D}{T \cdot s} \cdot (1 - e^{-T \cdot s})$	$\begin{cases} \frac{D}{T} & \text{for } 0 < t \le T \\ 0 & t > T \end{cases}$
k_{01} → First Order	$\frac{f \cdot D \cdot k_{01}}{s + k_{01}}$	$f \cdot D \cdot e^{-k_{01} \cdot t}$
(b)		
Disposition type	**Laplace transform**	**Time function (δ-response)**
 One compartment	$\frac{1}{s + k_{10}}$	$e_{10}^{-k \cdot t}$
 Two compartments with central elimination	$\frac{s + k_{21}}{(s+\alpha) \cdot (s+\beta)}$ with $\alpha + \beta = k_{12} + k_{21} + k_{10}$ and $\alpha \cdot \beta = k_{21} \cdot k_{10}$	$\frac{k_{21} - \beta}{\alpha - \beta} \cdot e^{-\beta t} - \frac{k_{21} - \alpha}{\alpha - \beta} \cdot e^{-\alpha t}$
 Two compartments with peripheral elimination	$\frac{s + k_{21} + k_{20}}{(s+\alpha) \cdot (s+\beta)}$	$\frac{k_{21} + k_{20} - \beta}{\alpha - \beta} \cdot e^{-\beta t} - \frac{k_{21} + k_{20} - \alpha}{\alpha - \beta} \cdot e^{-\alpha t}$

Population Pharmacokinetics

Developed during the end of the Seventies, population pharmacokinetics finds today more and more applications. Its objective, in a statistical sense, is " *to describe quantitative information on the interindividual variability of drugs in organisms, as well as evaluate target population dose relative to route of administration*" [Steimer et al., 1983]. More precisely, population pharmacokinetics is the study of the sources and correlations of variability in drug concentrations in biological fluids between individuals in a target population. Since the pioneering work of Sheiner et al. in 1972, more and more evidence has been gained that knowledge only of average pharmacokinetics is insufficient to determine appropriate dosage for individual patients. Certain patient pathophysiological demographical, and therapeutic features (called *covariates*) can regularly alter dose-concentration relationships. Population pharmacokinetics seeks to discover which measurable factors cause changes in the dose-concentration relationship and to what degree so that the appropriate dosage can be recommended.

Based on the raw subject data and the assay error, population PK modeling seeks to evaluate the contributions of interindividual and intraindividual variability and to describe the findings in terms that are useful both for research and for optimal patient care. Population PK modeling can be carried out based on two different approaches: *Parametric* and *Nonparametric* .

1. Parametric Approach

In parametric population modeling, the probability distribution of the parameters is itself described by these other single-valued parameters such as parameters' mean, for instance. These other parameters impart an assumed shape to each pharmacokinetic parameter distribution, usually a Gaussian or lognormal distribution (*hypothesis*). The parameter values found are the single point parameter estimates such as measures of central tendency - means, medians, or modes, which are felt to be the best estimators of

each parameter. It is also capable of providing population standard deviations, covariances, and ranges. The main strength of this approach is the ability to separate "*inter-*" from "*intra-*" individual variability and from *assay* variability which provides the opportunity for testing the hypothesis. Parametric modeling can also provide confidence limits and the opportunity to implement significant tests, however, its behaviour is not consistent. Examples of such population parametric modeling approaches are the standard two-stage—STS [Rowland M, Sheiner LB, and Steimer JL et al., 1985], and nonlinear mixed-effects modeling—NONMEM [Beal S, Sheiner LB, 1979] which are the main methods being used in the present study.

2. Nonparametric Approach

First introduced independently by Lindsay and Mallet, nonparametric models, on the other hand, have a different flavor. This approach describes that the most likely parameter estimates are actually found to be in a discrete, not continuous, collection of sets of individual parameter values. This approach estimates essentially one set of parameter values for each subject, along with an estimate of the probability of that particular set of values. Nonparametric approach makes no parametric assumptions (such as normality or unimodality) about the actual shape of the population parameter distribution. The richness of the method is in the ability to obtain not simply a single estimate for the central tendency and one for the dispersion (i.e., means, SD's, etc.), but rather to estimate the entire population parameter joint density (*Discrete Joint Density*) with consistent behaviour. Using nonparametric modeling enables one to evaluate expected therapeutic precision and to discover unsuspected subpopulations. However, confidence limits or tests of significance will yet be accomplished in future by a validation method such as bootstrapping. No further explanation would be given on this approach for it is beyond the scope of this paper.

Aim of Population Pharmacokinetics

Briefly, the population pharmacokinetic approach encompasses some or all of the following features:

1. It seeks to obtain relevant pharmacokinetic information in patients who are representative of the target population to be treated with a drug;
2. It recognizes variability as an important feature that should be identified and measured during drug development or evaluation;
3. It seeks to explain variability by identifying factors of demographic, pathophysiological, environmental, or drug-related origin that may influence the pharmacokinetic behaviour of a drug;
4. It seeks to quantitatively estimate the magnitude of the unexplained part of the variability in the patient population.

The magnitude of the unexplained (random) variability is important because the efficacy and safety of a drug may decrease as unexplainable variability increases. In addition to interindividual variability, the degree to which steady state drug concentrations in individuals typically vary about their long-term average is also important. Concentrations appear to vary due to inexplicable day-to-day or week-to-week kinetic variability and due to errors in concentration measurement. Estimates of this kind of variability (residual, intrasubject, and interoccasion variability) are particularly important for therapeutic drug monitoring (TDM).

Applications

1. Industry: At a fundamental level, population pharmacokinetics is a tool to optimize the design of biological experiments with drugs. It has become increasingly important in the design and development of new drugs and in the reassessment of old drugs. Conducted during the course of drug development, these studies serve as a useful marker for the safety of the drug, provide integrated information that might be decisive

for future experiments. Population pharmacokinetics, hence, has been integrated into drug development and regulatory process.

2. Clinical Context: Furthermore, population pharmacokinetics, in a clinical context, have resulted in improvements in utilization and direct benefits to patients. The clinical applications of pharmacokinetics should end up not only with therapeutic advances, but also with guidelines for the optimal use on the drug in each patient which provide the desired pharmacological response without inducing toxicity. The knowledge of the relationship between concentrations, response, and physiology is essential to *design dosing strategies for rational therapeutics that may not necessarily require therapeutic drug monitoring.*

Study Design

The subjects of pharmacokinetic studies are usually healthy volunteers or highly selected patients. Traditionally, the average behaviour of a group (i.e., the mean plasma concentration-time profile) has been the main focus of interest. Moreover. interindividual variability in pharmacokinetics is incorrectly viewed by many as a nuisance factor that has to be overcome, often through complex study designs and control schemes. However, this variability can be reduced through restrictive inclusion criteria. Study design and selection of volunteers, that are rigidly standardized so that they are as homogeneous as possible, are typical features of pharmacokinetic investigations. These studies, therefore, are often performed under artificial conditions that do not represent the intended clinical use of the drug.

Population Pharmacokinetic Data

Since the last two decades, there has been much interest in population pharmacokinetic modeling to recognize drug characteristics in a group of healthy volunteers (experimental studies) or in a group of patients (observational studies).

The problem of describing and estimating interindividual variability can be more precisely stated with regard to the commonly available data from drug studies. In a broad sense, "population pharmacokinetic data" must express the evident interindividual variability in drug kinetics. It is obvious that one can expect very accurate inferences from sample population, which includes a significant number of individuals. The observed kinetics result mainly from two major sources of investigation: experimental studies in animals and human subjects on one hand, and routine monitoring of patients—clinical studies—on the other hand. The distinction between experimental and clinical data is important because the pharmacokinetic methods presently available differ depending on the **nature** of the data. Some methods are restricted to analysis of kinetic data from experimental studies, while others are designed for clinical studies. *Two-Stage Methods* are good examples for analyses of experimental data, however, *One-Stage Methods* are dedicated to data analysis from clinical studies. These methods have general applicability, as will be described later.

1. Pharmacokinetic Data From Experimental Studies

Experimental pharmacokinetic (**EP**) data arise from studies under controlled conditions of drug dosing and extensive blood sampling. Example of such data is phase I (and partly phase II) of drug development which serve to define the initial parameters of toxicity, tolerance, and general pharmacokinetic characteristics of a drug. For analysis of these data, two-stage approaches are proposed and seem promising.

Since they provide valuable basic information about drug disposition and absorption kinetics, EP studies are often implemented on normal volunteers, and sometimes on patients with diseases likely to cause pathological and therapeutic

problems (e.g., renal failure). Measurements in each individual include numerous blood samples and/or urine collections that are assayed for unchanged drug and/or metabolite(s). Short-term (often single) administration is the rule. The number of individuals in a study is generally small for practical, economic, and ethical reasons. The goal of EP studies is to establish a kinetic model for the distribution and the elimination of the active ingredient in the body. These kinetics are characterized by:

- Homogeneous time of sampling, i.e. according to a strictly respected protocol qualitatively and quantitatively, between the individuals;
- Data are obtained through frequent and extensive sampling per individual which allows the estimation of the individual kinetic parameters using the criterion of the Maximum Likelihood;
- Few individuals studied for ethical and economical reasons;
- Recording of demographic and/or physiopathological characteristics of the individuals (age, weight, sex, renal and hepatic functions...) and experimental conditions are well controlled to decrease any other kinetic variability. The problems of bioavailability or genetic polymorphism, particularly in metabolism, are often revealed on this level of the development.

At a first glance, the information content of EP data regarding pharmacokinetic variability appears very limited. Usually, a sample composed of healthy volunteers is far from being representative of a patient target population. The typical sample size for a given study is, from a statistical point of view, small (always less than 30, often less than 15). However, one should notice that kinetic differences may be striking even in normal healthy volunteers, because individual variations in pharmacokinetics may result not only from biological or environmental differences but also from clearly defined pathophysiological alterations. Even in a subpopulation of normal healthy volunteers, accurate description and estimation of variability is of interest.

2. Pharmacokinetic Data From Clinical Studies

Clinical (observational) pharmacokinetic data, however, arise from investigations in patients with variable amount of data collected from each patient, i.e., different conditions of drug dosing and small number of measurements per patient. These data are usually collected during the final stages of drug development (Phase III) or sometimes when the drug is already released on the market (Phase IV—routine therapeutic drug monitoring). The analysis of these data is possible only in a single stage and it requires an explicit mathematical model, including both pharmacokinetic and statistical features (*Pharmacostatistical Model*), in order to describe variability and to detect the influential covariates. The data obtained during clinical studies exhibit certain characteristics:

- The time of sampling may occur randomly with respect to the time of dosing. Also, the number of individual observations may be highly variable between subjects.
- Unbalanced data from clinical studies contain less "pure" kinetic information than the data from carefully designed EP studies. The number of samplings in a given patient is less than the number of parameters; hence, the model is actually unidentifiable and causes problems in estimation.
- A significant number of individuals included in the study.
- Many additional sources of variability are observed. Because the data are routinely collected, the level of "noise" is likely to be higher than in carefully controlled studies, incorporating:
    i. all inaccuracy related to routine collection, manipulation, and assay of the samples at the hospital and the laboratory, and that due to mistakes in the recording of the data;
    ii. patients noncompliance, leading to errors in the amount(s) of drug taken in previous administrations or errors in the timing of the blood sample relative to the previous dose(s).

Despite the difficulties involved, the observational pharmacokinetic data are worth the effort because they arise from patients receiving the drug for therapeutic purposes. If relevant *a priori* selection criteria are applied, and if the data are collected with reasonable accuracy, such a sample of patients may provide a fairly good picture of pharmacokinetic variability in the target population.

Population Pharmacokinetic Methods

There can be significant variability in dose-response in human populations. Conceptually, variability in dose- and concentration-response are caused by variability in pharmacokinetic and pharmacodynamic responses. Pharmacokinetics pertains to variability in tissue concentration-time profiles. In practice, pharmacokinetic parameters cannot be directly measured in a population of interest. The "measurement device" provides indirect data, namely levels of drugs and/or metabolites in some easily accessible biological fluids (i.e., typically plasma or blood and/or urine). This is often fulfilled as serial measures at different times after drug intake. Accordingly, the principal difficulty of the kinetic data is the heterogeneity between the individuals (a number of different sampling time, different routes of administration).

To solve this problem, one should describe the population pharmacokinetics in terms other than the raw data in order to get *reduced*, *standard*, and hence *more useful* information. The basic requirement is to produce some mathematical transformation of the original data to the attributes of interest, the **parameters**. The connection between the original data and the parameters is made through the pharmacokinetic modeling. A model is useful for a simplified and global description of drug pharmacokinetics. PK modeling is used to transpose the pharmacokinetic variability expressed in observational context in more homogeneous space of the kinetic parameters.

The methods used in this study are based on parametric population approach. These methods can provide estimates of central tendency and dispersion (e.g., the first two moments of the parameter distribution):

1. Two-stage method

When individuals provide sufficient information of comparable quality across subjects, it is often preferable to use the two-stage method to estimate kinetic parameters. In other words, it is one of the most applicable methods when there is a significant number of observations (kinetic points) per individual. In asymptotic conditions, one can assume that '*inter-*' and '*intra-*' variability are independent.

In this method, individual data are fitted in a first stage, and individual estimates are combined to derive population characteristics and statistically describe interindividual variability in a second stage. The latter can be performed through simple averaging (standard two-stage, STS), through optimization of an extended least-squares objective function (global two-stage, GTS), or may imply repeated fittings of individual data (iterated two-stage, ITS). Generally, the two-stage approach:

- allows the identification of certain aspects of the data or underlying process through parameter estimates, or competing models.
- allows individual modeling to be well implemented because it estimates the measurement error and the uncertainty associated to each parameters, which enables one to evaluate the respective credibility of the estimation;
- facilitates the development of the regression models with the knowledge of the kinetic parameters;
- allows any types of parameterization, for example, one can also study the variability of area under the curve (AUC) or of the maximum concentration (C_{max}). However, these methods are often criticized because they require a significant number of observations per individual (8 to 20 based on the complexity of the drug disposition).

2. One-stage method

When crucial measurements are missing, or in sparse sampling that provide insufficient information to obtain adequate individual parameter estimates, the data set will need to be analyzed as a whole. In this way, information may be shared across subjects taking the relative contribution of each individual into account. In this method, the population characteristics are estimated in a unique stage through global analysis of all data. The nonlinear mixed-effects modeling (NONMEM) is the application of this method used in this memoir. Some of the most important and useful features of NONMEM are as follow [Sheiner LB, Beal SL. *NONMEN Users Guides*, 1994]:

- It can provide estimates for both the individuals and the population.
- Has a menu of pharmacokinetic models from which the most appropriate one can be chosen.
- The user specifies the relationship of pharmacokinetic parameters to independent variables, selecting "population" parameters that will be estimated.
- The user also specifies which parameters vary between individuals, and the form (model) for this variability, as well as the form (model) for the differences between observations from an individual and their predictions for this individual.
- It estimates parameters describing both inter- and intra- variability.
- It provides estimates (standard errors) of the precision of its parameter estimates, including those describing variability.
- Provides a means of deciding whether one model (e.g., that including weight's effect on CL and V) fits the data better than another using the minimum objective function value, a goodness-of-fit statistic.
- Provides (limited) graphics, useful in judging the adequacy of the model currently fit to the data.

Comparison of Methods: STS *vs.* NONMEM

The traditional approach for estimating population pharmacokinetic parameters is the two-stage approach. In this approach, the pharmacokinetic parameters of an individual are estimated on the basis of the data for the individual. Population averages and standard deviations are estimated from the sample of individual parameters using standard statistical methods. Although this approach has been shown to give relatively unbiased estimates of population averages of pharmacokinetic parameters, estimates of variability have been shown to be biased towards higher values. The two-stage approach is strictly applicable to data from balanced experiments with repeated measurements, as in this study, and sufficient data from each individual (rich data) are needed to estimate the pharmacokinetic parameters of the individual. This approach cannot therefore utilize the often significant amount of sparse, unbalanced data that is routinely collected in clinical settings.

Alternative methods, one-stage analysis, however proposed in recent years to address the limitations of traditional population pharmacokinetic analysis methods, one of which is NONMEM (Nonlinear Mixed Effects Model). This method employs some form of maximum likelihood estimation. In NONMEM, the statistical model is specified by defining a parametric relationship between measured covariates (such as weight, height, and age) and the pharmacokinetic parameters to be estimated. One-stage method allows the analysis of data from a variety of unbalanced designs as well as from studies that are normally excluded from regular PK analysis because they do not lend themselves to the usual forms of PK analysis. This method is designed for implementing pharmacokinetic analysis on retrospective and sparse data particularly on data from therapeutic drug monitoring (TDM).

In the following chapter, we will present the appropriate methods for both individual and population estimations.

Methodology 2

Estimation Criterion

Pharmacokinetic models are and have been very successful although the basic ideas behind them like transfer into, between and out of compartments with immediate mixing, can only approximate the actual physiology [Rowland M and Tozer TN 1995]. The previous chapter has shown how a model-based approach is an important tool for the description, transformation, and analysis of pharmacokinetic data analysis. Once an explicit PK model has been specified, it is necessary to assign values to each of the parameters in order to make the model representative of the given data. This process is known as *Parameter estimation.*

Given a set of observations, one often wants to condense and summarize the data by fitting it to a "model" that depends on adjustable parameters, by which the closest predictions would be generated. Basically, the model is simply a class of functions, such as polynominals or Gaussians, and the fit supplies the appropriate coefficients. After selecting a mathematical model, one should choose or design an optimization function (*figure-of-merit function*) that measures the agreement between the data and the model with a particular choice of parameters. In other word, we need a criterion to measure the goodness-of-fit between the observations and the model predictions. This criterion—a function measuring the error between observations and predictions—can be defined by

$$e_j(t_j, \mathbf{p}) = y_j - y_m(t_j, \mathbf{p})$$

where we can find the prediction error for each observation $\mathbf{y_j}$ at time $\mathbf{t_j}$. The optimization function is conventionally arranged so that small values represent close agreement. The parameters of the model are then adjusted to achieve a minimum in this function, yielding *best-fit parameters*.

The exact structure of the model being proposed, when the parameters $\mathbf{p}$ of the model approach the true values of the parameters $\mathbf{p^*}$ resulting optimal fit where $e_j(t_j, \mathbf{p^*})$ tend towards ε_j (Fig. 2.1). The estimation function must take into account these modeling errors expressed as $e_j(t_j, \mathbf{p^*})$, at all time instants $(\mathbf{j} = 1, \cdots, \mathbf{n_t})$.

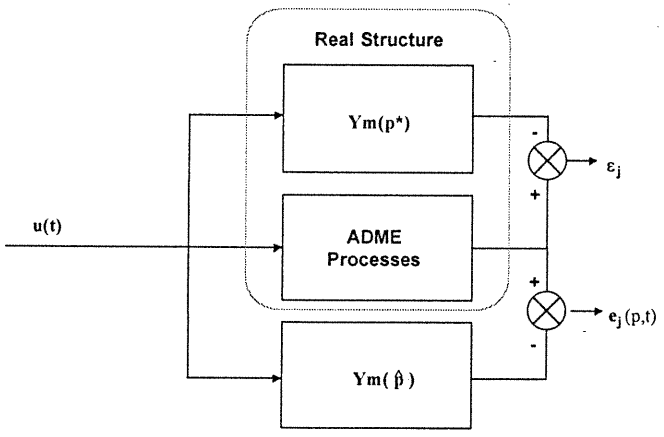


Figure 2.1– Illustration of measurement nuisance ε_j & $\mathbf{e_j}$ between the model and the real process.

Several optimization methods have been developed for estimating PK parameters. The choice of the optimization method depends upon the problem being considered and the required execution efficiency. In this section, different modeling techniques are described which provide a flexible framework for parameter estimation and the comparison of different candidate models. Model selection is an important part of the data modeling as it allows one to assess the ability of different models to describe the data whilst favoring economy above over-fitting. Here, we will present the optimization method used in our PK analysis: **The Maximum Likelihood (ML) Estimation**.

The Maximum Likelihood Estimation

Maximum likelihood is the most important and widespread method of estimation. Many well-known estimators such as the sample mean, the least-squares, and the extended least-squares estimators in regression are maximum likelihood estimators. Maximum likelihood is very useful in practice and tends to give more precise estimates than other methods of estimation (e.g., Least Squares method).

In particular cases where the number of observations in individuals under study are sufficiently large to identify all the parameters, the method of the maximum likelihood [Fisher, 1912] is the most appropriate choice. Based on this method, one wants to identify the maximum probability π_y of the observations y given the parameters **p**, as the *likelihood* of the parameters given the data. Hence, one must obtain $\mathbf{p}_{ML}$, by finding the values of the parameters **p** that produce a close approximation to the data (in other words, *maximize* the probability π_y of the observations to be occurred). This is important since knowledge of these parameters enables one to recreate the data, make further inferences about its characteristics, or perhaps transform the data by manipulation of the parameters. Therefore, we can define the maximum likelihood by

$$p_{ML} = \arg\max \pi_y(y / p)$$

If **p** is being fixed, π_y would be the probability density function (pdf) of the random vector y associated with a model parameters **p**. Contrary, in estimation problems, y is usually known or measured (observations) and **p** is required. We then define the likelihood function $L_y(\mathbf{p})$ by which one wants to maximize

$$p_{ML} = \arg\max L_y(p)$$

For a data sample y_j at time instant t_j, the model produces y_m that is a function of the parameters $\mathbf{p}^*$. As the model is deterministic and the structure is supposed to be exact, the error term b_j in the reconstruction can be expressed as

$$\varepsilon_j = \mathbf{y}_j - \mathbf{y}_m(\mathbf{t}_j, p^*)$$

therefore the error ε_j is a function of the true value p* being searched. Naturally, it is desired to reduce the error in the model's approximation by finding the set of values, which minimizes this error or, more specifically, the sum of the squares of the error terms over the length of the data. Introducing a vector notation to represent the sequence of values over the analysis interval, it is required to minimize the expression

$$\left(\varepsilon_j\right)^2 = \left(\mathbf{y}_j - \mathbf{y}_m(\mathbf{t}_j, \mathbf{p}^*)\right)^2$$

with respect to all possible values of the model parameters **p***,

$$\hat{\mathbf{p}}_{LS} = \arg\min\left(\varepsilon_j\right)^2$$

The least-squares parameter produced by this criterion is unsatisfactory in many situations. Success is measured solely by the model's proximity to the observation. The model is therefore susceptible to over fitting by allowing more parameters to be added to the model in order to reduce the error term. In the extreme case, when the number of parameters is equal to **p**, the data can be represented with zero error.

Over-fitting is undesirable for several reasons:

- Errors in the data are modeled (e.g., measurement error) and so sensitivity to noise is increased;
- The model loses the ability to generalize since minor variations in the data are modeled;
- The model produced is much larger (and therefore more expensive to represent and calculate) than the 'true' underlying model.

To obtain better parameter estimation criteria, the task is posed in a probabilistic framework. Suppose that each data point **y** has a measurement error $\mathbf{b}_j$ that are independently random and identically distributed as a normal (Gaussian) distribution

around the "true" model $y_m(.)$. Then the conditional probability density of ε_j knowing $\mathbf{p}^*$ can be written as

$$\pi_\varepsilon(\varepsilon/\mathbf{p}^*) = \pi_\varepsilon(\varepsilon_1, \varepsilon_2, .., \varepsilon_{n_t} / \mathbf{p}^*) = \prod_{j=1}^{n_t} \pi_{\varepsilon j}(\varepsilon_j / \mathbf{p}^*) = \prod_{j=1}^{n_t} \pi_{\varepsilon j}\left[y_j - y_m(\mathbf{t}_j, \mathbf{p}^*)\right]$$

where n_t is the number of measurements and $\pi_{\varepsilon j}$ is the conditional probability (pdf) of ε_j given $\mathbf{p}^*$. The preceding relationship shows that $\pi_\varepsilon(\varepsilon/\mathbf{p}^*)$ is also the conditional pdf $\pi_y(\mathbf{y} / \mathbf{p}^*)$ of observations y given $\mathbf{p}^*$. The conditional pdf of the observations knowing $\mathbf{p}^*$ is thus written as

$$\pi_y(\mathbf{y} / \mathbf{p}^*) = \prod_{j=1}^{n_t} \pi_{\varepsilon j}\left[y_j - y_m(t_j, \mathbf{p}^*)\right]$$

Let's recall that ε_j is the limit of e_j, the goodness-of-fit between the model and the observations, when $\mathbf{p}$ tends towards $\mathbf{p}^*$. The pdf $\pi_y(\mathbf{y} / \mathbf{p}^*)$of $\mathbf{y}$ for all the values $\mathbf{p}$ close to the true value $\mathbf{p}^*$ can be written as

$$\pi_y(y / \mathbf{p}) = \prod_{j=1}^{n_t} \pi_{\varepsilon j}\left[y_j - y_m(t_j, \mathbf{p})\right]$$

The likelihood expression is sometimes written as $L_y(\mathbf{p})$, to emphasize that it is a function of the parameters. The maximizing values of $\mathbf{p}$, indicated as the maximum likelihood estimates $\mathbf{p}^*$ also maximize the probability that the measurement sequence will actually occur. In fact, strictly speaking, the interpretation is that it is a function of the observations. In estimation process, instead of maximizing $L_y(\mathbf{p})$, usually $\ln \mathbf{L}_y(p)$ is maximized, resulting in most cases in an easier optimization problem. Maximizing this expression is equivalent to maximizing its logarithm, or minimizing the negative of its logarithm, because the logarithm is a monotonic function. Eventually

$$\mathbf{p}_{ML} = \arg\max \ln\left[L_y(\mathbf{p})\right] = \arg\max \sum_{j=1}^{n_t} \ln\left\{\pi_{\varepsilon j}\left[y_j - y_m(t_j, \mathbf{p})\right]\right\}$$

and the log-likelihood becomes a sum of terms of which each one is associated with the measurement error observed for each time t_j.

Now, we have to define the structure of the probability distribution $\pi_{\varepsilon j}$ of the associated error (noise). There are different type of distribution. However, based on the fact that the probability distribution of the sum of a very large number of very small random deviations almost always converges to a normal distribution and also according the *central limit theorem*, the Gaussian distribution seems most plausible and thus

$$\pi_{\varepsilon j}\left[y_j - y_m(\mathbf{t}_j, p)\right] = (2\pi\sigma_j^2)^{-1/2} \exp\left\{-\frac{1}{2}\left[\frac{y_j - y_m(\mathbf{t}_j, p)}{\sigma_j}\right]^2\right\}$$

Since the variance σ_j^2 is unknown and heteroscedastic (it means *non-stationary* in the sense that the local variance and to a lesser extent the local mean undergo changes on a time scale that is long compared to the fluctuations of the series itself), we could think of including n_t variance σ_j^2 in the parameter vector **p**. Under these conditions, the estimate of all the parameters becomes impossible since the dimension of **p** is higher than the total number of data. An alternative way exist where the variance is expressed in the form of a model function of the observations [Box and Hill, 1974], such that

$$\sigma_j = a \cdot \mathbf{y}_m^{\alpha}(t_j, \mathbf{p})$$

In this model, **a** represents a proportionality constant, and α is an exponent element allowing the choice of error model most adapted to the experimental conditions. More particularly, if $\alpha = 0$ the error is absolute and if $\alpha = 1$ the error is relative. Therefore, this model can adapt various forms of σ_j, previously discussed in **"Data Collection"** section.

The estimate of **p** in terms of the maximum likelihood estimation gives,

$$p_{ML} = \arg\max\left\{-2\mathbf{n}_t \ln \mathbf{a} - 2\alpha\sum_{j=1}^{n_t} \ln y_m(\mathbf{t}_j, p) - \frac{1}{\mathbf{a}^2}\sum_{j=1}^{n_t} \frac{\left[y_j - y_m(\mathbf{t}_j, p)\right]^2}{y_m^{2\alpha}(\mathbf{t}_j, p)}\right\}$$

which is equivalent to minimizing the criterion $j_{ML}(\mathbf{p})$

$$\mathbf{j}_{ML}(p) = 2\mathbf{n}_t \ln \mathbf{a} + 2\alpha \sum_{j=1}^{n_t} \ln y_m(\mathbf{t}_j, p) + \frac{1}{\mathbf{a}^2} \sum_{j=1}^{n_t} \frac{\left[y_j - y_m(\mathbf{t}_j, p)\right]^2}{y_m^{2\alpha}(\mathbf{t}_j, p)}$$

From this expression, we realize that when the partial derivative of this criterion in terms of **a** is equivalent to zero:

$$\frac{\partial \mathbf{j}_{ML}(p)}{\partial \mathbf{a}} = \frac{2\mathbf{n}_t}{\mathbf{a}} - \frac{2}{\mathbf{a}^3} \sum_{j=1}^{n_t} \frac{\left[y_j - y_m(\mathbf{t}_j, p)\right]^2}{y_m^{2\alpha}(\mathbf{t}_j, p)} = 0$$

One can then define a^2 as

$$a^2(\mathbf{p}, \alpha) = \frac{1}{n_t} \sum_{j=1}^{n_t} \frac{\left[\mathbf{y}_j - \mathbf{y}_m(t_j, \mathbf{p})\right]^2}{\mathbf{y}_m^{2\alpha}(t_j, \mathbf{p})}$$

By introducing this expression into the likelihood criterion and eliminating a term which does not depend on the parameters, $j_{ML}(\mathbf{p})$ [the maximum likelihood objective function] becomes

$$\mathbf{j}_{ML}(p) = \mathbf{n}_t \ln\left\{ \frac{1}{\mathbf{n}_t} \sum_{j=1}^{n_t} \frac{\left[y(\mathbf{t}_j) - y_m(\mathbf{t}_j, \mathbf{p})\right]^2}{y_m^{2\alpha}(\mathbf{t}_j, \mathbf{p})} \right\} + 2\alpha \sum_{j=1}^{n_t} \ln y_m(\mathbf{t}_j, p)$$

This choice of the error model can be verified *a posteriori* by the study of residuals in the observation space [D'Agostino and Stephens, 1986]. When the optimal values of parameters $\mathbf{p}_{ML}$ are determined, the value for constant **a** is calculated by

$$\mathbf{a}_{ML}^2(p_{ML}, \alpha) = \frac{1}{\mathbf{n}_t} \sum_{j=1}^{n_t} \frac{\left[y_j - y_m(\mathbf{t}_j, p_{ML})\right]^2}{y_m^{2\alpha}(\mathbf{t}_j, p_{ML})}$$

If the error is associated mainly to measurements, the comparison of this constant with the value provided by the dosing method enables one to evaluate the modeling process.

Maximum Likelihood estimates are asymptotically unbiased, as well as asymptotically efficient (non-skewed), i.e. when $\mathbf{P}_{mv}$ tends to being distributed according to a Gaussian law $N\left[\mathbf{p}^*, \mathbf{F}^{-1}(\mathbf{p}^*)\right]$ when $n_t \to \infty$. An error bound on the estimation errors can be derived from the so-called **Fisher information matrix**, where F_{p^*} is given by

$$F_{p^*} = \mathbf{E}_{y/p}\left[\frac{\partial}{\partial p}\ln \pi_y(y/p)\frac{\partial}{\partial p^T}\ln \pi_y(y/p)\right]_{p=p^*} = -\mathbf{E}_{y/p}\left[\frac{\partial^2}{\partial p \partial p^T}\ln \pi_y(y/p)\right]_{p=p^*}$$

where $\frac{\partial}{\partial \mathbf{p}}\ln\pi_y(\mathbf{y}/\mathbf{p})$ and $\frac{\partial^2}{\partial \mathbf{p}\partial \mathbf{p}^T}\ln\pi_y(\mathbf{y}/\mathbf{p})$ are the ***gradient*** and the ***Hessian*** $(\mathbf{H}_i)$ matrix of the log-Likelihood [Gill et al., 1981, p. 47-52]. The Fisher matrix F_{p^*} is of particular interest, for its inverse matrix provides a lower limit of the **precision matrix of the estimator $\mathbf{p}_{ML}$**. The latter, shortly called the precision matrix, makes it possible in turn to calculate the standard deviation of the estimate, square root of the k^{th} diagonal element of the matrix $\mathbf{p}_{mv}$.

The maximum likelihood estimation provides the optimal values for the parameter estimation, as well as their perspective precision. One can define the precision of the estimation in terms of the **Covariance-Correlation Matrix**.

$$\begin{array}{c|ccccc} & \mathbf{p}_1 & \mathbf{p}_2 & \cdots & \mathbf{p}_n \\ \mathbf{p}_1 & \sigma^2_{p_1} & \rho_{p_1p_2} & \cdots & \rho_{p_1p_n} \\ \mathbf{p}_2 & \sigma^2{}_{p_1p_2} & \sigma^2_{p_2} & \cdots & \rho_{p_2p_n} \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \mathbf{p}_n & \sigma^2{}_{p_1p_n} & \sigma^2{}_{p_2p_n} & \cdots & \sigma^2_{p_n} \end{array}$$

where $\sigma^2{}_{pn}$ is the variance of the estimated parameter $\mathbf{p}_n$ and $\sigma^2{}_{p1pn}$ is the covariance of the estimated parameters $\mathbf{p}_1$ and $\mathbf{p}_n$. The correlation between the parameters are expressed as

$$\rho_{p1p2} = \frac{\sigma^2_{p1p2}}{\sigma_{p1}\sigma_{p2}}$$

Numerical Aspects

Among the numerical approaches to the minimization of the objective function, approximate solutions have been used. The **Simplex** algorithm [Nelder, Mead, 1965] is based on the geometry of the parameter space. Gradient methods require the first derivatives as e.g., the **Gauss-Newton** method. Second derivatives are used by **Quasi-Gauss-Newton** [Goldfarb, 1970] type methods, which is used in our calculations.

It is the optimization algorithm which allows, starting from available information, to obtain the parameter values **p**. In all cases, optimization leads to numerical values of **p**, but it is misleading to believe that this result corresponds to the best possible model. Even in the ideal case where the structure of the model is exact, the uncertainty of the observations causes an uncertainty on the parameters. It is thus desirable to add a confidence interval to these numerical values. The minimization is implemented on all dimensions of the parameters' vector **p**.

In principle, any numerical optimization scheme may be applied for the solution of the maximum likelihood problem. In the Newton-Raphson algorithm, a sequence of steps $\Delta\mathbf{p}$ is calculated according to the following equation until a stationary value of $\mathbf{p}^*$ is reached.

$$\Delta p = -\left[\frac{\partial^2}{\partial p \partial p^T} \ln \pi_y(y/p)\right]^{-1}_{p=p^*} \frac{\partial}{\partial p} \ln \pi_y(y/p)$$

It is very time consuming to exactly evaluate the Hessian matrix of second derivatives in this expression. A good approximation results when the Fisher information matrix is substituted instead, resulting in the well-known **Gauss-Newton** algorithm:

$$\Delta p = \mathbf{F}^{-1}_{p^*} \frac{\partial}{\partial p} \ln \pi_y(y/p)$$

Now only first-order derivatives are required for the calculation of the information matrix F_{p^*}, as well as of $\Delta\mathbf{p}$. The convergence of this method towards the minimum value is very fast but it requires an initial estimate close to the final result. Where the iteration must necessarily start from a bad initial guess of **p** or when the information matrix is ill conditioned, the algorithm may not converge. This method can be applied on one-dimensional minimization.

In this thesis, a similar approximation to that of Gauss-Newton is used, called **Quasi-Gauss-Newton** method that can be well applied to multi-dimensional minimization problem. The difference is that the second derivatives are used for this type of approximation. This method has many advantages:

- The inversion matrix is not needed ;
- If $\mathbf{H}_1$ (**H**essian) is a positive definite matrix, so will be $\mathbf{H}_2$, ... ;
- Only gradients of functions must be evaluated at each iteration.

Model Selection Criterion

A need for *model selection* techniques arises from the need to compare several candidate models and assess their suitability in a manner consistent with *Occam's razor*: where model parsimony is traded off against the goodness-of-fit. This principle states that one should not make more assumptions than the minimum needed and entities are not to be multiplied beyond necessity. In this thesis, model order selection will be used to describe the selection between models of the same type which differ in the number of parameters. A model is chosen so that, based on the then available knowledge, it can best elucidate the relevant phenomena in the simplest way.

In pharmacokinetics, sum of exponentials are often used to provide compartmental descriptions of concentration-time profiles. When sums of exponentials

are applied, one is often confronted with choosing between two or more possible descriptions, for example, a bi- vs. a tri-exponential equation. Among different methods available, the method of minimum Akaike information criterion (AIC) [Yamaoka K, 1978] was used to determine the model that most adequately describe the kinetic data in our data analysis. This criterion is based on the number of blood levels, the residual sum of squares of the specific pharmacokinetic model, and the number of parameters in the same model. The AIC numerically expresses the amount of information in a group of experimental data. It can be defined as

$$\mathbf{AIC} = (\mathbf{N} \,.\, \ln \mathbf{R} + 2\mathbf{p})$$

where **N** is the number of designed points (i.e., blood levels), **R** is the mean residual sum of squares, and **p** is the number of estimated parameters in the model.

In fact, AIC is a function of the likelihood with some modifications that penalize model complexity by adding terms, which are dependent on the number of parameters in the model. It can hence be written as

$$\mathbf{AIC} = -2 \log (\text{maximum likelihood}) + 2\mathbf{p}$$

When AIC applied, the model producing the lowest value is selected. This measure when considered in a probabilistic setting, however, appear to make restrictive assumptions about the nature of the parameters. Principally, that is all model order coefficients carry equal weight and the cost of a model is function only of the number of terms.

The performance of AIC is evaluated using

1. percentage of correct model selection. That is how frequent the model is being chosen among alternative models;

2. mean (parameter estimation) error (**ME**) and mean absolute (parameter estimation) error (**MAE**) expressed as a percentage of the true value of the parameters such as clearance (**CL**), steady state volume of distribution ($\mathbf{V_{ss}}$), mean residence time (**MRT**), … etc.; and
3. prediction error as measured by the mean overall sampling times of the squared deviations between natural logarithms of the predicted and true concentration values.

In this thesis, the evaluation of model selection criterion (AIC) is mainly based on percentage of correct model selection in our data analysis.

Estimation of Population Pharmacokinetics

As stated in the precedent chapter, population pharmacokinetics is performed to estimate population characteristics (i.e., mean and variance) of PK parameters, and to discover the sources and correlations of the variability in response. Difficulties in population estimation arise mainly from the heterogeneity of the kinetic data between individuals (i.e., different sampling schedules and routes of administration). It is thus necessary, using modeling, to transpose this variability expressed in space of the observations in more homogeneous space of the kinetic parameters. This transposition is not easily performed for, on the one hand, the equation $\mathbf{y}_m(t, \mathbf{p})$ which binds the two spaces is nonlinear; and on the other hand, the often sparse nature of data (observational) makes it difficult to assess the kinetic parameters.

To overcome these problems, various methods were developed for the determination of population characteristics during the last years. In all cases, the description of variability comprises three levels:

- the choice of a pharmacokinetic model $\mathbf{y}_m(.)$ of the drug, which is generally selected at the time of early phases of the development. The model's structure is common to all individuals;
- development of a statistical model to describe the variability of the kinetic parameters;
- to find the correlations between the parameters **p** and the characteristics associated with demographic, physiopathological and environmental factors. It is usually assumed that the population under study is made up of more homogeneous subpopulations (where the variability is smaller), so that one can discover these correlations by using these characteristics called **covariates**, **q**, such as age, weight, gender, or creatinine clearance…

In the next sections, we will present methods widely used in population pharmacokinetics to estimate these characteristics, and to express sources of variability in parameters' space in terms of parametric modeling. The essence of parametric

modeling is that a common regression model is usually applied between the kinetic parameters and the covariates.

To estimate the variability of the kinetic parameters in the simplest case where the covariates are missing, it is assumed that all the parameters $\mathbf{p}$ are independent random variables following the same distribution law $\pi_p(\mathbf{p})$ in $\Re^{n_p}$ (n-dimensional Euclidean space). For example, in the case of a Gaussian distribution, we restrict our concern to the estimation of the first two moments calculation, the mean vector $\mathbf{p_0}$, which accounts for the central tendency in the population, and the variance Ω. If the covariates $\mathbf{q}$ are present, one can express $\mathbf{p_0}$ as a function of the covariates by traditional methods of linear regressions of the type

$$\mathbf{p}_0 = \Theta\mathbf{q} + \theta_0$$

where Θ and θ_0 are the regression parameters.

Examples of such population parametric methods are the *Standard Two-Stage* (STS) that is adapted to the experimental data and is primarily used to identify the individual kinetic parameters, and *NONlinear Mixed-Effects Modeling* (NONMEM) where $\mathbf{p_0}$ and Ω are simultaneously estimated from the observations of all individuals. Particular emphasis, in the following sections, will be placed on the procedures used in each method, in order to facilitate precise comparison in the subsequent discussion.

1. Standard Two-Stage Method

In this method, the first stage is to identify the kinetic parameters for each individual in the population. For this purpose, the criterion of the maximum likelihood is used. At the end of this stage one can obtain:

- $\mathbf{n}_i$ is a vector of estimated kinetic parameters, p^i_{ML} (of dimension n_p) and $\mathbf{n}_i$ sequence precision matrices $\mathbf{P}^i_{ML}$ with dimension equal to those of p^i_{ML}.
- *a posteriori* estimation of the constant a^i_{INV} for the variance model of the measurement error.

After estimating the parameters for each individual, these estimates may be combined and compared between individuals. This will produce adequate results if each individual provides estimates of comparable accuracy. However, if estimates are not based on (roughly) the same number of measurements for each individual, some form of weighting of the different estimates is appropriate [Matthews JNS 1993]. This weighting of the relative contribution can also be obtained by the mixed-effects models described in the next section.

By assuming that p^i_{ML} is a correct estimate of the true parameter values, $\mathbf{n}_i$ data samples are analyzed to establish the characteristics of the parameters' distribution and the covariates (if any).

If $\pi_p(\mathbf{p})$ follows a Gaussian law with mean parameters $\mathbf{p}_0$ and dispersion Ω, it can be written as

$$\pi_p(\mathbf{p}) = \left[(2\pi)^{n_p} \det\Omega\right]^{-1/2} \exp\left[-\frac{1}{2}(\mathbf{p}-\mathbf{p}_0)^T \Omega^{-1}(\mathbf{p}-\mathbf{p}_0)\right]$$

$\mathbf{p}_0$ and Ω can be estimated in terms of p^i_{ML} by using the following equation

$$p_0 = \frac{1}{\mathbf{n}_i}\sum_{i=1}^{n_i} p^i_{ML} \quad \text{and} \quad \Omega = \frac{1}{\mathbf{n}_i}\sum_{i=1}^{n_i}\left(p^i_{ML}-p_0\right)\left(p^i_{ML}-p_0\right)^T$$

If $\pi_p(\mathbf{p})$ follows a lognormal law, we can express the equations as

$$\pi_p(\mathbf{p}) = \left[(2\pi)^{n_p} \det\Omega_{ln}\right]^{-1/2}\left(\prod_{k=1}^{n_p} p_k\right)^{-1} \exp\left[-\frac{1}{2}(\ln\mathbf{p}-\mathbf{p}_{ln})^T \Omega_{ln}^{-1}(\ln\mathbf{p}-\mathbf{p}_{ln})\right]$$

$$p_{ln} = \frac{1}{\mathbf{n}_i}\sum_{i=1}^{n_i} \ln p^i_{ML} \quad \text{and} \quad \Omega_{ln} = \frac{1}{\mathbf{n}_i}\sum_{i=1}^{n_i}\left(\ln p^i_{ML}-p_{ln}\right)\left(\ln p^i_{ML}-p_{ln}\right)^T$$

with $\ln\mathbf{p} = \left[\ln p_1,\ldots,\ln p_{n_p}\right]^T$. Please recall that $\mathbf{M}^T$ is the transpose of the matrix $\mathbf{M}$.

When the data is highly erroneous (sampling errors, measurement errors, or insufficient sampling ...), uncertainty on the numerical value of the kinetic parameters becomes large and the matrix of covariance, Ω, is at risk of over-estimation. In order to avoid this influence, Gaillot et al. (1979) offered a particular way to weight the estimated parameters p^i_{ML} by their respective matrix of precision $\mathbf{P}^i_{ML}$. While knowing p^i_{ML} and $\mathbf{P}^i_{mv}$, it is possible to estimate the mean parameter p_0 and the covariance Ω by minimizing the criterion

$$\mathbf{j}(\mathbf{p}_0, \Omega) = \sum_{i=1}^{n_i} \left[\left(\mathbf{p}^i_{ML} - \mathbf{p}_0\right)^T \left(\mathbf{P}^i_{ML} + \Omega\right)^{-1} \left(\mathbf{p}^i_{ML} - \mathbf{p}_0\right) + \ln \det\left(\mathbf{P}^i_{ML} + \Omega\right) \right]$$

2. One-Stage Method (NONMEM)

The statistical class of mixed-effects models combines the information across individuals, providing an estimation method called *Nonlinear Mixed-Effects Modeling*. The models are called '**mixed**' because they describe the data using a mixture of fixed and random effects. Fixed effects are under the control of the investigator like the time of measurement and the dose administered, while random effects describe the variability in the measurements within (**intra-**) or between (**inter-**) subjects.

The power of the mixed-effects models comes from the fact that differences in parameters between subjects are modeled using distributions for these parameters. The task of estimating individual parameters for each subject is replaced by simultaneously estimating a single mean parameter and inter-individual variability estimate for each parameter. However, the total variability of the observations comes from two concomitant sources. The dispersion that exists between individuals on one hand, and the residual error specific to each individual (the experimental error, modeling error, or

intra-individual variability), on the other. the estimation is carried out using a least squares criterion

$$\mathbf{j}_{LS}(p_0,\Omega)=\sum_{i=1}^{n_i}\left\{\left[y^i-\mathbf{E}(y^i)\right]^T\left(\Psi^i\right)^{-1}\left[y^i-\mathbf{E}(y^i)\right]+\ln\det\Psi^i\right\}$$

with $\mathbf{E}(\mathbf{y}^i)$ of dimension n_t and the sequence matrices $\Psi^i=\mathrm{Var}(y^i)$ of order n_t. One should now express $\mathbf{E}(\mathbf{y}^i)$ and Ψ^i as a function of $\mathbf{p}_0$ and Ω. However, these two spaces, the observations and the parameters, are bound non-linearly. It means that the model for individuals is nonlinear in its parameters. Therefore

$$y^i=y_m^i(\mathbf{p}^i,t)+\varepsilon^i$$

Initially, Sheiner et al. proposed the mixed-effects modeling. The term *mixed-effects modeling* is the statistical term used in the situation where a combination of fixed and random effects is studied. Hence, it can be defined as the sum of fixed-effects parameters, expressed as a function of covariates, and random-effects parameters concerning the dispersion of the parameters Ω and the residual error of the observations σ^2, quantifying inter- and intra-individual variability, respectively. In turn, fixed-effects parameters are part of the experimental design and under control of the investigator such as the time of measurement or the treatment (dose) applied.

For an individual i, we can consider the model of an observation y_j^i at time $\mathbf{t}_j$ as

$$\mathbf{y}_j^i=\mathbf{y}_m^i(p_0+\eta^i,\mathbf{t}_j)+\varepsilon_j^i$$

The fixed effect $\mathbf{p}_0$ is the moving average of the population, dependent on the covariates or the subpopulation. The random effect η^i is the displacement of individual i around $\mathbf{p}_0$, and ε_j^i is the additive noise or the residual error. Hypothetically, we can

assume that η^i and ε_j^i follow the Guassian normal distribution, then $\eta^i \sim N_{\eta,n_p}(0,\Omega)$ and $\varepsilon_j^i \sim N_\varepsilon\left(0,\sigma_j^2\right)$.

Because of the nonlinear nature of nonlinear mixed-effects modeling, exact and analytic results are unavailable, and the problem must be approached through approximations and by the use of iterative techniques. The most extensively developed and widely used is a **first-order Taylor approximation** which results in a linearization of equation $y_m^i(\mathbf{p}_0 + \eta^i, t_j)$ in terms of η^i while successive iterations are evaluated at the mean value of the parameters. This means that during a step in the search for the best parameter combination, all subjects have the same parameter values. This so-called *'first-order method'*, introduced by Beal and Sheiner (1982), is used to obtain the first two moments of the pdf $\pi_p(\mathbf{p})$.

$$\mathbf{y}_j^i = \mathbf{y}_m^i(p_0, \mathbf{t}_j) + \frac{\partial \mathbf{y}_m^i(p_0, \mathbf{t}_j)}{\partial \eta^i} \cdot \eta^i + \xi_j^i + \varepsilon_j^i$$

Note that $\mathbf{g}_j^i(\mathbf{p}_0)$ is the vector of dimension n_P of the partial derivative of the model $y_m^i(\mathbf{p}_o, t_j)$ which is compared to η^i and $G^i(p_0) = \left\{ g_1^i(p_0),\ldots, g_{n_t}^i(p_0) \right\}$, matrix with dimensions n_p x n_t that binds these vectors together. ξ_j^i represents the error of the first-order approximation. If the first order approximation is adequate for individual i, $\xi_j^i = 0$, and the preceding expression becomes

$$y^i = y_m^i(p_0, \mathbf{t}) + G^{i^T}(p_0) \cdot \eta^i + \varepsilon^i$$

which is equal to

$$\mathbf{E}(y^i) = y_m^i(p_0, \mathbf{t}) + G^{i^T}(p_0) \cdot \mathbf{E}(\eta^i) + \mathbf{E}(\varepsilon^i) = y_m^i(p_0, \mathbf{t})$$

by definition $E(\eta^i) = 0$, hence the assumption becomes $\mathbf{E}(\varepsilon^i) = 0$. Therefore, we can obtain the estimation of $\mathbf{E}(\mathbf{y}^i)$ as a function of mean population parameters $\mathbf{p}_0$. The same approximation would be carried out to obtain the covariance matrix Ψ^i in the space of observations as a function of Ω and σ^2. Since the random-effects are assumed to be independent, we can write

$$\Psi^i = \mathbf{Var}(y^i) = \mathbf{Var}\left[y^i - y^i_m(p_0, \mathbf{t})\right] = \mathbf{Var}\left[G^{i^T}(p_0)\cdot\eta^i\right] + \mathbf{Var}\left[\varepsilon^i\right]$$

with the variance of the interindividual random-effects as

$$\mathrm{Var}\left[\mathbf{G}^{i^T}(\mathbf{p}_0)\eta^i\right] = \mathrm{E}\left[\mathbf{G}^{i^T}(\mathbf{p}_0)\eta^i\eta^{i^T}\mathbf{G}^i(\mathbf{p}_0)\right] = \mathbf{G}^{i^T}(\mathbf{p}_0)\cdot\Omega\cdot\mathbf{G}^i(\mathbf{p}_0)$$

and the variance of the residual error as

$$\mathbf{Var}(\varepsilon^i) = I_{n_t}\sigma^2$$

Consequently

$$\Psi^i = \mathbf{G}^{i^T}(\mathbf{p}_0)\cdot\Omega\cdot\mathbf{G}^i(\mathbf{p}_0) + I_{n_t}\sigma^2$$

Ω and $I_{n_t}\sigma^2$ represent the random-effects and I_{n_t} is the identity matrix by the order n_t. The equations of $\mathbf{E}(\mathbf{y}^i)$ and Ψ^i are implemented in the least-squares criterion to obtain a global minimization criterion to estimate $\mathbf{p_0}$ and Ω.

Additionally, explanatory covariates, $\mathbf{q}$, are sought to explain part of the inter-individual variability (discrepancy between individuals) of the parameters. In other words, we can explain the parameters in terms of the covariates, and hence $\mathbf{p_0}$ becomes a function of $\mathbf{q}$. These relations are estimated by simple- or multiple-regression models.

This approximation is called *first-order estimation method.* This method of approximation works well if subjects provide only little information. There are other methods for obtaining the population characteristics as well. For example, a more accurate approximation to equation $y_m^i(\mathbf{p}_0 + \eta^i, t_j)$ is obtained if parameter values are calculated for each individual during each step of the parameter search. This method that was investigated by Lindstrom and Bates (1990) is called the *'first-order conditional estimation'* method. However, this method is quite complicated and it is beyond the scope of this thesis.

This method of population estimation are implemented in a software package called NONMEM (*Nonlinear Mixed-Effects Modeling*). This software was initially developed to investigate the kinetic behavior of drugs in the field of population analysis, where only small amounts of routine clinical data were available [Sheiner LB and Ludden TM 1992].

In the following section after a brief outline of NONMEM package, we will try to present the way that population modeling is being implemented in NONMEM through an example.

NONMEM

The core of the NONMEM program is a set of subroutines written in the ANSI FORTRAN programming language, which are linked with the model-defining subroutine to produce the NONMEM executable file. Because of this architecture, NONMEM can run on any platform supporting a FORTRAN compiler. Basically, it is designed to fit general statistical (nonlinear) regression-type models to data. This package was developed by the NONMEM Project Group at the university of California at San Francisco for analyzing population pharmacokinetic data in particular. NONMEM is equipped with a module for model and data definition called NM-TRAN and comes with an extensive library of model-defining subroutines for pharmacokinetic

applications called PREDPP. Models may be modified, and models not included in the library can be defined by the user. Figure 2.2 shows the relationship between NONMEM, PREDPP, and NM-TRAN.

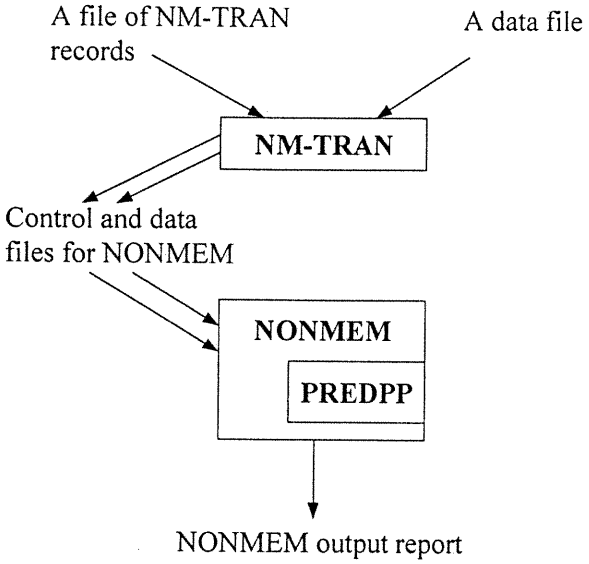


Figure 2.2 – NONMEM, PREDPP, and NM-TRAN.
A user-written PRED subroutine could be included instead of PREDPP
(Sheiner LB, Beal SL. *NONMEN Users Guides-Part V.* 1994, p 2)

Population Model in NONMEM

Proper modeling of population data involves accounting for both unexplainable inter- and intra-individual effects (random effects), as well as measured concomitant effects (fixed effects). NONMEM allows this mixed effect modeling. These models describe observations from a number of individuals sampled from the population. If the data come from **N** numbers of individuals **i**, then the general mixed effects model for y_j^i in a one-compartment model can be given, for the $\mathbf{j}^{th}$ observation from the $\mathbf{i}^{th}$ individual, as

$$\mathbf{y}_j^i = \mathbf{y}_m(\mathbf{t}_j^i, \mathbf{p}_i) + \varepsilon_j^i$$

This equation is now part of a population model because it is explicitly recognized, through the superscript, **i**, that the data come from distinct individuals. Also, the random effects in the residual errors are denoted by ε with individual variances of σ^2. When dealing with population data, the symbol η is reserved for random effects influencing the vector $\mathbf{p}_i$. A general model, then, for $\mathbf{p}_i$ can be written

$$\mathbf{p}_i = f(\mathbf{q}_i, \theta) + \eta_i$$

It is called the **parameter model**. Here, vector-valued function ***f*** is a structural (though non-kinetic) type model, which is a function of covariates, **q**, and a vector of population (fixed effects and possibly random effects) parameters, θ. The fixed effects function **q** is used for the particular fixed effects (covariates) in ***f***, such as the individual's height, weight, and so forth.

The simplest form that ***f*(.)** can take, and the most common, is one that is linear in θ. In this case all elements of θ appear as linear coefficients of terms involving data items. The data items can appear nonlinearly, without affecting the linearity with respect to θ.

Population Random Effect Models

There are two levels of random effects in NONMEM called η and ε, first and second level random effects, respectively. With data from a single individual, only first-level random effects are needed. However, with data from a population of individuals, both first- and second-level random effects are considered. First-level effects are used in the parameter model to help model unexplainable *interindividual* differences in **p**, and second-level effects are needed in the *intraindividual* error model.

Models for Interindividual Errors

The scalar difference between $\mathbf{p}_i$ and $\mathbf{g}(\mathbf{q},\theta)$ is called *interindividual error*. It arises from a few sources: the function **g** may be only approximate, and/or **q** may be measured with error. It is regarded as a random quantity, and it may be modeled in terms of η variables. This random effect satisfies the property that there exists a number ω, such that each value of the random effect arises in accord with a probability distribution whose mean and variance are zero and ω^2, respectively. This variance describes biological population variability.

The scalar difference between $\mathbf{y}_j^i$ and $\mathbf{y}_m(\mathbf{t}_j^i,\mathbf{p}_i)$ is called an *intraindividual* error. It is modeled in terms of ε variables in population data. Each ε variable is assumed to have zero mean and a variance denoted as σ^2. Both variances may be estimated by NONMEM. Each pair of elements in η has a covariance, and NONMEM is also able to estimate it, although often it is assumed that the covariance is zero. A covariance between two elements of η, η_k and η_m, is a measure of statistical association between these two random variables. Their covariance is related to their correlation, ρ_{km} ($\rho_{km} \equiv \rho_{mk}$) by

$$\mathrm{cov}(\eta_k,\eta_m) = \rho_{km}\omega_k\omega_m$$

The variances and covariances among the elements of η are laid out in a *variance-covariance matrix*, called Ω. The variance-covariance matrix of η, Ω, is the matrix whose dimension coincides with the length of η, whose diagonal terms are the variances of the elements η, and whose off-diagonal terms give the pairwise covariances between these elements. This (symmetrical) matrix then is just an array in which these parameters (i.e., all random interindividual effects), describing the variability and covariability of the elements of η, are organized. If η has, for example, 3 elements, Ω has the following form

$$\Omega = \begin{bmatrix} \omega_{11} & \omega_{12} & \omega_{13} \\ \omega_{21} & \omega_{22} & \omega_{23} \\ \omega_{31} & \omega_{32} & \omega_{33} \end{bmatrix}$$

Here, ω_{kk} is another way of writing the variance ω_k^2, and ω_{km} ($k \neq m$) is the covariance between η_k and η_m. The elements ω_{11}, ω_{22}, ω_{33} are called the *diagonal elements* of the matrix of precision. If the nondiagonal elements (covariances) are all zero, i.e. the correlation among all pairs of η elements is zero, the matrix is called a diagonal matrix.

Similarly, there is defined a single variance-covariance matrix, Σ, that includes all the variance and covariance parameters of all the random intraindividual effects.

Example of A Population Mixed Effects Model

All of the parts needed to fully define a population model have already been presented. It may be useful to recall this information by stating the entire general model:

$$\mathbf{y}_j^i = \mathbf{y}_m(\mathbf{t}_j^i, \mathbf{p}_i) + \mathbf{H}'(\mathbf{t}_j^i, \mathbf{p}_i)\varepsilon_j^i$$

$$\mathbf{p}_i = f(\mathbf{q}, \theta) + \eta_i$$

$$\text{cov}(\varepsilon_j^i) = \Sigma \ ; \ \text{cov}(\eta_i) = \Omega$$

$$\varepsilon_j^i, \varepsilon_{kl} \text{ independent for } (\mathbf{i}, \mathbf{j}) \neq (\mathbf{k}, \mathbf{l})$$

$$\eta_i \ , \ \eta_j \text{ independent for } \mathbf{i} \neq \mathbf{k}$$

$$\varepsilon_j^i \ , \ \eta_k \text{ independent for all } \mathbf{i}, \mathbf{j}, \mathbf{k}$$

where $\mathbf{H}$ is a vector valued function of $\mathbf{t}_j^i$ and parameters $\mathbf{p}$. ε_j^i is a vector, along with $\mathbf{t}_j^i$, $\mathbf{p}_i$, θ; and η_i, Σ and Ω are sequence matrices with dimensions equal to these of ε_j^i

and η_i. To try to represent the relationship between all the fixed and random effects of a population model graphically, consider figure below (Fig.2.3). Considering the relation of $\mathbf{k_i} = \frac{\mathbf{CL_i}}{\mathbf{V_i}}$, the model corresponding to this is:

$$\mathbf{y_j^i} = \frac{\mathbf{D}}{\mathbf{V_i}} \exp[-(\frac{\mathbf{CL_i}}{\mathbf{V_i}})\mathbf{t_j^i}] + \varepsilon_j^i$$

$$\mathbf{CL_i} = \theta_1 + \theta_2 \mathbf{q_i} + \eta_i^{CL}$$

$$V_i = V$$

$$\text{var}(\varepsilon_j^i) = \sigma^2 \quad ; \quad \text{var}(\eta_i^{CL}) = \omega_{CL}^2$$

Where the $\mathbf{V_i}$ are all equal to a constant **V**, i.e. there is no random interindividual variability in the volume of distribution, so that η_i is just a scalar.

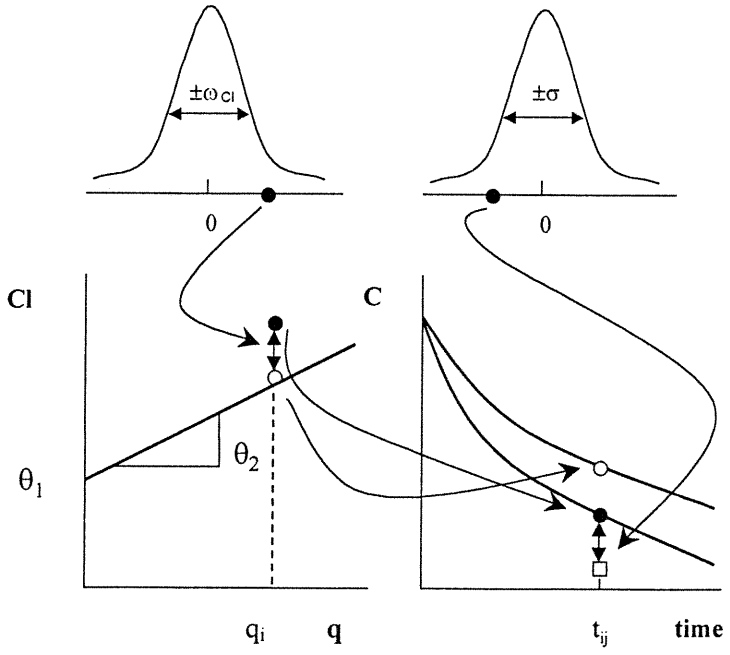


Figure 2.3 – The relationship between all the fixed- and random-effects of a population model (Sheiner LB, Beal SL. *NONMEN Users Guides-Part V.* 1994, p 40).

Application 3

Background

Cyclosporine A (CsA) is a potent immunosuppressive agent, widely used to prevent graft rejection of transplanted organs and to treat some autoimmune diseases [Kahan BD, 1989]. This drug is a lipophilic cyclic oligopeptide (Fig 3.1) which is derived from extracts of Tolypocladium Inflatium Gams, a member of the fungi Imperfekti family [Canafax DM, Ascher NL, 1983].

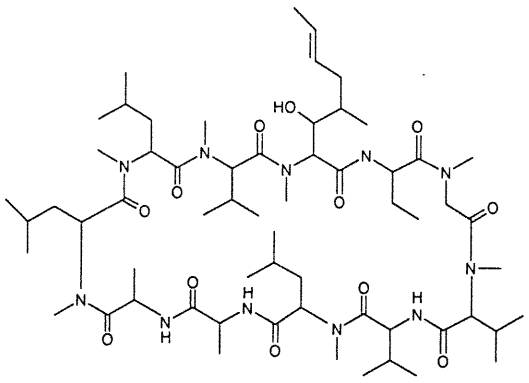


Figure 3.1 – Cyclosporine A.

The pharmacokinetic behavior of CsA varies among different patients with organ transplants and healthy volunteers [Lemaire M, Fahr A, 1990], and estimates of pharmacokinetic parameter values differ depending on the analytical method used [Karlsson MO et al., 1990]. Because many pharmacodynamic methods to monitor cyclosporine therapy have failed to fulfill clinical criteria of acceptability [Awni WM,

1992], information concerning the behavior of cyclosporine has been based on blood level measurements. However, CsA pharmacokinetics are typified by large variations in blood level after oral or intravenous administration [Ptachcinski RJ, 1986; Shaw LM, 1987] not only between patients but also within patients especially after oral dosing [Lindholm A, 1988]. This variability appears to be particularly evident in patients administered oral CsA in the first few weeks after transplantation [Kahan BD, 1984]. If blood concentrations of CsA are too low graft rejection usually ensues, whereas nephrotoxicity or hepatotoxicity are associated with high blood CsA concentrations (Shaw LM, 1987; Kahan BD, 1984; Lindholm A, 1990]. For these reasons—CsA narrow therapeutic index, it has been recommended that dosing schedules should be guided by routine pharmacokinetic monitoring for individualized therapy [Kahan BD, 1990].

Introduction

Bone marrow transplantation (BMT) provides a valuable therapeutic modality for several malignant and nonmalignant disorders [Storb R, 1983; O'Reilly RJ, 1983]. However, as patients begin to engraft, they are at risk of developing acute graft-versus-host disease (GVHD), caused by mature T lymphocytes reinfused with donor marrow. In turn, the risk of infection is increased as a result of prolonged immunosuppression required for GVHD treatment. A major complication of allogeneic BMT, acute to chronic GVHD, occurs in 30-50% of patients even after marrow grafts from HLA-identical sibling donors and more frequently in mismatched- or unrelated-donor transplants [Storb R, 1983; O'Reilly RJ, 1983; Ramsay NKC, 1982], and it continues to be the most common cause of morbidity and mortality following allogeneic BMT despite prophylactic immunosuppression after transplantation. Initially used by Powles [Powles, 1978], cyclosporine A (CsA) has been used as the main immunosuppressive treatment to prevent GVHD after BMT for decades. Moreover, prophylactic immunosuppressive therapy with CsA alone, CsA and methotrexate [O'Reilly RJ,

1983], and a combination of CsA and other drugs has been investigated in different studies and led to a significant reduction in the incidence of GVHD with improved survival rates. Given as GVHD prophylaxis, the administration of CsA is usually started intravenously before BMT in doses ranging from 1.5-5 $mg.kg^{-1}$ daily and is continued until patients tolerate oral administration after recovery from treatment-related gastrointestinal toxicity. Oral CsA is then given in the six following months (12 months in some cases) [Serre-Debeauvais F, Iliadis A et al., 1990].

Problem

Although CsA has been widely used in a clinical setting for decades, the application of CsA in the treatment of BMT patients is not optimal. The pharmacokinetic behavior of CsA varies among different patients with BMT and its kinetic profile is characterized by great variations in blood level concentrations. Identical CsA doses, approximately 4 $mg.kg^{-1}$, gave rise to considerable differences in CsA blood levels among patients during the course of study. This variability is even more pronounced in BMT candidates during the IV infusion period (the first two hours of the kinetic profiles). As pharmacokinetic profile of CsA exhibits wide inter- and intra-individual variability, it is hence essential to permanently maintain the CsA blood concentrations within a desired therapeutic window in order to preserve an optimal relationship between tolerance and therapeutic efficacy of this drug. Low blood CsA concentrations ensues acute rejection episodes, whereas nephrotoxicity, hepatotoxicity, and infections are associated with high blood CsA concentrations, increasing undesirable risk events.

Several approaches are useful in order to overcome these potential complicating factors, one of which is to optimize the CsA dosage regimen to reach a target blood CsA concentration. In doing so, it is crucial to choose the **best kinetic model**, which provides a good description of the pharmacokinetic behavior of CsA. A good description of the kinetic process is essential for proper account of the variability.

Hence, it has to be decided which compartmental model is best suited to minimize the discrepancy between the data and our mathematical predictions and reliably describe the time-concentration profile that yield accurate PK parameters to optimize the CsA dosage regimen as CsA pharmacokinetics have been documented as one- [Anderson JE, 1994], two- [Karlsson MO, 1990], and three-compartment models [Serre-Debeauvais F, Iliadis A, 1990; Karlsson MO, Lindberg-Freijs A, 1990], and even a physiological pharmacokinetic model including 14 tissue and two blood compartments [Bernareggi B, Rowland M, 1991].

Objective

The objectives of the present study were designed to:

- estimate cyclosporine population pharmacokinetic parameters, using standard two-stage (STS) and nonlinear mixed-effects modeling (NONMEM), and quantify the interindividual variability found with such experimental (rich) data;
- compare mean PK parameter estimates and interindividual variability obtained by the STS and NONMEM methods, and to determine which method is more appropriate for this data rich situation.

Preminary Observation

Pharmacokinetic analysis, in the current study, is based on observations in a peculiarity of the kinetic process of cyclosporine A (Sandimune®). By plotting the logarithm of drug concentration-time profile of CsA, the linearity attribute common in kinetic processes could be observed with time elapse (Figure 3.2). Using pharmacokinetic compartmental modeling, the most suitable seems to be a three-compartment model, however, the ambiguity still remains considering the third phase of

elimination. This phase seems to be redundant and hence inaccurate due to the CsA clinical data (i.e., not enough points available for accurate mathematical modeling of the 3rd phase of elimination). Accordingly, we proposed the use of a two-compartment open model in order to describe the kinetic profiles of blood CsA concentrations in our attempt to obtain mean parameter estimates using STS. In addition, data analysis should also be implemented using pharmacokinetic one-stage approach which allow us to describe the kinetic variability directly from the observational data. Hence, it can provide an acceptable method of comparison.

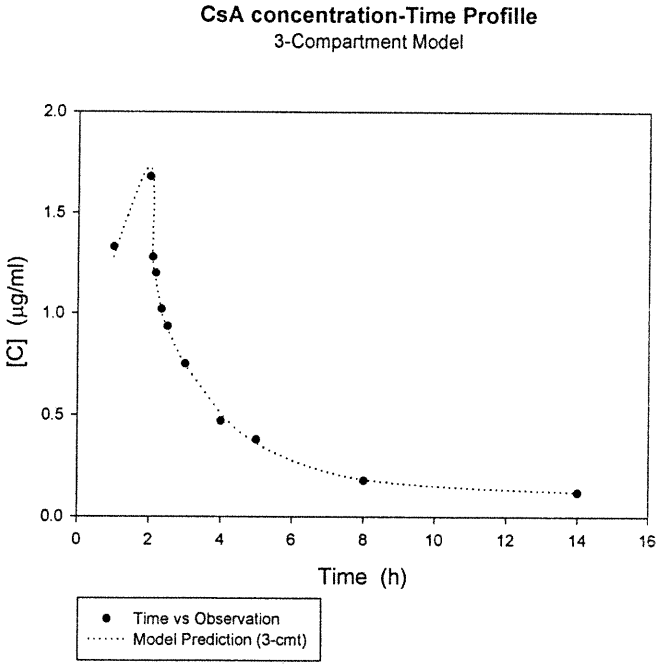


Figure 3.2a – CsA concentration-time profile (*Linear Representations*). Example of linear representation of kinetic profile of CsA (Sandimune). The observations (•) are superposed by a 3-cmt model.

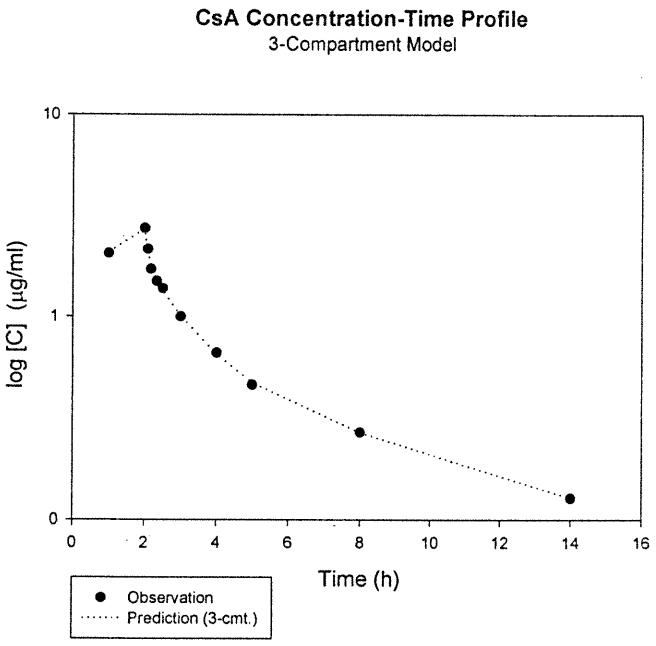


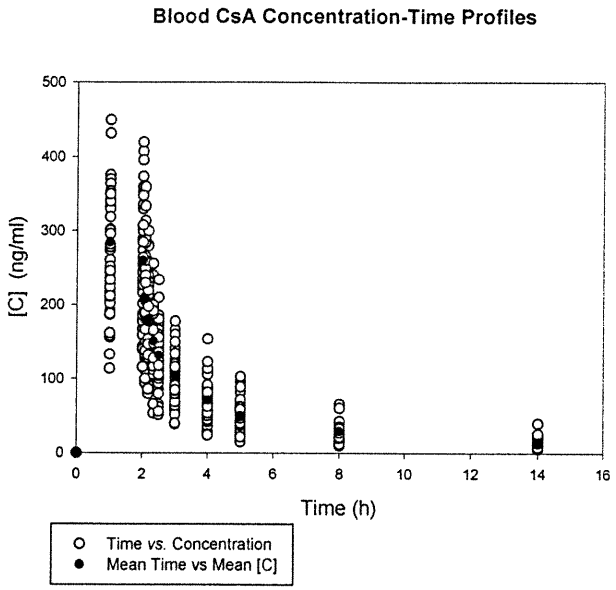
Figure 3.2b – CsA concentration-time profile (*Semilogarithmic Representations*). Example of semilogarithmic representation of kinetic profile of CsA (Sandimune). The observations (•) are superposed by a 3-cmt model.

Study Design

Data

CsA blood concentrations from 534 samples were measured after an intravenous (IV) single dose in 52 adult candidates for BMT aged 17-47 years [Serre-Debeauvais F, Iliadis A, 1990] (Appendix 1). Each patient received a two-hour intravenous (IV) infusion of CsA (4 $mg.kg^{-1}$) with constant infusion rate, 15 days before allogenic bone marrow transplant. Blood samples were collected just before the end of infusion and then at 5-, 10-, 20-, 30 minutes and 1, 2, 3, 6, and 12 hr after the end of infusion. The concentration levels were analyzed by HPLC analytical method [Serre-Debeauvais F, 1988]. Ten time-concentration pairs are available for each patient; and the observation sample included all 52 candidates, shown in Fig. 3.3, is considered for estimation of

mean population pharmacokinetic parameters by the maximum likelihood estimation using STS and NONMEM methods.



Mean concentrations (•) are shown with time elapse.

Figure 3.3 – Kinetic profile of CsA in 52 candidates before BMT.

Method

Two following methods were used to select a compartmental model which can well describe the kinetic profile of CsA and obtain its population characteristics in target patients:

1. Standard Two-Stage (STS):

Given the large number of blood samples drawn from each individual (average of 10 samples per candidate), we evaluated population characteristics by the STS

method. In the first stage, the blood CsA concentration-time profile in each of 52 pretransplant candidates of BMT taking the first course of CsA treatment was fitted by one-, two-, and three-compartment open models (Fig. 3.4) to obtain relevant individual pharmacokinetic parameter estimates. From the best model selected, population parameters (mean and interindividual variability) were estimated.

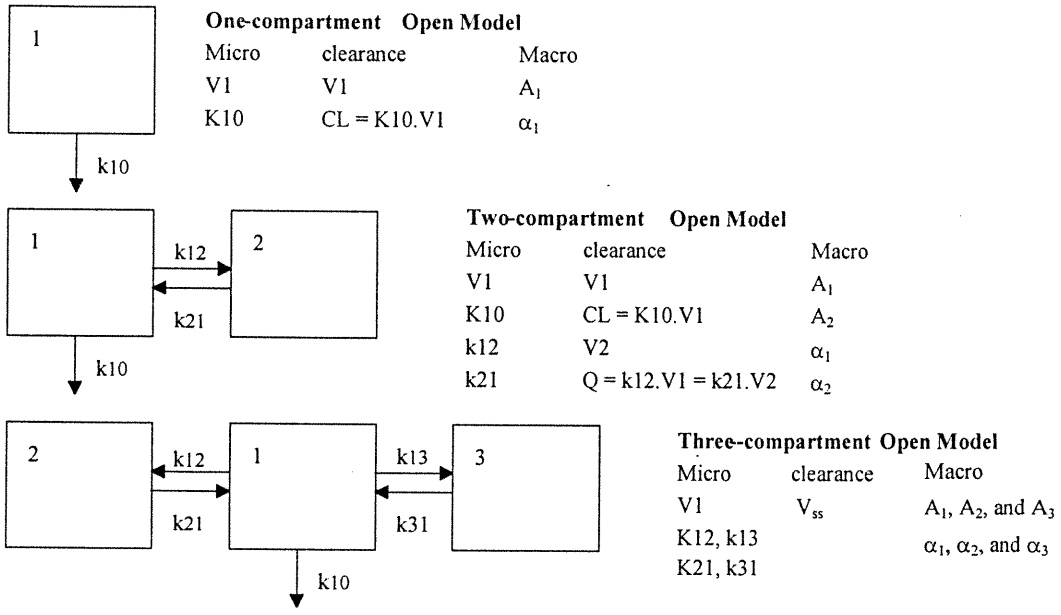


Figure 3.4 – Mammillary compartmental pharmacokinetic models.

The predictions of blood CsA concentrations by one-, two-, and three-compartment open models were evaluated for all individuals. All models and techniques were programmed within MATLAB program [The Math Works, Inc. 1999]; fitting and parameter estimation were carried out using a Quasi-Guass-Newton algorithm maximizing the likelihood function.

The experimental data were fitted to the equations of typical compartmental models following an infusion administration [Gibaldi, 1982], using MATLAB, run on an IBM-compatible Pentium® II under Microsoft Windows 95:

$$\mathbf{y_i = \frac{D}{T}\sum_{i=1}^{N}\frac{A_i}{\alpha_i}\left(1 - e^{-\alpha_i t}\right) \qquad for \quad t \le T}$$

where **N** is the number of compartment(s), **t** is the time after the start of the infusion, and **D** and **T** are the dose and the duration of infusion, respectively. Macroconstants $\mathbf{A_i}$ and α_i are the intercept and the slope for the i_{th} exponential term, respectively.

After the infusion stops (after time **T**), the decline in concentration is described by

$$\mathbf{y_i = \frac{D}{T}\sum_{i=1}^{N}\frac{A_i}{\alpha_i}\left(e^{-\alpha_i T} - 1\right).e^{-\alpha_i t} \qquad for \quad t > T}$$

As different initial parameters (guess values) may yield different parameter estimates, a duplicate analysis was always performed to rule out any potential bias in all models. This confirms that the convergence leads to a true minimum.

In our analysis, while the Akaike's information criterion (AIC) favored the three-compartment open model to describe CsA concentration-time profiles, the predicted concentrations of CsA showed that both two- and three-compartment models were better fitted than the one-compartment (Fig. 3.5). Although the three-compartment model has a slightly superior fit to that of the two-compartment model, its use (3-cmt) was rejected by the test of redundancy. The test of redundancy was performed using the relative covariance-correlation matrix of estimates and percent variation of the parameters. In evaluation of covariance-correlation matrix, any high correlation can be a sign of the redundancy of parameters. The three-compartment open model has many individuals with redundant parameters, describing similar elimination phases (see Appendix 2 for some examples).

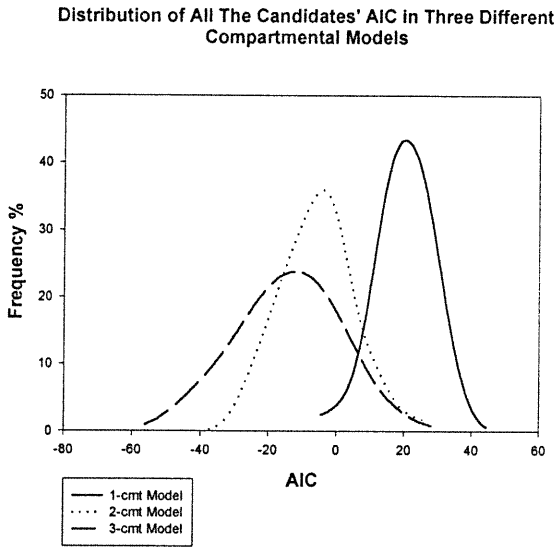


Figure 3.5 – Smooth histogram of all 52 pretransplant candidates' AIC for 3 different Models.

Although the AIC values in the three-compartment models are smaller, they have wider distribution (greater variation) and are shifted. In contrast, AIC values in the two-compartment model are evenly spread following normal Gaussian distribution with smaller variation.

Furthermore, the percent standard error of mean (%SE) for each parameter estimate in different compartmental models were calculated, which can be used for analyzing the precision of the parameter estimations. The precision at which the parameters were estimated is higher for the two- and three-compartment models, however, its distribution in the two-compartment model has smaller variance. Figure 3.6 represents the percent standard error of mean for parameter estimates in the three different models, which favored the use of the two-compartment model.

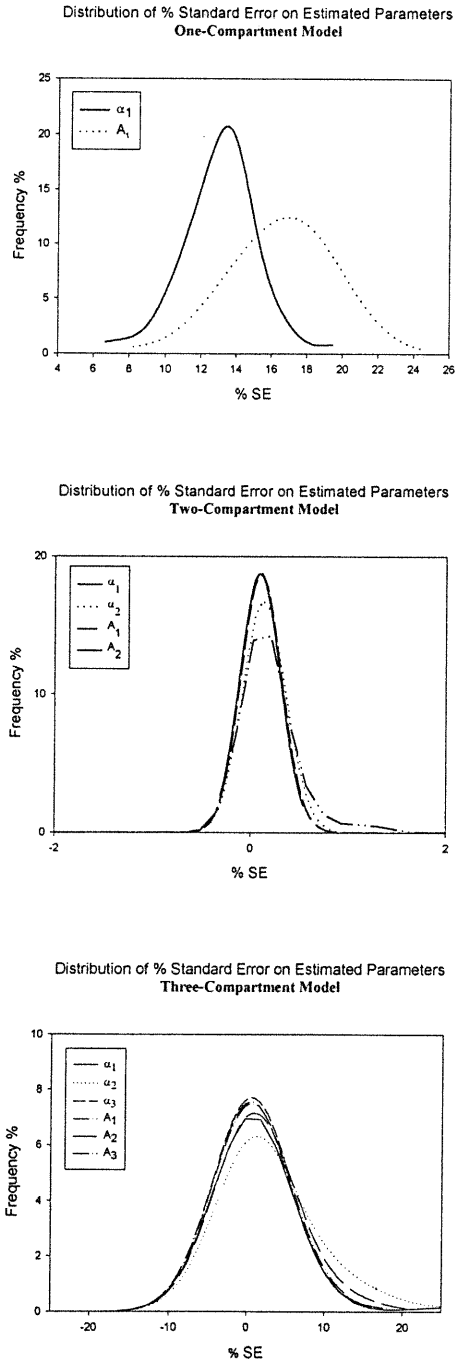


Figure 3.6 – Distribution of standard error of mean on estimated parameters for all candidates. Please **NOTE** that the extension of density plots beyond zero in the negative direction is a consequence of the smoothing function γ used in producing the plot: no precision value was negative.

The distribution of **%SE** of one-, two-, and three-compartment models using maximum likelihood estimation are shown. The range of distribution of **%SE** of the one-compartment model is wider than that of the two- and three-compartment models. It has also shown that the range of distribution for the two-compartment model is smaller. In addition, according to the ratio of frequency of negative **% SE** versus frequency of positive **% SE**, the variations of the two-compartment model are the most symmetrical, and the variations of the three-compartment model are less symmetrical as there are a larger number of over-estimated parameters in the analysis.

Consequently, as blood concentrations of CsA appeared to decline in a biphasic manner, the population analysis in the second stage was built on the assumption that CsA kinetic follows a linear two-compartment open model.

In the second stage, the mean population parameters and the covariance matrix were computed by combining the corresponding individual estimates of the two-compartment model. The computed mean population parameters and covariance matrix describe the fixed-effects and interindividual variability (random-effects), respectively. Population characteristics, i.e., mean parameter values and covariance matrix of macroconstant $\mathbf{A}_i$ and α_i, estimated by the STS method can be found in Appendix 3. In order to compare the result from the STS to that of the NONMEM, clearances and volumes were calculated from the macroconstants. This would in turn allow comparison with published results regarding CsA pharmacokinetics.

In a two-compartment open model (as shown in Fig. 3.7), the disposition kinetic parameters (microconstants) for disappearance of CsA from the central compartment are given by

$$\mathbf{k}_e = \frac{\alpha_1 + \alpha_2}{\mathbf{k}_{21}} \quad \text{and} \quad \mathbf{k}_{12} = \alpha_1 + \alpha_2 - \mathbf{k}_{21} - \mathbf{k}_e$$

where k_{21}, the constant rate of CsA disposition from the peripheral (second) compartment toward the central compartment is expressed as

$$\mathbf{k_{21}} = \frac{(\mathbf{A_1\alpha_2 + A_2\alpha_1})}{\mathbf{A_1 + A_2}}$$

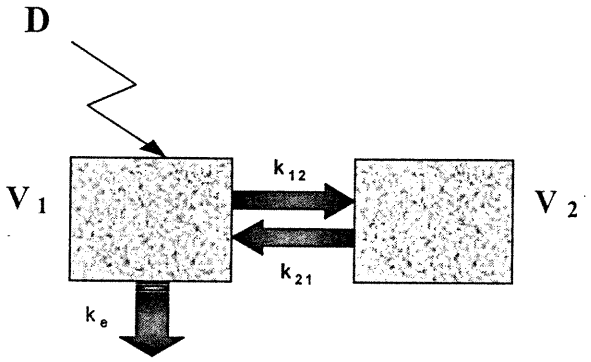


Figure 3.7 – A 2-compartment open model with elimination from the central compartment.

The actual parameters then calculated were clearance (**CL**), volume of distribution at central compartment ($\mathbf{V_1}$), intercompartmental clearance (**Q**), and volume of distribution at steady state ($\mathbf{V_{ss}=V_1+V_2}$). These parameters can be feasibly calculated as

$$\mathbf{V_1} = \frac{1}{\mathbf{A_1 + A_2}} \; ;$$

$$\mathbf{CL} = \mathbf{V_1 k_e} \; ;$$

$$\mathbf{V_2} = \mathbf{V_1}\frac{\mathbf{k_{12}}}{\mathbf{k_{21}}} \; ;$$

and $\mathbf{Q} = \mathbf{V_1 k_{12}} = \mathbf{V_2 k_{21}}$.

2. Nonlinear Mixed-Effects Modeling (NONMEM)

As previously implemented in STS method, the blood CsA concentration-time profiles of all pretransplant candidates of BMT taking the first course of CsA treatment were fitted by one-, two-, and three-compartment open models in order to determine the basic model that best described the data.

In the present study, maximum likelihood estimation known as first-order approximation (**FO**) was used for the nonlinear mixed-effects modeling and model discrimination was performed using the minimum value of the objective function among different models, which is estimated as twice the negative log-likelihood of the data. Using likelihood ratio theory, it can be shown that the difference between the minimum value of the objective function for two models follow a chi-square distribution with degrees of freedom equal to the difference in the number of parameters.

The population analysis was performed by the use of nonlinear mixed-effects modeling software package NONMEM (NONMEM Project Group, University of California, San Francisco). An IBM-compatible personal computer (Pentium® II) running under Microsoft Windows 95 was used with the Microsoft FORTRAN PowerStation 4.0 compiler and NONMEM version **V**, level 1.1 (double precision).

A one-compartment linear model with first-order elimination was fit to the population data using the **ADVAN1** and **TRANS2** subroutines to reparametrize the model. The mean pharmacokinetic parameters of clearance (**CL**) and volume of distribution (**V**) corresponding to the proposed model were determined. Also, population data were analyzed using a two-compartment linear model. The **ADVAN3** and **TRANS3** subroutines were selected to reparametrize the model in terms of clearance (**CL**), central volume ($\mathbf{V_1}$), intercompartmental clearance (**Q**), and volume of distribution at steady state ($\mathbf{V_{ss}}$). Fitting the three-compartment model to the data set was not successful because of the mis-specification of the third compartment (overparametrization). Furthermore, we were not able to assign interindividual variability (η) to all parameters, particularly on parameters describing the third phase of

elimination. Apparently, there was not enough kinetic points to describe the third phase of elimination in CsA kinetic profiles. However, failure of fitting the three-compartment model might probably due to the size of the population (52 candidates of BMT). The population is probably too small to allocate interindividual variability on the parameters describing the third phase of elimination.

Eventually, the two-compartment open model was selected after standard verification of its adequacy using two statistical parameters: AIC value and the coefficient of variation (**CV%**) of the parameter mean estimates (Appendix 4). The pharmacokinetic analysis was conducted using the classical two-compartment open model with input and elimination from the central compartment, which confirms the use of this model by the STS method. The following PK parameters were derived for population data: **V**, **CL**, **Q**, and $\mathbf{V_{ss}}$. As different initial parameters (guess values) may yield different parameter estimates, a duplicate analysis (i.e., different run with new initial values) was always performed to rule out any potential bias.

Statistical Model

We implemented statistical models that permitted the determination of interindividual variance of the pharmacokinetic parameters of the model and the intraindividual (residual) variance. Having yielded better result in terms of the **MOF** (Minimum Objective Function) value, exponential interindividual variability was modeled on all structural parameters as follows

$$\theta_i = \theta_\circ \times \exp(\eta_i)$$

where the operand, '$\times$', denotes element-wise multiplication of the vectors $\theta_\circ$ and $\exp(\eta_i)$; $\theta_\circ$ denotes the vector of population mean parameters, and η_i denotes a vector of interindividual random effects which are multivariate normal with a zero mean $\mathbf{E}(\eta_i) = 0$ and variance of Ω ($\mathbf{V}(\eta_i) = \Omega = \omega^2$). Initial modeling using a full covariance matrix (Ω) for the interindividual random effects proved difficult to

estimate due to over-parametrization. Subsequent modeling led to a reduced, blocked diagonal form for Ω. The block diagonal covariance matrix assumes that the random effects within each block are correlated.

Intraindividual variability representing deviations among pairs of observed responses (i.e., blood CsA concentrations) and those predicted by the population model, was also screened in conjunction with the base pharmacokinetic model. Smaller standard errors associated with parameter estimation and lower OF values were obtained when the proportional error model was used for modeling intraindividual (residual) variability, compared with the additive, exponential, or combined additive and proportional (i.e., slope/intercept) models. Using proportional error model can also be visually justified where the variation on CsA concentrations is greatly higher at the beginning of profiles than at the end. This error model can be shown as

$$\mathbf{y}_j^i = \mathbf{y}_m \times \left(1 + \varepsilon_j^i\right)$$

in which $\mathbf{y}_j^i$ is the ith observed CsA concentration for the jth individual, $\mathbf{y}_m$ is the CsA concentration predicted by the pharmacokinetic model, and ε_j^i are randomly distributed term in which each term has zero mean and variances σ^2 ($\mathbf{V}\left(\varepsilon_j^i\right) = \sigma^2$). The variance component in NONMEM is also shown by Σ. Such errors are caused by influences such as assay variability, choice of an inappropriate pharmacokinetic model, and timing errors in drug administration (incompliance) and blood sampling.

Finally, we can summarize the population pharmacokinetic model as

2 levels of random effects

- Residual variability

$$\mathbf{y}_j^i = \frac{\mathbf{k}_0}{\mathbf{CL}_i}\left[1 - \mathbf{e}^{-\frac{\mathbf{CL}_i}{\mathbf{V}_i}.\mathbf{T}_j^i}\right].\mathbf{e}^{-\frac{\mathbf{CL}_i}{\mathbf{V}_i}.\mathbf{t}_j^i} + \varepsilon_j^i$$

- Interindividual variability

$$\mathbf{CL_i = TVCL \,.\, e^{\eta_{CL,\,i}}}$$

- Nested random effects

$$\mathbf{y_j^i = \frac{k_0}{TVCL \,.\, e^{\eta_{CL,\,i}}}\left[1 - e^{-\frac{TVCL.e^{\eta_{CL,\,i}}}{TVV.e^{\eta_{V,\,i}}}.T_j^i}\right].e^{-\frac{TVCL.e^{\eta_{CL,\,i}}}{TVV.e^{\eta_{V,\,i}}}.t_j^i}} + \varepsilon_j^i$$

where:

$\mathbf{k_0}$ is the zero-order infusion rate constant;

$$\eta \sim \mathbf{i.i.d.}\ \mathbf{N}\left(0, \omega^2\right);$$

$$\varepsilon \sim \mathbf{i.i.d.}\ \mathbf{N}\left(0, \sigma^2\right).$$

Results

Fitted by different compartmental models, CsA whole blood concentration-time profiles are well described by a linear two-compartment open model. This seems reasonable as most of the pharmacokinetic studies using CsA have been conducted using the two-compartment open model [Ptachcinski RJ, 1986; Mallet A, 1988; Fahr A, 1993; and Guang W, 1996]. The distribution of parameters among individuals of a population may be characterized in different ways, however for the purposes of this study, it suffices to characterize this distribution in terms of only its mean and percent coefficient of variation. The estimates of the population mean parameters and their relative percent coefficient of variation (CV%) obtained from both STS and NONMEM methods are given in Table 3.1.

Table 3.1 – *Estimated population characteristics of **CsA** (Sandimune®) in candidates before undergoing bone marrow transplantation (BMT).*

		NONMEM		STS	
		Mean	**%CV**	**Mean**	**%CV**
CL	(*L/hr*)	2.30E+01	37.82%	2.50E+01	27.43%
$\mathbf{V_1}$	(*L*)	1.74E+01	16.94%	2.19E+01	45.01%
Q	(*L/hr*)	3.25E+01	16.19%	3.06E+01	34.29%
$\mathbf{V_{ss}}$	(*L*)	9.08E+01	24.70%	9.97E+01	30.58%
$\mathbf{V_2}^*$	(*L*)	7.34E+01	18.20%	7.86E+01	32.01%
$\mathbf{t_{½\beta}}^*$	(*hr*)	4.1	25.11%	3.9	31.78%

* : derived parameters.
$t_{½\beta}$ is the terminal half life.

It may be seen from Table 3.1 that in terms of the parameter estimates, the STS and NONMEM methods are comparable and show a great agreement in the estimation process. The clearance (**CL**) and the volume of distribution at central compartment ($\mathbf{V_1}$) presented herein are greatly comparable to those previously reported for CsA using pharmacokinetic analysis.

The population mean model using NONMEM fit to the observed CsA blood concentrations is illustrated in Figure 3.8. As the blood concentrations have been

normalized by dose, the data from all the candidates can be plotted on the same graph. This dose normalization can be performed without loss of information since $\mathbf{y}_m(.)$ is a linear kinetic model and hence the expected blood concentrations are proportional to dose. From this plot, it can be observed that the population mean model fit generally goes through the middle of the data.

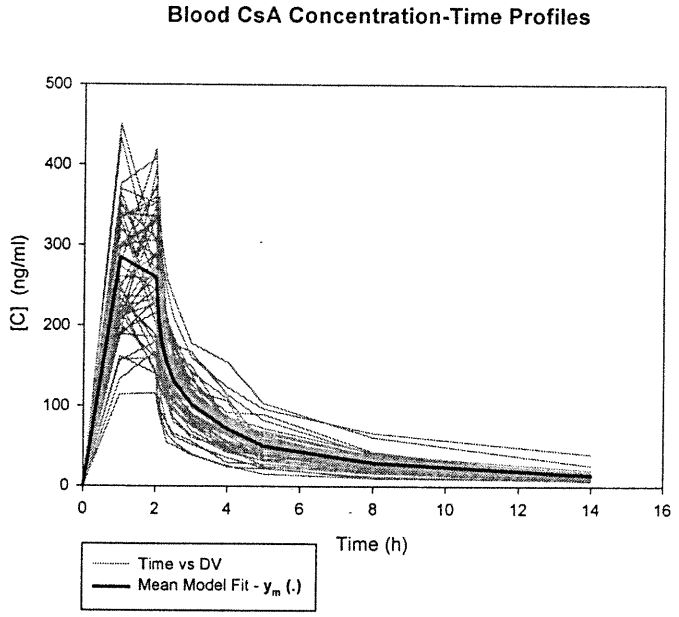
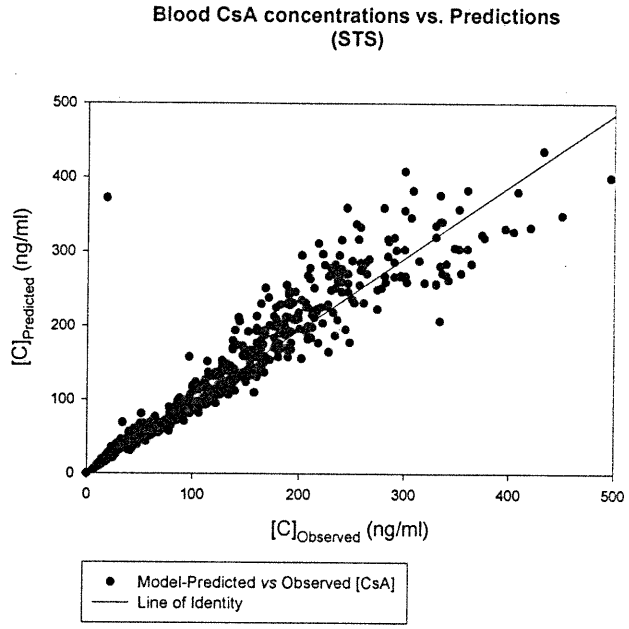


Figure 3.8 – The population mean model fit to the observed CsA blood concentrations (DV) using linear two-compartment open model with NONMEM.

Data regarding the assessment of the predictive performance of the model (goodness-of-fit) with both methods is contained in scatterplots of model-predicted versus observed CsA concentrations (Fig. 3.9) and weighted residual versus predicted concentrations (Fig. 3.10). Although most of the data points were distributed in an apparent random pattern around the line of identity (Fig. 3.9), there was some marked under-estimation from 300 to 400 ng/ml in both methods (higher variability in the first 2-hrs when the drug was being administered by infusion). It is also illustrated that the goodness-of-fit of the NONMEM method in our analysis is slightly better than STS.



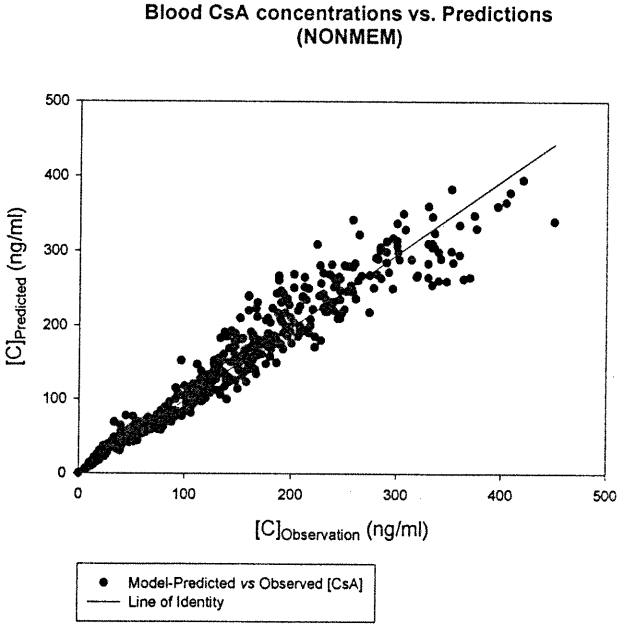
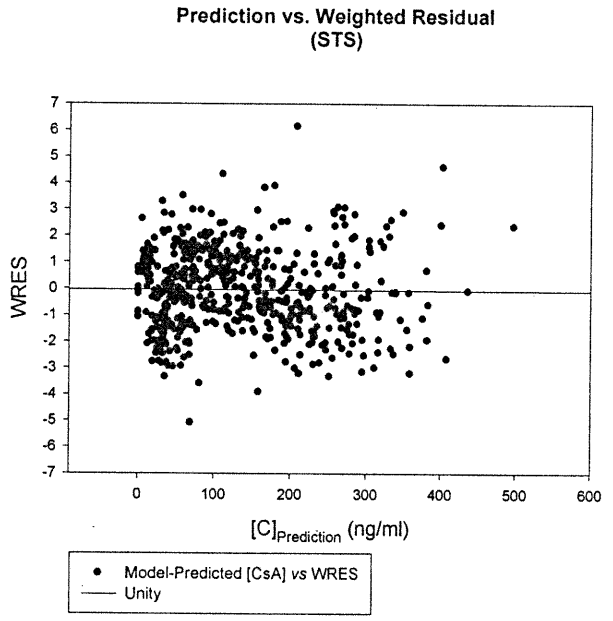


Figure 3.9 – Model-Predicted *vs.* Observed CsA concentration after population analysis using NONMEM.



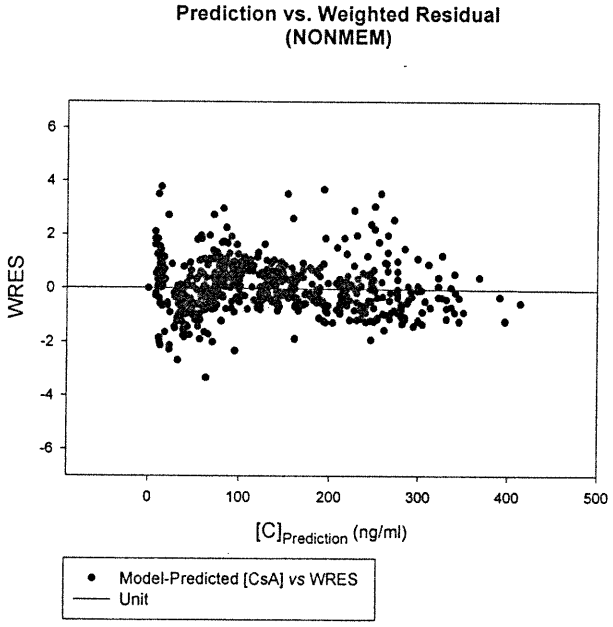


Figure 3.10 – Predictive performance of the 2-compartment open model after STS and NONMEM analysis.

As illustrated in Figure 3.10, residuals were randomly distributed and mostly lay within 2 Unit of the null ordinate in NONMEM results, which designates perfect agreement. However, wider distribution of weighted residual was seen in the STS method.

Another diagnostic scatterplot for the assessment of the goodness-of-fit of the final model is the plot of time versus weighted residual (Fig. 3.11). Most of the residuals were randomly distributed along the null ordinate. However, there was some marked under-estimation particularly at time 8 in NONMEM method and over-estimation at time 14 in the STS method (model mis-specification).

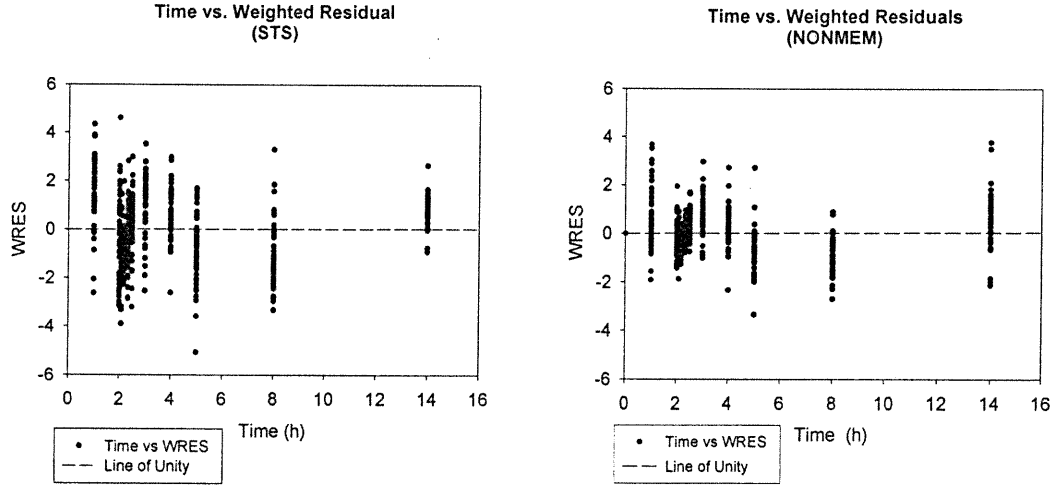


Figure 3.11 – Diagnostic plots after population analysis using STS and NONMEM. Goodness of fit (for different time-points). Model prediction using 2-cmt open model cannot fully describe the kinetic profile of CsA, particularly the last phase of elimination (WRES at time 8 and 14).

Examining the diagnostic scatterplots along with monitoring the changes in the value of objective function (**OF**) is one of the crucial steps in model selection process. Screening the value of **OF** could be potentially deceptive if it is being used as the sole

discriminator for choosing among population models without concurrent graphical analyses.

At the end of the analysis, individual first-order estimates of clearance (**CL**) conditional on the parameter values of the two-compartment model were calculated in NONMEM for all 52 candidates in the study. This provided a good comparison of the results obtained from NONMEM to that of STS method. It is noteworthy that the values of **CL** obtained from the STS method does not differ quantitatively from that of the results obtained from NONMEM. Figure 3.12 illustrates a good correlation between clearance obtained from both methods.

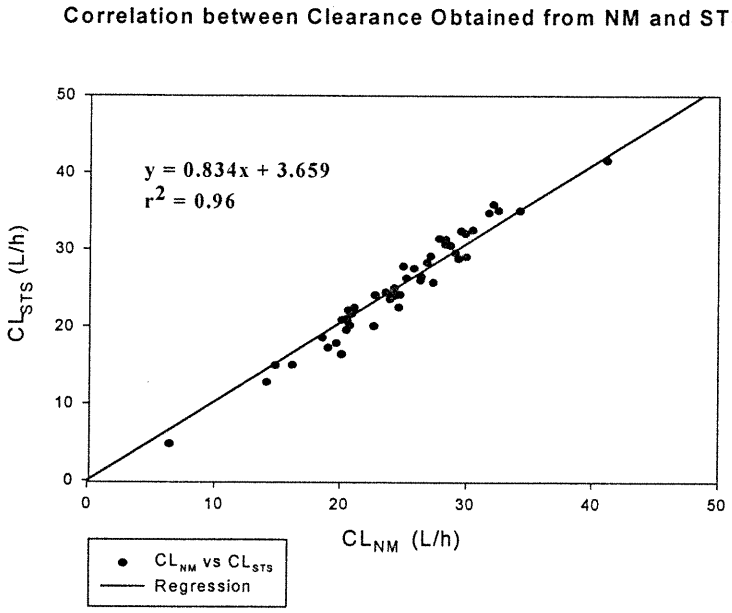


Figure 3.12 – High correlation between CL estimated by nonlinear mixed-effects modeling (X-axis) and CL obtained using standard two-stage (STS) method (Y-axis).

Discussion

The benefit of using CsA in the prevention of graft-versus-host disease (GVHD) after BMT is well established [Deeg HJ, 1985; Biggs J, 1986; Locatelli F, 2000]. Unfortunately, its use is associated with undesirable side effects namely nephrotoxicity and hepatotoxicity. Acute renal failure (nephrotoxicity) is one of the most frequent and potentially life threatening complications following BMT [Pulla B, 1998; Taler SJ, 1999]. CsA-induced renal dysfunction is related to reduced glomerular filtration rate and renal blood flow. This is generally thought to be secondary to vasoconstriction of the glomerular afferent arterioles, which causes a decrease in glomerular pressure [Myers BD, 1988; Hansen JM, 1997; Andoh TF and Bennett WM 1998]. However, liver dysfunction is another major complication encountered in CsA therapy, usually during the first month of therapy when higher dosages of the drug are used (preparative regimen) or in the case of early acute GVHD. Indeed, abnormalities of liver function test such as increased serum aminotransferase (transaminase), gamma-glutamyl transferase, and serum bilirubin concentrations are signs of cyclosporine hepatotoxicity [Canafax DM, 1983; Powles RL, 1980].

It has been shown that CsA adverse effects are concentration-dependent. "Several studies have demonstrated a relationship between nephrotoxicity and high CsA concentrations early after transplantation; on the other hand, the relationship between low CsA concentrations and GVHD is controversial" [Serre-Debeauvais F, 1990]. In our study, even identical CsA doses (≈ 4 mg.kg^{-1}) gave rise to considerable differences in CsA blood levels among patients. Also, population pharmacokinetic analysis of CsA in pretransplant candidates exhibited a wide interindividual variability. This requires the individualization of CsA dosage regimen in potential candidates of BMT, which would help preserving CsA blood levels in a therapeutic window at the time of transplantation. This may accordingly prevent GVHD since the immune recognition of the recipient's tissues by the bone marrow graft will occur once the transplant takes place [Serre-Debeauvais F, 1990].

A two-compartment open model is reportedly adequate for concentration predictions of CsA in the clinical setting and it proved satisfactory in describing CsA disposition in our study, although a three-compartment model has also been used on occasion to characterize the pharmacokinetic profile of CsA by others [Serre-Debeauvais F, 1990]. Multicompartment models are often used to describe CsA kinetics since it has been shown that the drug is widely distributed into body fluids and tissues, with most of the drug being distributed outside the blood volume.

Results of this study suggested that a less complex two-compartment open model approximation to the kinetic profile of CsA can yield relatively accurate fit for the actual observations. Its use resulted in substantially inflated estimates of the size and power of the likelihood ratio test. It is noteworthy however that the model selection was markedly based upon the comparison of three models and their relative goodness-of-fit criterion and redundancy of the parameters. Fitting a three-compartment model failed due to lack of sufficient kinetic points at the end of the cyclosporine A individual profiles. More kinetic data for individual profiles might in turn lead to the selection of a more complex model making parameters identifiable. Therefore, in view of CsA's whole elimination profile, an extensive blood sampling was deemed essential after the administration of an intravenous infusion dose.

Having a biexponential disposition in pretransplant adult candidates, CsA clearance and volume of distribution demonstrate considerable interindividual variability. This variability is more prominent using STS method. After applying the STS method to CsA data, undesirable interindividual variations were obtained in two out of six parameters. In fact, the percent coefficients of variation were higher on volume of distribution at central compartment $\mathbf{V_1}$ (CV=45.01%) and inter-compartmental clearance **Q** (CV=34.29%) compared to the other parameter estimates. It was however possible to derive lower interindividual variations of pharmacokinetic parameters for mean population estimates using the one-stage method (NONMEM). One might argue that this could simply be the result of a systematic overestimation of

interindividual variability due to larger errors on estimating the parameters in the first stage of the STS method.

Results of this study also revealed that almost all the parameters are normally distributed using NONMEM method. Density plots of parameter estimates calculated for both the STS and NONMEM methods are given in Fig. 3.13. A density plot of STS values of **Q** demonstrates a slightly non-normality in the distribution of the estimates of this parameter using the STS method which may be due in part to the nature of this method. However, density plots of **CL**, $\mathbf{V_1}$, $\mathbf{V_2}$, $\mathbf{V_{ss}}$, and $\mathbf{t}_{½}$ did not show any significant departure from normality (Fig. 3.13).

As shown in these plots (next page), NONMEM tends to narrow down and decrease the extent of the variability existed on each parameter estimates. This is due to the prior assumptions made in NONMEM through the process of model building. Basically, in NONMEM population modeling, the probability distribution of the parameters impart an assumed shape, as in our study the parameters are assumed to be lognormally distributed [$\theta_i = \theta_\circ \times \exp(\eta_i)$]. However, in the STS method, parameters are separately estimated for each individual in the first stage and no assumption is being made for their distribution.

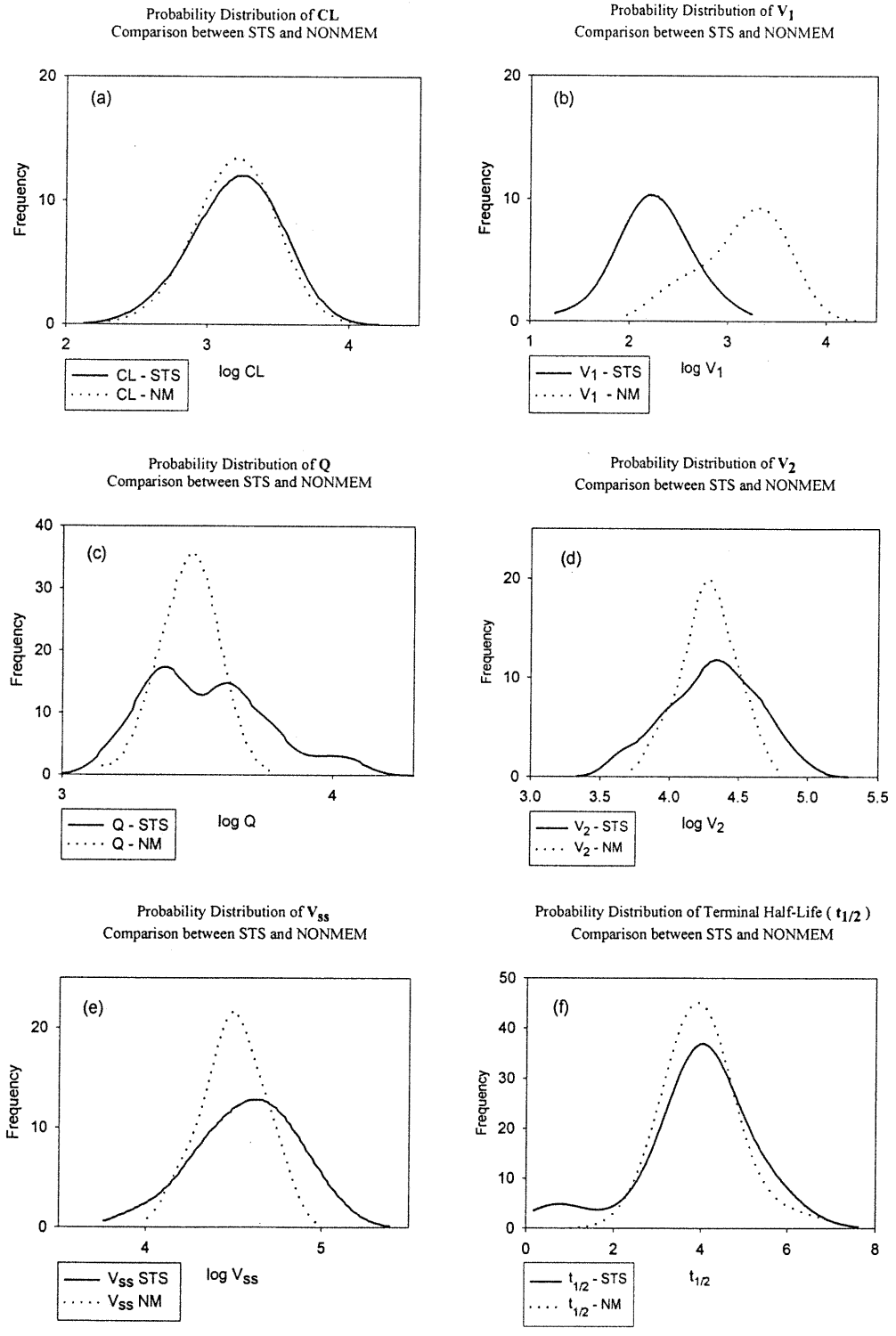


Figure 3.13 – Density plots showing the structure of the parameter estimates in both STS and NONMEM methods: (a) **CL**, (b) $\mathbf{V_1}$, (c) **Q**, (d) $\mathbf{V_2}$, (e) $\mathbf{V_{ss}}$ and (f) $\mathbf{t}_{1/2\,\beta}$.

Furthermore, as shown in Appendix 4, fixed-effects parameters (θs) were estimated with greater precision than the random-effects parameters (ω^2 and σ^2), which might be a characteristic of pharmacostatistical estimation with NONMEM, unless there are data from very large numbers of individuals [Boeckmann, 1992].

Table 3.2 shows the absolute predictive performance of the two-compartment model built by the STS and NONMEM methods. Although the results favor the two-compartment model built by the STS, it is unlikely that there is a significant difference between STS and NONMEM methods in this rich data situation. Therefore, it is difficult to determine which of the two methods is the best. It can also be seen that the two-compartment model fitted the data adequately (small bias) and the differences in predictions between the two methods are quite small.

Table 3.2 - *Absolute predictive performance for STS and NONMEM methods.*

	Two-compartment model	
	STS	**NONMEM**
ME (bias)	0.011 (0.005 – 0.017)	0.015 (0.001 – 0.029)
MAE (accuracy)	182.45 (165.84 – 199.06)	195.02 (178.49 – 211.56)
RMSE (precision)	182.45	195.02
Posterior Variance	14.53	16.30
n	534	534

- ***ME*** (mean prediction error) and ***MAE*** (mean absolute prediction error) are presented as the mean with 95% confidence intervals. The precision is presented as ***RMSE*** (root mean squared prediction error). The unit for all absolute predictive performances is **ng ml^{-1}**.
  n: number of samples.

In addition, Figure 3-14 shows the distribution of root mean squared prediction errors (**RMSE**) and posterior variances of the best compartmental model using NONMEM and STS methods. There was also found to be no significant difference in distributions of absolute predictive performances between NONMEM and STS in the two-compartment model.

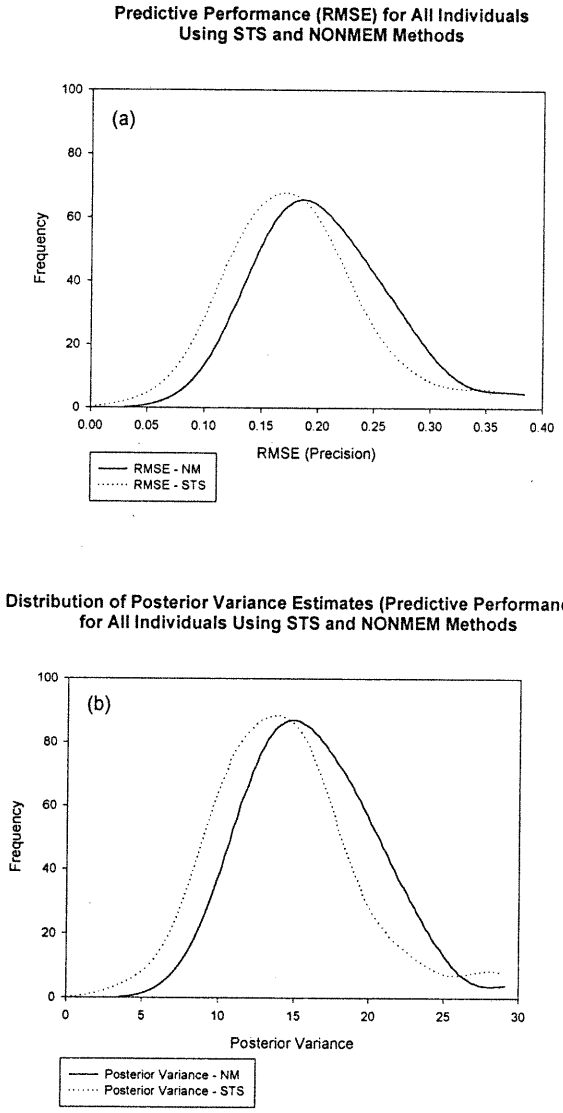


Figure 3.14 – Density plots showing distributions of (a) RMSE and (b) Posterior Variance for the two-compartment model assessing the predictive performance.

Finally, one of the most important aspects of population pharmacokinetic analysis is to reduce the interindividual variability by means of covariates. In our study, however, we were not able to screen the effect(s) of different covariates as they were

missing. Systematic deviations of observed and predicted levels may be caused by demographic and/or pathophysiologic factors that either have not been recognized or fail to achieve the statistical significance required for inclusion in the final population models.

Conclusion

Based upon AIC values and predictive performance analysis, a two-compartment open model best describes CsA population pharmacokinetics, as built by NONMEM and STS, and it is able to predict CsA levels equally well in our population of BMT candidates. Considering the coefficient of variation (%CV) on each parameter, the mean PK parameter estimates obtained by STS and NONMEM are comparable. However, NONMEM seemed to estimate parameters with lower interindividual variability, particularly on $\mathbf{V_1}$, **Q,** and $\mathbf{t_{½\beta}}$. This is due to a fundamental problem with the STS method, which arises in the way it estimates random interindividual variability. This method tends to be upward biased because each parameter is estimated from the original drug concentration versus time data with some error, and this error adds variability to the parameter estimates that is not biological in origin [Sheiner LB, 1984]. Moreover, the STS and NONMEM appeared to be both suitable methods of population pharmacokinetic analysis and for this purpose, they can equally offer reliable and accurate results. In conclusion, our results indicate that:

First, overall the NONMEM (first-order) method behaves about as well as the two-stage method when there are enough observations per individual for this set of data. In fact, asymptotic properties insure that an increase of the sample size will compensate inaccuracy of the parameter estimates in the two-stage method. This conclusion is consistent with previously reported comparisons between these two methods [Sheiner LB, 1981; Beal SL, 1984].

The great similarity found between the scatterplots of the STS and NONMEM first-order estimation (**FO**) methods also illustrates the utility of that traditional method

(STS). However, the NONMEM algorithm is mathematically superior and can include sparse data sets which the STS method cannot handle. Using first-order conditional estimation (**FOCE**) might result in a better parameter estimation, particularly in studies where a rich-data situation is involved.

The second conclusion is that, care should be taken in using minimal pharmacokinetic models (fewer parameters). Their limitations should be recognized, however, they can provide useful information under appropriate design conditions.

Future

In order to assess the predictive performance of the designs and investigate the limitations of using a less complex model (fewer parameters) in both methods, clinical trial simulations and Bayesian estimation are highly suggested on other data sets. They can provide information on the minimal structural model that can be supported by the design and data. In addition, they can be used to assess bias in parameter estimates and variance components when using such minimal models. They can also be used to make sample size recommendations for such population pharmacokinetic study.

As the use of the two-compartment open model approximation resulted in substantially inflated estimates of the size and power of the likelihood ratio test, clinical trial simulation is a good practice to simulate data under the null distribution to assess the type I or false positive error rate particularly when a minimal model is employed.

Finally, alternative PK model should therefore be applied to predict CsA blood levels more precisely as the compartmental modeling seemed to somehow misspecify the kinetic profile of this essential but highly variable drug.

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Appendix

Table A.1 – Blood CsA kinetic data in 52 candidates before bone marrow Transplantation.

ID	Dose (mg)	$C_{(t)}$ (ng/ml)											
		$C_{0.0}$	$C_{1.0}$	$C_{2.0}$	$C_{2.08}$	$C_{2.17}$	$C_{2.33}$	$C_{2.50}$	$C_{3.0}$	$C_{4.0}$	$C_{5.0}$	$C_{8.0}$	C_{14}
1	340	0.000	-	1.760	1.720	1.520	1.340	1.240	1.160	0.920	0.655	0.320	-
2	150	0.000	1.328	1.680	1.280	1.200	1.024	0.936	0.752	0.472	0.380	0.180	0.120
3	340	0.000	3.340	3.520	2.900	2.580	2.060	1.860	1.400	0.450	0.530	0.315	0.167
4	260	0.000	2.136	2.896	2.496	1.992	1.600	1.216	0.840	0.534	0.340	0.208	0.114
5	232	0.000	2.080	2.456	2.080	1.792	1.600	1.408	1.160	0.816	0.546	0.372	0.152
6	200	0.000	1.560	1.800	1.320	1.080	0.780	0.660	0.560	0.290	0.275	0.170	0.095
7	264	0.000	3.420	2.180	1.900	1.860	1.640	1.260	1.060	0.760	0.505	0.300	0.133
8	345	0.000	3.300	2.220	1.820	1.640	1.340	1.100	0.820	0.560	0.465	0.280	0.105
9	224	0.000	2.060	2.740	2.160	1.720	1.500	1.380	1.000	0.660	0.458	0.268	0.128
10	292	0.000	3.024	3.300	2.400	1.904	1.808	1.680	1.152	0.744	0.584	0.364	0.168
11	168	0.000	2.280	1.404	0.966	1.149	1.076	0.857	0.602	0.478	0.246	0.160	0.073
12	204	0.000	2.224	2.344	1.616	1.368	1.136	1.112	0.792	0.672	0.426	0.274	-
13	240	0.000	2.960	3.342	2.464	2.080	1.920	1.720	1.420	1.088	0.518	0.400	0.108
14	256	0.000	2.480	1.600	1.380	1.280	1.120	0.940	0.780	0.440	0.320	0.175	0.095
15	164	0.000	1.872	2.320	1.840	1.472	1.392	1.136	0.912	0.532	0.460	0.196	0.096
16	232	0.000	2.180	1.440	1.120	0.920	0.780	0.620	0.520	0.355	0.225	0.100	-
17	270	0.000	3.760	4.080	2.840	2.280	2.080	1.800	1.200	1.060	0.340	0.320	0.180
18	92	0.000	1.576	1.590	1.520	1.280	1.150	1.032	0.896	0.672	0.680	0.568	0.354
19	148	0.000	1.140	1.160	0.940	0.800	0.536	0.512	0.390	0.250	0.150	0.100	0.070
20	206	0.000	2.440	1.780	1.420	1.280	1.040	0.800	0.640	0.420	0.315	0.185	0.060
21	296	0.000	1.920	2.160	1.680	1.480	1.340	1.300	1.040	0.740	0.520	0.340	0.145
22	264	0.000	4.320	-	2.448	2.416	2.080	1.584	1.344	0.848	0.650	0.388	0.172
23	304	0.000	3.010	3.060	2.995	2.810	1.990	1.760	1.665	1.140	0.820	0.400	0.200
24	280	0.000	2.608	2.576	1.824	1.552	1.232	1.024	0.976	0.680	0.468	0.224	0.112
25	240	0.000	3.700	3.520	2.016	1.680	1.552	1.152	1.024	0.880	0.506	0.326	0.248
26	218	0.000	0.980	1.640	1.640	1.600	1.000	0.880	0.600	0.430	0.140	0.110	-
27	228	0.000	3.340	3.600	2.628	2.080	1.712	1.432	1.136	0.856	0.720	0.412	0.100
28	144	0.000	0.180	5.880	1.680	1.600	1.220	0.920	0.680	0.465	0.330	0.145	0.060
29	250	0.000	2.364	2.360	1.600	1.344	1.280	1.260	1.024	0.784	0.536	0.348	0.150
30	276	0.000	2.240	-	3.600	2.800	2.400	2.100	1.600	1.232	0.960	0.660	0.400
31	360	0.000	2.960	3.300	2.840	2.400	1.960	1.760	1.180	0.860	0.580	0.290	0.140
32	148	0.000	1.400	1.620	1.000	0.860	0.660	0.560	0.400	0.240	0.215	0.120	0.070
33	260	0.000	2.340	1.880	1.520	1.480	1.300	1.160	0.880	0.640	0.495	0.225	0.105
34	264	0.000	1.888	1.856	1.376	1.360	1.056	1.000	0.688	0.448	0.372	0.216	0.108
35	244	0.000	2.020	1.600	1.380	1.320	1.120	1.040	0.800	0.500	0.400	0.215	0.130
36	260	0.000	1.880	3.960	3.144	2.568	1.950	1.808	1.264	0.916	0.890	0.425	0.120
37	204	0.000	3.640	2.540	2.100	1.900	1.680	1.380	1.080	0.800	0.625	0.295	0.140
38	280	0.000	3.400	3.360	2.660	2.300	1.660	1.540	1.020	0.700	0.400	0.200	0.105
39	220	0.000	2.740	2.440	1.940	1.760	1.540	1.240	0.840	0.540	0.400	0.220	0.108
40	260	0.000	2.520	2.900	2.460	1.760	1.640	1.440	1.020	0.780	0.515	0.285	0.130
41	292	0.000	2.108	2.273	1.671	1.544	1.262	1.162	0.906	0.660	0.462	0.268	0.132
42	260	0.000	3.303	2.021	1.866	1.471	1.165	1.056	0.848	0.625	0.394	0.258	0.127
43	230	0.000	2.780	4.200	2.920	2.320	1.640	1.600	1.500	0.800	0.548	0.243	0.123
44	300	0.000	3.540	2.580	2.500	2.140	2.000	1.660	1.180	0.800	0.400	0.350	0.135
45	162	0.000	3.194	2.852	2.357	1.912	1.660	1.508	1.120	0.757	0.487	0.272	0.126
46	320	0.000	2.820	3.740	2.600	1.960	1.880	1.600	1.200	0.800	0.575	0.235	0.142
47	296	0.000	2.120	3.360	1.900	1.800	1.480	1.480	1.080	0.800	0.465	0.215	0.080
48	272	0.000	4.500	3.000	2.380	2.260	1.640	1.600	1.340	0.815	0.620	0.283	0.143
49	200	0.000	2.460	3.480	2.060	1.780	1.280	1.180	1.040	0.780	0.500	0.270	0.150
50	288	0.000	2.120	2.640	2.400	2.200	1.920	1.560	1.160	0.780	0.565	0.315	0.140
51	256	0.000	3.400	-	3.340	3.000	2.560	2.340	1.780	1.540	1.025	0.605	0.257
52	288	0.000	4.040	3.080	2.300	1.980	1.660	1.360	1.160	0.820	0.585	0.327	0.135

Bloob Cyclosporine A (Sandimune) Concentration-Time Data in 52 Patients before BMT.

Appendix 2 – Table A.2.

Covariance-correlation matrices of candidates resulted from **3-compartment** model using STS.

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
1	α_1	7.45E-03	**9.77E-01**	5.96E-01	-5.89E-01	**9.83E-01**	3.17E-01
	α_2	1.12E-02	1.75E-02	5.16E-01	-7.29E-01	9.99E-01	2.67E-01
	α_3	2.68E-01	3.56E-01	2.72E+01	1.51E-02	5.25E-01	8.85E-01
	A_1	-2.01E-05	-3.82E-05	3.12E-05	1.57E-07	-7.15E-01	6.05E-02
	A_2	6.36E-05	9.91E-05	2.05E-03	-2.12E-07	5.62E-07	2.72E-01
	A_3	2.34E-04	3.02E-04	3.95E-02	2.05E-07	1.75E-06	7.31E-05
2	α_1	6.37E-04	3.65E-01	**9.03E-01**	**9.94E-01**	2.09E-01	4.00E-01
	α_2	2.19E-02	5.66E+00	5.24E-01	3.81E-01	9.29E-01	7.11E-01
	α_3	1.26E-03	6.87E-02	3.03E-03	9.27E-01	3.20E-01	7.04E-01
	A_1	8.45E-06	3.06E-04	1.72E-05	1.14E-07	2.19E-01	4.33E-01
	A_2	9.21E-05	3.86E-02	3.07E-04	1.29E-06	3.04E-04	4.91E-01
	A_3	6.04E-06	1.01E-03	2.32E-05	8.71E-08	5.11E-06	3.57E-07
3	α_1	6.85E-04	6.89E-01	2.71E-01	9.49E-01	5.05E-01	5.81E-02
	α_2	7.10E-03	1.55E-01	5.92E-01	7.74E-01	9.36E-01	2.16E-01
	α_3	9.65E-02	3.18E+00	1.86E+02	3.10E-01	7.48E-01	8.55E-01
	A_1	1.24E-05	1.52E-04	2.11E-03	2.48E-07	5.79E-01	6.82E-02
	A_2	1.13E-04	3.15E-03	8.71E-02	2.46E-06	7.30E-05	3.56E-01
	A_3	8.95E-05	5.00E-03	6.85E-01	2.00E-06	1.79E-04	3.46E-03
4	α_1	1.46E-04	3.91E-01	7.51E-01	9.76E-01	3.03E-02	5.39E-01
	α_2	4.66E-03	9.76E-01	7.64E-01	4.32E-01	7.20E-01	8.93E-01
	α_3	1.25E-03	1.04E-01	1.90E-02	8.09E-01	2.24E-01	9.37E-01
	A_1	2.34E-06	8.46E-05	2.21E-05	3.93E-08	3.91E-02	5.94E-01
	A_2	2.05E-06	3.99E-03	1.73E-04	4.35E-08	3.15E-05	3.67E-01
	A_3	1.63E-05	2.21E-03	3.23E-04	2.95E-07	5.15E-06	6.25E-06
5	α_1	1.50E-04	2.76E-01	7.83E-01	9.66E-01	7.80E-02	4.37E-01
	α_2	1.35E-02	1.60E+01	5.39E-01	3.09E-01	8.85E-01	7.43E-01
	α_3	2.14E-03	4.81E-01	4.98E-02	8.51E-01	2.22E-01	8.52E-01
	A_1	6.29E-06	6.58E-04	1.01E-04	2.84E-07	8.93E-02	5.02E-01
	A_2	2.15E-05	8.00E-02	1.12E-03	1.07E-06	5.10E-04	4.07E-01
	A_3	1.15E-05	6.41E-03	4.11E-04	5.76E-07	1.99E-05	4.66E-06

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
6	α_1	1.89E-04	7.36E-01	2.78E-01	9.58E-01	5.00E-01	7.52E-02
	α_2	2.51E-03	6.14E-02	5.80E-01	8.13E-01	9.15E-01	2.38E-01
	α_3	9.24E-03	3.48E-01	5.85E+00	3.15E-01	7.55E-01	8.64E-01
	A_1	3.23E-06	4.94E-05	1.87E-04	6.01E-08	5.70E-01	8.70E-02
	A_2	1.53E-05	5.05E-04	4.06E-03	3.11E-07	4.95E-06	3.93E-01
	A_3	1.89E-05	1.08E-03	3.82E-02	3.90E-07	1.60E-05	3.34E-04
7	α_1	2.18E-01	**8.46E-01**	4.94E-01	6.97E-01	**8.92E-01**	2.61E-01
	α_2	2.22E-02	3.15E-03	2.83E-01	2.93E-01	9.82E-01	1.29E-01
	α_3	1.27E+00	8.73E-02	3.04E+01	7.07E-01	3.08E-01	9.06E-01
	A_1	1.04E-03	5.26E-05	1.25E-02	1.03E-05	3.44E-01	4.49E-01
	A_2	6.84E-04	9.04E-05	2.78E-03	1.81E-06	2.70E-06	1.42E-01
	A_3	8.62E-03	5.12E-04	3.53E-01	1.02E-04	1.65E-05	5.00E-03
8	α_1	5.01E-04	6.51E-01	2.94E-01	9.29E-01	4.94E-01	1.69E-02
	α_2	1.94E-02	1.77E+00	6.57E-01	7.59E-01	9.56E-01	1.46E-01
	α_3	3.97E-02	5.28E+00	3.64E+01	3.51E-01	7.84E-01	7.67E-01
	A_1	1.09E-05	5.28E-04	1.11E-03	2.73E-07	5.89E-01	2.24E-02
	A_2	1.09E-04	1.26E-02	4.66E-02	3.04E-06	9.73E-05	2.64E-01
	A_3	1.61E-05	8.25E-03	1.97E-01	4.98E-07	1.11E-04	1.81E-03
9	α_1	3.62E-05	7.80E-01	2.57E-01	**9.71E-01**	4.22E-01	**9.48E-02**
	α_2	2.68E-04	3.26E-03	5.19E-01	8.41E-01	8.43E-01	2.51E-01
	α_3	2.29E-03	4.40E-02	2.21E+00	2.85E-01	7.27E-01	9.02E-01
	A_1	1.02E-06	8.37E-06	7.40E-05	3.04E-08	4.79E-01	1.07E-01
	A_2	1.94E-06	3.67E-05	8.25E-04	6.38E-08	5.83E-07	4.31E-01
	A_3	5.97E-06	1.50E-04	1.41E-02	1.96E-07	3.45E-06	1.10E-04
10	α_1	2.23E-04	7.53E-01	2.68E-01	9.61E-01	4.76E-01	7.56E-02
	α_2	2.87E-03	6.50E-02	5.47E-01	8.27E-01	8.93E-01	2.26E-01
	α_3	1.44E-02	5.02E-01	1.30E+01	3.02E-01	7.33E-01	8.77E-01
	A_1	6.59E-06	9.68E-05	4.99E-04	2.11E-07	5.43E-01	8.70E-02
	A_2	2.07E-05	6.63E-04	7.70E-03	7.27E-07	8.50E-06	3.89E-01
	A_3	3.31E-05	1.68E-03	9.26E-02	1.17E-06	3.32E-05	8.59E-04

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
11	α_1	1.94E-01	8.29E-01	4.98E-01	7.64E-01	8.76E-01	2.72E-01
	α_2	2.65E-02	5.24E-03	2.76E-01	3.64E-01	9.82E-01	1.32E-01
	α_3	1.56E+00	1.42E-01	5.08E+01	7.05E-01	3.00E-01	9.08E-01
	A_1	1.37E-03	1.07E-04	2.04E-02	1.65E-05	4.13E-01	4.47E-01
	A_2	5.94E-04	1.09E-04	3.29E-03	2.58E-06	2.36E-06	1.45E-01
	A_3	1.09E-02	8.67E-04	5.89E-01	1.65E-04	2.02E-05	8.28E-03
12	α_1	7.50E-01	9.92E-01	5.48E-01	1.00E+00	-9.80E-01	2.41E-01
	α_2	6.21E-01	5.22E-01	5.99E-01	9.95E-01	-9.50E-01	2.78E-01
	α_3	1.45E+00	1.32E+00	9.29E+00	5.56E-01	-4.22E-01	8.80E-01
	A_1	6.80E-03	5.65E-03	1.33E-02	6.18E-05	-9.76E-01	2.46E-01
	A_2	-4.33E-03	-3.51E-03	-6.56E-03	-3.92E-05	2.61E-05	-1.46E-01
	A_3	7.04E-03	6.79E-03	9.06E-02	6.54E-05	-2.51E-05	1.14E-03
13	α_1	5.14E-01	8.32E-01	5.14E-01	7.15E-01	8.88E-01	2.28E-01
	α_2	2.05E-02	1.18E-03	2.88E-01	2.97E-01	9.75E-01	9.84E-02
	α_3	2.28E+00	6.14E-02	3.84E+01	7.34E-01	3.17E-01	8.92E-01
	A_1	2.35E-03	4.69E-05	2.09E-02	2.11E-05	3.55E-01	4.35E-01
	A_2	1.49E-03	7.85E-05	4.60E-03	3.82E-06	5.47E-06	1.10E-01
	A_3	1.06E-02	2.19E-04	3.58E-01	1.29E-04	1.67E-05	4.19E-03
14	α_1	8.11E-02	8.24E-01	5.00E-01	7.87E-01	8.70E-01	2.79E-01
	α_2	1.30E-02	3.06E-03	2.76E-01	3.91E-01	9.82E-01	1.36E-01
	α_3	7.54E-01	8.07E-02	2.80E+01	7.02E-01	3.00E-01	9.10E-01
	A_1	4.84E-04	4.67E-05	8.03E-03	4.66E-06	4.39E-01	4.48E-01
	A_2	1.78E-04	3.89E-05	1.14E-03	6.80E-07	5.14E-07	1.49E-01
	A_3	4.02E-03	3.80E-04	2.44E-01	4.89E-05	5.40E-06	2.55E-03
15	α_1	1.02E-03	8.89E-01	3.58E-01	9.91E-01	3.33E-01	1.94E-01
	α_2	3.10E-03	1.19E-02	5.40E-01	9.19E-01	6.74E-01	3.17E-01
	α_3	3.23E-02	1.67E-01	8.00E+00	3.78E-01	7.32E-01	9.19E-01
	A_1	2.49E-05	7.90E-05	8.43E-04	6.20E-07	3.72E-01	2.06E-01
	A_2	1.40E-05	9.69E-05	2.73E-03	3.86E-07	1.73E-06	4.98E-01
	A_3	1.82E-04	1.02E-03	7.64E-02	4.77E-06	1.93E-05	8.66E-04

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
16	α_1	1.49E+01	9.93E-01	5.36E-01	1.00E+00	-9.81E-01	2.59E-01
	α_2	4.42E+00	1.33E+00	5.84E-01	9.95E-01	-9.54E-01	2.94E-01
	α_3	8.15E+00	2.65E+00	1.55E+01	5.44E-01	-4.16E-01	8.85E-01
	A_1	3.00E-02	8.92E-03	1.66E-02	6.03E-05	-9.77E-01	2.64E-01
	A_2	-1.92E-02	-5.59E-03	-8.32E-03	-3.85E-05	2.57E-05	-1.70E-01
	A_3	4.23E-02	1.44E-02	1.48E-01	8.69E-05	-3.66E-05	1.80E-03
17	α_1	4.31E-03	8.28E-01	2.87E-01	9.84E-01	4.20E-01	1.48E-01
	α_2	1.43E-02	6.96E-02	5.07E-01	8.71E-01	8.02E-01	2.91E-01
	α_3	1.23E-01	8.75E-01	4.27E+01	3.10E-01	7.03E-01	9.15E-01
	A_1	7.71E-05	2.74E-04	2.42E-03	1.43E-06	4.65E-01	1.61E-01
	A_2	1.08E-04	8.27E-04	1.80E-02	2.17E-06	1.53E-05	4.55E-01
	A_3	9.15E-04	7.23E-03	5.63E-01	1.81E-05	1.67E-04	8.85E-03
18	α_1	1.03E-04	5.40E-01	2.66E-01	9.01E-01	4.16E-01	-1.46E-01
	α_2	8.69E-03	2.52E+00	7.45E-01	6.64E-01	9.71E-01	-1.76E-01
	α_3	2.78E-02	1.22E+01	1.07E+02	3.35E-01	8.47E-01	4.46E-01
	A_1	7.64E-06	8.82E-04	2.90E-03	7.01E-07	5.22E-01	-1.80E-01
	A_2	1.49E-04	5.46E-02	3.10E-01	1.55E-05	1.25E-03	-6.87E-02
	A_3	-9.04E-05	-1.71E-02	2.82E-01	-9.21E-06	-1.49E-04	3.75E-03
19	α_1	4.25E-04	7.75E-01	2.76E-01	9.77E-01	4.51E-01	1.31E-01
	α_2	1.84E-03	1.32E-02	5.57E-01	8.28E-01	8.65E-01	3.13E-01
	α_3	1.21E-02	1.37E-01	4.57E+00	3.03E-01	7.46E-01	9.05E-01
	A_1	3.94E-06	1.87E-05	1.27E-04	3.83E-08	5.02E-01	1.46E-01
	A_2	1.06E-05	1.13E-04	1.81E-03	1.12E-07	1.29E-06	4.75E-01
	A_3	4.64E-05	6.18E-04	3.32E-02	4.91E-07	9.25E-06	2.94E-04
20	α_1	4.82E-04	2.95E-01	6.94E-01	9.41E-01	4.38E-02	5.08E-01
	α_2	3.29E-02	2.58E+01	6.20E-01	3.44E-01	8.12E-01	7.63E-01
	α_3	1.44E-02	2.97E+00	8.88E-01	7.91E-01	1.82E-01	9.42E-01
	A_1	1.39E-05	1.18E-03	5.01E-04	4.52E-07	5.31E-02	5.95E-01
	A_2	4.67E-05	2.00E-01	8.31E-03	1.73E-06	2.36E-03	3.11E-01
	A_3	9.46E-05	3.29E-02	7.53E-03	3.39E-06	1.28E-04	7.19E-05

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
21	α_1	6.55E-02	8.42E-01	5.07E-01	6.88E-01	8.93E-01	2.46E-01
	α_2	4.33E-03	4.05E-04	2.88E-01	2.75E-01	9.79E-01	1.14E-01
	α_3	4.95E-01	2.21E-02	1.46E+01	7.27E-01	3.16E-01	9.06E-01
	A_1	2.03E-04	6.38E-06	3.20E-03	1.33E-06	3.30E-01	4.55E-01
	A_2	1.43E-04	1.23E-05	7.53E-04	2.38E-07	3.91E-07	1.26E-01
	A_3	1.21E-03	4.38E-05	6.63E-02	1.01E-05	1.51E-06	3.68E-04
22	α_1	1.09E-03	8.07E-01	2.60E-01	9.74E-01	3.88E-01	1.09E-01
	α_2	1.03E-02	1.48E-01	4.97E-01	8.65E-01	8.02E-01	2.51E-01
	α_3	2.71E-02	6.05E-01	9.98E+00	2.87E-01	7.06E-01	8.96E-01
	A_1	4.04E-05	4.19E-04	1.14E-03	1.58E-06	4.45E-01	1.22E-01
	A_2	4.99E-05	1.20E-03	8.69E-03	2.18E-06	1.52E-05	4.25E-01
	A_3	2.68E-04	7.23E-03	2.12E-01	1.14E-05	1.24E-04	5.61E-03
23	α_1	4.09E-02	9.74E-01	5.97E-01	9.99E-01	5.99E-02	3.79E-01
	α_2	3.19E-02	2.63E-02	6.86E-01	9.80E-01	2.59E-01	4.49E-01
	α_3	2.86E-01	2.64E-01	5.63E+00	6.07E-01	6.21E-01	9.14E-01
	A_1	3.35E-04	2.64E-04	2.39E-03	2.75E-06	8.00E-02	3.86E-01
	A_2	1.17E-05	4.04E-05	1.42E-03	1.28E-07	9.29E-07	4.91E-01
	A_3	1.58E-03	1.50E-03	4.47E-02	1.32E-05	9.76E-06	4.24E-04
24	α_1	1.58E-02	9.64E-01	4.26E-01	9.99E-01	-8.50E-02	2.59E-01
	α_2	1.17E-02	9.40E-03	5.17E-01	9.72E-01	1.43E-01	3.24E-01
	α_3	1.02E-01	9.52E-02	3.60E+00	4.35E-01	5.12E-01	9.21E-01
	A_1	7.87E-05	5.91E-05	5.17E-04	3.93E-07	-6.43E-02	2.65E-01
	A_2	-4.37E-06	5.68E-06	3.97E-04	-1.65E-08	1.67E-07	3.76E-01
	A_3	7.38E-04	7.12E-04	3.96E-02	3.77E-06	3.48E-06	5.13E-04
25	α_1	2.41E-02	9.22E-01	4.88E-01	5.54E-01	9.42E-01	2.63E-01
	α_2	1.00E-02	4.91E-03	3.54E-01	2.49E-01	9.95E-01	1.84E-01
	α_3	2.33E-01	7.64E-02	9.47E+00	6.35E-01	3.69E-01	8.92E-01
	A_1	9.39E-05	1.90E-05	2.13E-03	1.19E-06	2.81E-01	3.79E-01
	A_2	1.35E-04	6.44E-05	1.05E-03	2.84E-07	8.55E-07	1.92E-01
	A_3	2.22E-03	7.00E-04	1.49E-01	2.25E-05	9.65E-06	2.96E-03

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
26	α_1	1.17E+00	9.80E-01	7.92E-01	1.00E+00	9.13E-01	-4.49E-01
	α_2	1.44E+00	1.84E+00	8.76E-01	9.83E-01	9.73E-01	-3.88E-01
	α_3	4.49E+01	6.23E+01	2.75E+03	7.98E-01	9.48E-01	4.27E-02
	A_1	3.85E-03	4.75E-03	1.49E-01	1.27E-05	9.19E-01	-4.47E-01
	A_2	2.16E-02	2.90E-02	1.09E+00	7.18E-05	4.81E-04	-2.66E-01
	A_3	-9.33E-03	-1.01E-02	4.29E-02	-3.06E-05	-1.12E-04	3.69E-04
27	α_1	1.88E+04	1.61E+02	4.97E-06	-1.45E-04	-Inf	-1.03E-05
	α_2	1.22E+00	3.04E-09	-7.66E+07	6.53E+08	-Inf	1.60E+08
	α_3	3.22E-03	-2.00E+04	2.24E+01	-4.90E-02	-Inf	-1.31E-01
	A_1	-3.70E+01	6.69E+07	-4.30E+02	3.45E+06	-Inf	-1.55E-07
	A_2	-3.70E+01	6.69E+07	-4.30E+02	1.00E+00	0.00E+00	-Inf
	A_3	-7.77E-02	4.86E+05	-3.43E+01	-1.59E-02	-2.39E-08	3.05E+03
28	α_1	4.25E-02	9.08E-01	3.34E-01	9.93E-01	2.36E-01	1.93E-01
	α_2	1.18E-01	3.98E-01	4.82E-01	9.33E-01	5.67E-01	2.90E-01
	α_3	3.65E-01	1.61E+00	2.81E+01	3.51E-01	6.39E-01	9.03E-01
	A_1	8.80E-04	2.53E-03	8.00E-03	1.85E-05	2.73E-01	2.03E-01
	A_2	2.64E-04	1.94E-03	1.84E-02	6.36E-06	2.95E-05	4.26E-01
	A_3	1.11E-02	5.11E-02	1.34E+00	2.44E-04	6.46E-04	7.81E-02
29	α_1	5.66E-03	9.46E-01	3.68E-01	9.95E-01	-8.11E-01	1.97E-01
	α_2	2.87E-02	1.63E-01	4.89E-01	9.65E-01	-6.20E-01	2.78E-01
	α_3	9.96E-02	7.08E-01	1.29E+01	3.86E-01	6.19E-02	9.17E-01
	A_1	2.03E-04	1.05E-03	3.75E-03	7.33E-06	-7.91E-01	2.08E-01
	A_2	-1.07E-04	-4.37E-04	3.89E-04	-3.75E-06	3.06E-06	9.74E-02
	A_3	5.36E-04	4.05E-03	1.19E-01	2.03E-05	6.15E-06	1.30E-03
30	α_1	3.25E-03	8.13E-01	2.65E-01	9.75E-01	3.80E-01	1.10E-01
	α_2	3.10E-02	4.46E-01	5.00E-01	8.70E-01	7.91E-01	2.51E-01
	α_3	9.68E-02	2.14E+00	4.10E+01	2.93E-01	7.11E-01	9.00E-01
	A_1	1.37E-04	1.44E-03	4.64E-03	6.11E-06	4.36E-01	1.23E-01
	A_2	1.53E-04	3.74E-03	3.23E-02	7.64E-06	5.02E-05	4.33E-01
	A_3	8.35E-04	2.23E-02	7.68E-01	4.05E-05	4.08E-04	1.77E-02

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
31	α_1	1.00E-03	9.22E-01	5.52E-01	9.95E-01	5.59E-01	2.87E-01
	α_2	3.49E-03	1.43E-02	7.23E-01	9.43E-01	8.10E-01	4.10E-01
	α_3	1.80E-02	8.91E-02	1.06E+00	5.74E-01	8.65E-01	8.71E-01
	A_1	1.57E-05	5.61E-05	2.95E-04	2.48E-07	5.92E-01	3.00E-01
	A_2	1.71E-05	9.35E-05	8.61E-04	2.85E-07	9.33E-07	5.68E-01
	A_3	6.47E-05	3.48E-04	6.39E-03	1.06E-06	3.91E-06	5.06E-05
32	α_1	1.49E-04	2.57E-01	7.38E-01	9.59E-01	8.19E-02	4.86E-01
	α_2	6.29E-03	4.00E+00	5.42E-01	2.91E-01	8.78E-01	7.19E-01
	α_3	1.67E-03	2.01E-01	3.43E-02	8.13E-01	2.38E-01	9.06E-01
	A_1	2.60E-06	1.29E-04	3.35E-05	4.93E-08	9.41E-02	5.54E-01
	A_2	2.06E-05	3.62E-02	9.08E-04	4.31E-07	4.26E-04	3.87E-01
	A_3	1.08E-05	2.61E-03	3.05E-04	2.23E-07	1.45E-05	3.29E-06
33	α_1	2.42E-02	4.11E-01	9.58E-01	9.98E-01	2.43E-01	-4.33E-01
	α_2	2.88E-01	2.03E+01	5.14E-01	4.23E-01	9.23E-01	3.44E-01
	α_3	3.29E-02	5.11E-01	4.88E-02	9.69E-01	3.15E-01	-1.97E-01
	A_1	2.64E-04	3.24E-03	3.64E-04	2.89E-06	2.51E-01	-4.10E-01
	A_2	1.55E-03	1.70E-01	2.86E-03	1.75E-05	1.68E-03	2.74E-01
	A_3	-6.89E-05	1.58E-03	-4.45E-05	-7.12E-07	1.15E-05	1.05E-06
34	α_1	2.93E-04	7.41E-01	2.72E-01	9.57E-01	4.90E-01	6.99E-02
	α_2	4.09E-03	1.04E-01	5.60E-01	8.19E-01	9.07E-01	2.19E-01
	α_3	2.13E-02	8.24E-01	2.08E+01	3.09E-01	7.38E-01	8.69E-01
	A_1	5.71E-06	9.21E-05	4.92E-04	1.22E-07	5.60E-01	8.09E-02
	A_2	2.22E-05	7.73E-04	8.91E-03	5.17E-07	6.99E-06	3.76E-01
	A_3	2.83E-05	1.67E-03	9.40E-02	6.69E-07	2.35E-05	5.61E-04
35	α_1	2.40E-03	8.56E-01	3.01E-01	9.86E-01	3.42E-01	1.53E-01
	α_2	9.60E-03	5.25E-02	5.04E-01	8.95E-01	7.21E-01	2.86E-01
	α_3	7.70E-02	6.03E-01	2.73E+01	3.24E-01	7.09E-01	9.17E-01
	A_1	3.60E-05	1.53E-04	1.26E-03	5.55E-07	3.87E-01	1.66E-01
	A_2	2.65E-05	2.61E-04	5.85E-03	4.56E-07	2.50E-06	4.66E-01
	A_3	2.89E-04	2.52E-03	1.85E-01	4.76E-06	2.84E-05	1.49E-03

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
36	α_1	2.72E-04	3.81E-01	4.54E-01	8.96E-01	-4.23E-01	4.20E-01
	α_2	1.46E+00	5.44E+04	9.81E-01	4.82E-01	-9.92E-01	9.94E-01
	α_3	2.30E-01	7.03E+03	9.44E+02	5.70E-01	-9.98E-01	9.97E-01
	A_1	1.49E-05	1.13E-01	1.77E-02	1.02E-06	-5.33E-01	5.30E-01
	A_2	-1.47E-02	-4.87E+02	-6.45E+01	-1.13E-03	4.43E+00	-1.00E+00
	A_3	1.48E-02	4.94E+02	6.53E+01	1.14E-03	-4.49E+00	4.55E+00
37	α_1	2.30E-02	9.53E-01	3.94E-01	9.97E-01	-5.03E-01	2.30E-01
	α_2	4.16E-02	8.28E-02	5.02E-01	9.66E-01	-2.61E-01	3.04E-01
	α_3	2.41E-01	5.81E-01	1.62E+01	4.08E-01	3.03E-01	9.17E-01
	A_1	5.13E-04	9.43E-04	5.56E-03	1.15E-05	-4.78E-01	2.38E-01
	A_2	-1.57E-04	-1.55E-04	2.51E-03	-3.34E-06	4.25E-06	2.42E-01
	A_3	2.88E-03	7.24E-03	3.06E-01	6.69E-05	4.14E-05	6.87E-03
38	α_1	1.04E-02	8.12E-01	5.67E-01	8.52E-01	8.56E-01	3.32E-01
	α_2	2.08E-03	6.29E-04	3.16E-01	4.74E-01	9.84E-01	1.65E-01
	α_3	1.19E-01	1.63E-02	4.23E+00	7.49E-01	3.41E-01	9.08E-01
	A_1	1.36E-04	1.86E-05	2.41E-03	2.45E-06	5.19E-01	4.88E-01
	A_2	2.93E-05	8.27E-06	2.35E-04	2.72E-07	1.12E-07	1.80E-01
	A_3	8.80E-04	1.08E-04	4.85E-02	1.98E-05	1.57E-06	6.76E-04
39	α_1	4.17E-04	7.39E-01	2.55E-01	9.61E-01	4.75E-01	7.84E-02
	α_2	3.95E-03	6.88E-02	5.42E-01	8.12E-01	8.98E-01	2.36E-01
	α_3	2.41E-02	6.59E-01	2.15E+01	2.88E-01	7.26E-01	8.82E-01
	A_1	1.03E-05	1.12E-04	7.01E-04	2.76E-07	5.40E-01	9.00E-02
	A_2	3.99E-05	9.70E-04	1.39E-02	1.17E-06	1.69E-05	3.94E-01
	A_3	7.07E-05	2.74E-03	1.81E-01	2.09E-06	7.16E-05	1.95E-03
40	α_1	4.10E-04	8.42E-01	3.06E-01	9.83E-01	3.51E-01	1.42E-01
	α_2	2.42E-03	2.02E-02	5.34E-01	8.87E-01	7.45E-01	2.88E-01
	α_3	1.45E-02	1.78E-01	5.49E+00	3.33E-01	7.48E-01	9.07E-01
	A_1	1.12E-05	7.10E-05	4.39E-04	3.18E-07	4.00E-01	1.55E-01
	A_2	9.26E-06	1.38E-04	2.28E-03	2.94E-07	1.70E-06	4.83E-01
	A_3	6.17E-05	8.81E-04	4.57E-02	1.89E-06	1.36E-05	4.63E-04

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
41	α_1	4.46E-04	8.66E-01	3.02E-01	9.86E-01	2.38E-01	1.45E-01
	α_2	2.48E-03	1.84E-02	4.99E-01	9.06E-01	6.30E-01	2.72E-01
	α_3	1.51E-02	1.60E-01	5.62E+00	3.25E-01	7.04E-01	9.14E-01
	A_1	9.26E-06	5.46E-05	3.43E-04	1.98E-07	2.86E-01	1.58E-01
	A_2	3.56E-06	6.05E-05	1.18E-03	8.98E-08	5.00E-07	4.64E-01
	A_3	5.05E-05	6.07E-04	3.56E-02	1.15E-06	5.39E-06	2.70E-04
42	α_1	4.11E-03	3.00E-01	8.76E-01	9.87E-01	1.53E-01	1.98E-01
	α_2	7.41E-02	1.48E+01	4.85E-01	3.23E-01	9.07E-01	6.76E-01
	α_3	2.28E-02	7.58E-01	1.66E-01	9.13E-01	2.71E-01	5.85E-01
	A_1	8.64E-05	1.69E-03	5.07E-04	1.87E-06	1.65E-01	2.45E-01
	A_2	5.80E-04	2.06E-01	6.53E-03	1.34E-05	3.51E-03	4.43E-01
	A_3	2.46E-05	5.02E-03	4.60E-04	6.46E-07	5.07E-05	3.73E-06
43	α_1	2.59E-03	8.98E-01	3.60E-01	9.93E-01	4.53E-01	2.06E-01
	α_2	4.82E-03	1.11E-02	5.21E-01	9.22E-01	7.50E-01	3.15E-01
	α_3	5.98E-02	1.79E-01	1.06E+01	3.76E-01	7.03E-01	9.21E-01
	A_1	3.79E-05	7.26E-05	9.18E-04	5.60E-07	4.84E-01	2.16E-01
	A_2	3.67E-05	1.26E-04	3.65E-03	5.76E-07	2.53E-06	4.73E-01
	A_3	5.04E-04	1.60E-03	1.45E-01	7.76E-06	3.62E-05	2.32E-03
44	α_1	1.14E-03	7.30E-01	2.70E-01	9.56E-01	4.93E-01	6.81E-02
	α_2	1.43E-02	3.36E-01	5.64E-01	8.10E-01	9.13E-01	2.20E-01
	α_3	8.71E-02	3.13E+00	9.15E+01	3.07E-01	7.38E-01	8.67E-01
	A_1	3.04E-05	4.42E-04	2.77E-03	8.89E-07	5.64E-01	7.90E-02
	A_2	1.39E-04	4.43E-03	5.91E-02	4.45E-06	7.00E-05	3.72E-01
	A_3	1.65E-04	9.16E-03	5.97E-01	5.36E-06	2.24E-04	5.18E-03
45	α_1	3.20E-02	8.33E-01	5.01E-01	7.55E-01	8.79E-01	2.73E-01
	α_2	4.46E-03	8.95E-04	2.80E-01	3.56E-01	9.82E-01	1.33E-01
	α_3	3.01E-01	2.81E-02	1.13E+01	7.10E-01	3.05E-01	9.10E-01
	A_1	4.04E-04	3.18E-05	7.12E-03	8.92E-06	4.05E-01	4.53E-01
	A_2	1.86E-04	3.48E-05	1.21E-03	1.43E-06	1.40E-06	1.46E-01
	A_3	3.14E-03	2.56E-04	1.97E-01	8.71E-05	1.11E-05	4.15E-03

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
46	α_1	1.34E-03	3.89E-01	9.18E-01	9.96E-01	2.31E-01	5.37E-01
	α_2	2.44E-02	2.94E+00	5.28E-01	4.01E-01	9.24E-01	7.01E-01
	α_3	1.68E-03	4.52E-02	2.49E-03	9.34E-01	3.28E-01	7.85E-01
	A_1	8.22E-06	1.55E-04	1.05E-05	5.10E-08	2.39E-01	5.60E-01
	A_2	1.47E-04	2.76E-02	2.85E-04	9.39E-07	3.04E-04	4.76E-01
	A_3	1.03E-05	6.29E-04	2.05E-05	6.62E-08	4.34E-06	2.73E-07
47	α_1	6.65E-03	9.43E-01	3.98E-01	9.97E-01	-3.96E-02	2.30E-01
	α_2	1.08E-02	1.97E-02	5.18E-01	9.57E-01	2.45E-01	3.14E-01
	α_3	1.23E-01	2.75E-01	1.43E+01	4.11E-01	5.78E-01	9.24E-01
	A_1	8.54E-05	1.41E-04	1.63E-03	1.10E-06	-1.09E-02	2.39E-01
	A_2	-2.64E-06	2.82E-05	1.79E-03	-9.37E-09	6.69E-07	4.19E-01
	A_3	5.67E-04	1.33E-03	1.06E-01	7.58E-06	1.04E-05	9.15E-04
48	α_1	2.98E-02	9.49E-01	4.01E-01	9.98E-01	1.26E-01	2.38E-01
	α_2	3.65E-02	4.98E-02	5.10E-01	9.61E-01	3.88E-01	3.14E-01
	α_3	2.65E-01	4.37E-01	1.47E+01	4.12E-01	6.08E-01	9.17E-01
	A_1	3.07E-04	3.82E-04	2.81E-03	3.17E-06	1.51E-01	2.46E-01
	A_2	3.29E-05	1.31E-04	3.54E-03	4.08E-07	2.30E-06	4.26E-01
	A_3	3.16E-03	5.38E-03	2.70E-01	3.35E-05	4.96E-05	5.88E-03
49	α_1	5.59E-03	9.54E-01	4.08E-01	9.98E-01	-4.39E-02	2.44E-01
	α_2	6.52E-03	8.36E-03	5.12E-01	9.65E-01	2.13E-01	3.17E-01
	α_3	5.19E-02	7.97E-02	2.90E+00	4.19E-01	5.47E-01	9.20E-01
	A_1	6.41E-05	7.58E-05	6.13E-04	7.39E-07	-1.91E-02	2.51E-01
	A_2	-2.03E-06	1.20E-05	5.74E-04	-1.01E-08	3.81E-07	3.95E-01
	A_3	5.68E-04	9.02E-04	4.87E-02	6.72E-06	7.59E-06	9.67E-04
50	α_1	2.70E-02	8.80E-01	8.50E-01	9.35E-01	9.13E-01	-1.44E-01
	α_2	1.78E-03	1.51E-04	6.14E-01	6.85E-01	9.90E-01	-2.15E-01
	α_3	1.03E-01	5.57E-03	5.44E-01	9.47E-01	6.48E-01	2.98E-01
	A_1	3.10E-04	1.70E-05	1.41E-03	4.06E-06	7.25E-01	-5.43E-04
	A_2	5.68E-05	4.62E-06	1.81E-04	5.54E-07	1.44E-07	-2.19E-01
	A_3	-4.80E-05	-5.38E-06	4.46E-04	-2.22E-09	-1.68E-07	4.12E-06

Patient N°		α_1	α_2	α_3	A_1	A_2	A_3
51	α_1	3.51E-02	4.96E-01	**9.10E-01**	1.12E-01	2.72E-01	**9.40E-01**
	α_2	2.49E-01	7.17E+00	3.36E-01	5.42E-01	9.17E-01	3.57E-01
	α_3	4.70E-03	2.48E-02	7.59E-04	-2.45E-01	1.67E-01	9.91E-01
	A_1	3.00E-05	2.08E-03	-9.69E-06	2.06E-06	3.95E-01	-2.05E-01
	A_2	1.84E-03	8.91E-02	1.67E-04	2.06E-05	1.31E-03	1.79E-01
	A_3	3.20E-04	1.74E-03	4.96E-05	-5.35E-07	1.18E-05	3.31E-06
52	α_1	2.80E-03	8.94E-01	3.14E-01	9.89E-01	2.50E-03	1.58E-01
	α_2	1.76E-02	1.38E-01	4.84E-01	9.27E-01	3.84E-01	2.67E-01
	α_3	5.24E-02	5.66E-01	9.93E+00	3.35E-01	6.30E-01	9.07E-01
	A_1	8.94E-05	5.88E-04	1.81E-03	2.92E-06	4.70E-02	1.70E-01
	A_2	2.30E-07	2.48E-04	3.45E-03	1.40E-07	3.02E-06	4.25E-01
	A_3	4.41E-04	5.23E-03	1.51E-01	1.53E-05	3.90E-05	2.79E-03

Appendix 3 – Parameter estimates of all 52 pretransplant candidates using **STS**.

Table A.3-1a – One-Compartment Model (STS):

sujet	α_1	A_1	RMS	AIC
1	1.976E-01	5.199E-03	1.74E-01	-7.17E+00
2	2.256E-01	1.032E-02	4.45E-01	7.51E+00
3	2.682E-01	1.002E-02	5.88E-01	2.81E+01
4	2.714E-01	9.626E-03	5.43E-01	1.93E+01
5	2.409E-01	1.072E-02	4.01E-01	1.46E+01
6	2.517E-01	7.604E-03	6.27E-01	1.29E+01
7	2.791E-01	1.106E-02	6.77E-01	2.70E+01
8	2.946E-01	7.948E-03	7.24E-01	2.60E+01
9	2.568E-01	1.091E-02	4.65E-01	1.64E+01
10	2.581E-01	1.053E-02	5.52E-01	2.51E+01
11	2.938E-01	1.104E-02	7.42E-01	1.80E+01
12	3.868E-01	1.311E-02	4.76E-01	1.66E+01
13	2.928E-01	1.420E-02	4.44E-01	2.02E+01
14	2.803E-01	8.002E-03	7.23E-01	2.06E+01
15	2.705E-01	1.325E-02	4.69E-01	1.31E+01
16	5.284E-01	9.914E-03	6.35E-01	1.33E+01
17	2.695E-01	1.336E-02	6.38E-01	3.10E+01
18	1.451E-01	1.828E-02	4.21E-01	1.38E+01
19	2.492E-01	7.185E-03	6.54E-01	6.10E+00
20	3.164E-01	1.030E-02	6.93E-01	1.79E+01
21	2.355E-01	7.380E-03	4.21E-01	1.32E+01
22	2.880E-01	1.601E-02	6.96E-01	3.51E+01
23	2.474E-01	1.135E-02	4.32E-01	2.30E+01
24	2.763E-01	8.865E-03	6.08E-01	2.13E+01
25	1.918E-01	8.612E-03	4.91E-01	1.71E+01
26	4.599E-01	9.306E-03	3.28E-01	4.95E-01
27	3.056E-01	1.559E-02	5.21E-01	2.38E+01
28	3.355E-01	1.670E-02	9.48E-01	2.63E+01
29	2.444E-01	9.400E-03	5.29E-01	1.92E+01
30	2.077E-01	1.432E-02	5.42E-01	3.37E+01
31	2.749E-01	9.112E-03	5.02E-01	2.33E+01
32	2.662E-01	8.781E-03	6.85E-01	1.04E+01
33	2.711E-01	8.647E-03	5.60E-01	1.76E+01
34	2.543E-01	7.040E-03	5.66E-01	1.49E+01
35	2.422E-01	7.677E-03	5.91E-01	1.68E+01
36	2.752E-01	1.280E-02	3.20E-01	1.36E+01
37	2.806E-01	1.539E-02	6.71E-01	2.83E+01
38	3.043E-01	1.170E-02	6.41E-01	2.66E+01
39	2.832E-01	1.170E-02	6.26E-01	2.22E+01
40	2.674E-01	1.065E-02	5.03E-01	2.01E+01
41	2.489E-01	7.533E-03	5.12E-01	1.67E+01
42	2.787E-01	9.971E-03	7.79E-01	2.75E+01
43	2.869E-01	1.457E-02	5.16E-01	2.36E+01
44	2.833E-01	1.094E-02	6.16E-01	2.72E+01
45	2.826E-01	1.882E-02	6.00E-01	2.51E+01
46	2.716E-01	9.740E-03	5.38E-01	2.39E+01
47	3.006E-01	9.219E-03	4.48E-01	1.50E+01
48	2.950E-01	1.358E-02	7.37E-01	3.29E+01
49	2.560E-01	1.321E-02	5.68E-01	2.26E+01
50	2.542E-01	9.429E-03	3.92E-01	1.52E+01
51	2.418E-01	1.662E-02	4.03E-01	2.65E+01
52	2.919E-01	1.199E-02	6.97E-01	3.05E+01

Table A.3-1b – Population Result from one-compartment model **(STS)**.

	α_1	A_1
Mean Parameters	2.765E-01	1.114E-02
CV %	20.77%	27.60%

Covariance-Correlation Matrix

	α_1	**A 1**
α_1	**3.300E-03**	0.034
A_1	6.0764E-06	**9.450E-06**

The macroconstants are the model parameters: $\mathbf{A_i}$ is the intercept and α_i the slope of the **i**-*th* order phase. The covariance-correlation matrix is devided into three parts: in the upper-right triangular part are the interindividual correlations between two parameters. They were computed from covariances that are written in the lower-left triangular part. The variances of each parameter (squared standard deviation) are presented on the diagonal of the matrix.

Appendix 3 – Parameter estimates of all 52 pretransplant candidates using **STS**.

Table A.3-2a – Two-Compartment Model (STS):

sujet	α_1	α_2	A_1	A_2	RMS	AIC
1	3.751E-1	2.911E-2	5.982E-3	6.158E-4	7.69E-02	-22.588
2	1.266E-1	1.599E+0	3.838E-3	2.384E-2	1.20E-01	-17.484
3	1.128E-1	1.662E+0	2.071E-3	2.985E-2	1.60E-01	-0.427
4	1.308E-1	1.783E+0	2.275E-3	3.024E-2	1.12E-01	-18.505
5	1.621E-1	2.292E+0	5.172E-3	2.895E-2	8.13E-02	-15.307
6	1.263E-1	2.750E+0	2.294E-3	3.470E-2	1.48E-01	-20.548
7	1.777E-1	4.232E+0	4.544E-3	6.721E-2	7.38E-02	1.838
8	1.771E-1	4.548E+0	2.861E-3	5.643E-2	3.28E-02	-5.758
9	1.519E-1	1.885E+0	3.898E-3	3.001E-2	1.11E-01	-15.832
10	1.486E-1	2.475E+0	3.769E-3	3.847E-2	1.11E-01	-6.312
11	1.810E-1	3.526E+0	3.981E-3	5.807E-2	1.39E-01	-3.710
12	2.415E-1	8.932E+0	6.673E-3	1.255E-1	1.14E-01	-11.125
13	2.306E-1	5.609E+0	8.374E-3	8.353E-2	1.69E-01	-1.493
14	1.594E-1	2.820E+0	2.588E-3	3.462E-2	5.84E-02	-3.164
15	1.736E-1	2.076E+0	5.174E-3	3.775E-2	1.34E-01	-14.631
16	3.364E-1	**1.000E+1**	4.138E-3	1.174E-1	6.81E-02	-12.325
17	1.648E-1	3.196E+0	4.911E-3	6.363E-2	2.72E-01	10.975
18	**1.000E+0**	3.656E-2	2.739E-2	6.691E-3	5.05E-02	-0.028
19	1.149E-1	2.233E+0	1.902E-3	2.734E-2	1.60E-01	-22.749
20	2.030E-1	4.523E+0	3.819E-3	7.060E-2	6.17E-02	-14.605
21	1.635E-1	2.874E+0	3.861E-3	2.365E-2	9.47E-02	-12.738
22	1.794E-1	5.603E+0	5.952E-3	1.550E-1	2.26E-01	6.691
23	1.758E-1	2.875E+0	5.855E-3	3.725E-2	1.23E-01	0.140
24	1.911E-1	6.288E+0	4.219E-3	7.954E-2	1.66E-01	-8.021
25	1.262E-1	4.873E+0	4.648E-3	8.355E-2	2.22E-01	0.992
26	**1.000E-6**	9.006E-1	4.433E-4	1.426E-2	2.32E-01	-7.304
27	**1.000E+0**	1.697E-1	2.234E-2	4.357E-3	5.44E-02	15.099
28	2.231E-1	7.193E+0	5.937E-3	3.332E-1	2.78E-01	10.850
29	1.785E-1	**1.000E+1**	5.502E-3	1.164E-1	1.41E-01	-8.938
30	**1.000E+0**	6.174E-2	2.753E-2	3.138E-3	1.79E-01	24.458
31	1.719E-1	2.242E+0	3.356E-3	2.931E-2	1.08E-01	-8.955
32	1.343E-1	3.165E+0	2.496E-3	4.840E-2	1.49E-01	-25.501
33	1.854E-1	3.668E+0	4.052E-3	4.015E-2	9.13E-02	-6.768
34	1.441E-1	2.552E+0	2.510E-3	2.675E-2	8.02E-02	-17.965
35	1.363E-1	2.408E+0	2.843E-3	2.676E-2	5.97E-02	-7.751
36	2.120E-1	3.012E+0	7.149E-3	4.163E-2	6.63E-02	-3.835
37	1.811E-1	4.652E+0	6.471E-3	1.031E-1	9.65E-02	0.907
38	1.790E-1	2.524E+0	3.429E-3	4.705E-2	1.56E-01	-2.161
39	1.574E-1	2.468E+0	3.513E-3	4.595E-2	7.15E-02	-10.884
40	1.736E-1	2.775E+0	4.411E-3	4.050E-2	1.26E-01	-10.796
41	1.616E-1	3.168E+0	3.392E-3	3.137E-2	1.16E-01	-12.710
42	1.682E-1	5.933E+0	3.819E-3	9.612E-2	1.21E-01	-1.269
43	1.651E-1	1.521E+0	4.171E-3	3.494E-2	2.28E-01	3.669
44	1.651E-1	2.409E+0	3.623E-3	3.898E-2	1.22E-01	3.368
45	1.777E-1	2.895E+0	7.079E-3	7.779E-2	1.09E-01	-2.382
46	1.691E-1	2.374E+0	3.571E-3	3.380E-2	1.99E-01	-0.029
47	2.278E-1	3.151E+0	4.635E-3	3.421E-2	2.06E-01	-3.807
48	1.969E-1	6.154E+0	5.789E-3	1.259E-1	1.29E-01	7.588
49	1.730E-1	6.009E+0	6.242E-3	1.200E-1	1.82E-01	-4.828
50	1.560E-1	1.578E+0	3.562E-3	2.079E-2	4.73E-02	-26.241
51	**1.000E+0**	1.308E-1	2.494E-2	5.489E-3	1.17E-01	11.714
52	1.906E-1	5.981E+0	5.024E-3	1.086E-1	1.08E-01	-0.829

Table A.3-2b – Population Result from two-compartment model **(STS)**.

	α_1	α_2	A_1	A_2
Mean Parameters	2.377E-01	3.420E+00	5.923E-03	5.757E-02
CV%	96.00%	68.30%	100.41%	93.27%

Covariance-Correlation Matrix

	α_1	α_2	A1	A2
α_1	**5.205E-02**	-0.321	0.971	-0.215
α_2	-1.711E-01	**5.454E+00**	-0.298	0.762
A_1	1.318E-03	-4.141E-03	**3.538E-05**	-0.160
A_2	-2.632E-03	9.551E-02	-5.118E-05	**2.883E-03**

The macroconstants are the model parameters: A_i is the intercept and α_i the slope of the **i-*th*** order phase. The covariance-correlation matrix is devided into three parts: in the upper-right triangular part are the interindividual correlations between two parameters. They were computed from covariances that are written in the lower-left triangular part. The variances of each parameter (squared standard deviation) are presented on the diagonal of the matrix.

Appendix 3 – Parameter estimates of all 52 pretransplant candidates using **STS**.

Table A.3-3a – Three-Compartment Model (STS):

sujet	α_1	α_2	α_3	A_1	A_2	A_3	RMS	AIC
1	3.202E-1	**1.000E-6**	7.352E+0	5.503E-3	3.787E-4	1.264E-2	6.22E-02	-26.902
2	2.034E-2	**1.000E+1**	5.001E-1	1.018E-3	7.882E-2	1.041E-2	5.14E-02	-39.390
3	1.037E-1	1.243E+0	**1.000E+1**	1.870E-3	1.832E-2	5.504E-2	1.96E-01	1.072
4	9.085E-2	4.141E+0	8.369E-1	1.428E-3	4.045E-2	9.525E-3	4.64E-02	-32.177
5	1.385E-1	**1.000E+1**	9.050E-1	3.983E-3	6.907E-2	9.623E-3	5.87E-02	-24.241
6	9.814E-2	1.035E+0	8.900E+0	1.690E-3	7.625E-3	7.939E-2	9.51E-02	-32.435
7	6.918E-1	1.235E-1	**1.000E+1**	7.213E-3	2.449E-3	1.332E-1	6.90E-02	1.441
8	1.620E-1	1.493E+0	**1.000E+1**	2.457E-3	7.246E-3	9.535E-2	2.09E-02	-5.355
9	1.162E-1	8.425E-1	**1.000E+1**	2.585E-3	1.190E-2	8.089E-2	1.62E-01	-43.923
10	1.240E-1	9.918E-1	**1.000E+1**	2.881E-3	1.054E-2	9.597E-2	7.21E-02	-15.522
11	7.019E-1	1.110E-1	**1.000E+1**	8.484E-3	1.778E-3	1.309E-1	1.70E-01	-3.911
12	**1.000E-6**	4.614E-1	**1.000E+1**	1.011E-3	7.632E-3	1.276E-1	9.50E-02	-12.959
13	8.244E-1	1.984E-1	**1.000E+1**	7.558E-3	5.940E-3	1.208E-1	1.68E-01	-1.948
14	6.793E-1	8.390E-2	**1.000E+1**	6.554E-3	1.081E-3	9.714E-2	4.18E-02	-7.664
15	9.292E-2	5.815E-1	8.986E+0	1.922E-3	1.197E-2	1.017E-1	8.90E-02	-25.633
16	**1.000E-6**	4.523E-1	**1.000E+1**	2.213E-4	4.582E-3	1.123E-1	5.20E-02	-9.340
17	6.361E-2	6.494E-1	**1.000E+1**	1.494E-3	1.210E-2	1.480E-1	2.57E-01	7.686
18	6.865E-2	1.884E+0	**1.000E+1**	9.515E-3	2.348E-2	1.124E-1	5.98E-02	-17.489
19	4.195E-2	7.258E-1	8.351E+0	8.161E-4	6.997E-3	7.161E-2	8.30E-02	-39.157
20	1.829E-1	**1.000E+1**	1.338E+0	3.101E-3	1.207E-1	8.705E-3	1.90E-01	-14.955
21	7.175E-1	1.298E-1	**1.000E+1**	5.112E-3	2.648E-3	5.761E-2	5.69E-02	-21.777
22	1.265E-1	7.949E-1	**1.000E+1**	3.344E-3	9.801E-3	2.387E-1	1.75E-01	0.631
23	**1.000E-6**	3.556E-1	5.804E+0	5.643E-4	8.851E-3	5.941E-2	9.62E-02	-7.370
24	**1.000E-6**	3.736E-1	**1.000E+1**	3.502E-4	5.990E-3	1.155E-1	5.56E-02	-22.186
25	4.408E-1	**1.000E-6**	**1.000E+1**	7.904E-3	9.876E-4	1.492E-1	1.38E-01	-8.802
26	**1.000E-6**	8.624E-1	3.999E+0	4.372E-4	1.303E-2	4.107E-3	2.82E-01	-3.380
27	2.189E-1	2.189E-1	6.281E+0	3.460E-3	4.218E-3	1.286E-1	5.69E-02	-18.817
28	1.122E-1	5.685E-1	**1.000E+1**	1.592E-3	9.475E-3	4.433E-1	2.30E-01	12.265
29	1.145E-1	4.697E-1	**1.000E+1**	2.596E-3	4.529E-3	1.044E-1	8.92E-02	-11.611
30	7.617E-2	7.285E-1	**1.000E+1**	3.781E-3	1.046E-2	2.172E-1	1.03E-01	18.600
31	8.694E-2	5.433E-1	4.813E+0	1.182E-3	6.751E-3	4.403E-2	4.16E-02	-24.070
32	1.006E-1	**1.000E+1**	1.017E+0	1.718E-3	1.132E-1	8.560E-3	8.12E-02	-40.324
33	5.621E-2	**1.000E+1**	4.203E-1	7.705E-4	9.249E-2	6.288E-3	4.43E-02	-9.713
34	1.208E-1	1.045E+0	**1.000E+1**	1.939E-3	7.529E-3	6.246E-2	6.47E-02	-22.335
35	6.603E-2	6.068E-1	**1.000E+1**	1.234E-3	6.580E-3	7.788E-2	4.13E-02	-12.128
36	2.118E-1	4.206E+0	2.826E+0	7.139E-3	1.005E-2	3.246E-2	8.12E-02	0.164
37	6.989E-2	4.347E-1	**1.000E+1**	1.588E-3	9.420E-3	2.011E-1	3.53E-02	-0.515
38	6.978E-1	8.358E-2	8.218E+0	1.085E-2	1.104E-3	1.097E-1	6.70E-02	-17.983
39	1.242E-1	1.018E+0	**1.000E+1**	2.411E-3	1.354E-2	1.093E-1	4.42E-02	-14.813
40	1.184E-1	6.879E-1	8.801E+0	2.314E-3	8.710E-3	8.959E-2	7.90E-02	-21.919
41	1.029E-1	6.278E-1	**1.000E+1**	1.711E-3	5.695E-3	7.438E-2	3.72E-02	-26.953
42	9.805E-2	**1.000E+1**	6.037E-1	1.712E-3	1.492E-1	5.344E-3	8.04E-02	-3.499
43	5.918E-2	5.541E-1	**1.000E+1**	1.127E-3	1.349E-2	1.513E-1	1.02E-01	-11.418
44	1.367E-1	1.098E+0	**1.000E+1**	2.673E-3	1.294E-2	9.075E-2	1.49E-01	3.653
45	6.955E-1	1.118E-1	**1.000E+1**	1.558E-2	3.303E-3	2.014E-1	4.03E-02	-12.243
46	6.310E-3	**1.000E+1**	4.934E-1	4.642E-4	1.033E-1	8.903E-3	6.48E-02	-25.699
47	9.956E-2	5.003E-1	**1.000E+1**	9.367E-4	7.736E-3	8.353E-2	1.16E-01	-15.406
48	3.291E-2	4.350E-1	**1.000E+1**	7.441E-4	8.715E-3	1.899E-1	6.60E-02	2.765
49	1.263E-2	3.989E-1	**1.000E+1**	8.200E-4	9.212E-3	1.778E-1	7.51E-02	-22.131
50	6.684E-1	1.242E-1	3.010E+0	6.792E-3	2.431E-3	2.206E-2	2.44E-02	-38.749
51	5.628E-1	**1.000E+1**	1.260E-1	1.044E-2	1.477E-1	5.106E-3	7.51E-02	-11.196
52	1.317E-1	6.023E-1	**1.000E+1**	2.553E-3	5.967E-3	1.656E-1	3.72E-02	-3.912

Table A.3-3b – Population Result from three-compartment model **(STS)**.

	α_1	α_2	α_3	A_1	A_2	A_3
Mean Parameters	2.036E-01	2.165E+00	7.569E+00	3.407E-03	2.408E-02	9.709E-02
CV%	118.00%	159.99%	48.40%	96.80%	161.10%	81.38%

Covariance-Correlation Matrix

	α_1	α_2	α_3	A_1	A_2	A_3
α_1	**5.772E-02**	-0.169	0.087	0.816	-0.126	0.019
α_2	-1.409E-01	**1.200E+01**	-0.858	-0.084	0.948	-0.528
α_3	7.701E-02	-1.089E+01	**1.342E+01**	0.055	-0.800	0.650
A_1	6.463E-04	-9.634E-04	6.648E-04	**1.087E-05**	-0.058	0.076
A_2	-1.175E-03	1.274E-01	-1.136E-01	-7.471E-06	**1.505E-03**	-0.478
A_3	3.638E-04	-1.445E-01	1.880E-01	1.982E-05	-1.466E-03	**6.242E-03**

The macroconstants are the model parameters: A_i is the intercept and α_i the slope of the **i-*th*** order phase. The covariance-correlation matrix is devided into three parts: in the upper-right triangular part are the interindividual correlations between two parameters. They were computed from covariances that are written in the lower-left triangular part. The variances of each parameter (squared standard deviation) are presented on the diagonal of the matrix.

Appendix 1 – Calculated (derived) parameters for all 52 pretransplant candidates **(STS)**.

Table A.3-4 – Two-Compartment Model :

[* indicates the poor fit for candidates due to measurement error]

sujet	CL	V_1	Q	V_{ss}	V_2	$t\ ½_{\beta}$	MRT
1*	26.95	151.57	25.01	558.80	407.23	23.8	20.73
2	22.11	36.13	28.28	121.66	85.50	5.5	5.50
3	27.51	31.33	21.41	131.58	100.50	6.2	4.78
4	29.11	30.76	22.17	120.77	90.00	5.3	4.15
5	22.45	29.31	35.26	102.02	72.70	4.3	4.54
6	32.49	27.03	37.45	156.56	129.60	5.5	4.82
7	24.12	13.94	31.29	85.92	72.00	3.9	3.56
8	35.01	16.87	38.15	115.18	98.30	3.9	3.29
9	24.05	29.49	25.67	102.57	73.10	4.6	4.26
10	24.45	23.67	29.23	105.79	82.10	4.7	4.33
11	26.00	16.12	27.37	85.29	69.20	3.8	3.28
12	23.99	7.57	40.29	66.77	59.20	2.9	2.78
13	19.53	10.88	36.17	61.08	50.20	3.0	3.13
14	35.07	26.88	35.75	130.66	103.80	4.3	3.73
15	20.84	23.30	22.19	78.35	55.10	4.0	3.76
16	41.61	8.23	37.98	65.31	57.10	2.1	1.57
17	20.12	14.59	23.34	75.69	61.10	4.2	3.76
18*	4.75	29.34	19.04	113.70	84.40	0.7	23.92
19	34.73	34.20	36.93	180.37	146.10	6.0	5.19
20	29.05	13.44	28.75	81.13	67.70	3.4	2.79
21	31.40	36.35	59.24	145.27	108.90	4.2	4.63
22	16.44	6.21	17.12	51.30	45.10	3.9	3.12
23	21.62	23.20	36.58	90.64	67.40	3.9	4.19
24	28.80	11.94	42.61	97.47	85.50	3.6	3.39
25	18.53	11.34	33.89	101.37	90.00	5.5	5.47
26*	0.00	68.01	59.50	2255.80	2187.79	-	999964.28
27	20.83	37.46	11.56	75.33	37.90	0.7	3.62
28*	13.71	2.95	7.14	23.64	20.69	0.1	1.72
29	23.55	8.20	54.83	96.42	37.90	3.9	4.09
30	12.76	32.61	16.72	138.57	106.00	0.7	10.86
31	30.67	30.61	31.44	112.41	88.20	4.0	3.66
32	29.52	19.65	29.75	124.76	106.00	5.2	4.23
33	30.49	22.62	45.27	112.34	81.80	3.7	3.68
34	35.84	34.18	44.32	160.54	105.10	4.8	4.48
35	31.28	33.78	42.69	154.24	89.70	5.1	4.93
36	21.03	20.50	32.30	72.41	126.40	3.3	3.44
37	17.27	9.13	22.78	60.28	120.40	3.8	3.49
38	26.46	19.81	20.39	80.07	51.90	3.9	3.03
39	24.43	20.22	22.15	89.13	51.20	4.4	3.65
40	25.00	22.27	31.11	94.75	60.30	4.0	3.79
41	32.37	28.77	50.33	139.36	68.90	4.3	4.31
42	25.70	10.01	31.47	91.00	72.50	4.1	3.54
43	20.73	25.57	14.46	72.28	110.60	4.2	3.49
44	26.23	23.47	25.83	96.05	81.00	4.2	3.66
45	14.99	11.78	16.45	52.47	46.70	3.9	3.50
46	28.28	26.76	29.60	104.70	72.60	4.1	3.70
47	32.05	25.74	40.08	95.28	40.70	3.0	2.97
48	20.06	7.59	24.67	61.42	77.90	3.5	3.06
49	17.84	7.92	27.47	67.45	69.50	4.0	3.78
50	27.77	41.06	28.48	119.37	53.80	4.4	4.30
51	14.95	32.86	12.76	77.27	59.50	0.7	5.17
52	22.46	8.80	27.92	71.29	78.30	3.6	3.17

Appendix 4

Table A.4 – Population characteristics for one- and two-compartment models using **NONMEM**.

Parameters				
	CL	**V1**	**Q**	**Vss**
1-cmt	39.60	77.50		
SE	1.72	3.24		
% CV	***30.22%***	***20.90%***		
2-cmt	23.00	17.40	32.50	90.80
SE	1.37	1.36	1.71	3.72
% CV	***37.82%***	***16.94%***	***16.19%***	***24.70%***
Interindividual Variability				
	η_{CL}	η_{V1}	η_{Q}	η_{Vss}
1-cmt	9.13E-02	4.37E-02		
SE	2.71E-02	1.36E-02		
2-cmt	1.43E-01	2.87E-02	2.62E-02	6.10E-02
SE	7.47E-02	1.15E-02	1.56E-02	1.56E-02
Intraindividual Variability				
	ε_1	ε_2		
1-cmt	3.17E-02	2.08E-02		
SE	3.82E-03	2.41E-03		
2-cmt	3.06E-02			
SE	3.81E-03			
Objective Function				
	CL			
1-cmt	-730.931			
2-cmt	-1084.281			

1-cmt : One-compartment Model;
2-cmt : Two-compartment Model;
SE : Standard Error of Estimate;
%CV : Percent Coefficient of Variation;
OF : Minimum Objective Function.