Pharmacokinetic population model for ganciclovir in solid organ transplant recipients administered valganciclovir

Levin Løssfelt

Department of Pharmaceutical Biosciences

School of Pharmacy

Faculty of Mathematics and Natural Science

UNIVERSITY OF OSLO

May 2009

Acknowledgement

I would like to address thanks to Anders Åsberg, for his guidance and vast knowledge of everything pharmacokinetic. Without him this thesis would not have been possible.

I would also like to thank Lê Thanh Trúc Vân for his brilliant thesis, which provided a guiding light from the beginning to the end of my work on this thesis, as well as advice when the model seemed beyond salvation.

In addition, thanks to Trủởňg-Sỏn Mạnh Trần for suffering the whims of NONMEM along with me.

And finally, my family and Caroline, for unwavering and merciless belief in me even when I had none myself.

Oslo, May 2009

Levin Løssfelt

Table of Contents

ACKNOWLEDGEMENT **2**

TABLE OF CONTENTS **3**

ABBREVIATIONS **6**

ABSTRACT **8**

1. BACKGROUND **10**

1.1 PHARMACOKINETICS 10

1.1.1 Introduction *10*

1.1.2 The compartment concept *11*

1.2 POPULATION PHARMACOKINETICS 12

1.2.1 Naïve pooled data approach *14*

1.2.2 The two-stage approach *15*

1.2.3 Nonlinear mixed-effects modeling *15*

1.2.4 Sampling designs in population pharmacokinetic investigations *16*

1.2.5 Population PK modeling using NONMEM *17*

1.3 CYTOMEGALOVIRUS 21

1.4 VALGANCICLOVIR 22

1.4.1 History of valganciclovir *22*

1.4.2 Applications and mechanism of action *22*

1.4.3 Adverse effects and drug interactions *23*

1.4.4 ADME of valganciclovir *23*

1.4.5 Population PK models of valganciclovir in the literature *24*

1.5 AIM OF THE STUDY 25

2. METHODS & MATERIALS 26

2.1 DESIGN OF VICTOR STUDY 26

2.2 POPULATION FOR POPULATION PHARMACOKINETICS 27

2.3 BUILDING THE PK/PD MODEL 28

2.4 ANALYZING FOR COVARIATES 29

2.5 CRITERIA FOR CHOICE OF MODEL 31

2.6 VALIDATING THE MODEL 31

2.6.1 K-fold cross-validation 31

2.6.2 Confidence interval 33

3. RESULTS 34

3.1 MODEL BUILDING RESULTS 34

3.1.1 Graphical Comparison of Models 36

3.2 ANALYZING FOR COVARIATES 37

3.2.1 Covariate analysis based on visual graphical inspection 38

3.3 THE FINAL MODEL FOR VALGANCICLOVIR 43

3.4 VALIDATING THE FINAL MODEL 47

3.4.1 Internal validation 47

3.4.2 Examining the predictive performance of the population model 48

3.4.3 Confidence interval of population parameters 49

4. DISCUSSION 51

4.1 MODEL BUILDING 51

4.1.1 1-compartment, first-order absorption 52

4.1.2 2-compartment, first-order absorption 53

4.1.3 3-compartment, first-order absorption 54

4.2 ANALYZING FOR COVARIATES 55

4.3 THE FINAL MODEL FOR VALGANCICLOVIR....57

4.4 VALIDATING THE MODEL....58

5. CONCLUSIONS....63

6. REFERENCES....64

7. APPENDIX....66

7.1 COCKCROFT-GAULT EQUATION....66

7.2 PARTIAL INPUT-FILE FOR NONMEM....66

7.3 CONTROL FILES FOR NONMEM....69

7.3.1 1-compartment with first-order absorption....69

7.3.2 2-compartment with first-order absorption....71

7.3.3 3-compartment with first-order absorption....72

7.3.4 Jackknife....74

7.3.5 Scatterplot of WRES vs. PRED....77

7.3.6 Scatterplot of WRES vs. IPRE....77

Abbreviations

-2LL – -2 log likelihood

ADME – Administration Distribution Metabolism Elimination

ALAG – Absorption lag time

CI – Confidence interval

CL – Clearance

CMV – Cytomegalovirus

CONC - Concentration

CRCL – Creatinine clearance

DNA – Deoxyribonucleic acid

F – Bioavailability

FO – First-order

FOCE – First-order conditional estimation

FDA – Federal drug administration (USA)

GOF – Goodness of fit

HCMV – Human cytomegalovirus

IPRE – Individual predictions

KA – Absorption rate constant

ME – Mean prediction error

MSE – Mean squared prediction error

NONMEM – Nonlinear mixed effects modeling

NPD - Naïve pooled data

OFV – Objective function value

PD – Pharmacodynamics

PK – Pharmacokinetics

PRED – Population predictions

Q3 – Intercompartmental clearance from central to peripheral 1

Q4 – Intercompartmental clearance from central to peripheral 2

RMSE – Root mean squared error

SEX - Gender

SOT – Solid organ transplant

SPE – Standardized prediction error

Tx - Transplantation

V2 – Central volume

V3 – Peripheral volume 1

V4 – Peripheral volume 2

VICTOR – Valcyte in CMV-disease treatment of solid organ recipients

WGT – Weight

WRES – Weighted residuals

Abstract

Background:
Cytomegalovirus (CMV) is the most common pathogen responsible for morbidity and mortality in immunosuppressed individuals. One population at high risk is solid organ transplant (SOT) recipients.

Intravenous ganciclovir is the gold standard for treatment of CMV disease. Oral valganciclovir is a prodrug of ganciclovir, with an almost 60% oral bioavailability, and has recently been shown to be non-inferior to intravenous ganciclovir in the VICTOR study.

The purpose of this thesis was to develop a pharmacokinetic population model to describe the ganciclovir plasma concentration observations in the patient population of the VICTOR study, after receiving oral valganciclovir for treatment of CMV disease. Additionally the model was to be validated, in order to make it clinically applicable.

Methods:
Of the 321 patients who were included in the VICTOR study 164 were randomized into the valganciclovir arm, and in 108 was the plasma concentration of ganciclovir measured. The development of the population pharmacokinetic model was based on these 108 patients. The data contained information of demographical, physiological and pathophysiological nature, as well as the measured drug concentrations. The model was constructed by use of the NONMEM version VI computer program, as well as PREDPP and R for subroutines and graphs, respectively.

Results:

A 2-compartment model with first-order absorption was found to be the overall best model for the data set. The ADVAN4 routine was used in combination with the TRANS4 subroutine. After a forward inclusion and backwards deletion procedure creatinine clearance and gender were significant covariates in the model. The average

(± SD) parameter estimates for the final model were CL/F = 12±0.05 L/h, V2/F = 59.4±2.76 L, Q/F = 7.21±0.20 L/h, V3/F = 304±7.79 L.
The validation procedure employed a K-fold cross-validation, as recommended by the FDA, and the Jackknife technique. The model appeared stable, but susceptible to variations in the data-set (Central volume had a range of 57.1 – 93.8 L, while peripheral volume had a range of 270 – 357 L.

Conclusions:

The model fulfills the goal of predicting ganciclovir plasma concentrations to a satisfying degree, especially for individually predicted concentrations, although it has room for improvement. It is a good basis for the further development of a PK/PD model that compares ganciclovir pharmacokinetics with both therapeutic effects and adverse events.

1. Background

1.1 Pharmacokinetics

1.1.1 Introduction

Pharmacokinetics (PK) is the study of a drugs life in the body. From concentration measurements gathered from different samples, a model is constructed which describes how the body "treats" the drug, i.e. how it is metabolized and eliminated. The counterpart to pharmacokinetics is pharmacodynamics (PD), which in short is the study of the effects a drug has on the body [1].

Every drug has a so-called "therapeutic window", illustrated in Figure 1. What this term means is that for every drug there is a certain range of concentrations that promise successful treatment, while at the same time have a sufficiently low chance for adverse events. If the concentration in the patient is too low, the treatment will fail. If the concentration is too high, the risk of adverse events is unacceptably large. This window exists for the majority of drugs, but for some it is quite wide, while for others extremely narrow. It is in particular for the ones with the narrow windows that pharmacokinetic monitoring is of benefit, as it is extra important to keep plasma concentrations within the acceptable range of concentrations [1].

Figure 1: Interplay between drug-concentration and ineffective therapy, therapeutic effect and adverse events.

1.1.2 The compartment concept

The human body is an incredibly complex system. Trying to include every element in the body that might influence a drug's concentration and disposition in a model would quickly spiral out of both control and usefulness.
It is interesting, therefore, that very simple models have been found quite useful in representing and predicting drug concentrations in the body.

Figure 2 is an example of a 2-compartment model. A dose is given which is absorbed into the central compartment. From there the drug is either eliminated or distributed to the peripheral compartment. There is also transport back from the peripheral compartment and into the central one once distribution has started. Obviously, the body contains no such distinct compartments, but considering that the model predicts drug measurements in the plasma to an acceptable degree it is satisfactory. Furthermore, the predictions derived from using it, and its variations, are accurate.
It is also possible to add or subtract compartments to get models that better describe the drug at hand. Both 1, 2 and 3-compartmental models are quite common [1, 2].

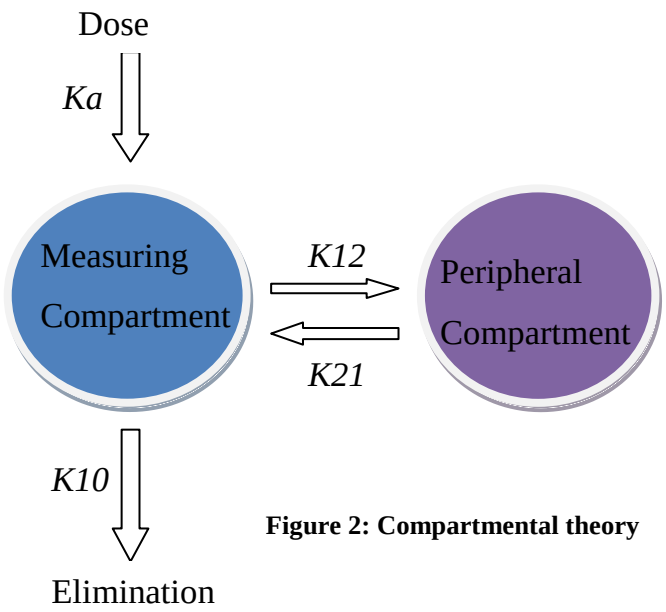


Figure 2: Compartmental theory

It is also useful to differentiate between transfer and chemical compartments for some drugs, but this does not include ganciclovir. A transfer compartment can be thought of as a "place" in the body. The blood, for instance, is such a place. When substances differ chemically from each other they are said to be in different chemical compartments. A metabolite is chemically different from its parent drug, and they are therefore in different chemical compartments [1].

Once again, it is not normally possible to measure the concentrations of drugs or metabolites at every location, or compartment, in the body. Usually one only has plasma-samples to work with, and has to extrapolate concentrations in other locations [1].

The site of effect of ganciclovir is within virus-infected cells. CMV usually lies dormant in endothelial cells, but can infect many different organs during a primary infection or reactivation.
Ideally, one would measure the concentration of active drug at the site of action. Regretfully for science, if not for the patient, this is not usually feasible. It is often too difficult or too risky, if not downright terminal, to take samples from the site of effect. Therefore one usually measures the concentration of the drug in the blood, and correlates this concentration with the success or failure of treatment.
Another bodily fluid often used for sampling is urine [1].

1.2 Population pharmacokinetics

Population pharmacokinetics can be defined as the study of variability in plasma drug concentrations in a population representative of the intended target group for the drug. This type of study focuses on certain demographical, pathophysiological, physiological, therapeutical and other kinds of features that vary between individuals, and which are known to possibly be responsible for the alterations in drug concentrations inter-individually. For example, obese patients with their increased body-mass will in all probability have a higher distribution volume. A given amount of drug will therefore give a lower apparent concentration than in a patient of more normal weight. The reason for this kind of study is that if we find the sources of the variability in concentration for a certain drug, and are able to measure them, we can modify the dosage given to each patient, ensuring optimal therapeutic concentration [3, 4].

Another positive side of the use of population pharmacokinetics is that it allows one to gain quite extensive and integrated information on pharmacokinetics from sparse data. It is also usable on dense data, and even mixed sparse/dense data. This makes it possible to analyze and gain information from studies of unbalanced design, and also some that would otherwise have been excluded because they do not normally lend themselves to pharmacokinetic analysis. It is also quite useful in situations where the drug in question has a very narrow window of therapeutic effect, and the dose will have to be adjusted individually [4].

Even though the term was not originally coined with this in mind, population pharmacokinetics has come to mean the design, execution and analysis of studies with limited pharmacokinetic data [3].
Traditionally, pharmacokinetic studies have had very strict exclusion criteria, wanting groups of subjects that were as homogenized as possible. This was because the main focus was on finding the mean drug concentration, and any inter-individual variability was seen as something that must be avoided. The study designs and control schemes were also similarly strict. The variability in concentration is of high importance in the clinical use of drugs, and as these studies suppressed them it was not possible to find the factors responsible for the differences, nor measure them.

It is usual to divide variability into inter-individual and residual variability. The inter-individual variability is a biological imperative, and stems from the simple fact that every person is biologically different from practically all others. This leads in most cases to variations in plasma drug concentrations which can be quite large.
Residual variability is a combination of sources for variation, such as intra-individual differences, inter-occasional differences, and errors made in measurement, dosing and modeling [1, 4, 5]. From this one can surmise that some of the more important differences between population pharmacokinetics (PK) and traditional pharmacokinetics are the collection of individual data relevant to the measuring of inter-individual variability, the measuring of said variability (both during development and the subsequent evaluation of drugs), the explanation of the true variability by pharmacological factors logically capable of inducing these changes,

and last but not least the estimation of the remaining unexplained variability. There will almost always be a remaining unexplained variability, both inter-individually and intra-individually. This may be because of time-dependent pharmacological variations within each patient, errors during sampling, or possibly other unforeseen events. One must also remember that all these mathematical calculations are just a simplification of reality, unable to perfectly explain everything. Regardless, it is imperative for the optimal treatment of patients to have a sense of understanding for how these unexplained differences behave, and the magnitude of them.

1.2.1 Naïve pooled data approach

As the name implies, the Naïve Pooled Data Approach (NPD) is a method where all the data from the different patients is treated as if it came from a single patient. This model is easy to use, and requires little computational power. It can also be used on a variety of data, from experimental data to routine pharmacokinetic data. There are of course drawbacks, and these lie mainly in the simplification process. By pooling, one looses the detailed information of each individual and variation between individuals. This can lead one to believe that the data is neat and simple, while the fact may be that each patient varies widely in their observed concentrations. There may also be trends in individual curves which are smothered in the multitude, and therefore become unrecognizable. The method works well when there are low variations between individuals, but this is rarely the case with humans, which limits its usefulness.
Parameter estimates will be means, and one will not gain any estimates of spread. In addition, if the number of observations varies between patients, this will negatively affect the reliability of the method [6].

This approach was not utilized in this thesis, and will therefore not be discussed further.

1.2.2 The two-stage approach

The two-stage approach is a traditional pharmacokinetic analysis, and was designed to be used in the data-rich environment of previously mentioned traditional pharmacokinetic investigations. The first part entails using nonlinear regression to estimate an individual's pharmacokinetic parameters from the concentration-time data gathered. As mentioned, this must be rich data. These estimates are then used in the second step to calculate statistics such as mean parameter estimates, variance and covariance of said parameter estimates, as well as for searching for dependencies between the parameters in question.
It is quite possible to get decent and relatively unbiased estimations of parameters using this two-stage approach, even though random effects are usually exaggerated in practical situations. The catch is the necessity of a data-rich situation [4, 6].

This method was not used in this thesis, therefore it will not be further discussed.

1.2.3 Nonlinear mixed-effects modeling

The reason for the words "mixed-effect" in the name of this method comes from the fact that a model built this way will contain a fixed structure and a randomness block. The fixed structure contains what we know, or suspect, of the models behavior and interaction with other parameters. Knowing that a drug is eliminated predominantly by the renal pathway might lead us to include renal clearance as a scaling parameter for the observed concentration of drug.
The randomness block is there to describe the residual variance. This variance will always be present, to a greater or lesser extent. It is the biological changes in a body from day to day, the effect a diet has on a person, the compliance of a patient, and any possible errors in the sampling procedure. The common factor is that they cannot be predicted in advance. Together, these fixed effects and random effects are called mixed-effects [2].

The use of nonlinear mixed-effects modeling on population pharmacokinetics makes it possible to design less extensive studies than what is needed for a two-stage approach, and still give valid results. These designs are less restrictive to the patients, which makes it easier both for the participants and the conductors. When one finds oneself in a situation with sparse or mixed data where a two-staged model cannot be used, nonlinear mixed-effects modeling is a very handy tool for bringing answers out of the material at hand.

The nonlinear mixed-effects approach uses the population as a whole, not the individual, as basis for the estimation of parameters, and how these interact with relevant covariates. Even so, the individual is not lost in the masses, and through the estimates of population parameters and their variability, and not least the covariates that influence these parameters, it is possible to make predictions regarding individual patients [4].

1.2.4 Sampling designs in population pharmacokinetic investigations

In population pharmacokinetics we talk about, roughly, three sampling approaches: Single-trough sampling, multiple-trough sampling and full population PK sampling. These different designs give an increasing amount of pharmacokinetic information in that order.

In single-trough, a single blood-sample is taken from each patient right before they get each new dose. At this point the drug concentration will be at its lowest in relation to the normal oscillation between doses. This design requires a larger mass of patients, and also a stricter regimen, to be able to supply valid data. It is, though, quite possible to estimate the mean trough concentration, and its variability, in the population. If the design-criteria are not met, the variability-estimate will be contaminated by other random factors than purely pharmacokinetic ones.

In multiple-trough design, two or more samples are required from each patient near the trough of his/her concentration curve. This design makes it possible to distinguish inter-individual variability from other random factors, and also allows the study of each patient in greater detail, for instance correlating drug-concentration with different patient characteristics. The design also necessitates a smaller patient-base than the single-trough design.

The full population PK sampling design is also known as “experimental population pharmacokinetic design” or “full pharmacokinetic screen”. It requires samples to be drawn at, usually, minimum 3-4 times following one dose interval. This gives an even more complete set of information regarding the patients, and allows not only the estimation of PK parameters and variability of drug in the population when using the nonlinear mixed-effects approach, but is also capable of delving into the relationships between the concentration and patient characteristics of both demographic, physiological an pathophysiological nature [4].

1.2.5 Population PK modeling using NONMEM

NONMEM is a computer program designed to help construct models utilizing the nonlinear mixed effects approach. In particular it was developed with pharmacokinetic models in mind. It was the first such program made to analyze large quantities of pharmacokinetic data, and to achieve linearization it employed a so-called first-order (FO) Taylor series expansion in regard to the random effect variables η_i and ε_{ij}. This is the default estimation method. NONMEM also uses two alternatives to this; first-order conditional estimation (FOCE) and the Laplacian methods. FOCE is a FO expansion about conditional estimates (empirical Bayesian estimates) of the inter-individual random effects. The Laplacian method on the other hand uses second-order expansions about the conditional estimates of the random effects [6].

A model built with NONMEM will contain a fixed structure and a randomness block, as mentioned earlier for models built using the nonlinear mixed-effects modeling

method. One does, however, differentiate between two types of variance. There is inter-individual variance, which is the result of the simple fact that we are not all alike, even physiologically. And then there is residual variance, which is the "noise", or true errors, as well as intra-individual variance [2].

NONMEM is, regardless of its many strengths and versatility, not a particularly user-friendly program [7]. To build a model for a dataset one will need to make two files. The first is the data-file, which is basically all the data written into a text-file. One must follow some guidelines on how it is written though.
The second is the control-file, and this is the true model. Here the parameters and errors are defined, as well as supplied with starting estimates [2].
The parameters and the inter-individual variance are coded like this:

Equation 1: $$P_{ij} = P_{TVj} \times EXP(\eta_{ij})$$

P_{ij} is the *j-th* parameter for the *i-th* individual, P_{TVj} is the population "typical value" of the *j-th* parameter, and η_{ij} is a random variable for the *i-th* individual on the *j-th* parameter. It has a distribution of 0 and a variance of ω_{ij}^2.

Intra-individual variance is coded similarly:

Equation 2: $$Y = Y_{TV} + \varepsilon$$

Here Y is the observed value of the parameter, Y_{TV} is its true value, and ε is a random variable representing the intra-individual error with a distribution of 0 and a variance of σ^2.

The residual variability is mainly described by three different types of equations: additive, proportional (also known as constant coefficient of variation) and exponential [2]. Here are examples, in order:

Equation 3: $Y = Y_{TV} + \varepsilon$

Equation 4: $Y = Y_{TV} \times (1 + \varepsilon)$

Equation 5: $P_{ij} = P_{TVj} \times EXP(\eta_{ij})$

It is also possible to combine these into mixed equations, like for instance:

Equation 6: $Y = Y_{TV} \times (1 + \varepsilon_1) + \varepsilon_2$

The Objective Function Value (OFV) describes how good a model is at fitting the observed data. It does this by assuming that the model is correct, and asks how probable is it to get data like that which has been observed if the model is true. It employs the -2 log likelihood, or -2LL equation:

Equation 7: $-2\log(L) = n\log(2\pi) + \sum_{i=1}^{n}\left(\log(\sigma_i^2) + \frac{(Y_i - \hat{Y}_i)^2}{\sigma_i^2}\right)$

By minimizing this value, one increases the likelihood of the model being a good fit for the data. To minimize -2LL one cannot do anything about the part: $n\log(2\pi)$, seeing as this is a constant. However it is possible to minimize the part: $\sum_{i=1}^{n}\left(\log(\sigma_i^2) + \frac{(Y_i - \hat{Y}_i)^2}{\sigma_i^2}\right)$ This part is also known as the "extended least squares" objective function. NONMEM looks for parameter estimates that will give the smallest possible -2LL.

Now, this number does not say anything of interest by itself. What it does is let us compare models trying to describe the same data. By subtracting the lowest OFV from the highest OFV from two models, one can see if it is significantly better than the other. This is because the difference follows a chi-squared distribution, with

degrees of freedom equal to the difference of parameters between the models. For instance, if there is 1 degree of freedom, a difference of 3.84 between the OFV's is significant at $p<0.05$.
This is what makes -2LL such a useful value, because it is quite simple to subtract to numbers in the search for significance. However, OFV's cannot be compared between datasets. A model with an OFV of 10.000 might fit a data-set better than a model with an OFV of 350 fits another data-set. Also, one should not add more than 1 or 2 parameters at a time, as this will make the comparison of models unjustified.

A final thought is that OFV is not a perfect guide. One must also evaluate the goodness of fit of the model, the complexity and the stability (as well as the run-time). A model may for instance be chosen because of its reliability, even though it is statistically less significant than another. There are also other ways to evaluate how well the model actually predicts the observations. This is essential for good model-building, one must not rely on a single method for evaluation [2, 6].

1.3 Cytomegalovirus

Human cytomegalovirus (CMV, or HCMV) is a double-stranded DNA virus from the Herpesviridae family of approximately 220 kb, found only in humans. It can be found in all secretions from the body. As a member of this particular viral family, it possesses their unique ability to cause a latent infection which can lie dormant until it reactivates later on. It is also possible to be re-infected with a new strain, so immunity is not granted [8-11].

CMV is a present infection in between 60-90% of the world's population, but in developed countries the rate is estimated at 30-70%. The exact rate is dependent on both social differences, as well as geographical location. In a healthy human being this will usually be a benign infection, with no visible symptoms [8, 11].

The virus can be transmitted from person to person in a number of ways. Since all bodily fluids contain the pathogen, the transmission may be both sexually and non-sexually. Blood transfusions may of course also contain CMV, although screening measures have reduced the likelihood of this happening. The virus can also be transferred from mother to child, either during pregnancy or after birth through breastfeeding. And lastly, it may be transferred from donor to recipient in a transplanted organ [11, 12].

CMV is a very common pathogen in populations with a reduced immune defense system. It is the most common virus to infect, or re-activate from latency, in patients who have undergone solid organ transplants, and who therefore have reduced defensive capabilities. In fact, it is the leading pathogen causing morbidity, mortality and graft rejection in this population. Those at highest risk are the so-called "D+/R-"-patients, those who get an organ from a seropositive donor, but are themselves seronegative. It can affect many parts of the body; pneumonitis, hepatitis, encephalitis and illness in the gastrointestinal tract are but some of the possibilities. The survival of both patient and graft are also negatively affected by the presence of this disease, and it is particularly in the first three months after transplantation that the risk of it developing is greatest [9-11, 13, 14].

1.4 Valganciclovir

1.4.1 History of valganciclovir

Valganciclovir (Ro 107 9070) was developed in an attempt to create a more easily administrable oral formulation of ganciclovir, because of the latter's low bioavailability when taken orally, and the resulting large dose required. This would reduce costs and presumably also increase compliance.
In this respect valganciclovir was a success. It is easier on the patients when compared to oral ganciclovir, because of the reduced size and frequency of dosage, not to mention when compared to intravenous ganciclovir.

Valganciclovir has also been proposed economically attractive in comparison with intravenous ganciclovir therapy. This is due to many factors, from a reduced requirement of health workers, to less risk of treatment-requiring secondary infections [9, 11, 13-15].

1.4.2 Applications and mechanism of action

Valganciclovir is a prodrug of ganciclovir. More specifically, it is a monovalyl ester. After oral administration it is transported from the intestine to the bloodstream via the PEPT1 peptide transporter. Thereafter it quickly reaches the liver, and both intestinal and liver esterases swiftly hydrolyze the prodrug to its active form, ganciclovir. It is however during pre-systemic absorption that most of the conversion to ganciclovir takes place [8, 9, 11, 13-17].

Ganciclovir is an antiviral agent, and achieves this by inhibiting the viral DNA-reproduction. This is due to the nature of ganciclovir being a 2'deoxyguanosine (purine) nucleoside analog. A virus-protein converts this to ganciclovir monophosphate by means of a phosphotransferase. This is the UL97 gene product of CMV. After several further human transformations, the current incarnation of the drug, called ganciclovir triphosphate, is ready to curb some viral replication. The molecule is incorporated in the growth of the viral DNA, specifically at the 3' site. This inclusion of ganciclovir causes the strand to lose stability, which results in

severe impediment of strand elongation.
There has been reported resistance to ganciclovir, through mutations resulting in reduced use of the drug in DNA replication [8, 9, 11].

1.4.3 Adverse effects and drug interactions

There have been observed several adverse effects in conjunction with valganciclovir. Anemia, diarrhea, headache, nausea, graft rejection and neutropenia have been observed in a few studies. Vomiting, abdominal pain, fever, thrombocytopenia, insomnia, peripheral neuropathy, paresthesias, tremor, back pain, hypertension, constipation, edema, urinary tract infection and retinal detachment, as well as several more, have also been reported. The last event is, curiously, also a possible event in retinitis caused by CMV [9, 10, 13-15, 17].

Since the conversion from valganciclovir to ganciclovir is very rapid, there is not expected to be any specific drug interactions between valganciclovir and other drugs. Therefore, any interactions that can take place will involve ganciclovir. Combination with imipenem-cilastatin may cause cramps. Probenecid reduces renal clearance of ganciclovir which causes significant increased exposure. Zidovudine may increase the risk for neutropenia. Combination with didanosine can lead to neuropathy, etc [9, 17, 18].

Finally, since ganciclovir is mainly eliminated from the body by renal function, any patients with reduced renal capacity will need to adjust the administered dose accordingly [9, 13, 15, 19].

1.4.4 ADME of valganciclovir

The absolute bioavailability of valganciclovir is reported to be ~60%. Oral formulations of ganciclovir have only been shown to give a bioavailability of 6-9%. A 900mg dose of valganciclovir is also reported to be equivalent to a 5mg/kg dose of ganciclovir intravenously, in terms of AUC [8-11, 13-17, 20].

Ganciclovir is minimally bound to plasma proteins (1-2%) after absorption, and has a large apparent volume of distribution (>120 L) after administration of oral valganciclovir [21].

Furthermore, it has been reported that valganciclovir reaches a maximum concentration in the blood after 1.75-2 hours, depending on dose given [20].

Valganciclovir is eliminated from the body as ganciclovir, almost exclusively by renal excretion, through glomerular filtration and active tubular secretion. Elimination of ganciclovir is biphasic with the mean terminal elimination half-life following oral administration of valganciclovir 900 mg once daily of 6.5 hours in solid organ transplant recipients [21].

It should also be noted that there has been observed a significant increase in bioavailability for valganciclovir when administered in a fed state, as opposed to a fasted state. This increase was measured to be 30% [9].

1.4.5 Population PK models of valganciclovir in the literature

There have in recent years been a few attempts made at making population pharmacokinetic models to describe ganciclovir after the administration of valganciclovir. 2-compartment models, in combination with 1.-order absorption, have been utilized in at least two studies [13, 15].
Apart from that, there has been observed large variations in regard to covariates employed in these models, seemingly without any one of them in particular rising up and above the rest. A notable exception is renal function, which is highly correlated with a good description of observations. This is not unexpected, seeing as ganciclovir is almost completely eliminated renally [13, 15, 19].

1.5 Aim of the study

The aim of the VICTOR study was to develop a population pharmacokinetic model describing the ganciclovir plasma concentrations in solid organ transplant recipients (SOT) receiving oral valganciclovir for treatment of CMV-disease. This model was to be internally validated to affirm its clinical applicability.

2. Methods & materials

2.1 Design of VICTOR study

The VICTOR (Valcyte in CMV-disease treatment of solid organ recipients) study is a randomized, open-label, parallel-group, active drug-controlled, multi-centre phase III/IV noninferiority trial in adult solid organ transplant recipients with CMV disease [14]. It was conducted in 42 centres in 20 different countries. Adult patients who had undergone solid organ transplants, and who had both virological and clinical evidence of CMV disease were candidates for inclusion in the study, regardless of the relative serostatus. CMV disease had to be confirmed by use of a local assay, following certain criteria. In addition, compatible symptoms had to be verified, following the recommendations from the American Society of Transplantation. Informed consent was given by each patient before inclusion in the study [14]. Exclusion criteria were if the disease was considered life-threatening, a history of significant adverse reaction to ganciclovir, valganciclovir, acyclovir or valacyclovir, if they had proven ganciclovir resistance, inability to take oral formulations, had received an investigational drug within the last 30 days or had very poor renal clearance (defined as a calculated creatinine clearance of <10mL/min, using the Cockcroft-Gault equation (appendix 7.1)).
There were also several other drugs participants were not allowed to use during the study [14].

The participants were randomly assigned to two different treatment groups. The valganciclovir group received 900 mg orally twice daily during the induction period, while the ganciclovir group received 5 mg/kg intravenously twice daily. The induction period lasted for 21 days. Afterwards, both groups received 900 mg valganciclovir once daily for 28 days, which means until day 49. The oral doses were given in the form of Valcyte® by Roche, each tablet containing 450 mg valganciclovir.
Seeing how ganciclovir is almost exclusively eliminated renally, doses were adjusted according to renal function.

The study concluded that valganciclovir was non-inferior to ganciclovir by showing that viral eradication was reached in 45.1% of the valganciclovir group versus 48.4% in the ganciclovir group by day 21 [14].

2.2 Population for population pharmacokinetics

Of the 333 patients originally screened for the study, 321 actually received at least one dose of study drug. These were randomly divided so that the valganciclovir group contained 164 patients, and the ganciclovir group contained 157 patients. Ganciclovir plasma concentrations were measured in 108 of the 164 patients in the valganciclovir group. This is the population used to develop the population pharmacokinetic model during this thesis. Demographic data for the 108 patients at baseline is shown in table 1 [14].

Table 1: Baseline demographics of patients in the VGC-group included in the pop-PK analysis

Parameters	***Value***	***Mean***	***Range***		***Median***
Number of patients	108				
Male	68				
Female	40				
Age (years)		46	18	72	
Weight (kg)		66.5	36.0	129.6	
Height (cm)		166	143	190	
Creatinine Clearance* (mL/min)		62	7	214	
Dosage given (mg)		672	450	900	
Observed concentration (µg/mL)		3.3	0.0	22.2	
Number of samples taken (n)	636				
Average number of samples per patient (n)		5.9	1.0	11.0	
Time after transplant (days)		366	10	9257	69
Race (n)					
Caucasian/White	86				
Black	3				
Oriental	11				
Hispanic	4				
Other	4				

Organ transplanted (n)	
Heart	4
Kidney	83
Kidney and Pancreas	2
Liver	13
Liver and Kidney	1
Lung	4
Pancreas	1
CMV IgG serostatus at disease start (n)	
Positiv	61
Negativ	11
NA	36
D/R CMV IgG serostatus at Tx (n)	
D-/R-	3
D-/R+	7
D+/R-	16
D+/R+	53
Missing	29
*Cockcroft-Gault formula	

2.3 Building the PK/PD model

As previously mentioned, the computer program utilized was NONMEM. More specifically Version VI (GloboMax LLC, Hanover, MD, USA). The graphs were made using the computer program R (http://www.r-project.org/), as well as Excel 2007© (Microsoft Corporation, USA) and Minitab 15© (Minitab Inc., USA).

The first part of the building process was to create an input file. It contained all the data thought to be of interest to the model. This includes age, weight, creatinine clearance, sex and ganciclovir plasma concentration.
The next step was to create control files to test different structural models to find out an appropriate model for the dataset. The models tested were from one to three compartments, with first-order and zero-order absorption. Elimination was assumed to be first-order. The choice between these models was based on which one gave the

lowest OFV, and at the same time was stable, gave a good fit to the data, and had a short run time. To be considered a statistically significant better model, there had to be a change in OFV of more than 3.84, which corresponds to a significant level of $p<0.05$ [2, 22].

The parameters employed in these models were clearance (CL/F), intercompartmental clearances (Q_n/F),rate constant of elimination (K), absorption rate constant (KA), absorption lag time (ALAG), rate constant between central and peripheral compartments (K_{12}, K_{13}, K_{21}, K_{31}), central volume (V2/F) and peripheral volumes (V3/F, V4/F) [22].

The first-order conditional estimate (FOCE) method was used during development of the entire model [6].

2.4 Analyzing for covariates

After having built several structural models and chosen the most appropriate one, the inclusion of covariates was to be tested. A covariate is a patient-specific variable, which ideally will adjust a predicted parameter to give an even better individual fit for patient data. Covariates are usually divided into two groups, continuous and categorical covariates. It is often the case that continuous covariates are put into linear equations with the parameters in question, and categorical covariates are handled by one or more IF/ELSE statements. Continuous covariates are also often centred on the population mean [23, 24].

The first step in the covariate phase involves the stepwise inclusion of each covariate. The covariates are added to the structural model one at the time, and tested against every possible parameter with every possible equation. If a covariate is found to give a significant improvement to the OFV it is included in the model, and the search continuous to find more significant covariates. It is usual to set the significance at $p<0.05$, which translates to a ΔOFV of >3.84 [23, 24].

The covariates were tested with the following general equations:

Equation 8: $P = \theta_1 \times COV \times \theta_2$

Equation 9: $P = \theta_1 \times COV \times (1/\theta_2)$

Equation 10: $P = \theta_1 \times COV^{\theta_2}$

P is the typical value of the parameter, θ_2 is the fractional change in the parameter with each unit of the covariate, and θ_1 is the typical estimated value of the parameter in an individual. In addition, all continuous covariates were centred on their population average according to the following equation [25]:

Equation 11: $COV = Individual\ Covariate/Population\ Mean\ Covariate$

The categorical covariate was also tested with these IF/ELSE statements:

$IF\ (COV = 1)\ THEN$
$TV[parameter] = \theta_1$
$ELSE$
$TV[parameter] = \theta_2$
$ENDIF$

Or
$IF\ (COV = 1)\ THEN$
$TV[parameter] = \theta_1 \times \theta_2$
$ELSE$
$TV[parameter] = \theta_1 \times \theta_3$
$ENDIF$

Or

$IF\ (COV = 1)\ THEN$
$TV[parameter] = {\theta_1}^{\theta_2}$
$ELSE$
$TV[parameter] = {\theta_1}^{\theta_3}$
$ENDIF$

After inclusion of all significant covariates into the model, the backwards deletion step was initiated. This step involves deleting each covariate from the new covariate model one at a time, and if the change in OFV is not significant it is discarded. Then one deletes the next covariate, until one reaches the point where there are no more covariates left to delete that do not give a significant change in OFV. The criterion for significance is set stricter at this step, and the change in OFV had to be >6.63 for a significance of $p<0.05$, and >10.9, for a significance of $p<0.01$. This is then called the final model [23, 24, 26].

2.5 Criteria for choice of model

The choice of model was based upon evaluating a combination of the objective function value (OFV), the individual predictions versus the individual observations, stability of the model and the observed goodness of fit of the graphs [25, 26].

2.6 Validating the model

2.6.1 K-fold cross-validation

As advised in the FDA guideline [4], a form of data-splitting was chosen to validate the model. It was a K-fold cross-validation, where K was set to 10 [27, 28]. The data-set was randomly divided into 10 subsets containing 90% of the data, as shown in table 2. The randomization was achieved through use of the Random Sequence Generator at the webpage Random.org (http://www.random.org/sequences/) [29].

Table 2: Patients removed from cross-validation groups

Group	Patients removed from group										
	A	***B***	***C***	***D***	***E***	***F***	***G***	***H***	***I***	***J***	***K***
1	107	102	88	75	56	92	36	15	78	66	48
2	43	31	77	21	72	94	51	91	108	61	12
3	39	33	26	2	45	52	50	23	47	96	73
4	84	86	101	7	10	100	18	93	89	30	16
5	74	57	70	76	67	65	85	79	8	58	11
6	69	13	27	40	25	80	1	9	53	95	59
7	82	104	54	60	81	44	90	19	62	42	97
8	37	6	63	5	103	20	98	68	29	14	71
9	99	35	4	32	28	55	83	38	49	17	
10	34	24	105	46	22	41	87	106	3	64	

The final model was then run on all these 90%-data-sets. Afterwards, the parameter estimates obtained through these runs were compared with the parameter estimates from the full input-file. This was done to validate the parameter estimates of the model.

The 10 new models with parameter estimates from the 90%-groups were then used to predict the drug concentrations in the corresponding 10%-data-sets. This was done by running the models with the commands "MAXEVAL=0" and "POSTHOC" in the line $ESTIMATION in the control-file. To gain additional information on the predictive power of the models, they were each run on 4 variations of the 10%-data-sets. The 4 different sets contained, respectively: 1) all the ganciclovir concentration measurements per patient, 2) the two first measurements per patient, 3) the first measurement per patient, and 4) no measurements per patient. This was done to check the predictive performance of the model.

Finally, the 10 new models with parameter estimates from the 90%-groups were run on the full input-file, and the OFV's from these runs were compared to the OFV from the final model on the full input-file. This was done to evaluate the robustness of the model [4, 26, 30-32].

The equations used to check the performance of the model were as follows: 12) Mean prediction error (ME, μg/mL), 13) Mean squared prediction error (MSE, $(\mu g/mL)^2$), 14) Root mean squared error (RMSE, μg/mL). The mean prediction error checked the

bias in the model, the mean squared prediction error and the root mean squared error controlled the predictive performance. In addition, Standardized prediction error (SPE) was employed to evaluate predictive performance (15) [5, 24].

Equation 12: $$ME = \frac{1}{n}\sum_{i=1}^{n}(C_{Pred} - C_{Obs})$$

Equation 13: $$MSE = \frac{1}{n}\sum_{i=1}^{n}(C_{Pred} - C_{Obs})^2$$

Equation 14: $$RMSE = \frac{1}{n}\sum_{i=1}^{n}\sqrt{(C_{Pred} - C_{Obs})^2}$$

Equation 15: $$SPE = \frac{C_{Obs} - C_{Pred}}{SD_{Cpred}}$$

C_{Obs} is the observed concentration, C_{Pred} is the predicted concentration, SD_{Cpred} is the standard deviation of the predicted concentration and *n* is the total number of observations in the validation group.

2.6.2 Confidence interval

The Jackknife method was chosen to calculate the confidence interval for the parameters in the model. 108 sets of control-files and input-files were created for this, and after running them the parameter estimates from each sub-model were gathered. Confidence interval, standard error and bias were computed, and the parameter estimates were also compared to those from the final model on the full input-file.

3. Results

3.1 Model building results

The different structural models were compared on the criteria OFV and residual variability as shown in table 3.

As can be seen, more sophisticated models decrease the variability and increase the descriptive power up to a certain point. This comes at the expense of increased complexity, which results in a larger required computational power and longer run-time.

Table 3: Comparison of structural models by OFV and residual variability

Compartment model	ADVAN routine	TRANS subroutine	Objective function value (OFV)	Δ OFV	Residual variability	
					Proportional (%)	Additive (µg/mL)
1-compartment	2	1	324.64	-	-	5.33
1-compartment	2	2	265.26	59.38	-	3.01
2-compartment	4	4	238.64	86	-	2.42
2-compartment	4	4	238.64	26.62	31.4	0.413
3-compartment	12	4	233.05	5.59	29.8	0.416

In table 4 and 5 the different structural models are compared on their parameter estimates. The models show large variations in parameter estimations. Some of these variations can be considered to be extreme and non-physiological.

Table 4: Comparison of different structural models by parameter estimates

Compartment model	ADVAN routine	TRANS subroutine	CL/F	Q/F	
				1	2
1-compartment	2	1			
1-compartment	2	2	11.3		
2-compartment	4	4	3.54	1.58	
3-compartment	12	4	6.33	5.71	3.73

Table 5: Comparison of different structural models by parameter estimates

Compartment model	V/F			K	KA
	C	P1	P2		
1-compartment				1.99	0.278
1-compartment	0.06				0.0154
2-compartment	10.9	99.4			2.14*10^21
3-compartment	0.1	6130	0.0501		0.0249

Abbreviations: CL=clearance, Q_1=intercompartmental clearance for peripheral compartment 1, Q_2=Intercompartmental clearance for peripheral compartment 2, V_C=central volume, V_{P1}= peripheral volume 1, V_{P2}=peripheral volume 2, F=bioavailability, K=elimination constant, K_A=absorption rate constant.

Various error models were tested during the investigation, both intra-individually and inter-individually. An exponential error model gave the best fit for the inter-individual variances, measured by an improvement of ΔOFV by 0 to 23.1, in addition to enhanced stability. The intra-individual error was best explained through a combined proportional and additive equation because the model would not run successfully with any other equation after advancing to a 2-compartmental model [2, 25].

3.1.1 Graphical Comparison of Models

Patient number 34 (randomization number) was chosen to demonstrate the difference between the different structural models in graphical terms.

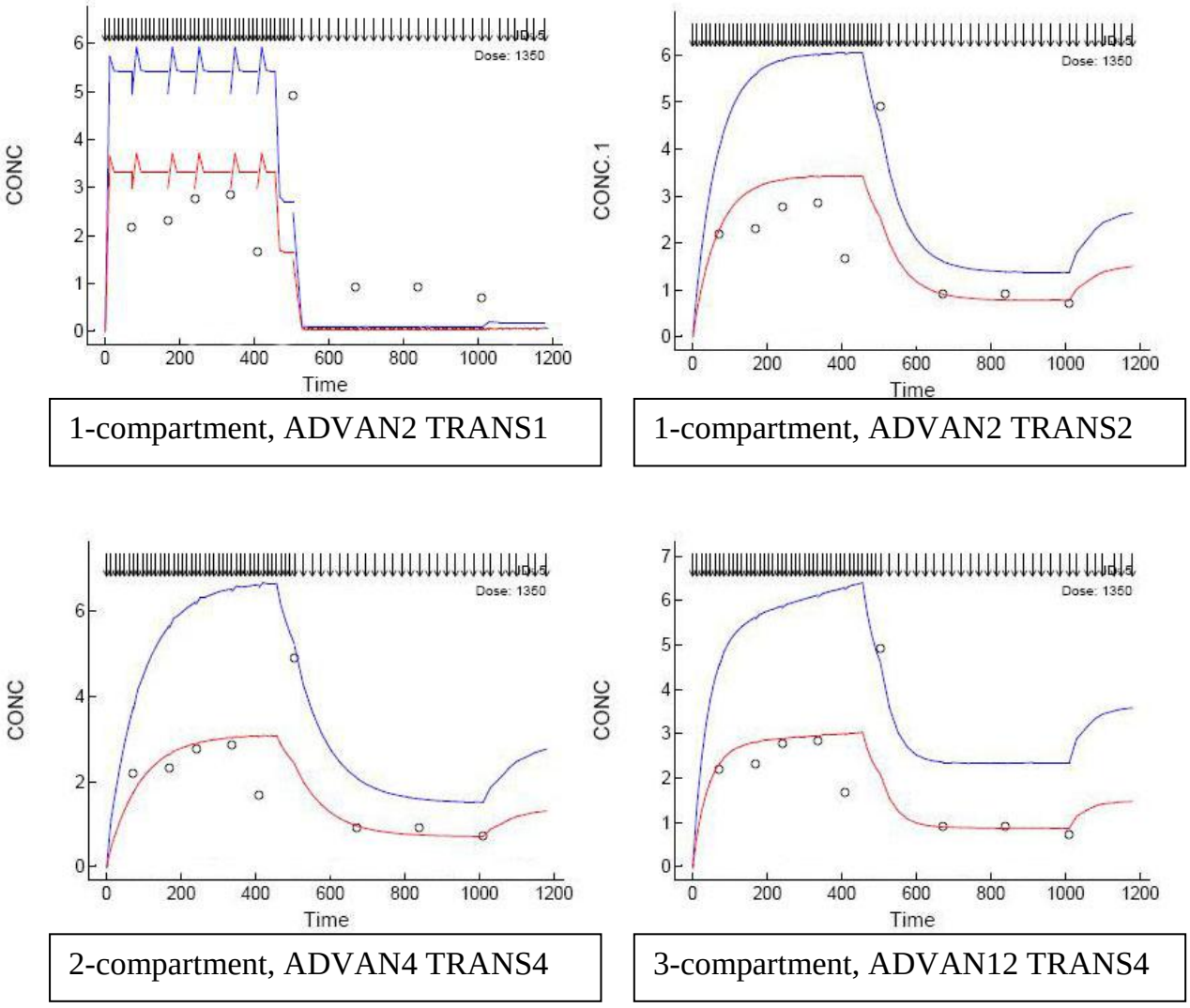


Figure 3: Comparison of different structural models by graphs. Red lines are individual predictions, blue lines are population predictions and circles are measured drug concentrations.

3.2 Analyzing for covariates

The 2-compartment model with first-order absorption chosen as the structural model to further improve with addition of covariates contained the variables clearance (CL/F), central volume (V2/F), intercompartmental clearance (Q/F), peripheral volume (V3/F), absorption rate constant (KA) and absorption lag time (ALAG). These were coded as θ_1 to θ_6, respectively.
The starting values for the parameter estimates were all based on data from the PV16000 study [25]. The estimates for KA and ALAG were fixed at the values in the PV16000 study.

The parameters were tested in all combinations with the covariates creatinine clearance (CRCL), weight (WGT), gender (SEX) and age (AGE), as described in section 2.4.

The covariate SEX was coded as both a linear statement and as an IF/ELSE statement. Seeing as none were significantly better than the other, the linear code was chosen for simplicity.

Table six contains the covariates found significant during forward inclusion, while table seven shows those that remained significant after backwards deletion. The criteria were $\Delta OFV>3.84$ ($p<0.05$) and $\Delta OFV>10.9$ ($p<0.01$) for forward inclusion and backwards deletion, respectively.

The original model, without any covariates, had an OFV of 1175.71. After the forward inclusion of all significant covariates into the full model, the OFV decreased to 1048.03. Ultimately, after the backwards deletion had led to the final model, the OFV had risen to 1064.77. This represented a net decrease in OFV of 110.94 since the original model, and is highly significant.

Table 6: Significant covariates after forward inclusion, equations used and OFV

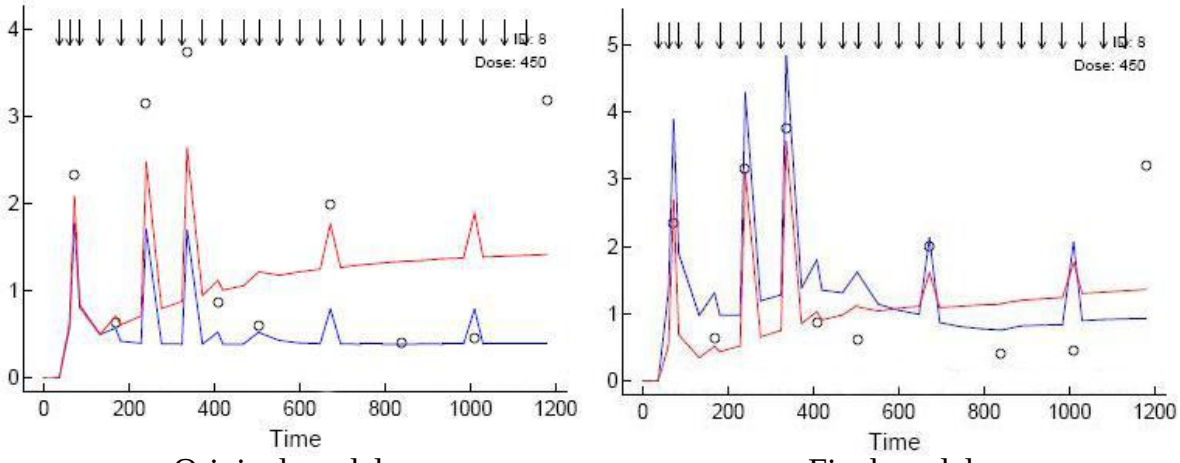
Covariate	Parameter	Model	OFV	ΔOFV	P
CrCl	CL	TVCL=THETA(1)*(CRCLM**THETA(7))	1087.40	88.31	p<0.01
CrCl	V3	TVV3=THETA(4)*(CRCLM**THETA(8))	1083.22	4.18	p<0.05
WGT	CL	TVCL=THETA(1)*(CRCLM**THETA(7))*(WGTM**THETA(9))	1074.00	9.22	p<0.01
SEX	KA	KA=THETA(5)*(SEX*(1/THETA(10)))	1059.42	14.58	p<0.01
SEX	ALAG	ALAG1=THETA(6)*(SEX*THETA(11))	1052.40	7.02	p<0.01
AGE	V3	TVV3=THETA(4)*(CRCLM**THETA(8))*(AGE**THETA(12))	1048.03	4.37	p<0.05

Table 7: Significant covariates after backwards deletion, equations used and OFV

Covariate	Parameter	Model	OFV	ΔOFV	P
CrCl	CL	TVCL=THETA(1)*(CRCLM**THETA(7))	1157.82	-93.05	p<0.01
SEX	KA	KA=THETA(5)*(SEX*(1/THETA(8)))	1113.00	-48.23	p<0.01
SEX	ALAG	ALAG1=THETA(6)*(SEX*THETA(9))	1080.80	-16.03	p<0.01

3.2.1 Covariate analysis based on visual graphical inspection

Patient number 97 (randomization number) showed a marked improvement from the original model to the final model, as shown in figure 4. The individual and population predictions display a greater consistency, and they are better able to "pick up" the points representing measured drug concentrations. The graphs are still not optimal, showing room for improvement, especially as time progresses.



Original model Final model

Figure 4: Comparison of original model without covariates and final model with covariates. Red lines are individual predictions, blue lines are population predictions and circles are measured drug concentrations.

The graphs below (figure 5) show three of the patients with the best visual fits in the original model (on the left), and three of the patients with the worst visual fits (on the right).

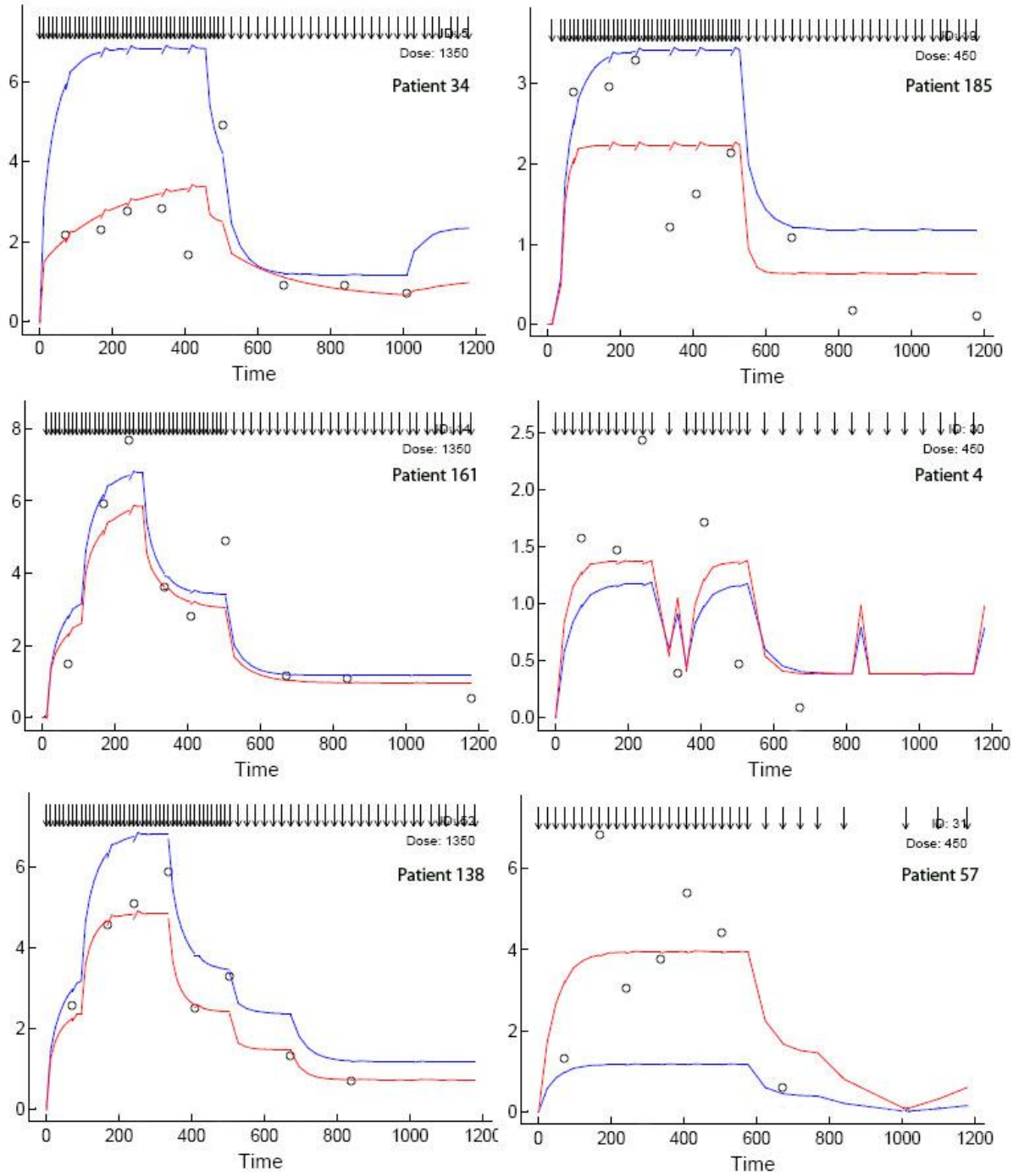


Figure 5: Three of the patients with the best (on the left) and the worst (on the right) visual fits in the original model. Red lines are individual predictions, blue lines are population predictions, and circles are measured drug concentrations.

Figure 5 shows some of the patients with the best and worst visual fits in the original model (left and right, respectively), but this time they are a result of the final model, after both forward inclusion and backwards deletion. The improvement in predictive power is shown in the graphs below.

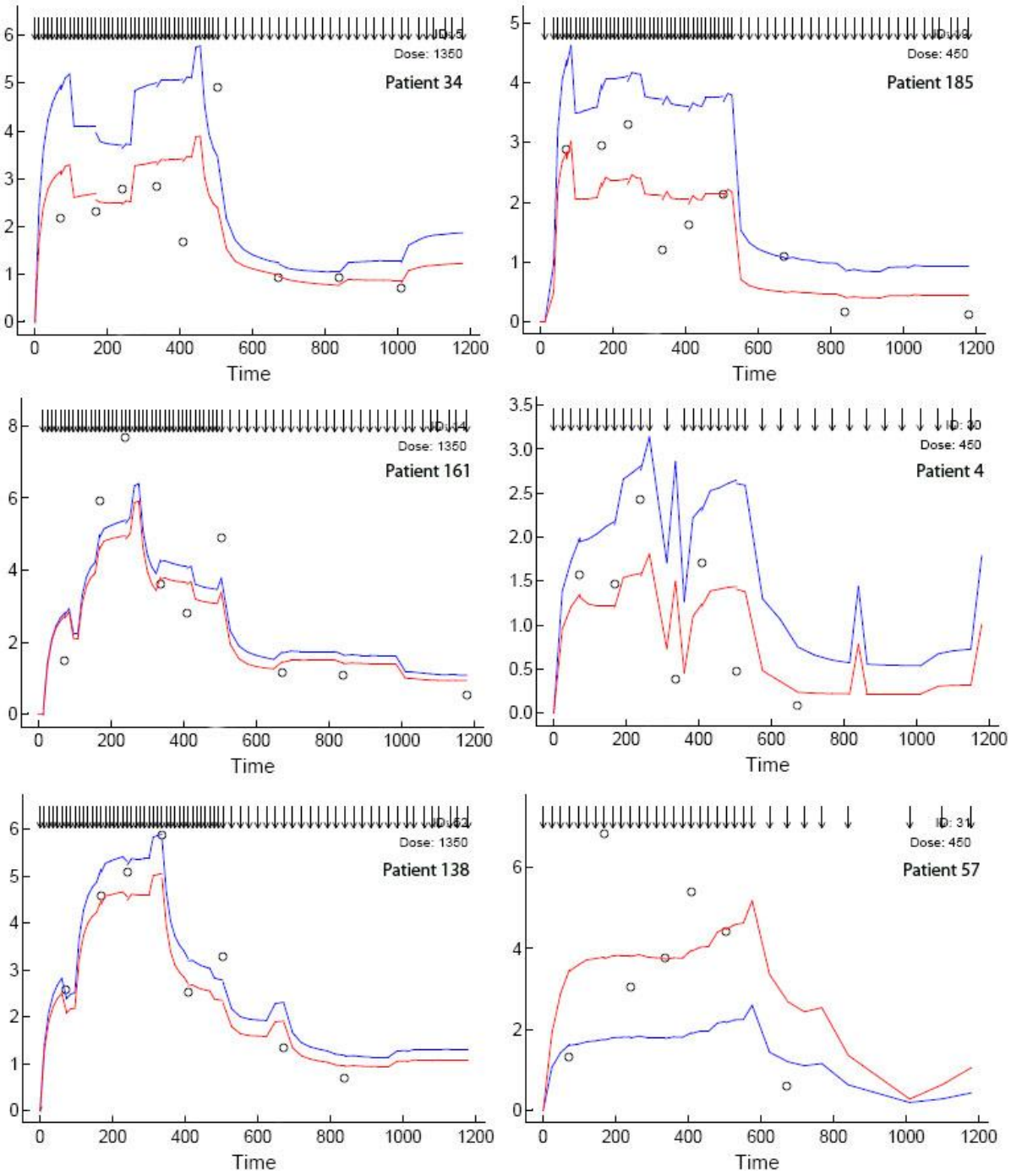


Figure 5: Three of the patients with the best (on the left) and the worst (on the right) visual fits in the final model

Goodness of fit plots shown in figures 6-9 below also indicate that the model is fair but needs further improvement (which can be readily understood through the OFV). The original model has a far wider distribution around the center than the final model.

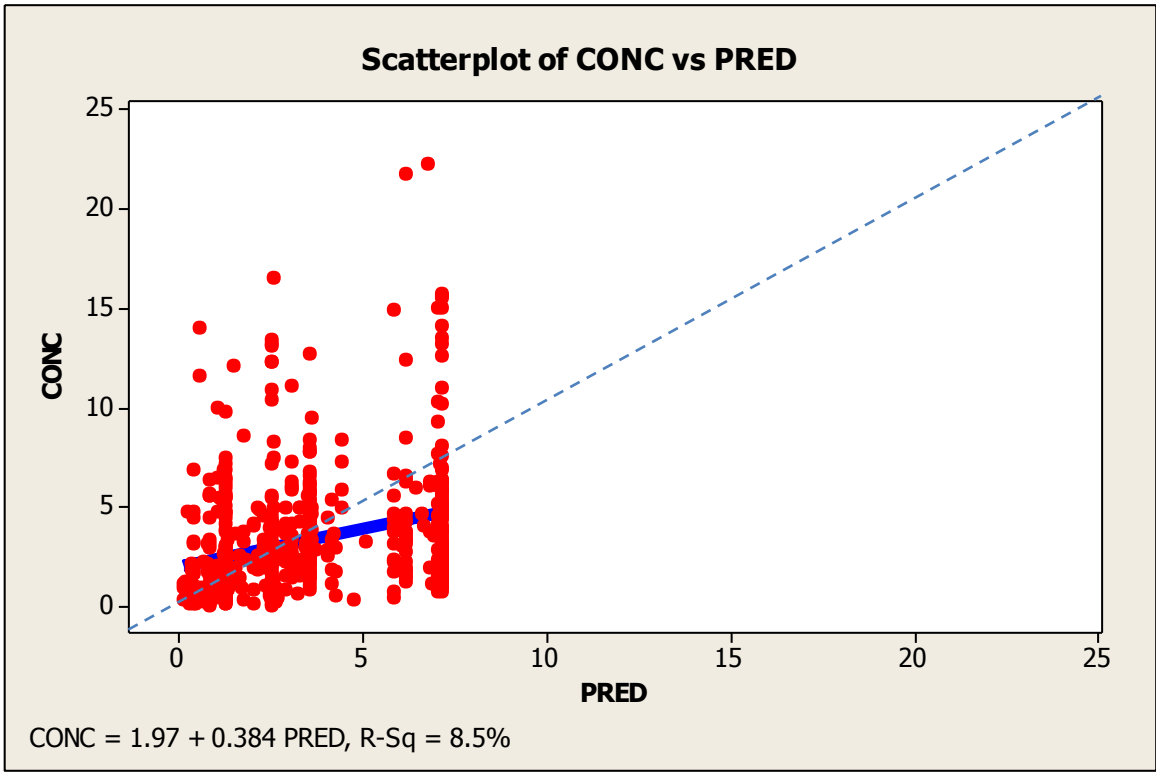


Figure 6: GOF plot of concentration vs. population prediction for original model. The dotted line is the union, the whole line is the regression line.

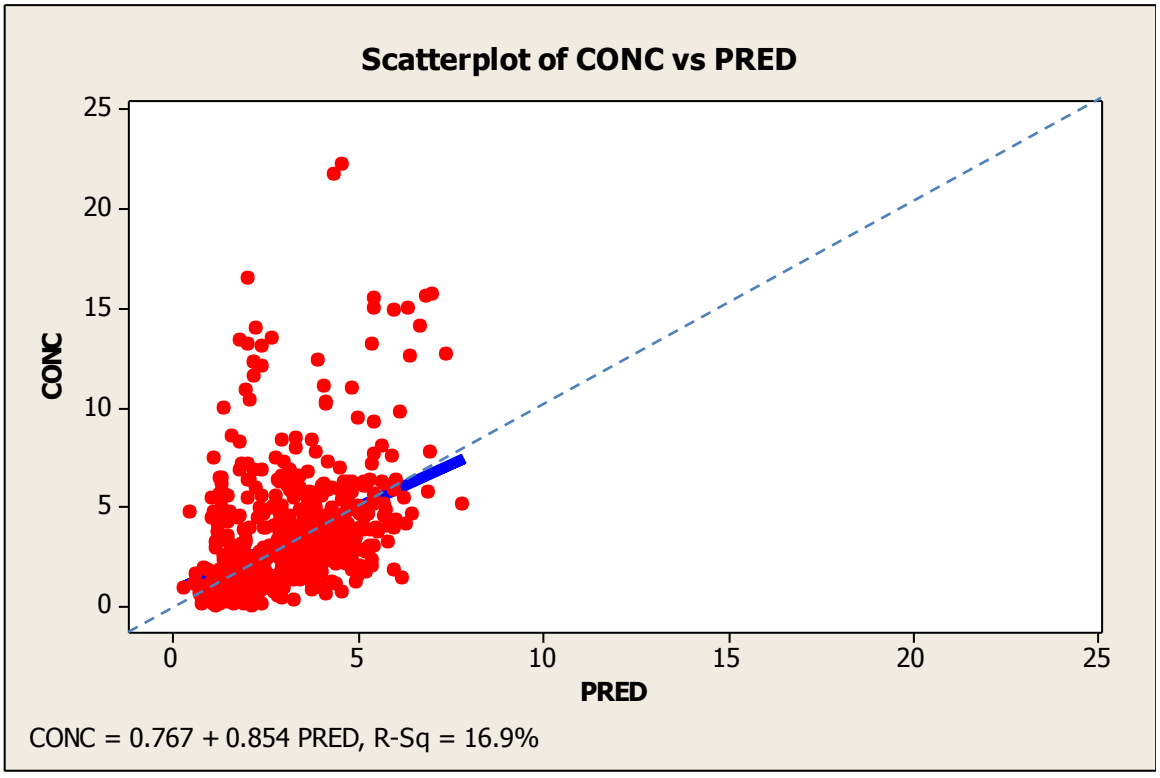


Figure 7: GOF plot of concentration vs. population prediction for final model including covariates. The dotted line is the union, the whole line is the regression line.

In addition to the more centered “look” of the plot for the final model (figure 7), the minimum/maximum WRES range has decreased from -2.42/13.50 of the original model (figure 8) to -2.22/9.69 of the final model (figure 9).

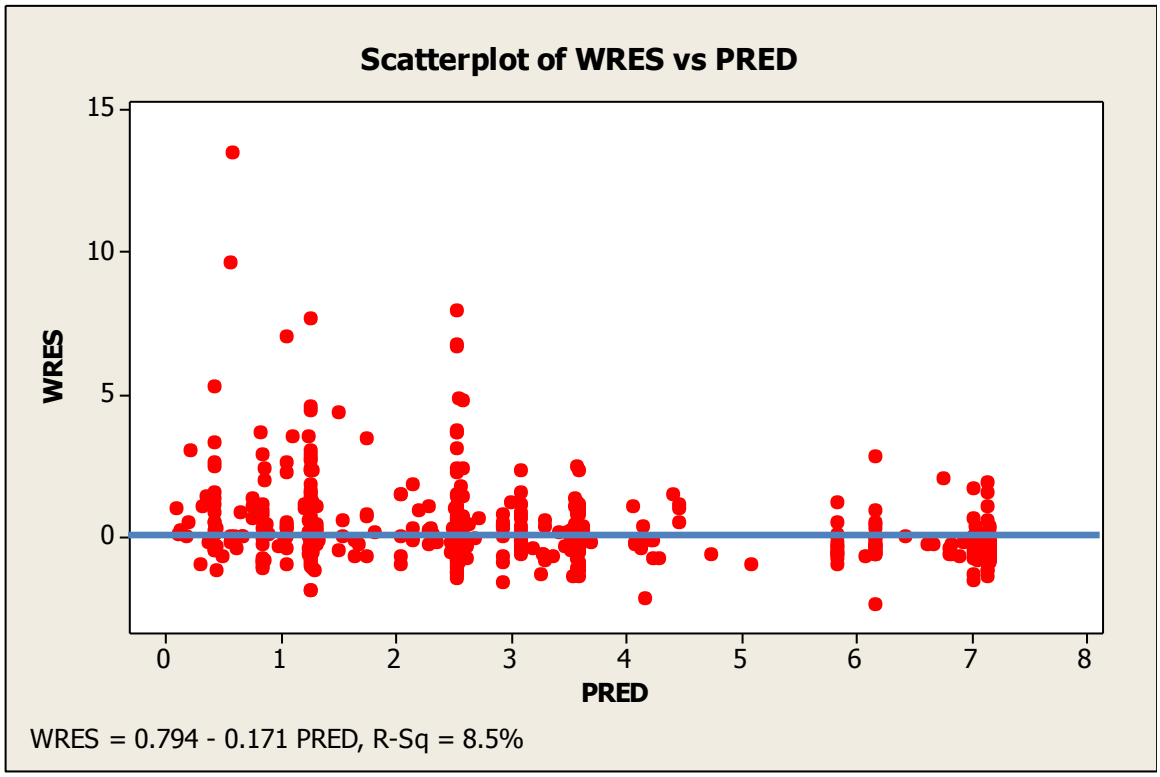


Figure 8: GOF plot of weighted residual vs. population prediction for original model

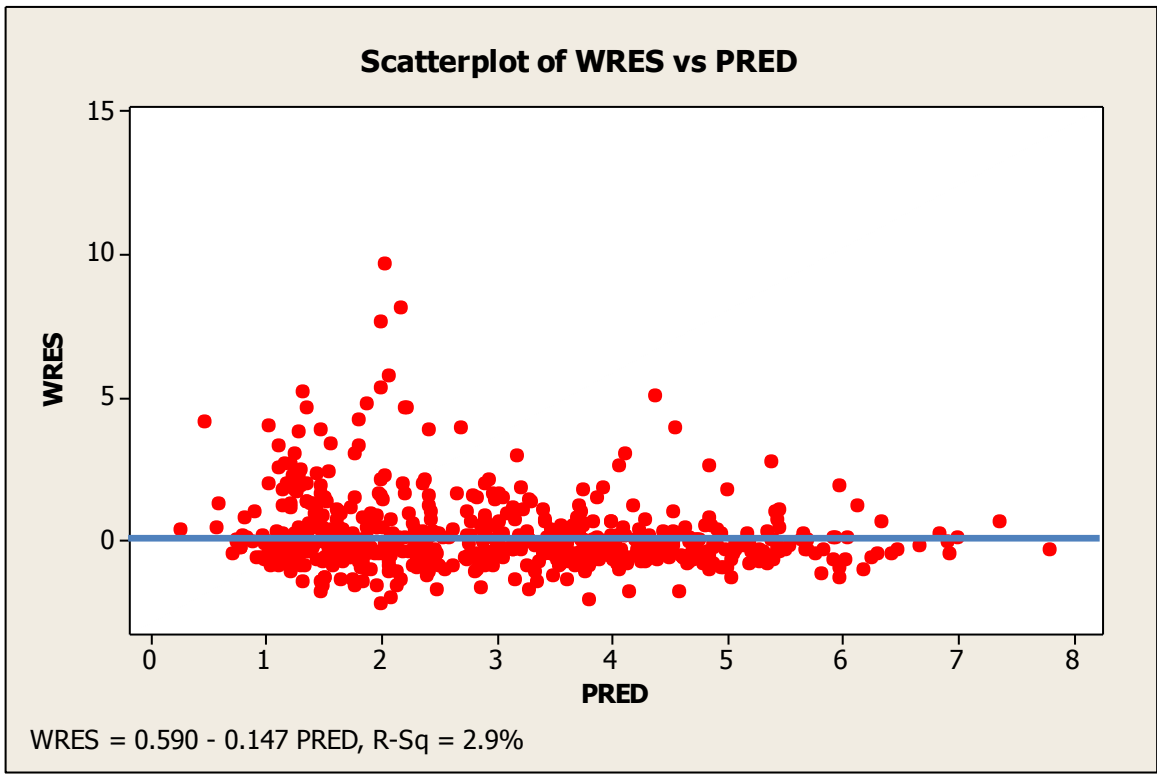


Figure 9: GOF plot of weighted residual vs. population prediction for final model

3.3 The final model for valganciclovir

We concluded that a 2-compartment model with first-order absorption best described the data at hand. The estimation method employed was the first-order conditional estimate (FOCE).
The inter-individual error was modeled with an exponential function, while a combined proportional and additive error-model best described the residual variability.

The model in table 8 below was the final model used. It also contains all starting estimates for THETA, OMEGA and SIGMA. These are, respectively, parameter estimates, inter-individual error variance and intra-individual error variance.

Table 8: The final model for valganciclovir

```
$PROB final model, faar vi se hvordan det gaar

$DATA alleValPOalder-1.txt

$INPUT ID AMT RATE DATE=DROP TIME CRCL WGT CONC=DV MDV
DOSE=DROP SEX DAY=DROP PASN=DROP AGE

$SUBROUTINE ADVAN4 TRANS4

$PK
CRCLM=CRCL/57.65 ; median
WGTM=WGT/66 ; median

TVCL=THETA(1)*(CRCLM**THETA(7))
CL=TVCL*EXP(ETA(1))
TVV2=THETA(2)
V2=TVV2*EXP(ETA(2))
TVQ=THETA(3)
Q=TVQ*EXP(ETA(4))
TVV3=THETA(4)
V3=TVV3*EXP(ETA(3))
KA=THETA(5)*(SEX*(1/THETA(8)))
ALAG1=THETA(6)*(SEX*THETA(9))
```

```
; THE FOLLOWING ARE REQUIRED BY PREDPP

K=CL/V2
K23=Q/V2
K32=Q/V3
S2=V2
S3=V3

$ERROR
IPRED=F
Y=F+F*ERR(1)+ERR(2)

$THETA
(0, 12, 50)
(0, 59.4, 100)
(0, 7.21, 100)
(0, 304, 400)
(0, 0.287) FIXED
(0, 0.661) FIXED
1.12
3.55
4.77

$OMEGA
0.329 4.11 3.42 0.533

$SIGMA
0.181 0.354

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=2000 PRINT=0
POSTHOC NOABORT

$TABLE ID DV TIME
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID KA K AMT TIME IPRED V2 V3
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

The graphs in figures 10 and 11 below show the Goodness of Fit scatterplots of CONC/PRED and CONC/IPRED for the final model. There is considerable spread in the population estimate, as well as some bias towards under-prediction. This can be seen in figure 10 because the bulk of the measurements lie under the line of union, while there are quite a few measurements in the left part of the plot high above the

line. The individual prediction however shows an acceptable precision of prediction. In figure 11 the measurements are more evenly distributed around the union, and the high concentrations are relatively well predicted, and thereby centered along the line.

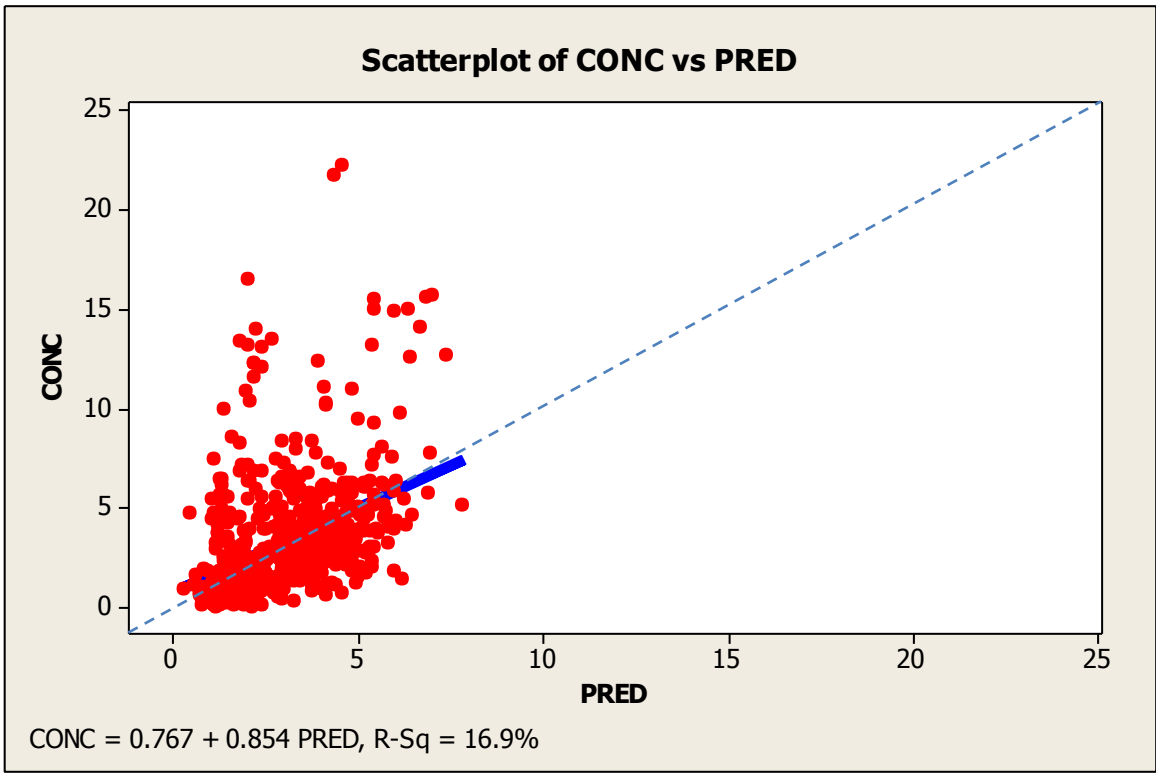


Figure 10: Goodness of fit plot of measurements vs. population predictions for the final model. The dotted line is the union, the whole line is the regression line.

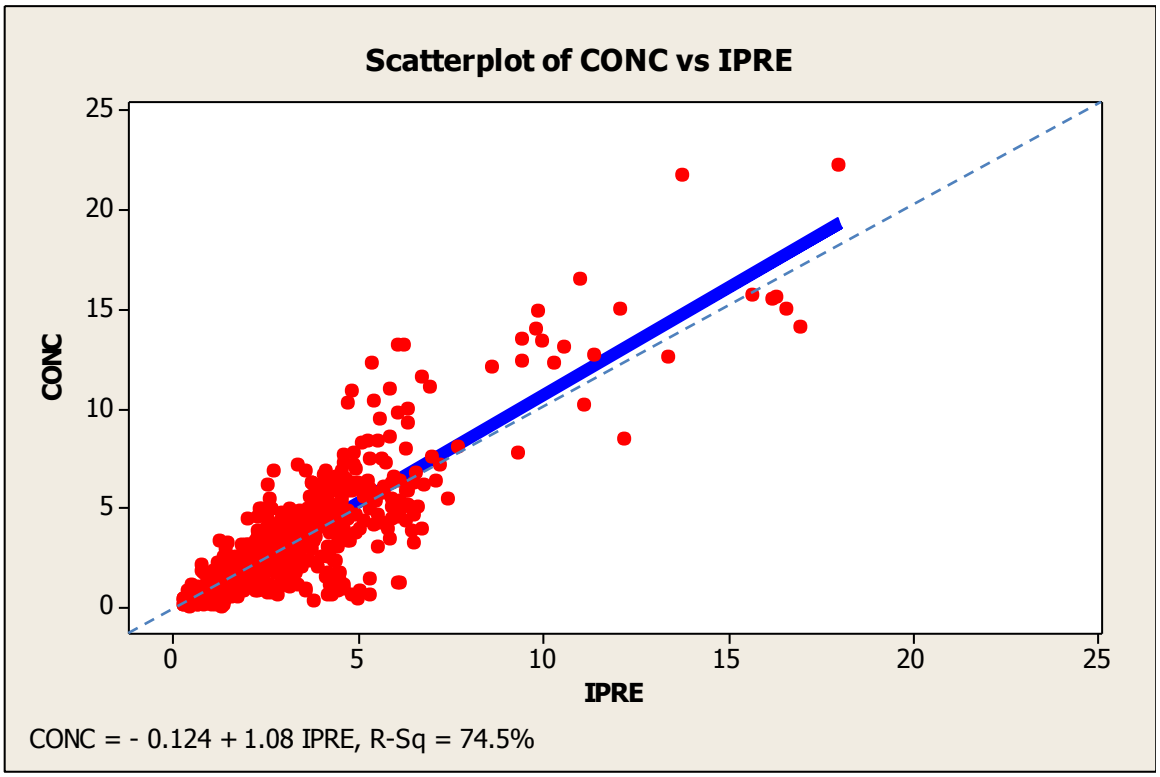


Figure 11: Goodness of fit plot of measurements vs. individual predictions for the final model. The dotted line is the union, the whole line is the regression line.

As can be seen from the plot of WRES vs. TIME (figure 12), there is a slight trend towards positive residuals. This also seems to first decrease somewhat with time, but then increase again after about 25 days.

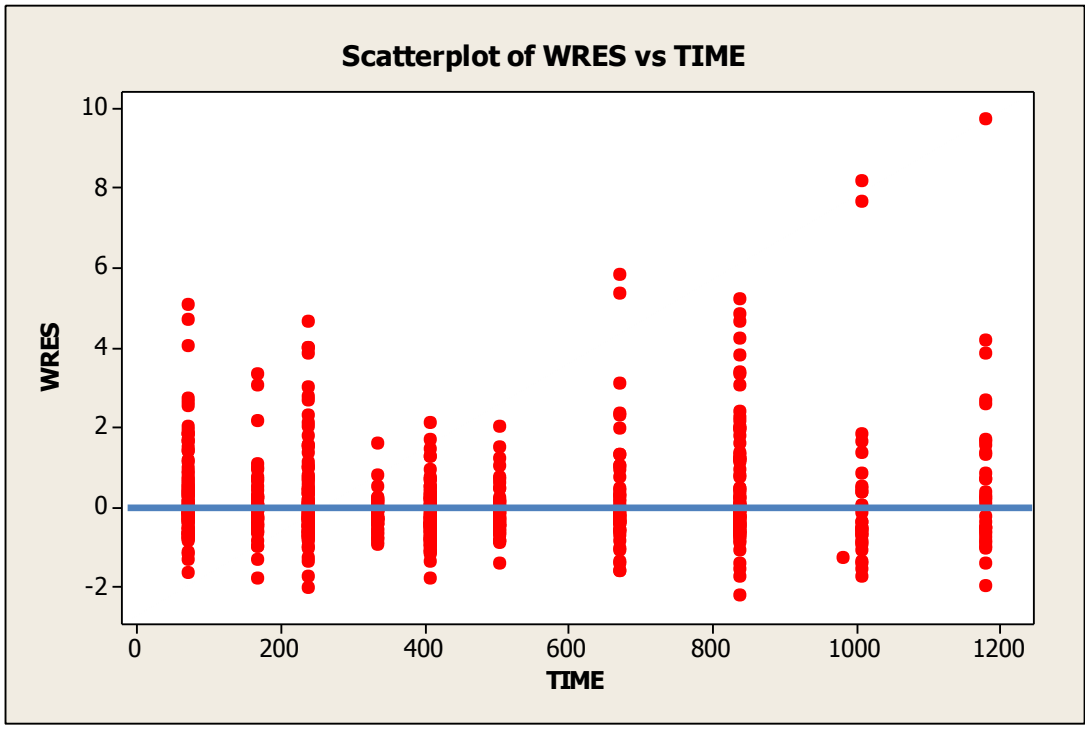


Figure 12: Plot of weighted residuals vs. time for the final model

The WRES vs. PRED plot (appendix 7.3.5) shows a very large positive WRES bias, which seems to decrease somewhat with increasing PRED values.
This bias is maintained in the WRES vs. IPRE plot (appendix 7.3.6), but here it is more spread out. Apart from this, the weighted residuals are fairly well centered on the zero-line, with a slight majority of the bulk on the negative side.

3.4 Validating the final model

3.4.1 Internal validation

The plots below (figure 13) illustrate the parameter estimates from each of the ten 90%-groups. Each plot shows that between three and seven estimates are more than two standard deviations away from the full input-file estimates.

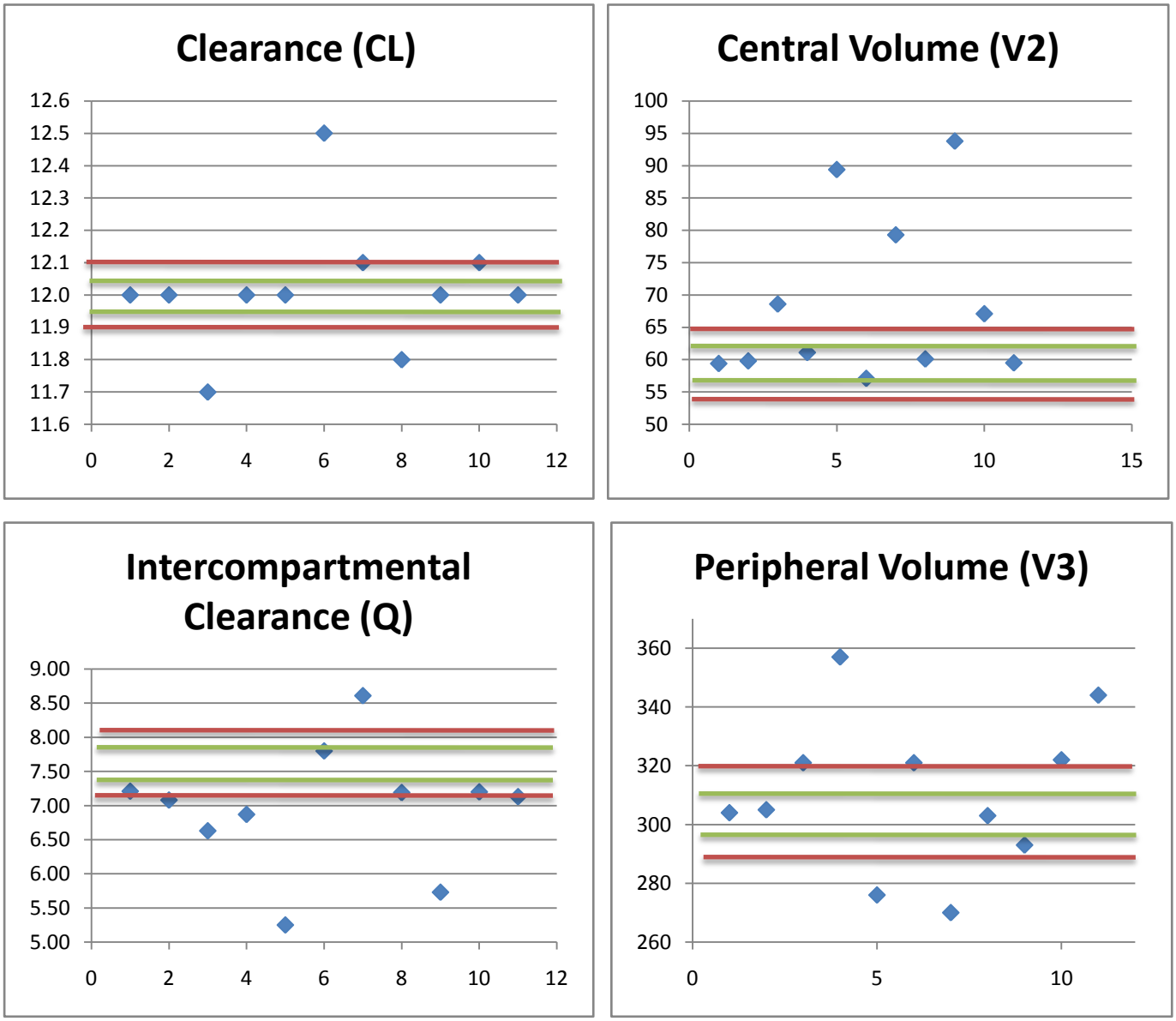


Figure 13: Parameter estimates from 90%-groups compared to full input-file. The green lines are one standard deviation away from the estimate of the final model on the full input-file, while the red lines are two standard deviations away.

3.4.2 Examining the predictive performance of the population model

Table 9 displays the prediction errors of the final model when run on the full data-set, with only the first and second observation, only the first observation and finally no drug concentration measurements at all.

Mean prediction error is a measure of bias, and the table shows that the model slightly under-predicts the concentrations.
Mean squared prediction error and Root mean squared error both ascertain the models precision in its predictions. It is apparent that the input-file with all measurements included gives the best predictions, while they become incrementally worse as the number of observations decreases.
The Average standardized prediction error was fairly close to zero on all input-files, which strengthens the validity of the models fit to the data. The 95% CI also contained zero for all input-files but the one without any observations.

Table 9: Validation of final model by statistical comparison of predictive error when measurements are excluded from the input-file

		All	One & Two	None	Only One
Mean Prediction Error (ME)	Mean	-0.15	-0.29	-0.36	-0.28
	SD	1.58	1.97	2.80	2.15
	95% CI min.	-0.27	-0.44	-0.57	-0.45
	95% CI max.	-0.03	-0.14	-0.14	-0.12
Mean Squared Prediction Error (MSE)	Mean	2.51	3.95	7.94	4.68
	SD	6.21	12.83	25.71	14.31
	95% CI min.	2.39	3.79	7.72	4.52
	95% CI max.	2.63	4.10	8.15	4.85
Root Mean Squared Error (RMSE)	Mean	1.06	1.25	1.80	1.38
	SD	1.18	1.55	2.17	1.67
	95% CI min.	0.94	1.10	1.59	1.22
	95% CI max.	1.18	1.40	2.02	1.55

Average Standardized Prediction Error (SPE)	Mean	-0.06	-0.13	-0.24	-0.13
	SD	0.64	0.87	1.85	1.00
	95% CI min.	-0.18	-0.28	-0.45	-0.30
	95% CI max.	0.06	0.02	-0.02	0.03

ME and RMSE have the unit μg/mL, while RMSE has the unit $(\mu g/mL)^2$

Table 10 contains OFVs for the 90%-groups compared to the OFV from the full input-file. All the OFVs were centered very well on the OFV from the full input-file, and none deviated more than 2.39 away from it. This is well inside the cut-off value of 3.84 for a 95% CI, and is highly significant. This indicates that the model is quite robust.

Table 10: Validation of final model by statistical comparison of OFVs when is stabilized on 90% of the population, then run on the full input-file

OFV Value	Final model		1362.63	Mean	Minimum
	Subgroups				
	Fixed parameter values from included subsets (90%) on full input file	1	1362.74		
		2	1363.01	1362.998	1362.11
		3	1363.32	Median	Maximum
		4	1362.11		
		5	1363.00		
		6	1365.02	1362.87	1365.02
		7	1362.71	Standard deviation	Standard error of mean
		8	1362.16		
		9	1363.24		
		10	1362.67	0.81641221	0.25817221

3.4.3 Confidence interval of population parameters

Table 11 contains the results from the Jackknife method.

As can be seen from the results in the table below, the Jackknife method showed that no single patient had any significant influence on the parameter estimates gained through use of the final model.

Table 11: Validation of final model by statistical comparison of parameter estimates after use of the Jackknife method

PK Parameters	Mean	Median	SD
Clearance (CL)	12.013	12.000	0.048
Central Volume (V2)	60.206	59.400	2.761
Intercompartmental Clearance (Q)	7.199	7.210	0.199
Peripheral Volume (V3)	307.093	304.000	7.794

PK Parameters	Minimum	Maximum	95% CI min.	95% CI max.
Clearance (CL)	12.000	12.300	12.004	12.022
Central Volume (V2)	58.400	79.800	59.686	60.727
Intercompartmental Clearance (Q)	5.540	7.940	7.162	7.237
Peripheral Volume (V3)	273.000	346.000	305.623	308.562

4. Discussion

4.1 Model building

Building the model was a challenging task. Searching for literature on population modeling of valganciclovir divulged disappointingly little. Very few studies on similar problems had been performed, and none on patients treated for active CMV-disease as in the VICTOR trial. There did however turn up one study that was very similar to ours during the last weeks of the thesis-writing, by Perrottet et al. [15], but it was not available during the development phase of the model. Wiltshire et al. had conducted the PV16000 study which was available for reference during the building of our model, and it was used as a template and guide in this process. The main difference between PV16000 and VICTOR was that PV16000 was conducted as a prophylactic study, where patients were given valganciclovir immediately after transplantation to prevent viral replication. VICTOR was a treatment study, where only transplant recipients with active CMV-disease were given valganciclovir. In addition, VICTOR had a secondary goal which was the investigation for a correlation between ganciclovir plasma concentration, viremia concentration and adverse effects. Both the PREDPP routines used, as well as the initial parameter estimates, were useful in choosing a place to start.

It was also noted that both Perrottet et al. and Wiltshire et al. had chosen to employ the 2-compartment set-up with first-order absorption. This lends strength to the argument that the 2-compartment is the optimal choice [13, 15].

Model building was a time consuming process, with much trial and error. The challenges lay mainly in getting the models to run, and getting the parameters stable. In the end, not many different models were tested before a two-compartment model was chosen. Only first-order absorption was tested, based on data gathered in a previous study [25].

It was decided that a 2-compartment model with first-order absorption was a reasonable assumption. This was based upon the OFV which was far better than the 1-compartment models, the better stability and shorter run-time than the 3-compartment model and the visual inspection of the plots. It also seemed appropriate considering the chemical and pharmacokinetic profile of valganciclovir.
In addition, other approaches to structural models would be tested and compared to the 2-compartmental model with first-order absorption.

The work of developing a model was dependent on a good input-file, the creation of which was a time-consuming task. The finished Excel worksheet contained 14 columns and 7622 rows of data.

The control-files were designed based on earlier studies by Wiltshire et al. [13] as well as Trúc Vân Lê [33]. The NONMEM guides [22] were also essential to the construction of different models.

During the initial development and the search for the optimal type of structural model to build our own model on, we used a minimal data-set with 11 patients to get a feel for the different models. We then increased the number of patients to 30 to see how this affected the initial assumptions on differences between models. After running some further tests on this set, we finally included all patients in the input-file.

After having built the full data-set, some of the first runs showed that a few of the more extreme observations caused stability problems. We therefore trimmed the data-set and used this new version alongside the full version during forward inclusion and backwards deletion. After that we discarded the trimmed set and only used the full set. No data-points were excluded in the final testing of the model, seeing as we had no rational for judging them as sampling- or calculation-errors.

4.1.1 1-compartment, first-order absorption

This model was written with ADVAN2 and two different subroutines: TRANS1 and TRANS2. The different numbered ADVANs instruct NONMEM as to how many compartments the model should have, and whether or not it has an absorption

compartment. The TRANS subroutines instruct NONMEM which basic PK parameters the user will supply starting estimates for. Here TRANS1 means the user must supply an estimate of the rate constant of elimination (K), while in TRANS2 he must supply estimates of clearance (CL) and volume of distribution (V).

TRANS1: This model showed a very poor fit to the data. The plots had few curves, mostly hard edges, and a distinctly unnatural look. The elimination was also much too fast, as seen by how the concentration-curve fell vertically to zero immediately after the dosage decreased or ceased. The plots of observed/predicted vs. time also showed a severe bias of firstly under-prediction which then turned to over-prediction with time. The OFV was 324.64. This model was discarded because of its obvious uselessness.

TRANS2: This approach displayed a much smoother attempt at plotting the predictions. There was still a certain amount of under-prediction, but far less than with TRANS1. The model was also much more consistent in its estimations. Particularly the individual predictions were showing an improved fit to the measured data. The OFV of 265.26 was also far superior to the TRANS1 model. Clearance (CL/F) was here estimated at 11.3 L/h and central volume (V2/F) at 0.06 L. Not ideal by any means.

4.1.2 2-compartment, first-order absorption

The control-file here utilized the ADVAN4 routine with the TRANS4 subroutine. ADVAN4 means the model is 2-compartment with first-order absorption, and TRANS4 means here that the user must supply clearance (CL), central volume (V2), intercompartmental clearance (Q), peripheral volume (V3) and absorption rate constant (KA). Visually there was not much improvement to the plots as compared to the 1-compartment model with TRANS2. The OFV though did sink to 238.64, a betterment of $\Delta 26.62$. The under-prediction also persisted as in the prior attempts. At this point in the development, the model experienced serious problems with stability. After some research and experimentation, a change of the intra-individual error model from additive to combined proportional and additive proved to be the solution.

This error model was then kept as the standard in the models further development. The estimate of clearance (CL/F) was now 3.54 L/h and central volume (V2/F) was 10.9 L, with a peripheral volume (V3/F) of 99.4 L and an intercompartmental clearance (Q/F) of 1.58 L/h. This seemed more plausible.

4.1.3 3-compartment, first-order absorption

This step in the building phase used the ADVAN12 routine with the TRANS4 subroutine. ADVAN12 means it is a 3-compartment model with first-order absorption. TRANS4 means here the user must input estimates for the parameters clearance (CL), central volume (V2), intercompartmental clearance between central and peripheral 1 (Q3), peripheral 1 volume (V3), intercompartmental clearance between central and peripheral 2 (Q4), peripheral 2 volume (V4) and absorption constant (KA). There was some visual change in the plots, a more rapidly decreasing curve, but not by much. The under-prediction stayed the same though. The slight improvement was noticeable in the OFV, as it sank by 5.59, to 233.05 points. While this was statistically significant, there arose stability problems which kept returning. The parameter estimations in this model: clearance (CL/F) was 6.33 L/h, intercompartmental clearance one (Q1/F) and two (Q2/F) were 5.71 L/h and 3.73 L/h respectively, central volume (V2/F) was 0.1 L, and peripheral volumes one (V3/F) and two (V4/F) were 6130 L and 0.05 L respectively. The volumes in particular were improbable.
This, in addition to the increased complexity, longer run-time and minimal improvement in prediction- and goodness of fit-plots, led us to decide not to continue development of this file.

The 2-compartmental model with first-order absorption was chosen for further development. It showed far superior OFV and visual graphical fit over the two 1-compartmental models, and a superior stability as well as run-time over the 3-compartmental model. In addition it complied with previous findings [13].

4.2 Analyzing for covariates

During the forward inclusion we found it somewhat strange that neither weight nor sex had any significant influence on either central or peripheral volume. This was surprising, as we expected gender-based divergences to stem from differences in liquids and fat. The fact that gender had such a strong effect on absorption constant (KA) and absorption time lag (ALAG) was also unexpected. This might warrant further investigation, either with a different kind of data set or with different kind of coding.

The estimation-values for KA and ALAG were fixed at the values from the PV16000 study. We chose to do this because all our drug measurements were trough-measurements. This did not enable us to estimate these parameters with sufficient accuracy to establish our own estimates.

When preparing to do the backwards deletion, we chose the very strict criterion of $\Delta OFV > 10.9$, giving $p<0.01$. We did this to be quite sure that the covariates chosen were indeed significant, but in hindsight this was perhaps too stringent. $P<0.05$ would still have been significant, and would have let some more covariates be included in the model. This could possibly have improved the predictive capability of the model.

During the writing of the input-file the time for administration of morning dose and taking of blood samples was set to a uniform 08:00, and 08:20 when there had been taken a sample. The administration of the evening dose was set to 20:00.
Later on, during the writing of the thesis, information was found that detailed deviations from the planned administration and sampling schedule. Some tests were run on a few patients that were affected by this, to ascertain if it would have any serious impact on the results of the final model, and the validity of it. These tests showed a negligible effect on the models predictions after the inclusion of this data. Nevertheless, it should be kept in mind that the inclusion of these corrections on the data of the patients in question could prove significant for the predictive precision of the population model.
Because all the measurements were trough-concentrations, we were not able to

estimate absorption lag time (ALAG) nor the absorption constant (KA) to any reasonably stable degree. Should this additional time data prove diverse enough, it might allow the estimation of KA and ALAG from the data at hand. This could improve the models predictive power.

During the decision-making process where factors were evaluated for inclusion in the testing for covariates, time after transplant was considered not to be a probable candidate. During the finishing work on the thesis, however, it was discovered that time after transplant was included in some population models on ciclosporin A were sampling had been done on different occasions [33]. Considering that our data had been sampled over a period of 50 days, and that patients had been included in the study on varying times after receiving transplants, the decision about testing time after transplant was reconsidered. We now believe it merits testing for significance as a covariate in the model, and that this should be done in a possible further refinement of this model.

Gender was modeled with both linear equations and IF/ELSE statements. This was done because some literature and previous studies [23, 33] had coded gender in that way. This did however not significantly improve our model, and the linear function was therefore chosen. This made the model slightly simpler by reducing the number of thetas by 1-2, depending on equation.

Comparing the original model and the final model shows that inclusion of covariates made a definite improvement. Especially in the individual predictions a much better fit to the data at hand can be observed. The population predictions were not that much better, compared to the individual predictions. The under-prediction was also still quite evident. The refinement of the entered time-data might be worth testing to see if it would mitigate the state of the model, as well as the reevaluation of certain factors regarding possible inclusion as covariates.

4.3 The final model for valganciclovir

The model chosen as original model after testing of different possibilities was the 2-compartment model with first-order absorption. The plots described the data reasonably well compared to the other models tested. The stability was also adequate, particularly after adjustment of the residual error function. In addition the run-time was short, which is not only good during the building and validation of a population model, but also later on should it be chosen for use in a clinical setting. While the 2-compartment model was not as good as the 3-compartment when compared on OFV, it sported superior run-time, simplicity and most important, stability.

As has been mentioned earlier, the final model shows a certain positive bias in the plot of its residuals. One can also see that there is a tendency towards a decrease in residuals with higher concentrations, although slight. This might indicate a problem with either predictions or measurements at lower concentrations, or simply a result of the fact that there are more low concentration measurements than there are high. The plot of weighted residuals vs. time displays an increase in the spread of residuals from around 600 hours (25 days). This coincides roughly with the end of the induction period and the beginning of the maintenance period (day 21). Whether or not this is a coincidence, and if there are any other factors that might be responsible for this, might merit further investigation.

Under-prediction has plagued our model since the very beginning, and we were not able to eliminate it to a satisfying degree. Exactly why this persists remains therefore undetermined, but may be correlated with the high estimations of V2/F and V3/F. It may be an error stemming from divergences in measurement or protocol deviations. It could also be non-compliance on the patient's part. Lastly, it may be factors we did not consider, and that would have had an influence on our model.

The data-set contained a number of measurements that seemed strangely high compared to different measurements in the same patient. Inter-occasional variability could of course be responsible for this, but it might be advisable to investigate if these concentrations are real or if they are erroneous. Should the latter be the case it could

possibly mitigate some of the problems the model has with large residuals. Then again, many of these measurements were predicted with a high enough degree of accuracy.

The goodness of fit plot for concentration vs. population prediction (figure 10) shows a drastic amount of spread. It also shows once more the under-prediction, as well as the measurements that diverge widely from the others in the population.
The plot for concentration vs. individual prediction (figure 11) is much more accurate. It shows a large degree of consistency, that is particularly impressing when compared to the previous plot. It also shows that most of the drug concentration measurements in the high end of the scale can be predicted to a certain degree. This supports the view that these are real measurements, not errors.

4.4 Validating the Model

When validating a model one is basically checking how good it really is. Taking off the training-wheels, so to speak. A model may be developed which looks very good. It can have a high predictive precision, be simple, fast and stable, and yet still come short at the validation step. If it is too fragile to cope with changes in the data-material, it is not a very versatile model. In some cases, where the model only seeks to explain the data in a given set, this may not be a problem. But when one designs a model to make predictions on a population beyond the available data, one must make sure it makes valid predictions for said population.

There are many different methods and approaches for validation, and since there are none that are clearly superior in every case, a researcher is at the mercy of his own decision. There may also be situations where one model has clear advantages over another [4, 5].

Validating a model can be defined as testing to see if a model developed on a set of data called developmental data manages to accurately predict the observations made in a set of data called the validation data.
Whether or not a model is judged valid depends largely on the purpose of the model,

meaning that models are seldom good all-round solutions, but rather developed specifically for a single task. There is no correct answer to the question: “what is the right model?”. Neither is there a single correct way to develop aforesaid model, nor to test it. Therefore personal opinion is a deciding factor when it comes to both the developing and testing of a model. As can be easily derived from this, there does not exist any consensus at the time of writing regarding what is the single best approach to validation. There are different validation methods, and some may be more prudent to use than others when it comes down to each single model. There is simply too much complexity possible in models for any one method to be able to reliably validate them all [4].

I will briefly mention some types of validation.

External validation is when one develops a model on a certain data-set, and then afterwards validates the model on a different but relevant data-set. This is the most rigorous method for testing a model.

Internal validation is when only one set of data is being used for both development and validation of the model. This term contains some different methods and techniques.

Data splitting is when one splits the data set in two parts. One is used for model building, and the second is used for validation. It is often recommended to make a 2-1 split. Two thirds are used for building, one third for testing.

Cross-validation is essentially a repeated data-splitting process. The results from each data-split give a prediction-error estimate, and these are averaged across all the repeats. The splits can be done randomly or not, and with different organizational approaches. A common method which will be used in this thesis is the K-fold cross-validation. The data is split into K parts. Then the model is fitted to K-1 of the parts, before it is validated on the last part. This is done K times, and the results are then averaged. One of the benefits is a reduced variability because one does not rely on a

single sample split [27, 28].

Bootstrapping is a form of resampling. After having taken a sample of size N from a population, one then repeatedly takes samples of size N from this first sample, but with replacement. Since the first sample is representative of the population, this will give the effect of having taken many samples from the population instead of just one. One can then calculate for instance a parameter in each second sample, and get a mean, variance and interval from looking at them all [28, 34].

Jackknife is a method for inferring the bias and the standard error of a particular statistic, for instance a parameter in a PK model. It is a resampling technique quite similar to bootstrapping, and basically consists of re-computing the statistic in question a number of times equal to the number of observations in the data set. Each time, though, one removes a different observation. From this new set of estimates one can then calculate bias and standard error for the parameter derived from the original full data set. Jackknife is not as widely applicable as the Bootstrap, but requires mostly less computational power. While the results gained from the two methods are often similar, the numbers yielded will still usually be different to varying degrees [28, 31].

In this thesis the decision to use a K-fold cross-validation and the jackknife method was based on recommendations from the Food and Drug Administration (FDA), as well as the work done in the thesis by Trúc Vân Lê [4, 33].

The results from the validation of the final model showed that OFV remained almost constant across the variations in sub-population groups. This indicates great robustness in the model. On the other hand, the parameter estimates from the sub-population groups varied widely. This might indicate that the original population was on the verge of how small it could be while still having enough power for predictive significance. When we further decreased the number of individuals in a group, it may have brought it below the limit necessary to give stable parameter estimates. This might have been circumvented by increasing the number of folds in the cross-

validation to for instance K-20.
The wide variations in estimates could of course also be indicative of a lack of stability across differing data-sets, or possibly hidden covariates.

The parameter estimates of the final model were quite acceptable for clearance (CL/F = 12.00 L/h) and intercompartmental clearance (Q/F = 7.21 L/h). The central volume (V2/F = 59.40 L) and peripheral volume (V3/F = 304.00 L) estimates were however somewhat unexpected. The previous study by Wiltshire et al. [25] had found 18.5 L and 44.4 L respectively, and the study by Perrottet et al. reported 28 L and 19.5 L respectively. While these showed some variation, our estimates were still far higher, particularly for the peripheral volume (V3), indicating a reason for the under-prediction by the model.

When performing the cross-validation on the four different 10%-groups, we observed that the sets with all the data points gave the best predictions, and that through the sets with two data points and one data point the predictions became gradually worse, ending up at the worst predictions on the data-set with no data points. This was what we had expected.
It should also be mentioned that the first data point used in set two and three was from day 3 in the study. This was done because some patients had minute concentrations measured in their samples on day 0. They should not have had any drug in their blood at that time, and it might indicate that they already were on the drug at the start of the study, or that there had been deviations from the administration and measurement schedule.

The Jackknife method used to calculate the 95% confidence interval required the tedious creation of 108 control-files and 108 input-files. It was nevertheless chosen above the Bootstrap method because it requires less computational power.
The confidence intervals gained display a sufficient lack of variation, and show that no single patient had any significant influence on the parameter estimates.

The validation showed both the apparent robustness of the model through the stableness of the OFVs after the cross-validation, and the apparent instability after the

comparison of the parameter estimates of the 10 sub-groups. It also showed that the model requires at least a few data points to give any meaningful predictions, and that although the population predictions are not particularly impressive, the individual predictions still are quite accurate.

Even so, there is much room for improvement. There is the possibility of additional significant covariates, and there are measurements that seem strange compared to the others. The data-set might need to be embellished with more detailed time-data, and the residuals point towards decreased predictive accuracy with time as well as with low concentrations. All in all, the final model is a good start, but it needs further development.

5. Conclusions

A pharmacokinetic population model was developed by means of the nonlinear mixed effects modeling computer program NONMEM for ganciclovir after administration of the prodrug valganciclovir in solid organ transplant recipient patients. After further refinement and combination with a model for CMV, this model may be of clinical use in devising dosage regimes as well as investigating associations with therapeutic effect and adverse events. This is the overall goal of developing the population pharmacokinetic model in this thesis.

A 2-compartment model with first-order absorption, combined with the covariates creatinine clearance on clearance and gender on absorption rate constant and absorption lag time, was found to be the best model.

The validation of the model showed that it was apparently robust, but at the same time, however, the parameter estimates produced differed greatly on the same varied population-base. This indicates that the model has trouble dealing with different populations.
Nevertheless, the model delivered good individual predictions for all population sets, provided it had at least a single drug-concentration measurement per individual to work with.

The model shows potential for further refinement, but manages regardless to fulfill the goals as set down for this thesis.

6. References

1. Rowland M., T.N.T., *Clinical Pharmacokinetics Concepts and Applications*. Third ed. 1995, Philadelphia: Lippincott Williams & Wilkins. 601.
2. D. Fisher, S.S., *Fisher/Shafer NONMEM Workshop Pharmacokinetic and Pharmacodynamic Analysis with NONMEM - Basic Concepts*, in *NONMEM Workshop*. 2007: Het Pand, Ghent, Belgium. p. 115.
3. Aarons, L., *Population pharmacokinetics: theory and practice.* Br J Clin Pharmacol, 1991. **32**(6): p. 669-70.
4. *Guidance for industry on Population Pharmacokinetics; availability. Food and Drug Administration, HHS. Notice.* Fed Regist, 1999. **64**(27): p. 6663-4.
5. Williams, R., et al., *Population Pharmacokinetics: A Regulatory Perspective.* Clinical Pharmacokinetics, 1999. **37**: p. 41-58.
6. Ette, E.I. and P.J. Williams, *Population pharmacokinetics II: estimation methods.* Ann Pharmacother, 2004. **38**(11): p. 1907-15.
7. Csajka, C. and D. Verotta, *Pharmacokinetic-pharmacodynamic modelling: history and perspectives.* J Pharmacokinet Pharmacodyn, 2006. **33**(3): p. 227-79.
8. Beatrice Mercorelli, E.S., Arianna Loregian, Giorgio Palù,, *Human cytomegalovirus DNA replication: antiviral targets and drugs.* Reviews in Medical Virology, 2008. **18**(3): p. 177-210.
9. Cocohoba, J. and I. McNicholl, *Valganciclovir: an advance in cytomegalovirus therapeutics.* Ann Pharmacother, 2002. **36**(6): p. 1075-1079.
10. Carlos Paya, A.H., Ed Dominguez, Kenneth Washburn, Emily Blumberg, Barbara Alexander, Richard Freeman, Nigel Heaton, Mark D. Pescovitz,, *Efficacy and Safety of Valganciclovir vs. Oral Ganciclovir for Prevention of Cytomegalovirus Disease in Solid Organ Transplant Recipients.* American Journal of Transplantation, 2004. **4**(4): p. 611-620.
11. Biron, K.K., *Antiviral drugs for cytomegalovirus diseases.* Antiviral Research, 2006. **71**(2-3): p. 154-163.
12. Aslam, M., et al., *CMV-Induced Neonatal Thrombocytopenia: A Case Report and Review of the Literature.* Amer J Perinatol, 2007. **24**(07): p. 429-434.
13. Wiltshire, H., et al., *Pharmacokinetic Profile of Ganciclovir After its Oral Administration and From its Prodrug, Valganciclovir, in Solid Organ Transplant Recipients.* Clinical Pharmacokinetics, 2005. **44**: p. 495-507.
14. A. Åsberg, A.H., H. Rollag, A. G. Jardine, H. Mouas, M. D. Pescovitz, D. Sgarabotto, M. Tuncer, I. L. Noronha, A. Hartmann,, *Oral Valganciclovir Is Noninferior to Intravenous Ganciclovir for the Treatment of Cytomegalovirus Disease in Solid Organ Transplant Recipients.* American Journal of Transplantation, 2007. **7**(9): p. 2106-2113.
15. Perrottet, N., et al., *Population pharmacokinetics of ganciclovir in solid organ transplant recipients receiving oral valganciclovir.* Antimicrob. Agents Chemother., 2009: p. AAC.00836-08.
16. D Salmon-Ceron, *Cytomegalovirus infection: the point in 2001.* HIV Medicine, 2001. **2**(4): p. 255-259.
17. Martin, D.F., et al., *A Controlled Trial of Valganciclovir as Induction Therapy for Cytomegalovirus Retinitis.* N Engl J Med, 2002. **346**(15): p. 1119-1126.
18. Roche, *Cymevene SPC*.

19. W. Vaudry, R.E., P. Jara, G. Varela-Fascinetto, M. R. Bouw, J. Ives, R. Walker,, *Valganciclovir Dosing According to Body Surface Area and Renal Function in Pediatric Solid Organ Transplant Recipients.* American Journal of Transplantation, 2009. **9**(3): p. 636-643.
20. C. E. Chamberlain, S.R.P., R. M. Alfaro, R. Wesley, C. E. Daniels, D. Hale, A. D. Kirk, R. B. Mannon,, *Pharmacokinetics of Low and Maintenance Dose Valganciclovir in Kidney Transplant Recipients.* American Journal of Transplantation, 2008. **8**(6): p. 1297-1302.
21. Cvetkovic, R.S. and K. Wellington, *Valganciclovir: a review of its use in the management of CMV infection and disease in immunocompromised patients.* Drugs, 2005. **65**(6): p. 859-78.
22. S. L. Beal, A.J.B., L. B. Sheiner, *NONMEM Users Guide*, ed. N.P. Group. 1992, San Francisco: University of California at San Francisco.
23. Jonsson, E.N. and M.O. Karlsson, *Automated Covariate Model Building Within NONMEM.* Pharmaceutical Research, 1998. **15**(9): p. 1463-1468.
24. Wählby, U., E. Jonsson, and M. Karlsson, *Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis.* The AAPS Journal, 2002. **4**(4): p. 68-79.
25. Wiltshire H, H.S., Farrell C, Paya C, Pescovitz MD, Humar A, Dominguez E, Washburn K, Blumberg E, Alexander B, Freeman R, Heaton N, *Pharmacokinetic profile of ganciclovir after its oral administration and from its prodrug, valganciclovir, in solid organ transplant recipients.* Clinical Pharmacokinetics, 2005. **44**(5): p. 495-507.
26. Saint-Marcoux F, M.P., Jacqz-Aigrain E, Bernard N, Thiry P, Le Meur Y, Rousseau A, *Patient characteristics influencing ciclosporin pharmacokinetics and accurate Bayesian estimation of ciclosporin exposure in heart, lung and kidney transplant patients.* Clinical Pharmacokinetics, 2006. **45**(9): p. 905-922.
27. Kohavi, R., *A study of cross-validation and bootstrap for accuracy estimation and model selection.* Proceedings of the Fourteenth International Joint Conference on Artificial Intelligence, 1995: p. 1137-1143.
28. Efron, B. and G. Gong, *A Leisurely Look at the Bootstrap, the Jackknife, and Cross-Validation.* The American Statistician, 1983. **37**(1): p. 36-48.
29. Haahr, M. *Random.org*. 2009 [cited 2009 04.04.2009]; Available from: http://www.random.org/.
30. Ette, E.I., *Stability and performance of a population pharmacokinetic model.* J Clin Pharmacol, 1997. **37**(6): p. 486-95.
31. EFRON, B., *Nonparametric estimates of standard error: The jackknife, the bootstrap and other methods.* Biometrika, 1981. **68**(3): p. 589-599.
32. Storehagen, L., *Ciclosporin A - Development of a Pharmacokinetic Population model*, in *Department of Pharmaceutical Biosciences, School of Pharmacy, Faculty of Mathematics and Natural Science*. 2007, University of Oslo: Oslo. p. 81.
33. Lê, T.V., *A Pharmacokinetic Population Model for Cyclosporin A in Renal Transplant Recipients*, in *Department of Pharmaceutical Biosciences, School of Pharmacy, Faculty of Mathematics and Natural Science*. 2008, University of Oslo: Oslo. p. 95.
34. T. C. Hesterberg; D. S. Moore, S.M., A. Clipson, and R. Epstein, *Bootstrap methods and permutation tests*, in *The Practice of Business Statistics*. 2005, W. H. Freeman and Company: New York.

7. Appendix

7.1 Cockcroft-Gault equation

$$GFR = \frac{(140 - age\ in\ years) \times weight\ in\ kgs}{72 \times Serum\ creatinine\ in\ {}^{mg}/_{dL}} \times 0.85\ for\ women$$

7.2 Partial input-file for NONMEM

#ID	AMT	RATE	DATE	TIME	CRCL	WGT	CONC =DV	MDV	DOSE	SEX	DAY	PASN	AGE
1	0	0	8/1/2006	08:00	84.53	89	0	0	900	2	0	6	40
1	900	0	8/1/2006	20:00	84.53	89	0	1	900	2	0	6	40
1	900	0	8/2/2006	08:00	84.53	89	0	1	900	2	1	6	40
1	900	0	8/2/2006	20:00	84.53	89	0	1	900	2	1	6	40
1	900	0	8/3/2006	08:00	84.53	89	0	1	900	2	2	6	40
1	900	0	8/3/2006	20:00	84.53	89	0	1	900	2	2	6	40
1	0	0	8/4/2006	08:00	84.53	89	2.23	0	900	2	3	6	40
1	900	0	8/4/2006	08:20	82.63	89	0	1	900	2	3	6	40
1	900	0	8/4/2006	20:00	82.63	89	0	1	900	2	3	6	40
1	900	0	8/5/2006	08:00	82.63	89	0	1	900	2	4	6	40
1	900	0	8/5/2006	20:00	82.63	89	0	1	900	2	4	6	40
1	900	0	8/6/2006	08:00	82.63	89	0	1	900	2	5	6	40
1	900	0	8/6/2006	20:00	82.63	89	0	1	900	2	5	6	40
1	900	0	8/7/2006	08:00	82.63	89	0	1	900	2	6	6	40
1	900	0	8/7/2006	20:00	82.63	89	0	1	900	2	6	6	40
1	0	0	8/8/2006	08:00	82.63	89	0.68	0	900	2	7	6	40
1	900	0	8/8/2	08:20	76.61	89	0	1	900	2	7	6	40

			006										
			8/8/2										
1	900	0	006	20:00	76.61	89	0	1	900	2	7	6	40
			8/9/2										
1	900	0	006	08:00	76.61	89	0	1	900	2	8	6	40
			8/9/2										
1	900	0	006	20:00	76.61	89	0	1	900	2	8	6	40
			8/10/										
1	900	0	2006	08:00	76.61	89	0	1	900	2	9	6	40
			8/10/										
1	900	0	2006	20:00	76.61	89	0	1	900	2	9	6	40
			8/11/										
1	0	0	2006	08:00	76.61	89	2.03	0	900	2	10	6	40
			8/11/										
1	900	0	2006	08:20	83.58	89	0	1	900	2	10	6	40
			8/11/										
1	900	0	2006	20:00	83.58	89	0	1	900	2	10	6	40
			8/12/										
1	900	0	2006	08:00	83.58	89	0	1	900	2	11	6	40
			8/12/										
1	900	0	2006	20:00	83.58	89	0	1	900	2	11	6	40
			8/13/										
1	900	0	2006	08:00	83.58	89	0	1	900	2	12	6	40
			8/13/										
1	900	0	2006	20:00	83.58	89	0	1	900	2	12	6	40
			8/14/										
1	900	0	2006	08:00	83.58	89	0	1	900	2	13	6	40
			8/14/										
1	900	0	2006	20:00	83.58	89	0	1	900	2	13	6	40
			8/15/										
1	0	0	2006	08:00	83.58	89	2.99	0	900	2	14	6	40
			8/15/										
1	900	0	2006	08:20	84.53	89	0	1	900	2	14	6	40
			8/15/										
1	900	0	2006	20:00	84.53	89	0	1	900	2	14	6	40
			8/16/										
1	900	0	2006	08:00	84.53	89	0	1	900	2	15	6	40
			8/16/										
1	900	0	2006	20:00	84.53	89	0	1	900	2	15	6	40
			8/17/										
1	900	0	2006	08:00	84.53	89	0	1	900	2	16	6	40
			8/17/										
1	900	0	2006	20:00	84.53	89	0	1	900	2	16	6	40
			8/18/										
1	0	0	2006	08:00	84.53	89	5.32	0	900	2	17	6	40
			8/18/										
1	900	0	2006	08:20	85.48	89	0	1	900	2	17	6	40
			8/18/										
1	900	0	2006	20:00	85.48	89	0	1	900	2	17	6	40
			8/19/										
1	900	0	2006	08:00	85.48	89	0	1	900	2	18	6	40

1	900	0	8/19/2006	20:00	85.48	89	0	1	900	2	18	6	40
1	900	0	8/20/2006	08:00	85.48	89	0	1	900	2	19	6	40
1	900	0	8/20/2006	20:00	85.48	89	0	1	900	2	19	6	40
1	900	0	8/21/2006	08:00	85.48	89	0	1	900	2	20	6	40
1	900	0	8/21/2006	20:00	85.48	89	0	1	900	2	20	6	40
1	0	0	8/22/2006	08:00	85.48	89	6.98	0	900	2	21	6	40
1	900	0	8/22/2006	08:20	78.36	89	0	1	900	2	21	6	40
1	900	0	8/23/2006	08:00	78.36	89	0	1	900	2	22	6	40
1	900	0	8/24/2006	08:00	78.36	89	0	1	900	2	23	6	40
1	900	0	8/25/2006	08:00	78.36	89	0	1	900	2	24	6	40
1	900	0	8/26/2006	08:00	78.36	89	0	1	900	2	25	6	40
1	900	0	8/27/2006	08:00	78.36	89	0	1	900	2	26	6	40
1	900	0	8/28/2006	08:00	78.36	89	0	1	900	2	27	6	40
1	0	0	8/29/2006	08:00	78.36	89	4.89	0	900	2	28	6	40
1	900	0	8/29/2006	08:20	77.48	89	0	1	900	2	28	6	40
1	900	0	8/30/2006	08:00	77.48	89	0	1	900	2	29	6	40
1	900	0	8/31/2006	08:00	77.48	89	0	1	900	2	30	6	40
1	900	0	9/1/2006	08:00	77.48	89	0	1	900	2	31	6	40
1	900	0	9/2/2006	08:00	77.48	89	0	1	900	2	32	6	40
1	900	0	9/3/2006	08:00	77.48	89	0	1	900	2	33	6	40
1	900	0	9/4/2006	08:00	77.48	89	0	1	900	2	34	6	40
1	0	0	9/5/2006	08:00	78.36	89	5.44	0	900	2	35	6	40
1	900	0	9/5/2006	08:20	78.36	89	0	1	900	2	35	6	40
1	900	0	9/6/2006	08:00	78.36	89	0	1	900	2	36	6	40
1	900	0	9/7/2006	08:00	78.36	89	0	1	900	2	37	6	40
1	900	0	9/8/2006	08:00	78.36	89	0	1	900	2	38	6	40

1	900	0	9/9/2006	08:00	78.36	89	0	1	900	2	39	6	40
1	900	0	9/10/2006	08:00	78.36	89	0	1	900	2	40	6	40
1	900	0	9/11/2006	08:00	78.36	89	0	1	900	2	41	6	40
1	0	0	9/12/2006	08:00	78.36	89	4.25	0	900	2	42	6	40
1	900	0	9/12/2006	08:20	80.1	89	0	1	900	2	42	6	40
1	900	0	9/13/2006	08:00	80.1	89	0	1	900	2	43	6	40
1	900	0	9/14/2006	08:00	80.1	89	0	1	900	2	44	6	40
1	900	0	9/15/2006	08:00	80.1	89	0	1	900	2	45	6	40
1	900	0	9/16/2006	08:00	80.1	89	0	1	900	2	46	6	40
1	900	0	9/17/2006	08:00	80.1	89	0	1	900	2	47	6	40
1	900	0	9/18/2006	08:00	80.1	89	0	1	900	2	48	6	40
1	0	0	9/19/2006	08:00	80.1	89	3.71	0	900	2	49	6	40
1	900	0	9/19/2006	08:20	80.1	89	0	1	900	2	49	6	40

7.3 Control files for NONMEM

7.3.1 1-compartment with first-order absorption

Trans 1:

```
$PROB En-kompartment med absorpsjon

$DATA test7.txt

$INPUT ID AMT RATE DATE=DROP TIME CRCL WGT CONC=DV MDV
DOSE SEX=DROP DAY=DROP PASN=DROP

$SUBROUTINE ADVAN2 TRANS1

$PK
K=THETA(1)*EXP(ETA(1))
KA=THETA(2)*EXP(ETA(2))
```

```
$ERROR
IPRED=F
Y=F+ERR(1)

$THETA
(0, 0.5, 2)
(0, 0.4, 2)

$OMEGA
0.00002 0.01

$SIGMA
3

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=2000 PRINT=0
POSTHOC NOABORT

$TABLE ID DV TIME
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID KA K AMT TIME IPRED DV
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

Trans 2:

```
$PROB En-kompartment med absorpsjon

$DATA test7.txt

$INPUT ID AMT RATE DATE=DROP TIME CRCL=DROP WGT=DROP
CONC=DV MDV DOSE SEX=DROP DAY=DROP PASN=DROP

$SUBROUTINE ADVAN2 TRANS2

$PK
TVCL=THETA(1)
CL=TVCL*EXP(ETA(1))
TVVD=THETA(2)
V=TVVD*EXP(ETA(2))
TVKA=THETA(3)
KA=TVKA*EXP(ETA(3))

; THE FOLLOWING ARE REQUIRED BY PREDPP

K=CL/V
```

```
S2=V

$ERROR
IPRED=F
Y=F+ERR(1)

$THETA
(0, 3)
(0, 6)
(0, 0.05)

$OMEGA
0.00001 0.00001 0.00001

$SIGMA
0.5

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=2000 PRINT=0
POSTHOC

$TABLE ID DV TIME
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID KA K AMT TIME DV IPRED
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.3.2 2-compartment with first-order absorption

```
$PROB To-kompartment med absorpsjon

$DATA test7.txt

$INPUT ID AMT RATE DATE=DROP TIME CRCL=DROP WGT=DROP
CONC=DV MDV DOSE=DROP SEX=DROP DAY=DROP PASN=DROP

$SUBROUTINE ADVAN4 TRANS4

$PK
CL=THETA(1)*EXP(ETA(1))
V2=THETA(2)*EXP(ETA(2))
Q=THETA(3)*EXP(ETA(3))
V3=THETA(4)*EXP(ETA(4))
KA=THETA(5)*EXP(ETA(5))

; THE FOLLOWING ARE REQUIRED BY PREDPP
```

```
K=CL/V2
K23=Q/V2
K32=Q/V3
S2=V2
S3=V3

$ERROR
IPRED=F
Y=F+F*ERR(1)+ERR(2)

$THETA
(0, 12, 50)
(0, 18.5, 100)
(0, 9.82, 50)
(0, 44.4, 100)
(0, 0.287)

$OMEGA
0.01 0.01 0.01 0.01 0.01

$SIGMA
0.15 0.001

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=2000 PRINT=0
POSTHOC NOABORT

$TABLE ID DV TIME
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID KA K AMT TIME IPRED DV
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

7.3.3 3-compartment with first-order absorption

```
$PROB Tre-kompartment med absorpsjon

$DATA test7.txt

$INPUT ID AMT RATE DATE=DROP TIME CRCL=DROP WGT=DROP
CONC=DV MDV DOSE=DROP SEX=DROP DAY=DROP PASN=DROP

$SUBROUTINE ADVAN12 TRANS4

$PK
```

```
CL=THETA(1)*EXP(ETA(1)) ;Clearance
V2=THETA(2)*EXP(ETA(2)) ;central volume
Q3=THETA(3)*EXP(ETA(3)) ;intercompartmental clearance (central and periph 1)
V3=THETA(4)*EXP(ETA(4)) ;peripheral 1 volume
Q4=THETA(5)*EXP(ETA(5)) ;intercompartmental clearance (central and periph 2)
V4=THETA(6)*EXP(ETA(6)) ;peripheral 2 volume
KA=THETA(7)*EXP(ETA(7)) ;absorption rate constant

K=CL/V2
K23=Q3/V2
K32=Q3/V3
K24=Q4/V2
K42=Q4/V4
KA=KA

S2=V2
S3=V3
S4=V4

$ERROR
IPRED=F
Y=F+F*ERR(1)+ERR(2)

$THETA
(0, 1)
(0, 10)
(0, 0.5)
(0, 15)
(0, 2)
(0, 5)
(0, 0.1)

$OMEGA
0.01 0.01 0.01 0.01 0.01 0.01 0.01

$SIGMA
0.5 0.5

$ESTIMATION SIG=3 METHOD=1 INTER MAXEVAL=2000 PRINT=0
POSTHOC NOABORT

$TABLE ID DV TIME
NOPRINT ONEHEADER FILE=table.txt

$TABLE ID KA K AMT TIME IPRED DV
FIRSTONLY NOPRINT ONEHEADER NOAPPEND FILE=etatable.txt
```

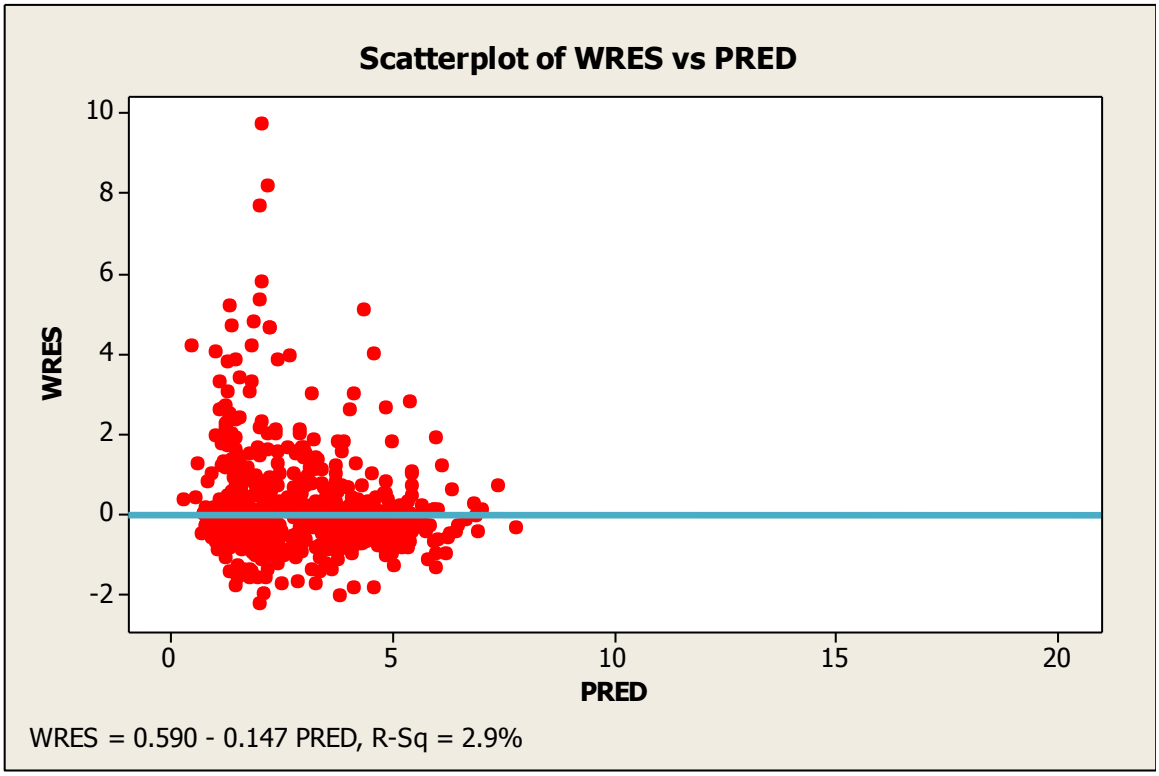
7.3.4 Jackknife

Pasient fjernet	Variabler					
	Clearance (CL)	Central Volume (V2)	Intercompartmental Clearance (Q)	Peripheral Volume (V3)	Absorption rate constant (KA)	Absorption time lag (ALAG)
1	12	63.1	7.19	314	0.287	0.661
2	12	61.7	7.21	316	0.287	0.661
3	12	59.4	7.21	304	0.287	0.661
4	12	59.4	7.21	304	0.287	0.661
5	12	59.4	7.21	304	0.287	0.661
6	12.1	63.5	7.07	317	0.287	0.661
7	12.2	67	7.57	301	0.287	0.661
8	12	59.4	7.21	304	0.287	0.661
9	12	59.4	7.21	304	0.287	0.661
10	12	59.4	7.21	304	0.287	0.661
11	12	59.4	7.21	304	0.287	0.661
12	12.1	65	7.18	312	0.287	0.661
13	12	59.4	7.21	304	0.287	0.661
14	12	59.4	7.21	304	0.287	0.661
15	12	59.4	7.21	304	0.287	0.661
16	12.3	60	6.88	346	0.287	0.661
17	12	67.1	6.93	304	0.287	0.661
18	12.2	79.8	5.54	273	0.287	0.661
19	12	59.4	7.21	304	0.287	0.661
20	12	59.4	7.21	306	0.287	0.661
21	12	59.4	7.21	311	0.287	0.661
22	12	59.4	7.21	304	0.287	0.661
23	12	59.1	7.07	328	0.287	0.661
24	12	59.4	7.21	304	0.287	0.661
25	12.1	68.4	7.69	314	0.287	0.661
26	12	59.4	7.21	304	0.287	0.661
27	12	59.4	7.21	304	0.287	0.661
28	12	58.4	6.74	312	0.287	0.661
29	12	59.4	7.21	304	0.287	0.661
30	12	59.4	7.21	323	0.287	0.661
31	12	59.4	7.21	317	0.287	0.661
32	12	59.4	7.21	319	0.287	0.661
33	12	59.4	7.21	304	0.287	0.661
34	12.1	64.2	7.21	319	0.287	0.661
35	12	59.4	7.21	304	0.287	0.661

36	12	59.4	7.21	309	0.287	0.661
37	12.2	63.9	7.55	311	0.287	0.661
38	12	59.4	7.21	304	0.287	0.661
39	12	59.5	7.2	319	0.287	0.661
40	12	63.1	7.17	316	0.287	0.661
41	12	59.4	7.21	304	0.287	0.661
42	12	59.4	7.21	304	0.287	0.661
43	12	59.4	7.21	304	0.287	0.661
44	12	59.4	7.21	304	0.287	0.661
45	12	59.4	7.21	304	0.287	0.661
46	12	59.4	7.21	304	0.287	0.661
47	12	59.4	7.21	323	0.287	0.661
48	12	59.4	7.21	308	0.287	0.661
49	12	71.3	7.94	325	0.287	0.661
50	12.1	59.7	7.16	322	0.287	0.661
51	12	61.5	7.35	318	0.287	0.661
52	12	59.4	7.21	304	0.287	0.661
53	12	59.4	7.21	304	0.287	0.661
54	12	59.4	7.21	312	0.287	0.661
55	12	59.4	7.21	304	0.287	0.661
56	12	59.4	7.21	304	0.287	0.661
57	12	59.4	7.21	304	0.287	0.661
58	12	59.4	7.21	304	0.287	0.661
59	12	59.4	7.21	304	0.287	0.661
60	12	59.4	7.21	304	0.287	0.661
61	12	59.4	7.21	304	0.287	0.661
62	12	59.4	7.21	304	0.287	0.661
63	12	59.4	7.21	304	0.287	0.661
64	12	59.4	7.21	304	0.287	0.661
65	12	59.4	7.21	304	0.287	0.661
66	12	59.4	7.21	304	0.287	0.661
67	12	59.4	7.21	304	0.287	0.661
68	12	59.4	7.21	304	0.287	0.661
69	12	59.4	7.21	304	0.287	0.661
70	12	59.4	7.21	304	0.287	0.661
71	12	59.4	7.21	304	0.287	0.661
72	12	59.4	7.21	304	0.287	0.661
73	12	59.4	7.21	304	0.287	0.661
74	12	59.4	7.21	304	0.287	0.661
75	12	59.4	7.21	304	0.287	0.661
76	12	59.4	7.21	304	0.287	0.661
77	12	59.4	7.21	304	0.287	0.661
78	12	59.4	7.21	304	0.287	0.661
79	12	59.4	7.21	304	0.287	0.661
80	12	59.4	7.21	304	0.287	0.661

81	12	59.4	7.21	304	0.287	0.661
82	12	59.4	7.2	326	0.287	0.661
83	12	59.4	7.21	304	0.287	0.661
84	12	59.4	7.21	304	0.287	0.661
85	12	59.4	7.21	304	0.287	0.661
86	12	59.4	7.21	316	0.287	0.661
87	12	59.4	7.21	305	0.287	0.661
88	12	59.4	7.21	304	0.287	0.661
89	12	59.4	7.21	304	0.287	0.661
90	12	59.4	7.21	304	0.287	0.661
91	12	59.4	7.21	304	0.287	0.661
92	12	59.4	7.21	304	0.287	0.661
93	12	59.4	7.21	304	0.287	0.661
94	12	59.4	7.21	304	0.287	0.661
95	12	59.4	7.21	304	0.287	0.661
96	12	59.4	7.21	304	0.287	0.661
97	12	59.4	7.21	304	0.287	0.661
98	12	59.4	7.21	304	0.287	0.661
99	12	59.4	7.21	304	0.287	0.661
100	12	59.4	7.21	304	0.287	0.661
101	12	59.4	7.21	304	0.287	0.661
102	12	59.4	7.21	311	0.287	0.661
103	12	59.4	7.21	304	0.287	0.661
104	12	59.4	7.21	309	0.287	0.661
105	12	59.4	7.21	304	0.287	0.661
106	12	59.4	7.21	304	0.287	0.661
107	12	59.4	7.21	304	0.287	0.661
108	12	59.4	7.21	304	0.287	0.661

7.3.5 Scatterplot of WRES vs. PRED



7.3.6 Scatterplot of WRES vs. IPRE