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# DEVELOPMENT OF A POPULATION PHARMACOKINETIC MODEL FOR VANCOMYCIN USING EXPECTATION-MAXIMIZATION ALGORITHM

# RAZVOJ POPULACIJSKEGA FARMAKOKINETIČNEGA MODELA ZA VANKOMICIN Z ALGORITMOM PRIČAKOVANJE-MAKSIMIZACIJA

MASTER'S THESIS

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I performed my master's thesis at the University of Ljubljana, Faculty of Pharmacy and at the Faculty of Pharmacy, University of Lisbon, under the mentorship of Assoc. Prof. Iztok Grabnar, PhD and Paulo Paixão, PhD.

**Declaration**

I declare that I performed the master's thesis alone under the mentorship of Assoc. Prof. Iztok Grabnar, PhD and Paulo Paixão, PhD.

Ljubljana, 2016 Jurij Zdovc

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# Contents

# Abstract

**Objectives:** Vancomycin is nowadays a relatively well known and an extensively used drug. However, due to the emergence of resistance among bacteria in the past years there is a need to furtherly optimize the treatment with this antibiotic and to characterize the influence of (patho)physiologic characteristics of a patient on the therapy. We will apply the means of population pharmacokinetic modelling in order to investigate the drug's pharmacokinetic trends in a certain population. The aim of the study is therefore to develop a population pharmacokinetic model, that could potentially be useful in the individualization of the treatment. Another aim is also to develop the model using two independent methods. The studied method will be the expectation-maximization (EM) algorithm in the Adapt 5 package. The work process and the performance of the studied method will be compared to the gold standard method in that area, the first order conditional estimation with interaction (FOCE-I) in the NONMEM 7.3 package.

**Materials and methods:** This was a retrospective study of concentration-vs.-time data for vancomycin in plasma of a population of thirty-three critically ill patients from the Hospital Centre Tondela – Viseu. The data from the patients' medical histories were analyzed using above mentioned estimation methods to obtain the maximum likelihood of the parameters. Forward selection process was used to construct the covariate models. Bootstrap with replacement, visual predictive check and the external validation were used to validate the chosen final model. A Cockcroft-Gault equation was used to calculate the clearance of creatinine and to assess the renal function.

**Results:** The best pharmacokinetic model of both methods consisted of a one-compartment model with additive residual unknown variability. Both models included total body weight and renal function as covariates describing the between subject variability (BSV) of volume of distribution and clearance. Furthermore, both models included a coadministration of a diuretic as a covariate. Furosemide was significant in the model constructed with EM and spironolactone was a significant covariate in the reference method. The volume of distribution of a typical individual (ClCr = 120 ml/min, total body weight = 70 kg) was 58.7 L (relative standard error was 22.6 %) with the BSV of 61.5 % (34.3 %) and 62.6 L (0.197 %) with the BSV of 49.9 % (23.0 %) in Adapt and NONMEM, respectively. The clearance of vancomycin was 4.84 L/h (15.1 %) with the BSV of 28.2 % (28.8 %) and 4.07 (4.45 %)

with the BSV of 23.5 % (14.3 %) in Adapt and NONMEM, respectively. Residual unknown variability was 2.96 mg/L (6.66 %) and 2.38 (11.4 %) mg/L in Adapt and NONMEM, respectively. The models were shown to be robust, since no 95 % confidence intervals in bootstrap validation included zero, furthermore the results of the visual predictive check of NONMEM final model were within the simulated intervals.

**Conclusions:** The study shows that Adapt 5 with the EM algorithm is a valid tool in population pharmacokinetic modelling, since the results and the work process were similar to the NONMEM method. We can also conclude, that we successfully developed and validated a population pharmacokinetic model for individualized treatment with vancomycin. The results were comparable to already described in literature and we confirmed, that the renal function and the total body weight play an important role in adjusting the treatment with vancomycin.

**Key words:** Vancomycin, population pharmacokinetics, expectation-maximization algorithm, Adapt, NONMEM.

# Povzetek

**Uvod:** Vankomicin je dandanes zelo dobro poznan in široko uporabljan glikopeptidni antibiotik. Porazdeljevanje najbolje opiše eno, dvo ali triprostorni farmakokinetični model. Ob pomanjkanju podatkov se pogosto uporabi enoprostorni model. Izloča se v glavnem skozi ledvice, zato ledvična funkcija igra eno pomembnejših vlog pri prilagajanju odmerkov posameznemu pacientu. Veliko raziskav na področju farmakokinetike in farmakodinamike vankomicina je bilo že narejenih, saj je prišel na trg že v petdesetih letih prejšnjega stoletja. Kljub dobrem poznavanju značilnosti tega zdravila pa je zaradi pojava rezistence proti vankomicinu v zadnjih letih narasla potreba po nadaljnjih raziskavah vplivov (pato)fizioloških značilnosti na farmakokinetiko in farmakodinamiko vankomicina in po nadaljnji optimizaciji zdravljenja.

Mi bomo za raziskovanje farmakokinetičnih trendov v populaciji bolnikov uporabili metodologijo populacijskega farmakokinetičnega modeliranja. Ti principi se v zadnjih letih pospešeno razvijajo in uporabljajo in predstavljajo učinkovitejšo in cenejšo alternativo tradicionalnim farmakokinetičnim študijam. Naši metodi bosta algoritem pričakovanje-maksimizacija v programskem paketu Adapt 5 in pogojno ocenjevanje prvega reda z interakcijo v programskem paketu NONMEM 7.3.

**Namen dela:** Namen naše raziskave je razviti populacijski farmakokinetični model za vankomicin, ki bi bil potencialno uporaben pri individualnem prilagajanju zdravljenja pacientom. Nadalje je naš namen uporabiti zgoraj omenjeni neodvisni metodi za razvoj modela. Adapt 5 z algoritmom pričakovanje-maksimizacija bo bolj proučevana metoda, saj se veliko manj uporablja za populacijsko modeliranje. Rezultate dobljene po tej metodi in postopek dela bomo primerjali z metodo pogojnega ocenjevanja prvega reda z interakcijo v programskem paketu NONMEM 7.3, ki je zlati standard populacijske farmakokinetike in bo služila kot referenca.

**Materiali in metode:** Izvedli smo retrospektivno raziskavo koncentracij vankomicina v plazmi triintridesetih kritično bolnih oseb, hospitaliziranih v kliničnem centru Tondela – Viseu. Podatke smo dobili iz zdravniških kartotek bolnikov in jih nato analizirali po obeh metodah. Z algoritmom pričakovanje-maksimizacija in pogojnim ocenjevanjem prvega reda z interakcijo smo dobili oceno največjega verjetja parametrov. Objektivno funkcijo največjega verjetja in Bayesov informacijski kriterij smo uporabili za rangiranje modelov.

Najprej smo preizkusili katera oblika modela je primerna za opis rezidualne napake in kateri farmakokinetični model najbolje opiše porazdeljevanje vankomicina. Nato smo s postopnim dodajanjem sočasnih spremenljivk zgradili polni kovariatni model. Dobljeni model smo validirali. V programu Adapt 5 smo izvedli le interno validacijo – metodo ponovljivega vzorčenja »bootstrap«, v programu NONMEM pa smo dodatno izvedli še vizualno analizo »visual predictive check« in pa zunanjo validacijo modela na drugi populaciji bolnikov. Za izračun ledvičnega očistka kreatinina in oceno ledvične funkcije smo uporabili enačbo Cockcroft – Gault.

**Rezultati:** Najboljši model za opis porazdeljevanja vankomicina in rezidualne napake je bil po obeh metodah enoprostorni farmakokinetični model z aditivnim tipom napake. V kovariatni model so kot sočasne spremenljivke po obeh metodah vstopile ledvična funkcija, telesna masa in sočasna aplikacija enega izmed diuretikov. Sočasna aplikacija furosemida je bila značilna sočasna spremenljivka po metodi z Adaptom in sočasna aplikacija spironolaktona je bila značilna spremenljivka v programu NONMEM. V obeh primerih je sočasno zdravljenje z diuretikom znižalo očistek vankomicina, kar je v nasprotju s podatki dobljenimi v literaturi. Furosemid naj bi zvišal očistek vankomicina, za spironolakton pa nismo našli opisanih interakcij s preiskovanim antibiotikom.

Porazdelitveni volumen vankomicina pri tipičnem posamezniku (telesna masa = 70 kg, očistek kreatinina = 120 ml/min) je bil 58.7 L (standardna napaka je bila 22.6 %) z interindividualno variabilnostjo 61.5 % (34.3 %), ledvični očistek pa 4.84 L/h (15.1 %) z interindividualno variabilnostjo 28.2 % (28.8 %) v končnem modelu dobljenim s proučevano metodo. Po referenčni metodi se je vankomicin porazdeljeval po volumnu 62.6 L (0.197 %) z interindividualno variabilnostjo 49.9 % (23.0 %), ledvični očistek pa je bil 4.07 L/h (4.45 %) z interindividualno variabilnostjo 23.5 % (14.3 %). Rezidualna napaka končnega modela je bila 2.96 mg/L (6.66 %) oz. 2.38 mg/L (11.4 %) v Adaptu oz. NONMEM-u. Oba modela sta se izkazala za robustna, saj noben 95 % interval zaupanja dobljen z metodo ponovljivega vzorčenja »bootstrap« ni vseboval vrednosti 0. Rezultati so bili znotraj simuliranih intervalov pri vizualni analizi. Končni model razvit po referenčni metodi se je na zunanji populaciji izkazal zmerno dobro, saj je imel relativno visoko povprečno absolutno napako in povprečno kvadrirano absolutno napako. Vsi rezultati so primerljivi in podobni parametrom in modelom že opisanim v literaturi.

Proces dela z Adaptom in principi so bili v glavnem podobni delu s programom NONMEM. Koraki so si sledili v enakem zaporedju, rezultati in dobljeni modeli so si bili zelo podobni. Razlika pa je bila predvsem v preprostosti izvedbe korakov in analize. V programu Adapt 5 je bilo kovariatni model treba graditi postopoma in ročno zaganjati analizo modelov, vsakega posebej. NONMEM je kovariatni model zgradil avtomatično. Tudi možnosti za validacijo je manj v Adaptu. »Bootstrap« smo morali izvesti ročno, zato smo simulirali in analizirali le 50 virtualnih populacij. V tem smislu je Adapt 5 odlično orodje za učenje in globlje razumevanje modeliranja, imeli smo tudi večji nadzor in uvid v postopek, lahko smo kaj spremenili in interpretirali že vmes. Po drugi strani pa je NONMEM tudi validacijo izvedel samostojno v enem koraku in je tako mnogo manj časovno potraten, ima tudi večjo bazo uporabnikov in referenc, kar lahko olajša delo.

**Sklepi:** Zaključimo lahko, da smo uspešno razvili in validirali populacijski farmakokinetični model z dvema neodvisnima metodama. Adapt 5 z algoritmom pričakovanje-maksimizacija se je izkazal kot uporabna metoda za farmakokinetično modeliranje, saj so bili rezultati podobni dobljenim z referenčno metodo, NONMEM-om in parametrom in modelom opisanim v literaturi.

Študija je potrdila, da ledvična funkcija in telesna masa igrata pomembno vlogo pri individualnem prilagajanju zdravljenja z vankomicinom kritično bolnim. Sočasno zdravljenje s furosemidom oz. spironolaktonom je prav tako vplivalo na farmakokinetiko vankomicina, a zanesljivost tega vpliva je vprašljiva, saj je bila proučevana populacija premajhna. Možno je tudi, da so bolniki, ki so prejemali diuretika, imeli v osnovi slabšo ledvično funkcijo in nižji očistek vankomicina kot ostali, kar se je kasneje zmotno pokazalo kot posledica prejemanja diuretikov. Nadaljnje raziskave so potrebne za preučitev tega vpliva, v vsakem primeru pa je nujna večja pozornost pri bolnikih, ki se sočasno zdravijo z diuretikom.

Rezultati naše študije tako lahko služijo tudi kot osnova za morebitne nadaljnje študije farmakodinamike v povezavi s farmakokinetiko vankomicina. Na ta način bi lahko raziskali v kolikšni meri določen režim odmerjanja dosega želene koncentracije v plazmi pri zdravljenju okužb z različnimi bakterijami.

**Ključne besede:** Vankomicin, populacijska farmakokinetika, algoritem pričakovanje-maksimizacija, Adapt, NONMEM.

# List of abbreviations

| | |
|---|---|
| AIC | Akaike information criterion |
| AUC | area under the curve |
| BIC | Bayesian information criterion |
| BSV | between subject variability |
| $Cl_{VAN}$ | clearance of vancomycin |
| ClCr | creatinine clearance |
| Co/Fu | coadministration of furosemide |
| Co/Sp | coadministration of spironolactone |
| EM | expectation-maximization |
| FOCE-I | first order conditional estimation with interaction |
| IV | intravenous |
| ME | mean prediction error |
| MIC | minimum inhibitory concentration |
| MLEM | maximum likelihood expectation maximization |
| MRSA | methicilin-resistant *Staphylococcus aureus* |
| MSE | mean squared prediction error |
| NAG | N-acetylglucosamine |
| NAM | N-acetylmuramic acid |
| NLMEM | nonlinear mixed effects modelling |
| OFV | objective function value |
| PD | pharmacodynamics |
| PG | peptidoglycan |
| PK | pharmacokinetic, pharmacokinetics |
| POPPK | population pharmacokinetics, population pharmacokinetic |

| | |
|---|---|
| RSE | relative standard error |
| RMSE | square root of mean squared prediction error |
| SD | standard deviation |
| SE | standard error |
| TBW | total body weight |
| VAN | vancomycin |
| Vd | volume of distribution of vancomycin |
| VISA | intermediate vancomycin-resistant *Staphylococcus aureus* |
| VPC | visual predictive check |
| VRSA | vancomycin-resistant *Staphylococcus aureus* |
| $\phi$ | phi, individual parameter |
| $\theta$ | theta, fixed effects |
| $\eta$ | eta, random effects |
| $\Omega$ | omega, between subject variability |
| $\varepsilon$ | epsilon, residual variability, intraindividual variability |
| $\sigma$ | sigma, residual variability |

# 1 Introduction

## 1.1 Vancomycin

### 1.1.1 Brief overview

Vancomycin (VAN) is nowadays a relatively well known and an extensively used drug. It was first introduced in 1956, and it was initially meant to control infections with *Staphylococcus aureus* that had acquired resistance to natural penicilins. Shortly after the discovery, it was pushed into the background and became reserved only for patients with serious β-lactam allergies. That happened mainly because of the toxicity related to the impurities (it was called »Mississippi mud« because of its brown colour) but also due to the subsequent development of penicilinase-resistant penicilins and cephalosporins. The increase in the use of VAN happened again in the early 1980s with the significant spread of methicillin-resistant staphylococci (MRSA) and penicilin-resistant *Streptococcus pneumoniae*. The product was then also purified, which lead to a rather low toxicity profile (1, 2, 3). Since then, VAN has been considered a drug of the last resort but still an effective treatment against Gram-positive bacteria. Even more so against MRSA, since it is only one among a few options available for treating infections caused by this pathogen, which is common in hospitals as well as intensive-care units, and it causes a high mortality rate (4-7).

However, the overuse of VAN has led to the emergence of resistance even in *Staphylococcus aureus* strains. In 2002, a strain of *Staphylococcus aureus* that was fully resistant to VAN was reported (VRSA), and an intermediate vancomycin-resistant *Staphylococcus aureus* (VISA) was reported some years earlier (8).

Therefore, in order to avoid or minimize the spread of resistance, enhance the outcomes of the clinical cures and improve the cost/benefit ratio, the optimization and individualization of treatment (in terms of dose and frequency) is of the utmost importance (9). In the following work, we try to provide a population pharmacokinetic model that could potentially contribute to the individualization of the treatment with VAN.

### 1.1.2 Mechanism of action

VAN is a tricyclic glycopeptide antibiotic with molecular weight of approximately 1450 Da. It acts by inhibiting cell wall synthesis of Gram-positive bacteria, which consists of many

layers of peptidoglycan (PG). PG layers are synthesized with the addition of monomer units of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG). NAM also has a pentapeptide linked to it. When new monomers are linked to the existing rows of PG, transpeptidase enzymes form a peptide bridge that cross-links the pentapeptides coming off of NAM in rows next to each other. These links give PG its rigidity and strength. VAN binds to the D-alanil-D-alanin part of the pentapeptide of the PG monomers and prevents the formation of a cross-link next to the transpeptidase enzymes. Without forming new cross-links and with autolysins breaking the existing ones, PG becomes less rigid and more permeable, which results in osmotic lysis of the bacterium. Figure 1 presents the structure of VAN and an insight into this mechanism as well as the interaction of VAN with the peptide is enclosed in the appendix – figure 20 (3, 10).

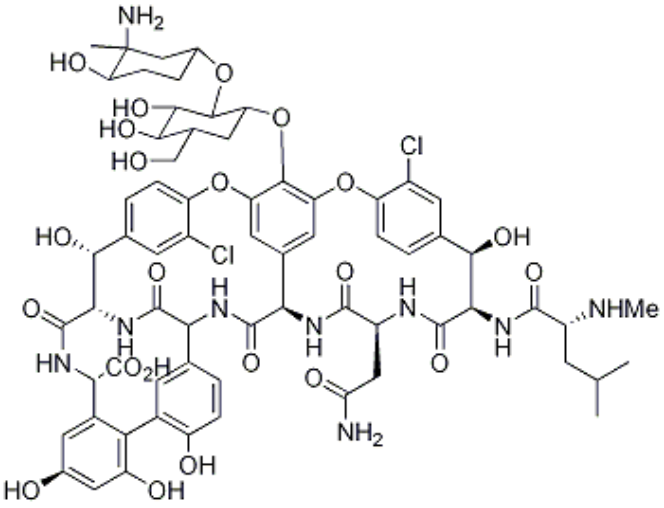


**Figure 1. Structure of Vancomycin (11).**

### 1.1.3 Spectrum of action and clinical indications

*Staphylococcus* spp., *Streptococcus* spp., enterococci (*faecalis*, *faecium*), *Listeria monocytogenes*, corynebacteria (*diphtheriae*, *jeikeium*), *Bacillus* spp., *Clostridum* spp., *Propionibacterium acnes*, *Actinomyces* spp., some *Lactobacillus* spp. and *Rhodococcus equi* are all susceptible to VAN. *Borrelia burgdorferi* and some strains of *Neisseria gonorrhoeae* are susceptible in vitro. Gram-negative and anaerobic bacteria are resistant to VAN (13).

However, to limit the spread of resistance of susceptible microorganisms, VAN's use is restricted, according to the guidelines, to the following situations (2):

- serious infections caused by MRSA and methicillin-resistant *Staphylococcus epidermidis*,

- infections caused by methicillin-susceptible *Staphylococcus aureus* in subjects who are allergic to penicilins,
- prophylaxis for major procedures involving the implantation of prostheses in hospitals with a high prevalence of MRSA,
- pseudomembranous colitis, in the case of a relapse or lack of response to metronidazole treatment,
- prophylaxis for endocarditis following high-risk procedures in penicilin-hypersensitive subjects.

### 1.1.4 Pharmacokinetics

#### *1.1.4.1 Absorption and distribution*

VAN's absorption from gastrointestinal tract is minimal if it exists at all. Therefore, it is administered intravenously (IV) in the form of a slow infusion (intermittent or continuous) for the treatment of systemic infections. It can be given orally in the treatment of pseudomembranous colitis caused by *Clostridium difficile* or *Staphylococcus aureus*. It is never administered intramuscularly because it causes severe pain (1, 3, 5, 14).

Serum concentration vs. time profile of VAN can be characterized as one-, two-, or three-compartment model, although one- and two- compartment models are more commonly used in the prediction of the substance's pharmacokinetic (PK) profile. In population pharmacokinetic (POPPK) studies, the use of the one-compartment model is preferable when there is a lack of data in the early (distribution) phase (3, 4, 15). Two-compartment model with infusion administration is presented in the figure 2.

According to the two-compartment PK profile, the distribution of VAN between tissues occurs in the initial α-phase that lasts from 30 min to 60 min. The penetration into tissues is low and depends on the degree of inflammation present. Protein-binding is around 50 % and the volume of distribution (Vd) is described to have a somewhat wide interval, varying from 0.4 to 1 L/kg of the total body weight (TBW) (4). High variability in Vd occurs due to various factors and conditions which will be discussed later on.

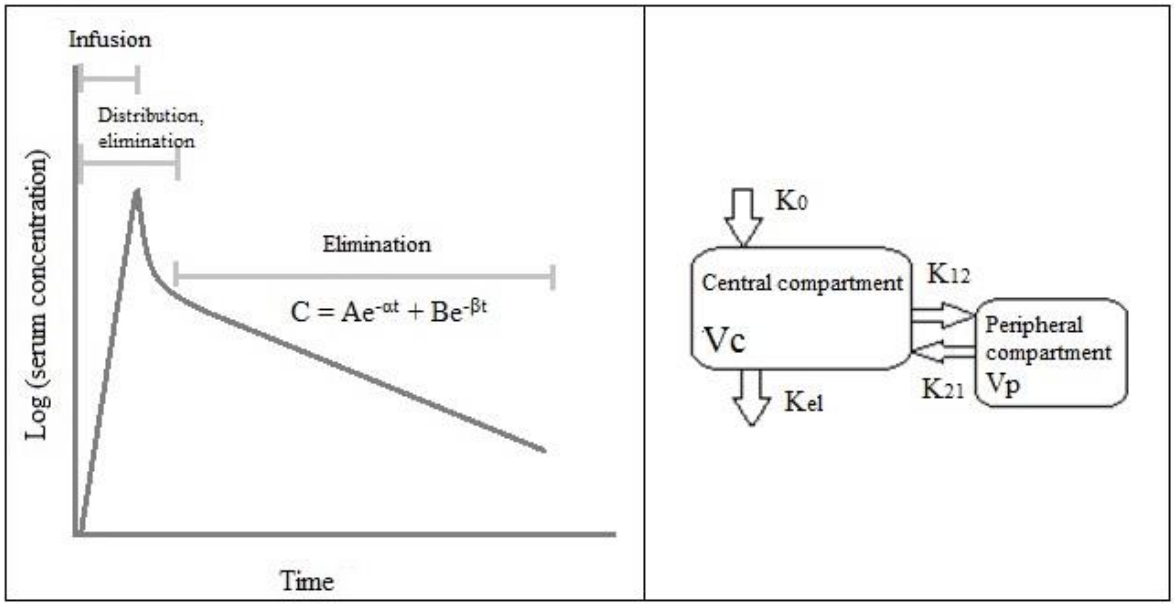


**Figure 2. Two-compartment model with infusion administration; left – Log(concentration)-time profile, right – compartments of a two-compartment model.**

### *1.1.4.2 Elimination*

VAN is almost entirely (80–90 %) excreted unchanged through kidneys via glomerular filtration and undergoes minimal metabolism. Consistent with this, the clearance of VAN ($Cl_{VAN}$) depends mostly on the renal function, even though some non-renal $Cl_{VAN}$ can occur. Elimination half time in β-phase of two-compartment model is 6-12 hours. In one-compartment models, $Cl_{VAN}$ is reported to be from 60 to 80 ml/min (3.6 to 4.8 L/h) (3, 4, 16).

$Cl_{VAN}$ shows a strong, linear correlation with creatinine clearance (ClCr) and is decreased in patients with renal impairment. The dosing should therefore be adjusted accordingly and serum creatinine should be monitored closely. However, the estimation of $Cl_{VAN}$ based only on serum creatinine could be misleading, and that is why it is necessary to follow the VAN concentrations in plasma/serum. Especially in critically ill patients, one should pay attention to other conditions that could potentially influence the renal function and $Cl_{VAN}$ (5, 9, 17, 18).

### *1.1.4.3 Toxicity*

In general, VAN is believed to be a relatively safe drug. The main issues appear to be nephrotoxicity and ototoxicity. On the contrary, »red-neck syndrome« as well as other adverse reactions, such as skin rashes, thrombophlebitis, chills and fever, are nowadays very rare due to the product being purified, and because VAN is administered in a form of a slow

infusion. Nephrotoxicity also does not occur very often, since the incidence is reported to be less than 5 %. In a large study that included 1750 patients, incidence was reported to be 1.4 %. Still, when administered with other nephrotoxic drugs, such as aminoglycosides, VAN potentiates the nephrotoxicity. Ototoxicity is mostly associated with very high serum concentrations (80–100 mg/L) and has the incidence of 2 % or less. The majority of experts even believe that the monotherapy with VAN is not ototoxic (3, 4).

#### *1.1.4.4 Pharmacokinetics in critically ill patients*

PK can be altered due to various conditions and this happens more often especially in critically ill patients, which is a very heterogeneous group. As a consequence, the concentration of drugs at the target site can vary. Since VAN is a hydrophilic antibiotic, it is more influenced by the day-to-day variations in Vd and (unstable) renal function than lipophilic antimicrobials. This can lead to an unsuccessful treatment of infection and emergence of resistance on one side, or to unwanted toxic effects on the other. The summary of recommendations in case of the presence of an altered PK is enclosed in the appendix – figure 21. In terms of VAN specifically, fluid therapy and ascites were recorded to increase the Vd. In respect to PK interaction in the elimination phase, concomitant therapy with drugs improving haemodynamics, such as furosemide, dopamine and dobutamine, were recorded to promote renal function. Leukaemia and burns were also reported to increase renal function and $Cl_{VAN}$. In the mentioned events, an increase of the dosage should be considered. On the contrary, in patients that are kept in hospitals for a longer period of time, muscle wastage (and lower creatinine production) can occur, which can lead to the overestimation of Cl and potentially too high and toxic dosing (9, 19, 20).

### 1.1.5 Therapeutic guidelines and individualization

Although our work will focus on PK of VAN, a short review of pharmacodynamic (PD) characteristics and therapeutic guidelines is provided since a possible continuation of this study would be a study of PD in relation to PK. The following recommendations cover the adult population.

The principal parameter to predict the efficacy of VAN is the area under the curve (AUC) divided by minimum inhibitory concentration (MIC). That parameter has to have a value of at least 400. Recommendations suggest that the initial dosing should be calculated on the basis of TBW and the subsequent dosage adjustments made according to serum samples of

VAN and renal function. The dosing can be intermittent or continuous although the latter has not shown significant improvements in treatment outcomes. The VAN sampling should begin just before the fourth dose (when the steady state conditions are established), and the trough concentrations are the best method of monitoring VAN. To avoid the development of resistance, the trough concentrations should not be lower than 10 mg/L. In general, trough concentrations of 15–20 mg/L are recommended when MIC is 1 mg/L to achieve the optimal treatment. For an individual with a normal renal function, the dosage of 15–20 mg/kg every 8–12 hours is adequate (ClCr more than 70 ml/min). However, guidelines suggest that the MIC of VAN in serum for MRSA is 2 mg/L, and for patients with a normal renal function the target value of 400 of AUC/MIC is often difficult to achieve when MIC is 2 mg/L (21).

Many attempts to address the individualization of VAN dosing have been made, and the factors that influence the treatment with VAN have been thoroughly studied. Several PK models and nomograms for VAN dosing have been constructed. In one study, fourteen nomograms for achieving the trough concentrations of VAN of 15–20 mg/L were assessed, and most of them determine the VAN dosing according to TBW and renal function. Renal function was estimated through ClCr (calculated with Cockroft-Gault Equation – equation *1*) or through the estimation of the glomerular filtration rate based on the Modification of Diet in Renal Disease Study equation (equation *2*) (22, 23, 24, 25)

$$ClCr\ (ml/min) = \frac{(140-age)*TBW}{72*Scr} * 0.85\ (if\ female) \qquad \text{Equation } 1$$

$$GFR\ (ml/min/1.73m^2) = 175 * Scr^{-1.154} * age^{-0.203} * 0.742(if\ female) * 1.212(if\ African\ American) \qquad \text{Equation } 2$$

An example of a nomogram for adjusting the therapeutic regime in critically ill patients according to renal function and TBW is enclosed in the appendix – table XVI. It was developed by Golenia et al. That nomogram was established to target the trough concentrations of VAN of 15–20 mg/L (26). Figure 3 presents the nomogram taken from the summary of product characteristics of Edicin (VAN) (13).

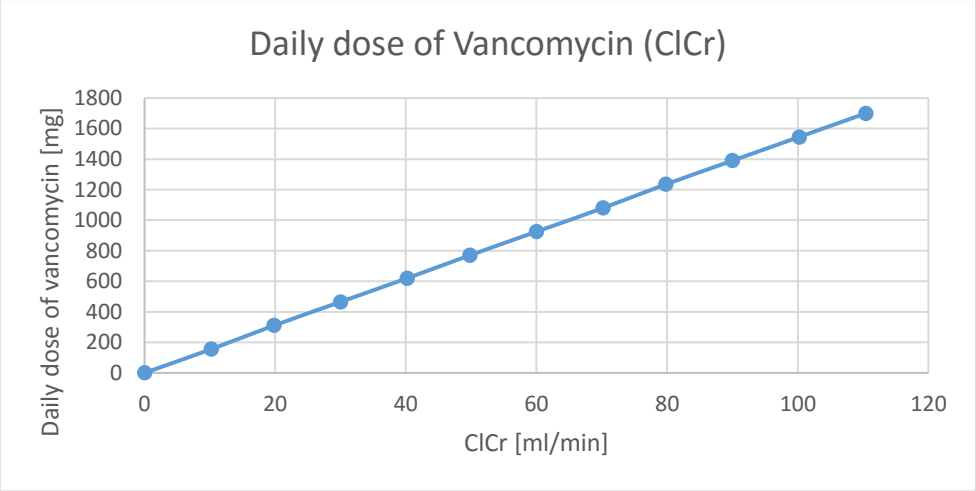


**Figure 3. VAN daily dose in relation to ClCr (13).**

However, further studies are needed if we are to understand the influence of personal characteristics on the treatment with VAN to a greater degree and refine the dosage guidelines as well as furtherly improve the safety and efficacy of the drug. In recent years, population pharmacokinetic approaches have been practiced to characterize the PK and PK in relation to PD and to contribute to the understanding of variability in PK of VAN among individuals (22, 27).

Although we will not construct and test a dosing nomogram, we are about to construct a population model and investigate the factors that influence the PK of VAN. Since we will apply the tools of population pharmacokinetics modelling, the basics of that approach are explained to a greater extent in the following chapter.

## 1.2 Population pharmacokinetics and modelling

Population pharmacokinetics (POPPK) is a field that connects knowledge of clinical pharmacology, pharmacometrics, statistics and computer science. It studies PK on a population level rather than individual. It seeks to explain the PK behaviour of drugs in a target population, and it tries to determine and quantitate the sources of variability between subjects (BSV) as well as the residual unpredictable variability in PK. POPPK models can be applied in various areas such as clinical care and drug development. From the therapeutic aspect, the aim of POPPK is to provide dosing guidelines and to serve as a tool for optimizing the treatment. In situations where no previous administrations or measurements of a drug have been made, clinicians are able to choose the appropriate therapeutic regime based on the individual characteristics, such as age, weight, renal function or previous therapy.

However, even when the sampling of a drug does happen, it is most often sparse and part of a routine monitoring. Here, the POPPK models are applied to estimate the PK parameters of a patient which are needed for dosage adjustment. However, all predictive techniques are imperfect and *»all models are wrong, but some are useful«*[1]. That means that predictions will always differ from the optimal to some degree because a human being is far more complex, and mathematical relations tend to simplify the reality (27, 28, 29).

»Population« in POPPK does not mean that the individual patient is less important. On the contrary, the significance of every subject is shown in the determination of BSV, where data from all individuals is studied. Furthermore, the trends of demographic, pathophysiologic and other factors are identified in the population of interest, allowing one to predict a PK profile of any patient with sufficient knowledge of the above-mentioned factors (27, 30).

Variability is generally characterized as interindividual and residual. Interindividual variability accounts for the differences between individuals, and it is the reason, why parameters in a certain individual differ from the expected, typical value. Residual variability includes the variability within the individual and between different occasions, error in measurement of a drug and misspecification of the model. BSV in POPPK studies is wider than in traditional PK studies, because the latter are based on strict inclusion/exclusion criteria of subjects. However, that is a big advantage of POPPK because it does not discard the variability but seeks to explain it and determine its magnitude. Furthermore, POPPK techniques allow one to study the population of interest, the population that is actually going to receive the drug. The PK of the drug in the target population can significantly differ to healthy volunteers (due to various conditions already discussed in previous chapter) who are generally studied in the traditional PK studies under artificial conditions (28, 30, 31).

Another important aspect is that POPPK approach can handle both, sparse and dense data sampling, whereas traditional studies usually involve dense data (6 or more samples per individual). Furthermore, various types of data (experimental, observational) from various sources, studies and populations can be combined. This allows us to study subpopulations that are otherwise difficult to study, such as neonates, critically ill patients, patients with AIDS or cancer patients, where the number of samples is limited. Besides, that means higher

[1] A quotation from George Box. (41)

cost-effectiveness of POPPK studies than expensive and patient-inconvenient traditional PK studies. A notable disadvantage of POPPK approach is that it requires a skilled pharmacometrician/pharmacokineticist because data and results could often be difficult to interpret and the analysis complex to perform (28, 30).

Although POPPK approaches include various modelling methods, such as naive pooled data, standard two-stage approach and iterative two stage Bayesian estimation (30), our work will comprise a comparison of two nonlinear mixed-effects modelling (NLMEM) methods and software. Therefore, a further review of NLMEM is provided.

### 1.2.1 Nonlinear mixed effects modelling

NLMEM is one of the most popular tools among POPPK approaches. »Nonlinear« means that there is a nonlinear relation between the dependent and independent variables. »Mixed effects« refers to the division of parameters into those that are the same for the whole population (fixed effects) and into those that are specific in a certain subject (random effects). Since there are usually few samples per patient, all data is analysed at the same time but the individual random effects are considered (29, 32, 33).

Fixed effects represent the typical parameter values of a population and are written as theta (θ). They have the same value for all the individuals. The difference between a typical value and an individual parameter is marked as eta (η), and it represents a part of the random effects. η vary between individuals and characterize the BSV. A typical value is generally a mean value and η are normally or log-normally distributed across population, centered around zero. η are non-measurable. The individual parameter value is marked as ϕ (27, 28).

Equation *3* presents an individual parameter value, where individual η are normally distributed with 0 mean, and equation *4* assumes log-normally distributed individual parameters, where η also has a mean 0. Log-normal distribution is preferred when the parameter values can be only positive (28, 32).

$$\phi_i = \theta + \eta_i \qquad \text{Equation } 3$$

$$\phi_i = \theta * e^{\eta i} \qquad \text{Equation } 4$$

The distribution of BSV is summarized as a standard deviation (SD) and is also referred to with an omega (Ω). Residual variability is expressed in a general equation of a model (equation *5*) as epsilon (ε) and is normally distributed with mean 0 and variance $\sigma^2$. Residual variability presents the other part of the random, unpredictable effects.

$$y_{ij} = f(\phi, x_{ij}, \eta_i) \pm \varepsilon_{ij}$$ Equation *5*

Equation *5* expresses a general function that describes a POPPK model. The function f describes a relation between a dependent (e.g. concentration of drug in plasma) and an independent variable (e.g. time) and it is an observed trend that the drug undergoes in the population, with a certain BSV. For example, it can be a one- compartment model with bolus administration. $y_{ij}$ is the observed dependent variable, the observed data in the jth subject, when the PK parameter takes the value $\phi$. $\varepsilon$ as the residual variability, expresses an error, a measurement noise that is always present and is unpredictable. Therefore, $\varepsilon$ cannot be explained. It is the difference between the model prediction and an observed value in the individual. Error model is often additive, proportional or a combination of both (28, 32, 34).

To explain the BSV, it is possible to introduce population characteristics to the model. In that manner we can include significant patients' factors like weight, age, renal function etc. to the fixed effects, where they act as covariates. For example, VAN is excreted renally, so the renal function markers can represent a covariate in relation to its clearance and explain a part of variability in $Cl_{VAN}$. Because renal function is included in fixed effects, it has an influence on clearance in the whole population. However, a part of BSV generally remains unexplained even after the inclusion of covariates to the fixed effects. There are various possibilities of inclusion of the covariates in the model. One of the simplest ones is a linear relation – an example is shown in the equation *6*. Equations *7* and *8* also assume log-normality and center Cl to the ClCr of 120 ml/min. That means that people with the ClCr of 120 ml/min will have a typical parameter value, $\theta_{Cl}$, and other individual values will be distributed log-normally around the typical value (32).

$$Cl_i = \theta_{Cl} + \theta_{ClCr} * ClCr + \eta i$$ Equation *6*

$$Cl_i = (\theta_{Cl} + \theta_{ClCr} * (ClCr - 120\ ml/min)) * e^{\eta i}$$ Equation *7*

$$Cl_i = (\theta_{Cl} * (1 + \theta_{ClCr} * (ClCr - 120\ ml/min)) * e^{\eta i}$$ Equation *8*

With that in mind, it can be summarized that there are five important aspects regarding NLMEM (32):

- data (further analysis depends highly on the quality and accuracy of the database),
- structural model (describes a typical concentration-time relationship of a drug in a population, represented as algebraic or differential equations),
- statistical model (describes the measurement error, BSV, residual variability),

- covariate model (explains a part of BSV with population characteristics),
- software (analyses and computes the data, solves the probability function using the estimation method).

#### *1.2.1.1 Estimation methods*

To find the suitable parameter values for the selected model related to a dataset, various estimation methods are used. Maximum likelihood approach is the most widely used approach for estimating the parameters in NLMEM. Assuming the predicted data values in relation to the observations have a possible range of values determined by the distribution, maximum likelihood means the highest probability that the estimated values of parameters will correspond to the true values. The estimation is expressed as »maximum likelihood objective function value« (OFV), which is calculated as minus twice the logarithm of likelihood (equation *9* and *10*). A higher probability yields lower OFV and one tries to minimize it (27, 33, 39).

$$L = \prod_{i=1}^{n} \frac{1}{\sqrt{2\pi\sigma_i^2}} e^{-\frac{1}{2\sigma_i^2}(Y_i-\hat{Y}_i)^2} \quad \text{Equation } 9$$

$$-2\,log(L) = nlog(2\pi) + \sum_{i=1}^{n}(log(\sigma_i^2) + \frac{(Y_i-\hat{Y}_i)^2}{\sigma_i^2}) \quad \text{Equation } 10$$

If the variance does not change from observation to observation, one has to minimize the $(Y_i - \hat{Y}_i)^2$ to minimize the -2log(L) – $Y_i$ is the measured concentration and $\hat{Y}_i$ is the predicted concentration. OFV is a single value representing the goodness of fit of the model and has approximately $\chi^2$ distribution in nested models, which is used to test statistical significance. The absolute value is not important, and it is rather used to compare the different models for the same database (27, 33, 39, 45).

OFV for two fitted parameters can be shown also as a 3D surface. When dealing with more complex problems, the risk of finding the local minimum is present during convergence. This means that for the same data and model, another set of parameters can have a comparably suitable fit for the data as the parameters associated with the global minimum. From that point of view, the introduction of adequate initial parameter estimates is important (27).

For solving the OFV of complex PK models and incomplete datasets, many optimization algorithms are used in order to find the minimum value of OFV. In our study, we will use two estimation methods within two software packages – expectation-maximization (EM) method in Adapt 5 software package and first order conditional estimation with interaction (FOCE-I) in the NONMEM 7.3 package. The focus will be on the work with Adapt 5 and the expectation-maximization method, since it is less used for estimating the POPPK parameters. FOCE-I and NONMEM will serve as a reference method since that method is a gold standard in POPPK modelling. The goal is to provide the user-evaluation of methods and a comparison of modelling workflow, and therefore the detailed mathematical backgrounds of estimation methods will not be the topic of our study.

*1.2.1.1.1 Adapt 5*

Adapt 5 was developed by Biomedical Simulations Resource in the department of Biomedical Engineering at the University of Southern California. It is intended for the use in PK and PD modelling, data analysis and simulation and is suitable for basic as well as clinical research scientists. Adapt software is designed to facilitate the study and application of PK and PD properties of drugs, and it provides a library of the fundamental models. It includes tools for individual and population analysis, and one of the estimation methods is also the parametric maximum likelihood estimation via the EM algorithm (35).

The EM algorithm addresses the problem of the incomplete data and various local minima by reducing the task of optimizing the OFV into simpler subproblems. It alternates between two steps: E-step (expectation) and M-step (maximization). In E-step, it generates the probabilities for all possible completions of data from the initial estimates of parameters according to the distribution. Then, in the M-step, it determines new parameters from these probabilities and repeats those two steps until convergence. It has shown a good performance in sparse and in rich data (36, 37).

*1.2.1.1.2 NONMEM 7.3*

NONMEM is a computer program implemented in Fortran 90/95 and was first developed cca. thirty years ago by the NONMEM Project Group at the University of California, mostly by Lewis Sheiner and Stuart Beal. From then, it has been continuously developed and widely used for solving the pharmaceutical statistical tasks. It has become a gold standard in POPPK and PK-PD modelling. NONMEM stands for NON-linear Mixed Effects Modelling and is a

well-validated program with a large group of users and a variety of interfaces that help and simplify the use (38, 39).

The software consists of three parts (38):

- NONMEM program, a basic and very general nonlinear regression program,
- PREDPP, which is a package of subroutines for handling the computation of predictions for POPPK data,
- NM-TRAN, a pre-processor allowing control and other needed inputs to be specified.

FOCE-I is one of the population analysis methods available in NONMEM 7.3. This method uses a first-order Taylor series expansion about conditional estimates (Bayesian estimates), with respect to the random effects – η and ε, to linearize the model. It takes into consideration the integral over all possible individual parameter values when determining the best fixed effects and also assumes the interaction between η and ε (33, 37)

#### *1.2.1.2 Comparing the models*

To rank the models from more to less appropriate, OFV is generally used – a lower OFV means a better fit. Models that are more complex and contain more parameters usually better describe the data. However, to avoid the overparametrization, the following criteria are used, and they penalize the complexity expressed in the higher number of parameters used in the model: Akaike information criterion (AIC) and Bayesian information criterion (BIC) (32). $n_p$ represents the number of parameters and N is the number of observations.

$$AIC = OFV + 2 * n_p$$ Equation *11*

$$BIC = OFV + n_p * Ln(N)$$ Equation *12*

Those criteria are preferable when the data are limited and like with the OFV, models with a lower value of AIC or BIC show a better fit. The interpretation of the differences in BIC are the following (32):

- Difference in BIC > 10; very strong evidence in the favour of the model with lower BIC,
- 6-10; strong evidence,
- 2-6; positive evidence,
- 0-2; weak evidence.

A drop of 2 is generally considered the threshold for picking one model over another (32).

The assessment of precision and bias is also advisable for describing and comparing the performance of different prediction methods and models. The mean prediction error (ME) is the measure of bias, and it is defined as a sum of the individual prediction errors, $pe_i$, divided by the number of observations, N (40).

$$ME = \frac{1}{N}\sum_{i=1}^{N} pe_i \qquad \text{Equation } 13$$

$$pe_i = \hat{Y}_i - Y_i \qquad \text{Equation } 14$$

ME estimates the magnitude of the systematic component of error. To measure precision, the mean squared prediction error (MSE) or its squared root (RMSE) is used. To calculate the MSE, one must first square the $pe_i$. The equations are shown below (40).

$$MSE = \frac{1}{N}\sum_{i=1}^{N} (pe_i)^2 \qquad \text{Equation } 15$$

$$RMSE = \sqrt{MSE} \qquad \text{Equation } 16$$

The confidence intervals for these predictors can also be obtained by assuming normal distribution of $pe_i$ (40).

#### *1.2.1.3 Evaluation and validation*

When the model construction is finished and the final model is selected, it has to be validated and evaluated to ensure that it is appropriate and robust. If the purpose of the model is only descriptive, the assessment of goodness of fit, model's reliability and its stability is sufficient. However, when one intends to use the model on populations other than the studied one (the one from which the model was constructed), a further validation based on the data of external patients is required (41).

The evaluation of goodness of fit covers graphical visualization of plots of:

- observed dependent variable versus predicted dependent variable,
- residuals versus predicted dependent variable,
- standardized residuals versus predicted dependent variable,
- residuals versus covariates.

The standardized residuals should be normally distributed and not biased. The residuals versus time should be centered around zero with most values being between -2 and +2 SDs (32, 41).

The reliability of a model can be evaluated with checking the uncertainty factors. Every parameter is estimated with a certain confidence that is expressed as a relative standard error (RSE) of a parameter estimate. A lower RSE means higher certainty – for fixed effects it should not be greater than 25 %, and for random effects it should not be greater than 50 % (32, 41).

$$Confidence\ interval = Parameter\ value \pm 1{,}96 * Standard\ error \quad \text{Equation } 17$$

For assessing reliability and stability, methods such as bootstrapping can be used. Bootstrapping is a method that generates new populations, where individuals are randomly drawn from the original population (and can be drawn several times, therefore it is also called »bootstrap with replacement«). Many virtual populations are created (usually more than 1000) and they all undergo the estimation of parameters using the final model. The upper 97.5 and the lower 2.5 percentiles are used for constructing the confidence intervals of parameter estimates. The confidence intervals of parameter estimates should not include a value zero (32).

In general, validation can be internal or external. Internal validation means that the model is validated using only the original set of data, whereas in external validation the final model is used on another population without estimating the parameters and with a subsequent evaluation of the predictive performance. A useful validation tool (that can be internal or external) is also the visual predictive check (VPC). It involves the simulation of the new data with the final model using the selected data-base design. Concentration-time profiles are used for constructing the confidence intervals that are compared with the observed data. VPC verifies that the simulated data and the observed data are consistent. Approaches, such as prediction- and variability-corrected VPC, are used to overcome different dosing regimes and different expected variability within the individuals (32, 41, 42).

With the validation of a model, one ensures that the deficiencies of a model will not turn out to be significant in the model's application. However, the model developing and evaluation are complex problems, and there is no correct way of facing them as there are no correct or false models (41).

# 2 Aim of the study

Resistance to antibiotics has become an increasing problem in the world. Optimizing the therapies and wise use of drugs is therefore of utmost importance. In the last thirty years, the techniques of POPPK have been continuously developed and have significantly contributed to the study of drugs in general, drug development, to understanding of PK and PD and to assess the characteristics that influence the PK and PD and the efficacy of the therapy.

The aim of the study is to develop and provide a validated population pharmacokinetic model that could be useful in the optimization and individualization of treatment with this antibiotic. This study could serve as a base for the potential PK-PD studies. The aim is also to develop a POPPK model using two different methods and compare the work with both.

The examined method will be the expectation-maximization in the Adapt 5 software package, and the first order conditional estimation with interaction in the NONMEM 7.3 software package will serve as a comparison and a reference method, since it is the gold standard in the PKPD modelling.

We will compare the work process, assess the differences and compare the results obtained using those two independent methods. We will also compare the obtained results with the models and parameters already described in the literature. Another important aim of the study is also to learn and upgrade my skills regarding the principles of a POPPK approach for being able to solve more complex problems and tasks in the future.

# 3 Materials and methods

The main material used in this respective study was the data obtained from medical treatment histories of a population of patients from the Clinical Center Tondela-Viseu, Portugal. The data were then analysed using two methods. The analysis included:

- preparation of the database,
- selection of the structural model,
- selection of the error model,
- construction of the covariate model through the forward selection process,
- validation of the model using Bootstrap, VPC or external validation
- comparison of the base model with the final model,
- comparison of the results obtained using both methods,
- comparison of the obtained parameter values with the values described in literature,
- comparison of the work with both methods.

## 3.1 Population and data

A cohort of thirty-three patients from a Clinical Centre Tondela-Viseu was investigated. The patients entered the hospital between 1.5.2010 and 16.10.2012 and they were all in critical condition when they began with the vancomycin therapy. All of them received intravenous infusions of VAN – intermittent or continuous. The patients' conditions differed (septic shock, nosocomial pneumonia, post-operative states, chest injury, respiratory insufficiency, pancreatitis, cancer) and the infection was not confirmed with the bacterial sample culture. However, the factors for suspecting the infection with MRSA were sufficient for choosing the VAN treatment. The vancomycin treatment was monitored and the following parameters were measured and recorded: total body weight, age, sex, height, vancomycin administrations (date and time, amount, infusion rate), vancomycin plasma concentrations, blood concentration of C-reactive protein, blood concentration of p-calcitonin, fluid balance, possible co-therapy with other drugs (diuretics, aminoglycosides, sympathomimetics), and renal and hepatic function markers (concentration of creatinine in serum, urine, ALT, AST, serum albumine). Possible exitus, mechanical ventilation status and cardiac, respiratory, renal or hepatic insufficiency were recorded, and the Acute Physiology and Chronic Health Evaluation score (APACHE II) and the Simplified Acute Physiology Score (SAPS II) were calculated. There were 24 men and 9 women in the studied population. For the

pharmacokinetic evaluation, renal function was quantified through creatinine clearance that was calculated using the Cockcroft-Gault equation (equation *1*).

All the patients were included in the analysis, even those who had only one or two measured samples of vancomycin in the plasma. In total, 166 points concentration-time were analysed.

## 3.2 Development of a POPPK model

### 3.2.1 Process in Adapt 5

The first step in the POPPK development was the preparation of the database. The data from the patients' medical histories were collected and put together in the file recognizable by Adapt. Potential errors were detected with the test runs and visual analysis of the individual plots. The interface of Adapt 5 is presented in the figure 4.

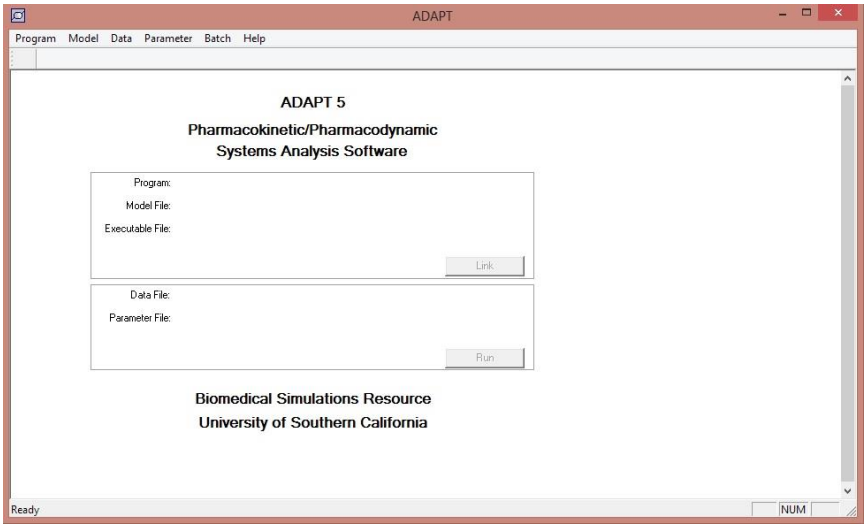


**Figure 4. Adapt 5 interface.**

#### *3.2.1.1 Base model*

Then, MLEM function was used for the fitting of the data. First, the selection of the structural model together with the model of error was performed. One- and two-compartment models with infusion administration from the Adapt library were tested (Figure 5). Equation *18* shows a one-compartment model, and equations *19* and *20* show a two-compartment model written in Fortran language in Adapt. Table I explains the variables.

**Table I. Symbols for one-compartment model.**

| | |
|---|---|
| XP(1) | dx/dt, central compartment |
| XP(2) | dx/dt, peripheral compartment |
| P(1) | Cl, total clearance |
| P(2) | Vd, volume of central compartment |
| P(3) | Cld, Cl between compartments |
| P(4) | Vp, volume of peripheral compartment |
| X(1) | x(t) in central compartment |
| X(2) | x(t) in peripheral compatrment |
| R(1) | r(t), rate of infusion |

$$XP(1) = -(P(1)/P(2))*X(1) + R(1)$$ Equation *18*

$$XP(1) = -(P(1)+P(3)/P(2)*X(1) + P(3)/P(4)*X(2) * R(1)$$ Equation *19*

$$XP(2) = P(3)/P(2)*X(1) – P(3)/P(4)*X(2)$$ Equation *20*

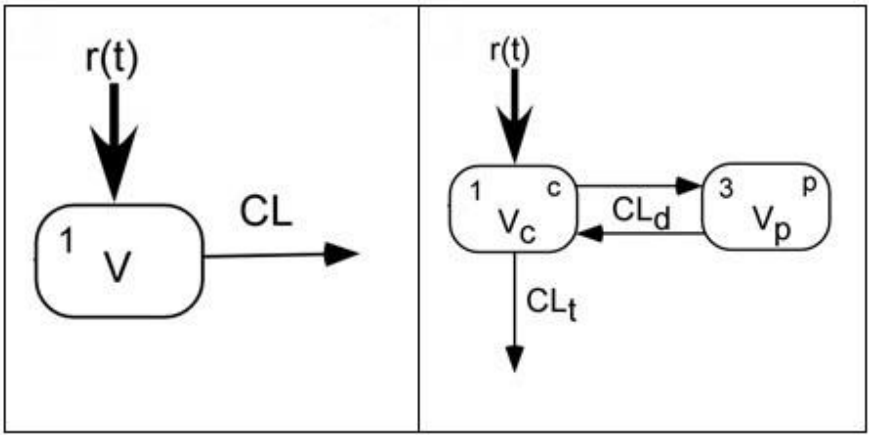


**Figure 5. Compartment models from Adapt 5 library; left - one-compartment model, right - two-compartment model.**

Additive error, proportional error and the combination of both were associated with the structural model, presented in equations *21*, *22* and *23* respectively.

$$y = f(\phi, x) + \varepsilon$$ Equation *21*

$$y = f(\phi, x) * (1 + \varepsilon)$$ Equation *22*

$$y = f(\phi, x) * (1 + \varepsilon_1) + \varepsilon_2$$ Equation *23*

The folowing settings for EM estimation were assumed:

- lognormal distribution of parameters,
- ful covariance matrix,
- 100 iterations,

- 1000 EM samples per iteration.

A drop in BIC was the criteria for model selection since the investigated population was relatively small and the data were sparse – therefore simpler models were favoured. Adapt 5 provides the BIC and AIC values.

#### *3.2.1.2 Covariate model*

To try to explain the BSV, the inclusion of covariates to the model was performed through the process of forward selection. To detect the potentially significant covariates, the ANOVA – general linear model analysis was performed, using mean values of covariates of every individual. Then we tested models step by step (forward selection), including covariates one by one. In the first step the covariate that caused the highest drop in BIC was included and then the process was repeated until no covariate inclusion caused a drop in BIC. In Adapt models had to be written manually and every run performed apart.

For testing their influence, chosen covariates and their relations associated with $Cl_{VAN}$ and Vd are shown in table II. Two types of covariates were tested: continuous and categorical. Continuous covariates were: ClCr, TBW, ALT, AST, height and age and the first four varied during the treatment. Categorical covariates were the following: coadministration of furosemide (Co/Fu), coadministration of spironolactone (Co/Sp) and sex. In those covariates, value 1 was assigned to men and when co-therapy was present, whereas 0 was assigned to women and when co-therapy was not present.

**Table II. Covariates tested in forward selection process and mode of inclusion.**

| Covariate | Equation |
|---|---|
| ClCr | $\phi = \theta + \theta_{ClCr} * (ClCr-120)$ |
| Weight (linear) | $\phi = \theta + \theta_{TBW} * (TBW-70)$ |
| Weight (proporcional) | $\phi = \theta * (TBW/70)$ |
| Weight (power) | $\phi = \theta * TBW^{\theta tbw}$ |
| ALT | $\phi = \theta + \theta_{ALT} * ALT$ |
| AST | $\phi = \theta + \theta_{AST} * AST$ |
| Coadministration of furosemide | $\phi = \theta + \theta_{co/fu} * Co/Fu$ |
| Coadministration of spironolactone | $\phi = \theta + \theta_{co/sp} * Co/Sp$ |
| Sex | $\phi = \theta + \theta_{sex} * Sex$ |
| Height | $\phi = \theta + \theta_{Height} * Height$ |
| Age | $\phi = \theta + \theta_{Age} * Age$ |

ClCr and TBW were centered to the typical individual of the population, with the values of 120 ml/min and 70 kg respectively.

#### *3.2.1.3 Validation and evaluation*

The final model was validated with the internal validation method, bootstrap with replacement to obtain the 95 % confidence intervals. Those confidence intervals should not include the value of zero. 50 new populations were constructed from the original population using Excel. The testing was performed manually, and only 50 samples were tested because running a greater number (e.g. 1000 samples) would be very time-consuming.

When we validated the chosen model, a comparison of the base model with the results obtained with the reference method and an overall comparison with the models and parameter values already described in literature, were made. We also observed a percentage of the BSV that we were able to explain with the covariate model. Precision and bias as measures of goodness of prediction were characterized with ME, MSE and RMSE.

Goodness of fit plots, individual plots, analysis of residuals and other graphs were drawn in R with the tool package for Adapt – AMGET (43) and Excel.

### 3.2.2 Process in NONMEM 7.3

#### *3.2.2.1 Base model*

The process was similar to working with Adapt. After the data preparation and cleaning, the structural model was tested together with the statistical model. Subroutines ADVAN 1/TRANS 2 (one-compartment model) and ADVAN 3/TRANS 4 (two-compartment model) were used to run the process. Additive, proportional and combination residual error models were tested and an estimation was performed using the FOCE-I method. For the residual variability, we fixed σ to 1 and estimated the factor »w« as a standard deviation of ε. The equation below represents the statistical model for the combined type of error. When we included only an additive or proportional type of error in the model, $\theta_{prop}$ or $\theta_{add}$ were fixed to 0 respectively.

$$w = \sqrt{(\theta^2_{add} + (\theta_{prop} * IPRED)^2)} \quad \text{Equation } 24$$

$$y = IPRED + \varepsilon * w;\ IPRED \ldots individual\ prediction \quad \text{Equation } 25$$

Shrinkage of the parameters was also observed. Shrinkage measures the extent to which the individual parameters »shrink« to population values (equation *26*). When it is above 30 %,

one does not have sufficient data to describe the variability. When it is 0, that means that parameter estimates represent the true values of the parameters (32).

$$Shrinkage(\eta) = (1 - \frac{SD(Individual\ estimates)}{Population\ variability}) * 100\ \%$$ Equation *26*

#### *3.2.2.2 Covariate model*

The covariate model was constructed automatically with the »stepwise covariate modelling« (scm) protocol in Perl-speaks-NONMEM modules. The forward selection was performed with the criteria for the inclusion of statistical significance, $p < 0.05$, which corresponds in a drop of OFV to more than 3.84 according to the $\chi^2$ distribution. The same covariates as in Adapt were tested and linear and power relations of all covariates associated with both parameters were tested. We also observed the BIC value, but we calculated it manually since NONMEM does not provide it.

For the time-varying covariates, like ClCr, intraindividual variability was studied in a separate step. Furthermore, the randomization protocol was performed for the covariates that were included in the final model, but we wanted to assess the actual significance of the data.

#### *3.2.2.3 Validation and evaluation*

The final model was validated with internal validation – bootstrap with replacement and VPC, and external validation – prediction analysis of an external set of data (population of 56 patients and 307 observations of vancomycin in plasma), obtained from the master's thesis of F. Sopotnik (44). A percentage of the explained BSV was assessed and the prediction performance analysed by calculating the ME, MSE and analysing the goodness of fit (observed variable versus predicted variable).

In the end, the results were compared to those obtained with Adapt 5 and the already existing literature. Graphs were drawn in R with the xpose4 package toolkit and Excel, and the Perl-speaks-NONMEM collection was used to aid the modelling with NONMEM.

# 4 Results and discussion

## 4.1 Population

The characteristics of the study cohort are presented in table III. The variability of covariates was not high in the population since the data were sparse. It is important to note that not all the subjects that received furosemide and spironolactone received those two drugs all the

time during VAN treatment, which is an important aspect when looking at the data. Moreover, some parameters varied during the treatment, therefore the mean value for every patient was calculated and then used to calculate the population mean presented in table III. The distribution of TBW and ClCr is shown in figure 6. The interval of ClCr seems wide, however, the higher values (above 160 ml/min) are questionable, since the lower concentration of creatinine in serum especially in older people often occur due to the lower muscle mass, rather than because of a high renal function. In that manner, it is possible that the value obtained with the Cockcroft-Gault equation (equation *1*) does not represent the true ClCr.

**Table III. Population characteristics.**

| Characteristic | Mean ± SD | Interval (min - max) |
|---|---|---|
| No of patients | 33 | / |
| No of conc-time points | 166 | / |
| Age [years] | 64 ± 15 | 21 - 82 |
| Sex | 24 ♂/ 9 ♀ | / |
| TBW [kg] | 78.82 ± 17.05 | 55.92 - 122.2 |
| Height [cm] | 164.4 ± 8.940 | 147.0 - 180.0 |
| ClCr [ml/min] | 127.5 ± 50.52 | 41.20 - 244.60 |
| ALT [U/L] | 58.61 ± 53.83 | 13.00 - 237.7 |
| AST [U/L] | 56.47 ± 41.52 | 18.50 - 204.8 |
| Co/Fu | 29 + / 4 - | / |
| Co/Sp | 8 + / 25 - | / |

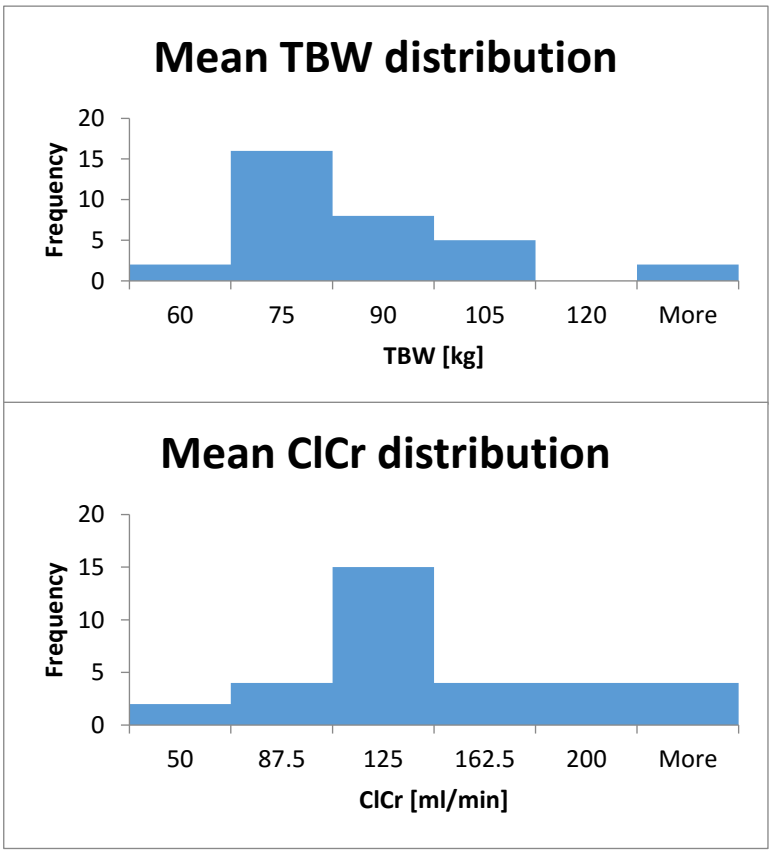


**Figure 6. Distribution of total body weight (above) and creatinine clearance (below) in the study cohort.**

## 4.2 Adapt 5 modelling

### 4.2.1 Base model

With the EM algorithm, the one-compartment model had a lower BIC value than the two-compartment one. One-compartment model was therefore used for the subsequent covariate model building, regardless of the lower OFV of the two-compartment model. In relation to the statistical model, an additive error model for describing the residual variability was the most adequate among the studied models, since it had the lowest BIC value. Results are presented in tables IV and V.

**Table IV. A comparison of one- and two-compartment model fits (with additive error).**

| | 1-compartment | 2-compartments |
|---|---|---|
| OFV | 965.523 | 922.439 |
| BIC | 996.195 | 999.119 |

**Table V. BIC values of statistical models (one-compartment model).**

| statistical model | BIC |
|---|---|
| additive | 996.195 |
| proportional | 1015.83 |
| combination | 998.042 |

### 4.2.2 Covariate model

The covariate model building in Adapt was time-consuming since all models had to be written manually and every run performed separately. However, the good side of the manual execution of forward selection is that we had a greater control and insight into the process than in NONMEM, where forward selection was run automatically. In the first step, TBW was associated with Vd. In the second step, ClCr was in correlation to $Cl_{VAN}$ and in the third step, the coadministration of furosemide was associated with $Cl_{VAN}$. Then, none of the covariates had a significant influence on the parameters. Detailed results are not presented since more than 70 models were tested. The BSV of $Cl_{VAN}$ and Vd was reduced from 33.4 % to 28.2 % and from 67.9 % to 61.5 % respectively – that means that the covariate inclusion explained cca. 15 % and 10 % of the BSV of $Cl_{VAN}$ and Vd respectively. Equations *27* to *30* describe the final model, and the comparison of parameter values of the base model and the final model is shown in table VI.

$$Cl_{VANi} = (\theta_{Cl} + (\theta_{ClCr}*(ClCr - 120) + \theta_{Co/Fu}*Co/Fu)) * e^{\eta i} \quad \text{Equation } 27$$

$$Vd_i = \theta_{Vd} * (TBW/70) * e^{\eta i} \quad \text{Equation } 28$$

$$Cl_{VANi} = (4.84 + (0.0155*(ClCr - 120) - 1.26*Co/Fu)) * e^{\eta i} \quad \text{Equation } 29$$

$$Vd_i = 58.7 * (TBW/70) * e^{\eta i} \quad \text{Equation } 30$$

**Table VI. Base model and final model fit summary and parameter values obtained in Adapt.**

| | Base model | Final model |
|---|---|---|
| Parameter | Estimate (RSE %) | Estimate (RSE %) |
| OFV | 965.523 | 947.774 |
| BIC | 996.195 | 988.67 |
| $\theta_{Cl}$ [L/h] | 3.85 (9.72) | 4.84 (15.1) |
| $\theta_{Vd}$ [L] | 67.2 (28.0) | 58.7 (22.6) |
| $\theta_{ClCr}$ [(L*min)/(ml*h)] | / | 0.0155 (43.3) |

| | | |
|---|---|---|
| $\theta_{Co/Fu}$ [L/h] | / | -1.26 (52.9) |
| BSV | | |
| $\Omega_{Cl}$ [%] | 33.4 (22.4) | 28.2 (28.8) |
| $\Omega_{Vd}$ [%] | 67.9 (58.9) | 61.5 (34.3) |
| Residual variability | | |
| SD [mg/L] | 2.98 (8.53) | 2.96 (6.66) |

### 4.2.3 Validation and evaluation

There is almost no difference in the individual predictions vs. observations of the base and final models (figure 7). The models are not biased and the precision is acceptable (figure 8).

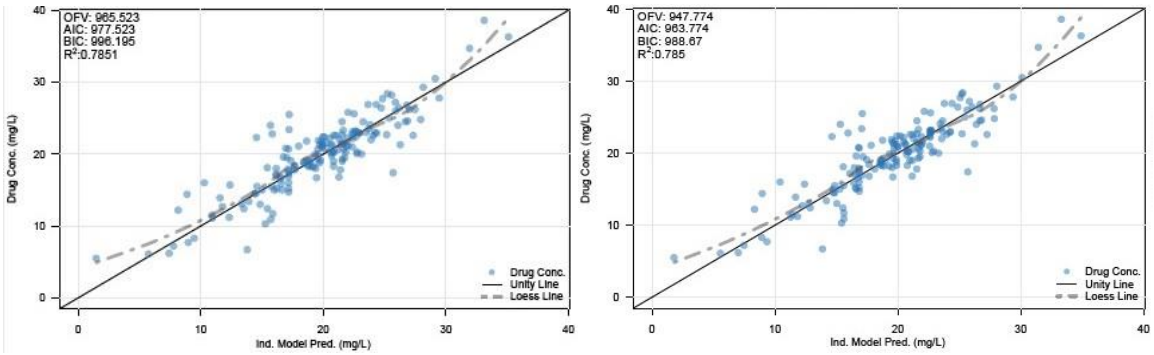


**Figure 7. Observations versus individual predictions; left - base model, right - final model.**

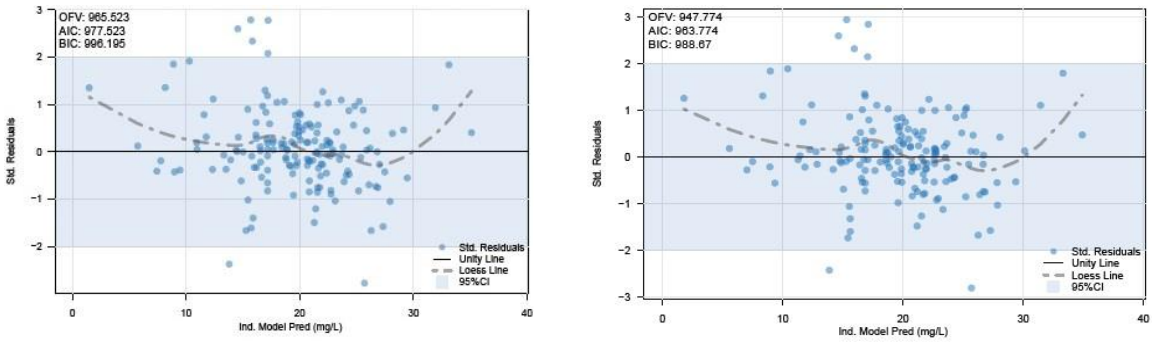


**Figure 8. Standardized residuals versus individual predictions; left - base model, right - final model.**

The population models are more important since they would potentially be used in the drug dosing if the clinician would not know the patient's characteristics, because the random effects in a specific individual are unknown. It is visible, that the final model has a significantly better trend and it predicts the concentrations much better than the base model, which means that the inclusion of covariates improves the prediction (figure 9). However, even the final model still overpredicts higher values and underpredicts lower values, and we were not able to find out why. One of the possible reasons is that our population consisted

of few patients. Moreover, the data were sparse and the deviation of characteristics was not high. The graphs of residuals vs. predictions do not show any trends, which means that the adequate model was used (figure 10).

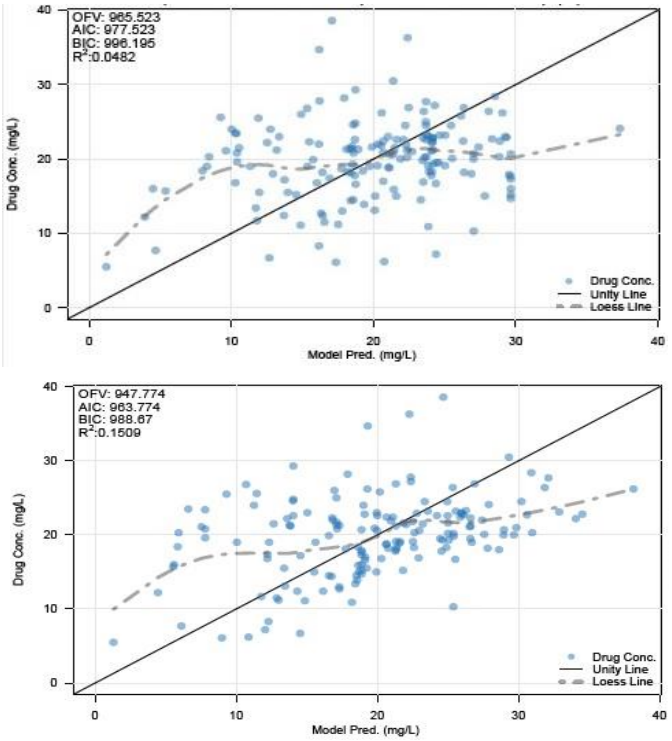


**Figure 9. Observations versus population predictions; above - base model, below - final model.**

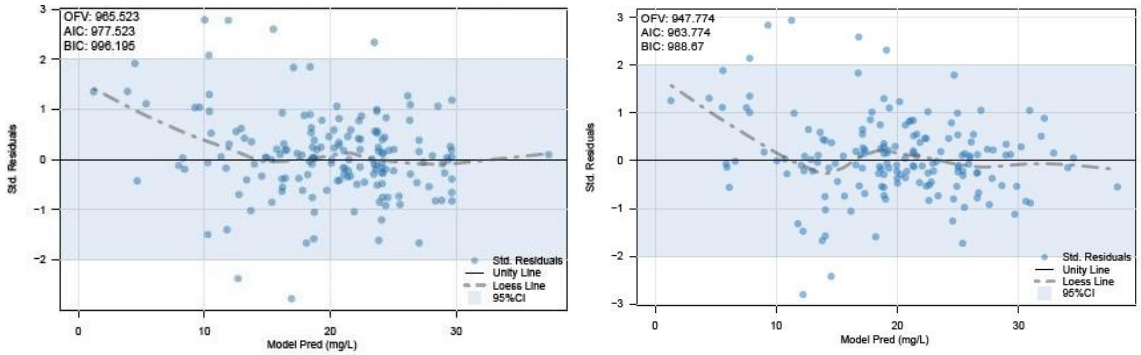


**Figure 10. Standardized residuals versus population predictions; left - base model, right - final model.**

The results of the bootstrap on 50 virtual populations provided the mean values, and the 95 % confidence intervals were obtained from the parameter estimates. All the parameters of the final model were within that interval and none contained value zero. That means that the model is robust. Results of the bootstrap are summarized in table VII.

**Table VII. Bootstrap results.**

| | Bootstrapping | | Final model |
|---|---|---|---|
| **Parameter** | **Mean ± SD** | **Confidence intervals (2.5 percentile – 97.5 percentile)** | **Mean** |
| $\theta_{Cl}$ [L/h] | 4.96 ± 0.388 | 4.14 – 5.64 | 4.84 |
| $\theta_{ClCr}$[(L*min)/(ml*h)] | 0.0172 ± 0.00724 | 0.00235 – 0.0297 | 0.0155 |
| $\theta_{Co/Fu}$ [L/h] | -1.30 ± 0.500 | -2.40 – -0.500 | -1.26 |
| $\theta_{Vd}$ [L] | 61.1 ± 12.3 | 36.7 – 87.9 | 58.7 |
| BSV | | | |
| $\Omega_{Cl}$ [%] | 25.5 ± 6.20 | 13.5 – 36.6 | 28.2 |
| $\Omega_{Vd}$ [%] | 55.5 ± 16.2 | 26.5 – 81.7 | 61.5 |
| Residual variability | | | |
| SD [mg/L] | 2.89 ± .509 | 1.77 – 3.77 | 2.96 |

The influences of TBW and ClCr on Vd and $Cl_{VAN}$ respectively have already been described in literature. A greater curiosity, however, exists regarding the coadministration of furosemide. The possible influences of this drug on Cl have been mentioned in the introduction of the thesis – supposedly furosemide increases Cl in patients. In our model it seems that it would decrease it. A possible explanation includes the fact that not all the patients received furosemide all the time during the treatment with VAN, and that $Cl_{VAN}$ varied due to the variation in renal function. Therefore, it could be possible that furosemide does not decrease the $Cl_{VAN}$, but that the patients who received it had lower $Cl_{VAN}$ at that moment which was the reason for the administration of this diuretic. From another point of view, it is also possible that the patients stopped receiving the diuretic because the Cl has normalized. A further examination is needed for any conclusions to be drawn. A distribution of $CL_{VAN}$ and Vd across the studied population is shown in figure 11.

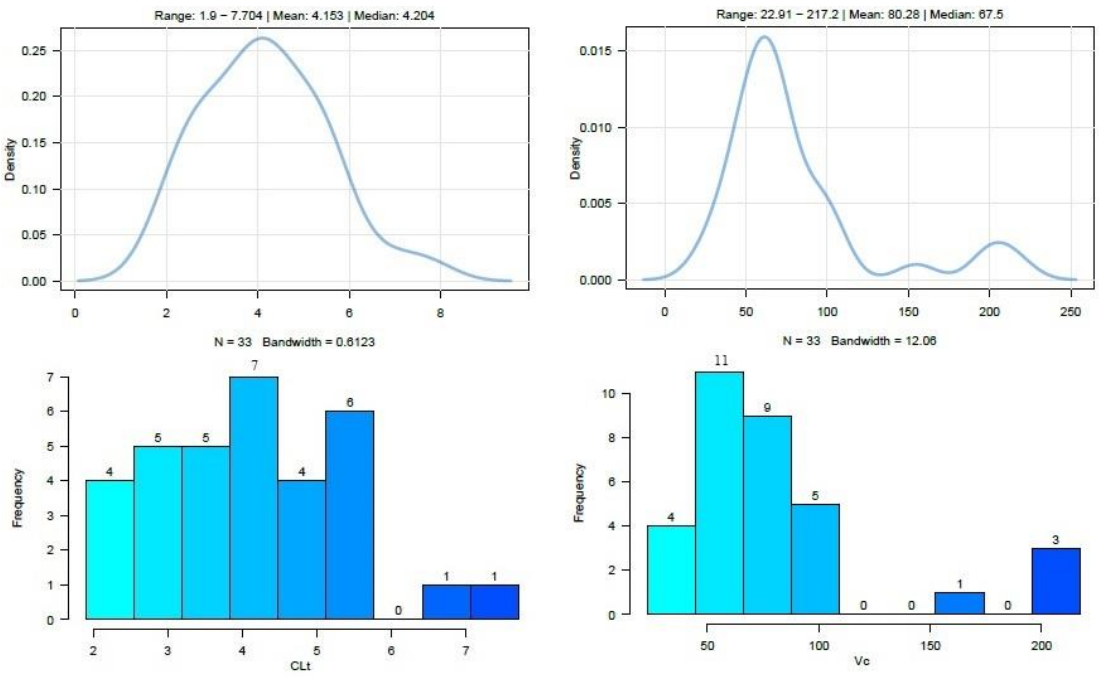


**Figure 11. Distribution of individual parameters; left - distribution of $Cl_{VAN}$, right - distribution of Vd.**

## 4.3 NONMEM 7.3 modelling

### 4.3.1 Base model

Modelling with NONMEM was far less time-consuming than modelling in Adapt, because the majority of functions and processes were automatized. The first step was the same as in Adapt – the structural model in relation with the model of error was assessed. As in Adapt, the one-compartment model with additive error has proved to be the most adequate, even though the two-compartment model had a much lower OFV. Here, a bigger doubt was whether to choose a one- or a two-compartment model, but in the end the latter was too unstable, and it had difficulties with convergence since we had to fix the BSV of intercompartmental clearance and the volume of the second compartment to 0. The results are presented in tables VIII and IX.

**Table VIII. a comparison of one- and two-compartment model fits (with additive error).**

| | 1-compartment | 2-compartments |
|---|---|---|
| OFV | 662.316 | 619.126 |
| BIC | 692.988 | 695.806 |

**Table IX. A comparison of statistical models (one-compartment model).**

| statistical model | OFV | BIC |
|---|---|---|
| additive | 662.316 | 692.988 |
| proportional | 680.703 | 711.375 |
| a combination | 662.316 | 698.1 |

### 4.3.2 Covariate model

The forward selection protocol included ClCr and the coadministration of spironolactone in respect to $Cl_{VAN}$ and TBW associated to Vd. At first TBW was associated linearly, however, we removed the intercept and the OFV stayed the same, but BIC diminished since there were fewer parameters. The definition of the final model in NONMEM is enclosed in Appendix – figure 22. The equations *31* to *34* express the constructed final model.

$$CL_{VANi} = (\theta_{Cl} * (1 + \theta_{ClCr} * (ClCr - 120)) * (1 + \theta_{Co/Sp})) * e^{\eta i} \quad \text{Equation } 31$$

$$Vd_i = (\theta_{Vd} * TBW/70) * e^{\eta i} \quad \text{Equation } 32$$

$$CL_{VANi} = (4.07 * (1 + 0.00462 * (ClCr - 120)) * (1 + (-0.203))) * e^{\eta i} \quad \text{Equation } 33$$

$$Vd_i = (62.6 * TBW/70kg) * e^{\eta i} \quad \text{Equation } 34$$

Additional comment is required in respect to the coadministration of spironolactone. When Co/Sp was 0, the $\theta_{Co/Sp}$ was also 0. When the Co/Sp was present and had the value 1, $\theta_{Co/Sp}$ had the value of minus 0.203. The summary of the parameter values of base model and final covariate model are presented in table X.

**Table X. Base model and final model fit summary and parameter values – NONMEM.**

| | Base model | | Final model | |
|---|---|---|---|---|
| | Estimate (RSE %) | | Estimate (RSE %) | |
| OFV | 662.316 | | 577.786 | |
| BIC | 692.988 | | 618.682 | |
| $\theta_{Cl}$ [L/h] | 3.93 (6.11) | | 4.07 (4.45) | |
| $\theta_{Vd}$ [L] | 70.5 (17.6) | | 62.6 (0.197) | |
| $\theta_{ClCr}$ | / | | 0.00462 (10.8) | |
| $\theta_{Co/Sp}$ | / | | -0.203 (13.8) | |
| BSV | | Shrinkage (%) | | Shrinkage (%) |
| $\Omega_{Cl}$ [%] | 34.0 (13.5) | 7.66 | 23.5 (14.3) | 8.17 |

| | | | | |
|---|---|---|---|---|
| $\Omega_{Vd}$ [%] | 67.1 (25.0) | 26.0 | 49.9 (23.0) | 23.1 |
| Residual variability | | | | |
| θwa [mg/L] | 2.98 (14.1) | 14.6 | 2.38 (11.4) | 15.0 |

### 4.3.3 Validation and evaluation

Goodness of fit plots are similar to those obtained with Adapt. Graphs comparing observations and individual predictions of the base and final models are very similar. However, in the figure 12 we can observe that the points are slightly more justified to the reference line in the final model (the red line represents the trend line and the black line is the reference line).

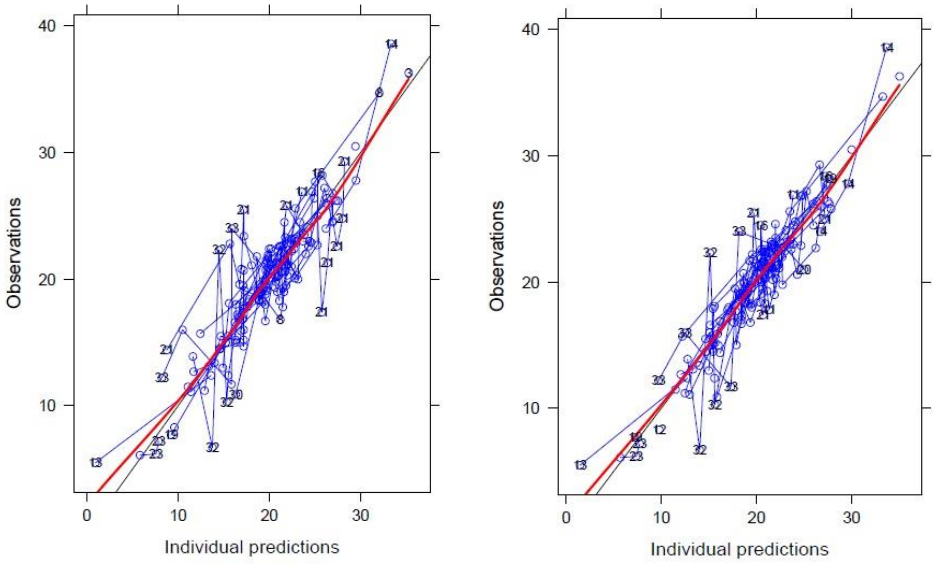


**Figure 12. Observations vs individual predictions, NONMEM; left - base model, right - final model.**

Population predictions meet with a greater difference. There is a significant improvement in the final model, as we can note that the points are closer to the reference line (figure 13). However, even the final model is not optimally predicting the concentrations since there is still deviation between the trend line and the reference line.

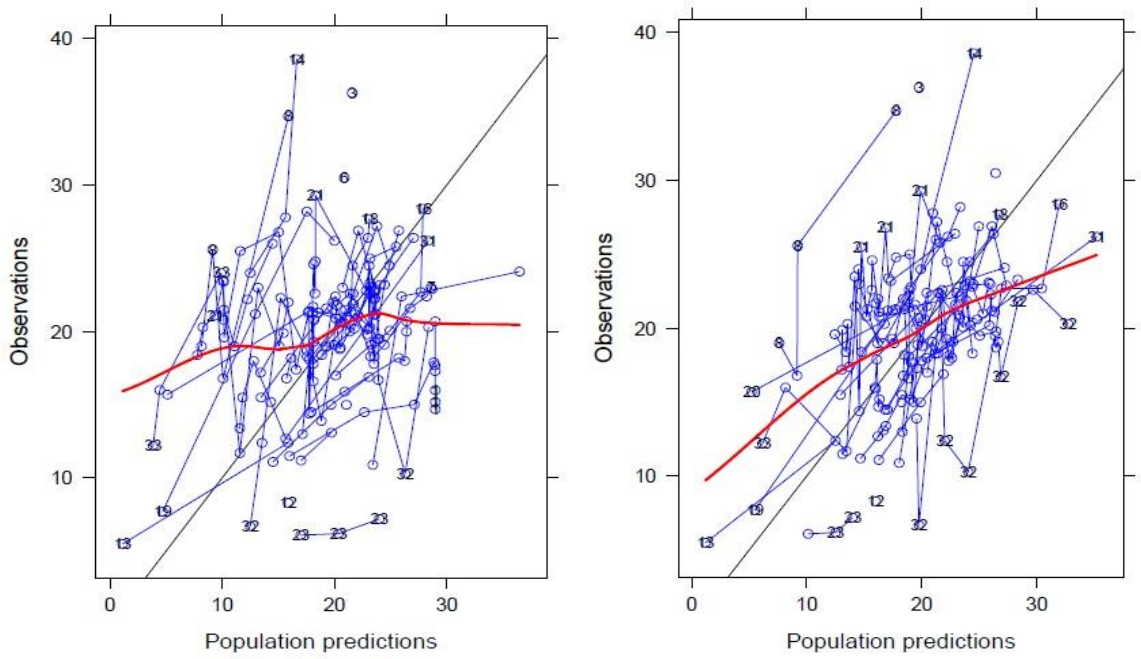


**Figure 13. Observations vs population predictions, NONMEM; left - base model, right - final model.**

A comparison of η and ClCr of the base and final models reveals that η is not (or is less) related to ClCr after the inclusion into the fixed effects, which was according to the expectations (figure 14).

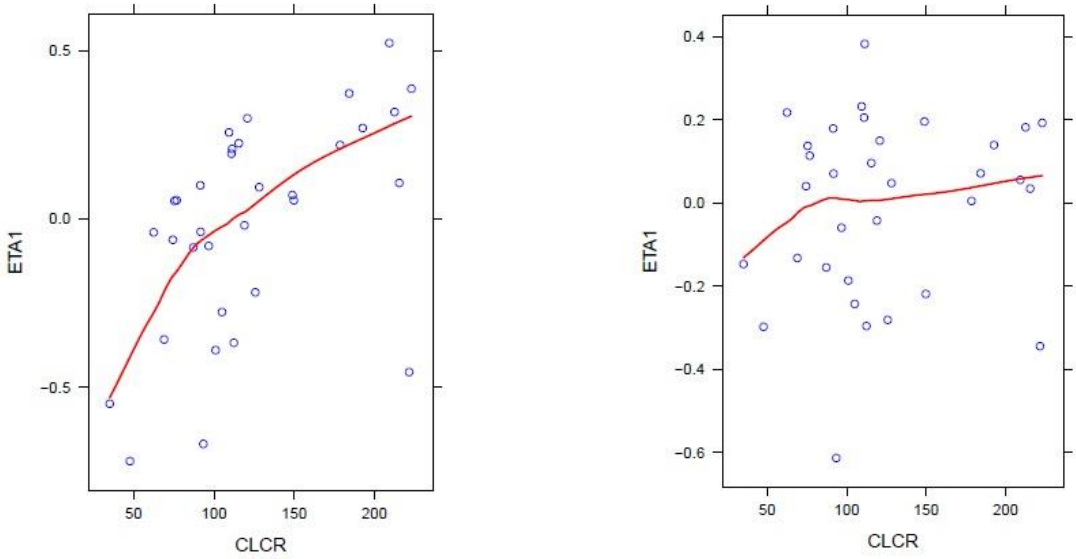


**Figure 14. Individual η in relation to ClCr; left - before inclusion to the fixed effects, right - after inclusion to the fixed effects.**

The distribution of parameter values is shown in the figure 15 and it is similar to Adapt. The distribution of $Cl_{VAN}$ in Adapt is moved slightly more to the right and has a normal distribution (figure 11), whereas here it seems to have a log-normal distribution. The

distribution of Vd is very similar – both methods display a few outliers towards the high values. Those outliers are also a possible reason for the fact that the BSV of Vd did not reduce to a greater extent. The individual random effects, η, seem to be distributed normally (figure 16).

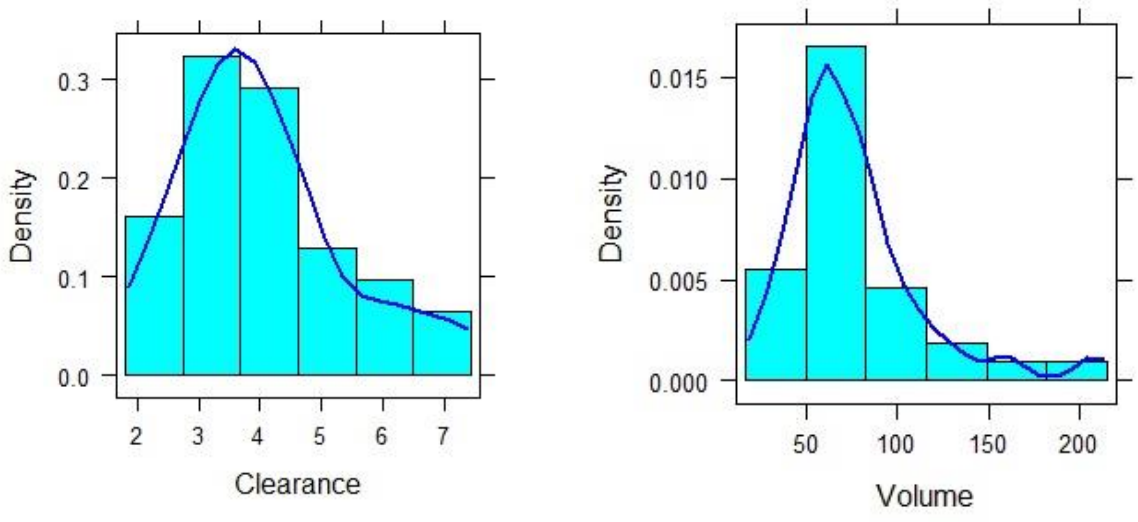


**Figure 15. Distribution of individual parameters; left - $Cl_{VAN}$, right - Vd.**

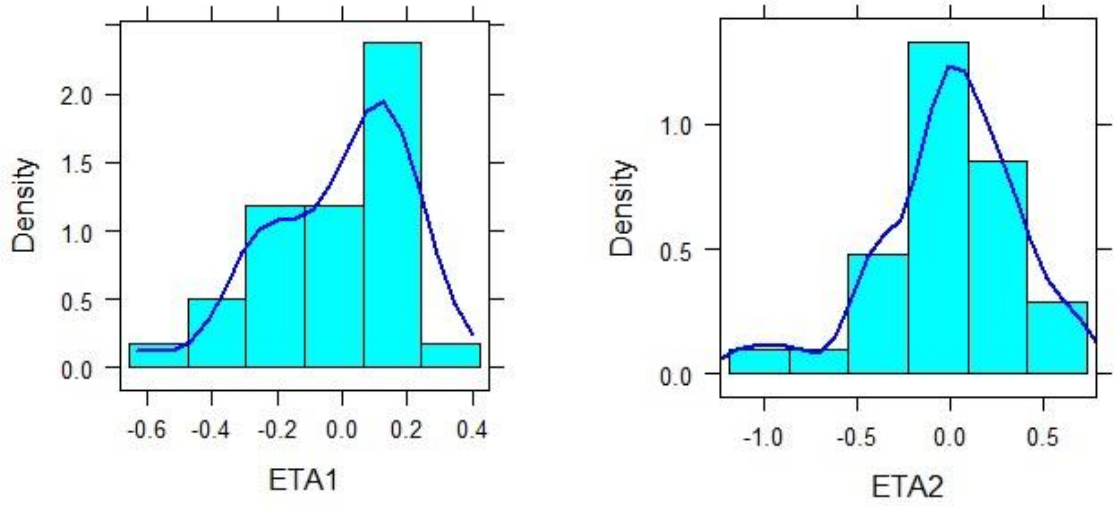


**Figure 16. Distribution of random effects; left - $Cl_{VAN}$, right - Vd.**

As was the case with modelling in Adapt, the coadministration of a diuretic (spironolactone in that case) also entered the final model and in a similar manner, in NONMEM – supposedly it lowers the clearance of vancomycin. However, no reports of spironolactone-vancomycin interactions were found in literature, therefore this relationship is highly questionable and

the suspicion about co-founding is present. The inspection of the distribution of parameters in relation to the administration of spironolactone was made and is presented in the figure 17. There is no detectable pattern, although two out of three outliers, in respect to Vd, have received spironolactone. The administration of diuretics in patients with a high volume of distribution is reasonable, however, spironolactone was associated to $Cl_{VAN.}$

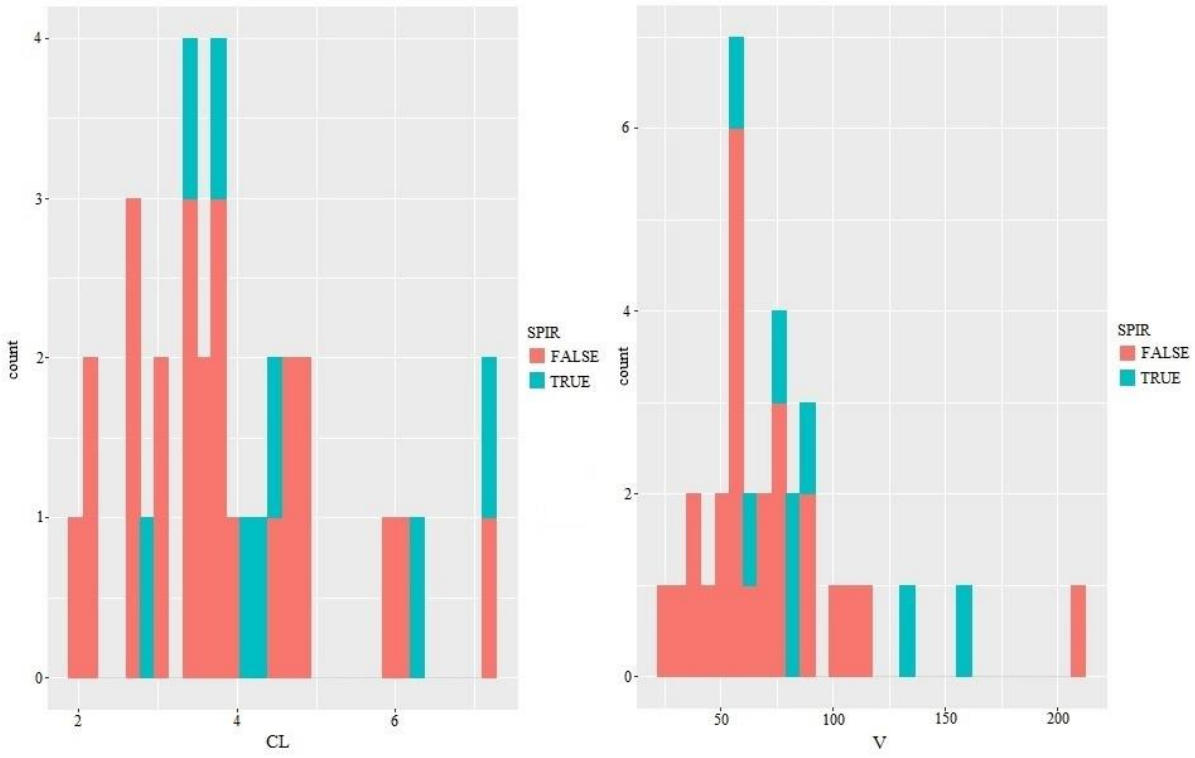


**Figure 17. Distribution of individual parameters in relation to coadministration of spironolactone; left - $Cl_{VAN}$, right - Vd.**

Since this relation was questionable, the assessment of an actual significance was performed with the randomization protocol, as described in an article by Wählby, et al (45).

#### *4.3.3.1 Assessment of actual significance of spironolactone as a covariate*

Covariate randomization in relation to PK is a protocol that tests the hypothesis which assumes that when a covariate and a parameter are not really related, it makes no difference which covariate value is assigned to a certain subject. In our case, the covariate is the coadministration of spironolactone and the parameter is $Cl_{VAN}$. Therefore, assigning any of the Co/Sp values to a certain individual would be equally likely. That is why the covariate values are first randomly assigned to individuals, and then a full model is again fitted to the data. This is repeated so that a large number of samples can be tested (e.g. n = 1000) in order to obtain the empirical reference distribution for the difference in OFV. Then, a comparison

with the observed difference in the OFV is done – a fraction of difference in the OFV that is greater in empirical distribution than in observed difference is assessed, and the actual *p* level is obtained (45). The Co/Sp has proved to be a significant covariate since the *p* value that corresponds to the observed ΔOFV is less than 0.01 (table XI).

**Table XI. Randomization results for assessment of actual significance – comparison of ΔOFV of model without Co/Sp and a model containing Co/Sp among covariates.**

| *p* value | Actual ΔOFV | ΔOFV for $\chi^2$, 1 degree of freedom | Observed ΔOFV |
|---|---|---|---|
| 0.001 | -17.43029758 | -10.828 | -16.61969575 |
| 0.01 | -12.78486918 | -6.6349 | |
| 0.05 | -8.001260227 | -3.8415 | |
| 0.1 | -6.451962102 | -2.7055 | |
| 0.15 | -4.993346749 | -2.0723 | |

#### *4.3.3.2 Time varying covariates*

Time-varying covariates in relation to the final model were also investigated. Critically ill patients are subjected to possible rapid variations in condition, and some parameters can significantly change during the treatment. Therefore, a time-varying covariate assessment can provide extra information about the population.

We studied the influence that the changes of ClCr during the VAN treatment have on $Cl_{VAN}$. This was assessed with the construction of a new, extended model (equation *35*). In the final model, (equation *31*) we assumed that $Cl_{VAN}$ changes according to ClCr in the same way across the whole population. However, in the extended model we separated the parameter $\theta_{ClCr}$ into two different parameters - $\theta_{BCOV}$ and $\theta_{DCOV}$. BCOV represents the baseline value of ClCr, the value in the beginning of the treatment (time = 0), and DCOV represents the difference from the baseline, the difference from the initial ClCr (when time $\neq$ 0). That way we were able to estimate the difference between and within individual effects. $\theta_{BCOV}$ describes the BSV, and $\theta_{DCOV}$ expresses the effect of covariate variation within the subject and is a fractional change in population clearance with individual changes in ClCr (46).

$$CL_{VANi} = (\theta_{Cl} * (1 + \theta_{BCOV} * (BCOV - 120) + \theta_{DCOV} * DCOV) * (1 + \theta_{Co/Sp})) * e^{\eta i} \quad \text{Equation } 35$$

However, in our case $\theta_{BCOV}$ and $\theta_{DCOV}$ turned out to be similar (0.00433 and 0.00468 respectively), which means that there is no indication of using the extended model for the detection of intraindividual variability. Final model was maintained in its basic form (equation *31*) and underwent a subsequent validation.

#### *4.3.3.3 Internal validation*

Bootstrap with replacement and VPC were performed as a part of an internal validation. In regard to bootstrap, 1000 samples were generated and fitted to the data. Since a larger number of samples was run, the reliability of results is greater than with the bootstrap performed in Adapt. The results of validation prove that the model is robust (table XII). None of the confidence intervals contain zero and the final model estimates are within the intervals.

**Table XII. Bootstrap with replacement results - NONMEM.**

| | **Bootstrapping** | | | **Final model** |
|---|---|---|---|---|
| **Parameter** | **Mean** | **Bias** | **Confidence intervals (2.5 percentile – 97.5 percentile)** | **Mean** |
| $\theta_{Cl}$ [L/h] | 4.08 | 0.01 | 3.70 – 4.46 | 4.07 |
| $\theta_{ClCr}$ | 0.00462 | 0 | 0.00297 – 0.00587 | 0.00462 |
| $\theta_{Co/Sp}$ | -0.19 | 0.0133 | -0.262 – -0.0676 | -0.203 |
| $\theta_{Vd}$ [L] | 62.3 | -0.3 | 46.0 – 79.9 | 62.6 |
| BSV | | | | |
| $\Omega_{Cl}$ [%] | 22.8 | -0.7 | 15.9 – 29.2 | 23.5 |
| $\Omega_{Vd}$ [%] | 49.4 | -0.5 | 24.7 – 70.7 | 49.9 |
| Residual variability | | | | |
| θwa [mg/L] | 2.34 | -0.0196 | 1.78 – 2.91 | 2.38 |

Also the VPC validation showed that the observed mean and confidence intervals were within the intervals obtained with VPC (figure 18).

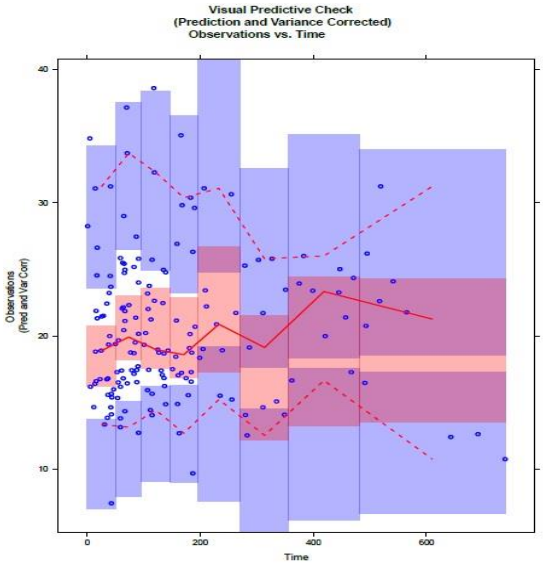


**Figure 18. Prediction and variability corrected visual predictive check**

### *4.3.3.4 External validation*

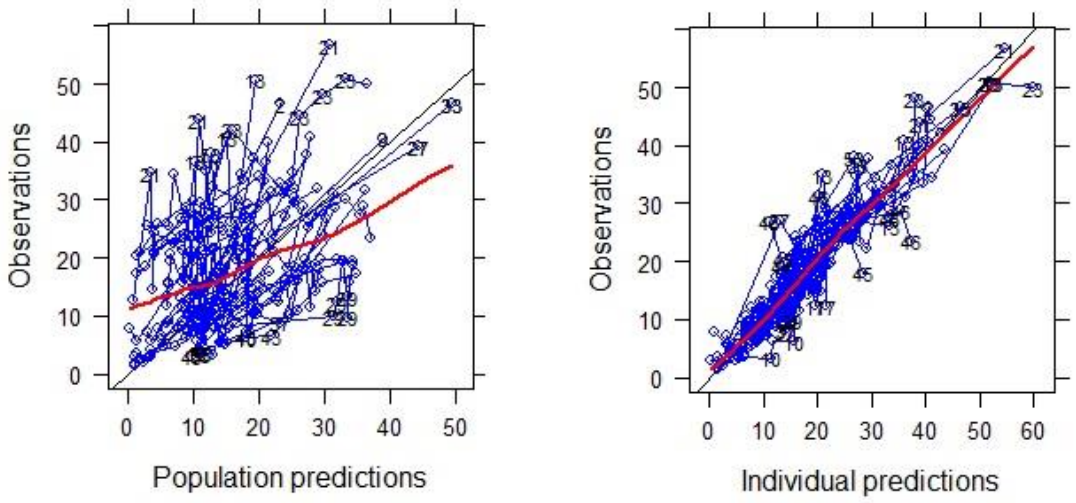


**Figure 19. Goodness of fit for external set of data; left - observations vs population predictions, right - observations vs individual predictions.**

The final model was tested on an external set of data. The data were fitted without estimation, and the goodness-of-fit graphs are shown above in the figure 19. The individual predictions are well predicted, however, the predictive performance of a population model in fair as well.

## 4.4 Predictive performance

To summarize the development process and the comparison of the modelling methods, the presentation of the overall predictive performance is provided. The accuracy and precision

of the constructed models were compared – individual prediction performance (BSV included) is shown in table XIII, and population prediction features (assuming all the individual are typical) are presented in table XIV. Mean values and confidence intervals are provided. A comparison of the estimated values of parameters is provided in table XV.

The final model constructed in NONMEM was the most precise one among all models in both the individual and population predicting concentrations, because it had the lowest MSE. However, the final model from Adapt was slightly less biased than the one from NONMEM since it had lower ME.

The worst model for fitting and predicting the data proved to be the final model from NONMEM, which fitted the external set of data, especially the population predictions. It was quite biased and also not very precise, which could mean the model is not good enough to be used in other populations. It is also true that all the constructed models overpredicted higher concentrations and underpredicted lower concentrations.

**Table XIII. Mean predictive error, mean squared predictive error and square root of mean squared predictive error - individual predictions.**

| Software | Model | Data | $ME_{ind}$ | $MSE_{ind}$ | $RMSE_{ind}$ |
|---|---|---|---|---|---|
| NONMEM | base | intern | 0.1297 ± 0.3867 | 6.518 ± 0.9856 | 2.553 |
| NONMEM | final | intern | 0.02357 ± 0.3077 | 4.092 ± 1.239 | 2.023 |
| NONMEM | final | extern | 0.3081 ± 0.4285 | 14.77 ± 3.409 | 3.843 |
| Adapt | base | intern | 0.2414 ± 0.3839 | 6.427 ± 1.926 | 2.535 |
| Adapt | final | intern | 0.2156 ± 0.3840 | 6.421 ± 1.955 | 2.534 |

**Table XIV: Mean predictive error, mean squared predictive error and square root of mean squared predictive error - population predictions.**

| Software | Model | Data | $ME_{pop}$ | $MSE_{pop}$ | $RMSE_{pop}$ |
|---|---|---|---|---|---|
| NONMEM | base | intern | 0.4001 ± 1.092 | 51.72 ± 11.46 | 7.192 |
| NONMEM | final | intern | 0.2553 ± 0.8532 | 31.52 ± 7.369 | 5.614 |
| NONMEM | final | extern | 2.759 ± 1.178 | 118.5 ± 19.18 | 10.89 |
| Adapt | base | intern | -0.05166 ± 1.108 | 53.03 ± 11.46 | 7.282 |
| Adapt | final | intern | 0.1401 ± 1.039 | 46.64 ± 9.655 | 6.829 |

The base models are very similar in mean errors as well as in parameter values. Both are one-compartment models with the additive type of error, and the typical values of parameters and variabilities are very similar (table XV). That indicates that the main differences in the model constructions and in the estimation came from building the covariate model.

However, the evaluation of both final models provides many similarities but also some curious differences. The estimates of $\theta_{Cl}$ and $\theta_{Vd}$ are comparable. Also the $\theta_{ClCr}$ is very similar when we interpret both relations in the same manner: in the Adapt model, $Cl_{VAN}$ increases/decreases for 0.0155 L/h for every ml/min of deviation in ClCr. In the NONMEM model, the $Cl_{VAN}$ increases/decreases for $(1+0.00462) * \theta_{Cl}$ for every ml/min of deviation in ClCr. If we convert this to the absolute value and then compare it to the relation expressed in Adapt, $Cl_{VAN}$ increases/decreases for 0.0188 L/h (for 1 ml/min of difference in ClCr), which is similar to the parameter value of the Adapt model. If we convert this to a percentage calculated in relation to the typical value, $Cl_{VAN}$ changes for 0.32 % or 0.46 % in Adapt and NONMEM models respectively for 1 ml/min of deviation in ClCr.

**Table XV. Summary and comparison of all model parameters, obtained from both methods.**

| | **Adapt 5 EM Estimate (RSE %)** | **NONMEM 7.3 FOCE-I Estimate (RSE %)** |
|---|---|---|
| **Base model** | | |
| OFV | 965.523 | 662.316 |
| BIC | 996.195 | 692.988 |
| $\theta_{Cl}$ [L/h] | 3.85 (9.72) | 3.93 (6.11) |
| $\theta_{Vd}$ [L] | 67.2 (28.0) | 70.5 (17.6) |
| BSV | | |
| $\Omega_{Cl}$ [%] | 33.4 (22.4) | 34 (13.5) |
| $\Omega_{Vd}$ [%] | 67.9 (58.9) | 67.1 (25.0) |
| Residual variability | | |
| SD/$\theta_{Wa}$ [mg/L] | 2.98 (8.53) | 2.98 (14.1) |
| **Final model** | | |
| OFV | 947.774 | 577.786 |
| BIC | 988.67 | 618.682 |
| $\theta_{Cl}$ [L/h] | 4.84 (15.1) | 4.07 (4.45) |
| $\theta_{Vd}$ [L] | 58.7 (22.6) | 62.58 (0.197) |
| $\theta_{ClCr}$ | 0.0155 (43.3) | 0.00462 (10.8) |
| $\theta_{Co/Sp}$ | / | -0.203 (13.8) |
| $\theta_{Co/Fu}$ [L/h] | -1.26 (52.9) | / |

| | | |
|---|---|---|
| BSV | | |
| $\Omega_{Cl}$ [%] | 28.2 (28.8) | 23.5 (14.3) |
| $\Omega_{Vd}$ [%] | 61.5 (34.3) | 49.9 (23.0) |
| Residual variability | | |
| SD/$\theta_{Wa}$ [mg/L] | 2.96 (6.66) | 2.38 (11.4) |

Regarding the coadministration of diuretics affecting the $Cl_{VAN}$, both methods detected the influence of coadministration but with different drugs. Furosemide was significant in Adapt and Spironolactone in NONMEM. Both drugs supposedly lower the $Cl_{VAN}$ and to a similar degree: in the Adapt model, furosemide lowered the ClVAN for 1.26 L/h and in NONMEM for 0.83 L/h, which is 26 % and 20 % respectively. However, this relation is questionable because no plausible mechanism for reducing the clearance was found. The influence of furosemide as a haemodynamically active drug is described in literature, but in the opposite way – furosemide should increase the $Cl_{VAN}$ since it promotes the blood flow, which could increase the GFR and the elimination of VAN. Spironolactone is also a diuretic but no correlation with PK of VAN was found in literature. Therefore, the most plausible reason lies in the small size of the population and data, and that those drugs do not decrease the $Cl_{VAN}$. Rather than that, the patients who received those two diuretics had a lower $Cl_{VAN}$ when they received them than when the coadministration was not present.

The inclusion of covariates in the final model fitted with the reference method, FOCE-I, explained more of the BSV, since the variability of $Cl_{VAN}$ and Vd lowered from 34 % to 23.5 % and from 67.1 % to 49.9 % respectively. The covariates in NONMEM therefore explained approximately 30 % and 25 % of the BSV of $Cl_{VAN}$ and Vd respectively, which is at least twice as much as in the modelling with the EM algorithm. Furthermore, the residual error in reference method also reduced after the inclusion of covariates, whereas it remained almost the same in Adapt. The relative standard errors were significantly lower when obtained with FOCE-I than those obtained with the EM algorithm.

In general, we hoped for a greater reduction in BSV, but apparently the data were not sufficient for describing the variability, and the population consisted of a small number of individuals.

# 5 Conclusion

The development of a POPPK model is a complex task and there is no »correct« way of doing it. An experienced pharmacist/pharmacometrist is required to plan and perform a POPPK study, and more importantly, to critically evaluate and assess the data and the results of the analysis since the interpretation is the most important aspect.

Adapt 5 with the EM algorithm proved to be a useful tool in POPPK modelling, since we were able to construct and validate a similar POPPK model as in NONMEM, which is a package that is a lot more researched and used. However, there is no doubt that modelling with NONMEM proved to be a lot less time-consuming and more straightforward than modelling with Adapt. On the other hand, Adapt, with its rigidity and manuality, is a perfect tool for learning and understanding the core of POPPK modelling from the beginning, since few functions are automated.

We can conclude that we successfully developed and validated two POPPK models using two independent methods. One of the models also behaved more or less well on another group of patients, however, its use on other populations is questionable because the fitting was not optimal. The parameter values obtained with both methods also correspond to the parameter estimates already described in literature.

Furthermore, the study shows and confirms that renal function plays a significant role in estimating the $Cl_{VAN,}$ and it confirms that TBW has an impact on Vd. Concomitant therapy with furosemide and spironolactone supposedly also has some influence. However, due to the data being sparse and a small number of patients, no conclusions regarding the co-therapy with those two drugs can be made, and further studies and clarifications are necessary. Still, more attention should be given to PK of VAN when concomitant therapy is used.

A possible next step in our study would now be a PD study and a simulation of a population of patients. That way one could assess a possible treatment regime and verify if the adjustment of treatment according to our model presents a useful tool in therapy with VAN, and if the concentrations achieve the required level.

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# 7 Appendix

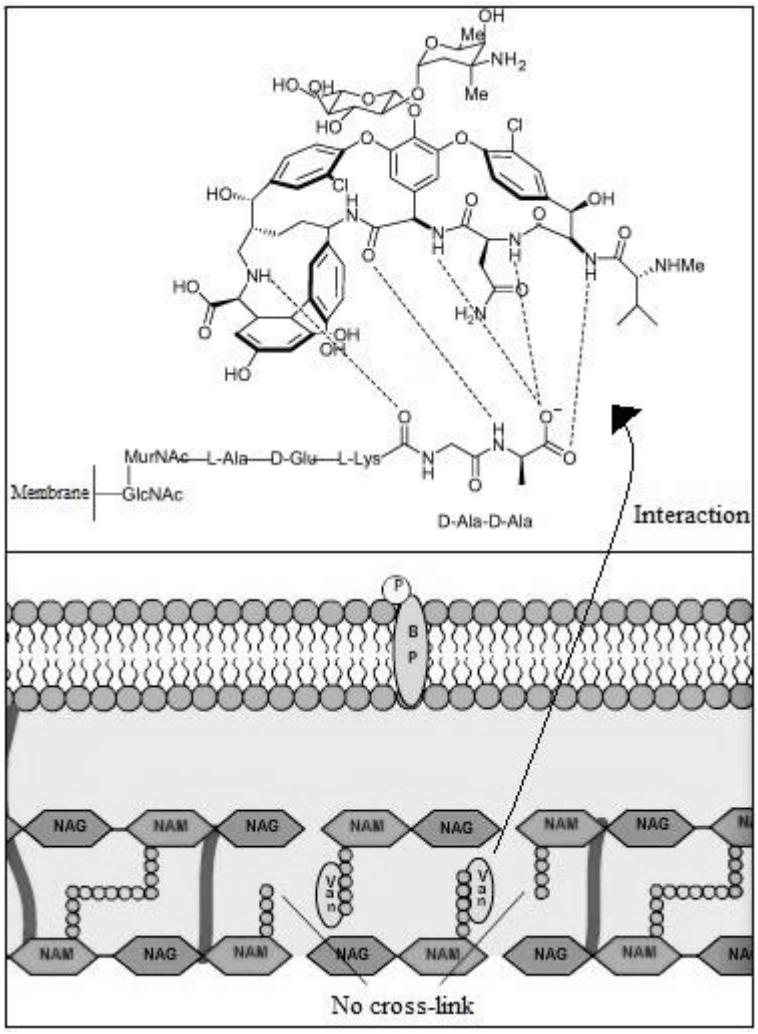


**Figure 20. Mechanism of action of vancomycin and interaction with D-ala-D-ala part of pentapeptide (10, 12).**

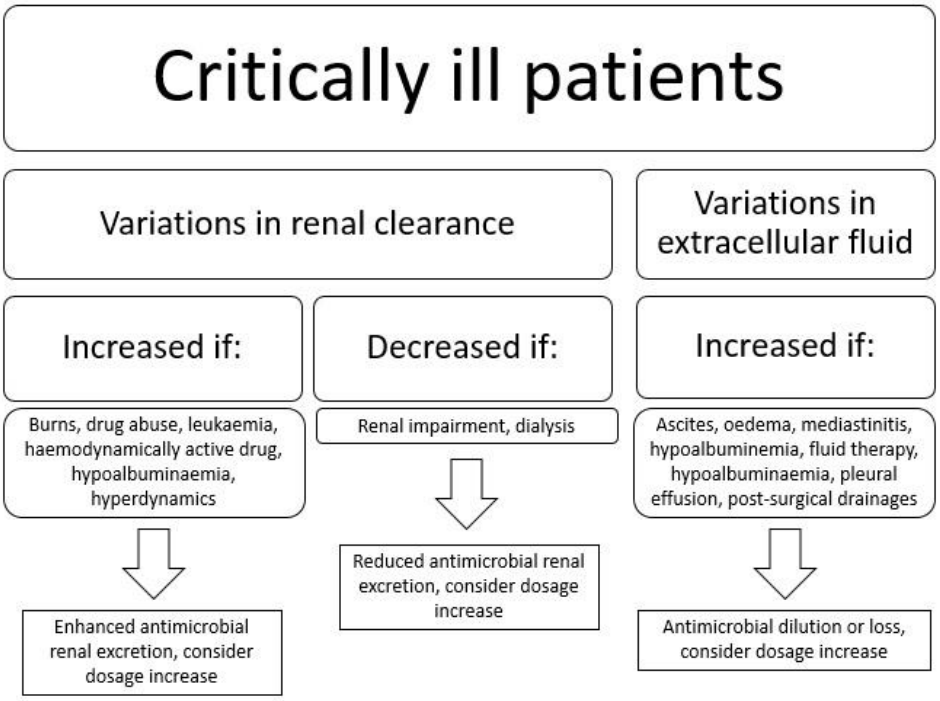


**Figure 21. Recommendations for dose alteration in relation to conditions occurring in critically ill patients (9).**

**Table XVI. Nomogram for adjusting VAN dosing (26).**

| Actual body weight [kg] | Loading dose [mg] | Maintenance Dose based on estimated GFR [ml/min/1.73 $m^2$] | | |
|---|---|---|---|---|
| | | 31-40 | 41-60 | >60 |
| | | every 24h | every 12h | every8h |
| 40-49 | 1000 | 750 | 750 | 750 |
| 50-59 | 1250 | 1000 | 1000 | 1000 |
| 60-69 | 1500 | 1000 | 1000 | 1500 |
| 70-79 | 1750 | 1250 | 1250 | 1500 |
| 80-89 | 2000 | 1500 | 1250 | 1500 |
| 90-99 | 2250 | 1500 | 1500 | 2000 |
| 100-109 | 2250 | 1750 | 2000 | 2000 |
| 110-119 | 2250 | 2000 | 2000 | 2000 |
| >120 | 2250 | 2000 | 2000 | 2000 |

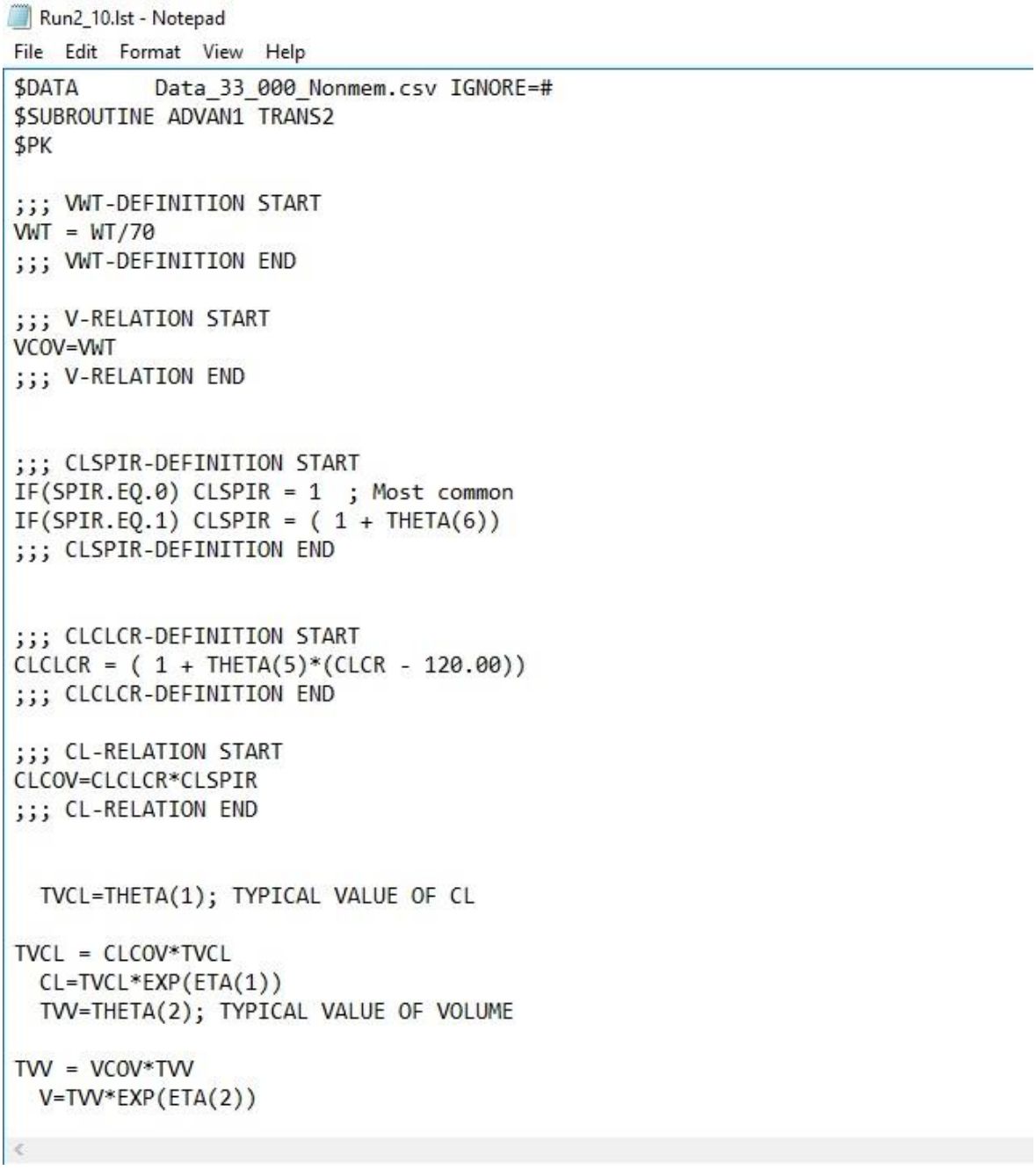


**Figure 22. Final model code in NONMEM language.**