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BIOESTATÍSTICA E BIOINFORMÁTICA APLICADAS À SAÚDE

Bayesian Pharmacokinetics/ Pharmacodynamics Modeling & Simulation

Verónica Mendonça

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Resumo

Para que um fármaco entre no mercado farmacêutico, é necessário realizar estudos que demonstrem a sua eficácia e segurança. Estes estudos permitem estudar interações e influência de determinados fatores fisiológicos (idade, dieta) e patológicos (ex.: problemas hepáticos) na absorção do fármaco no corpo humano. A farmacocinética (PK) tem como objetivo o estudo do processo cinético de absorção do fármaco – como também a biotransformação e eliminação, ou seja, estuda o que o corpo faz ao fármaco – usando parâmetros PK para medir a extensão do componente ativo, desde a fase de absorção até ao local de efeito. Por outro lado, a farmacodinâmica (PD) estuda o que o fármaco faz ao corpo (ex: após ocorrer a ligação entre o fármaco e o recetor), isto é, estuda o efeito produzido. A análise individual em contraste com análise populacional não tem em conta fatores que podem explicar alguma da variabilidade observada no fármaco. Daí que, a abordagem populacional é muitas vezes útil quando se deseja identificar e quantificar fatores que influenciam o comportamento ou que expliquem a variabilidade (variabilidade inter-sujeitos) numa determinada população de interesse. Pois, é possível estimar o efeito do fármaco desprovido de outros fatores que possam interferir e deste modo estimar a dosagem apropriada a sub-população específica, tais como crianças e idosos.

Para a análise PK/PD usou-se modelo de efeitos fixos e de efeitos mistos não-lineares com recurso à modelação Bayesiana. A modelação Bayesiana é particularmente atraente do ponto de vista biológico, uma vez que permite a incorporação de distribuições informativas prévias. Como também é preferível, do ponto de vista da estimação, pois consegue lidar com um grande número de parâmetros e; com a não-linearidade dos processos cinéticos. Na modelação Bayesiana recorreu-se aos métodos de *Markov chain-Monte Carlo*, algoritmos que permitem gerar amostras cuja distribuição se vai moldando e estabilizando, à medida que o número de simulações aumenta, em torno de uma distribuição, na qual se estima os parâmetros de interesse com um determinado grau de credibilidade.

O estágio na BlueClinical teve como objetivo caracterizar e perceber a análise individual e populacional da farmacocinética/farmacodinâmica para aceder à segurança e eficácia de um fármaco inovador num estudo ficcional de fase I e fase IIa de ensaios clínicos (conteúdo acedido em *Metrum Institute*). Assim foi possível explorar diferentes modelos, identificar e validar o modelo que melhor explica o estudo em causa. Neste caso, conclui-se que o modelo que melhor explica PK/PD é o modelo de dois compartimentos e para modelar os efeitos secundários foi considerado o modelo semi-mecânico de *Friberg and Karlsson*.

Palavras-chave: Análise Bayesiana, Farmacocinética/Farmacodinâmica, Bioestatística Computacional, Análise Individual e Análise Populacional.

Abstract

Clinical trials are conducted before a drug comes into the market to evaluate its safety and its efficacy. These studies evaluate the drug's interactions and the influence of various physiological factors such as age and diet and pathological factors such as diseases, for example, hepatic impairments. Pharmacokinetics (PK) aims to study the kinetic processes of a drug's absorption, as well as its distribution, biotransformation and elimination. PK parameters are commonly used to measure the rate and the extent of the drug's active ingredient or active moiety, from its absorption to when it becomes available at its target site. Pharmacodynamics (PD) studies what the drug does to the body when the drug-receptor interaction occurs.

In fact, the PK/PD analysis can be conducted at two different levels, the individual and the populational level. On one hand, in the individual PK/PD analysis, factors that can explain some of the variability observed in the study are not taken into consideration. On the other hand, the population PK/PD analysis takes this into consideration and is useful to identify and quantify factors that affect drug behaviour or to explain the variability in the target population. It also allows the estimation of its effect, regardless of other factors that can interfere, and thus, perceive the drug dosage that is appropriate to a specific sub-population like the elderly and children.

In this work, regarding PK/PD analysis, two different models were employed: a fixed effects model and a nonlinear mixed effects model by Bayesian modeling. The Bayesian model is particularly interesting because it allows to incorporate previous informative distributions. In addition, it also preferred from an estimation of point of view, due to the large number of parameters and because of the nonlinearity of the individuals specific kinetic process. We have also recurred to the use of Markov chain-Monte Carlo methods in Bayesian modeling. These algorithms allow for the generation of samples whose distribution stabilizes and shapes itself, as the number of simulations increases, around a distribution in which the parameters of interest are estimated with a certain degree of credibility.

The main objective of this internship at BlueClinical was to characterize and understand the Individual analysis compared to the Population analysis by PK/PD modeling, and to assess the efficacy and safety of a new drug from fictional phase I and phase II case studies (this data was retrieve from the Metrum Institute). With this report we applied different models and identified which model was capable of explaining this case better. The two-compartmental model described the PK/PD model better while the semi-mechanistic model proposed by Friberg and Karlsson was observed to best describe the side effects.

Keywords: Bayesian Analysis, Pharmacokinetics/Pharmacodynamics, Computational Biostatistical, Individual Analysis and Population Analysis.

Abbreviations and Acronyms

2ME2	2-Methoxyestradiol
ADME	Absorption, Distribution, Metabolism, Elimination
AUC	Area Under the Curve
C_{max}	Peak/ maximum plasma concentration
CrI	Credible Interval
CRO	Contract Research Organization
DIC	Deviance Information Criterion
EMA	European Medicines Agency
E_{max}	Maximum Effect
EC_{50}	Concentration at which 50% of maximum effect
FDA	Food and Drug Administration
Gut	Gastrointestinal tract
HIF	Hypoxia-Inducible Factors
IIV	Interindividual Variability
MCMC	Markov chain Monte Carlo
MIC	Minimum Inhibitory Concentration
NDA	New Drug Application
NLME	Non-Linear Mixed Effects
O_2	Oxygen
PD	Pharmacodynamics
PK	Pharmacokinetics
PK/PD	Pharmacokinetics/Pharmacodynamics
post-op	Post-operative
PR	Progesterone Receptor
PoC	Probe-of-concept
SD	Standard Deviation
t_{max}	Time of occurrence of the peak concentration
VEGF	vascular endothelial growth Facto
VTE	Venous Thromboembolism

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1. Introduction

Drug development is a complex and lengthy process that can take from 7 to 15 years for a single drug. This long development time can be extremely expensive, sometimes costing millions. This process is mostly divided into three sequential main parts: discovery and decision (based on proof-of-concept studies), preclinical studies and clinical studies.

BlueClinical is a private Portuguese Contract Research Organization (CRO), that provides services of scientific consultancy, and research and development studies in many healthcare areas, i.e. medicines, medical devices, and diagnostics. The company was founded in 2012 and has been growing ever since, in number of employees and in search of new services to provide a better and highly-customized client service. My internship at BlueClinical's offices, located in Matosinhos, was focused on Bayesian Pharmacokinetics/Pharmacodynamics (PK/PD) modeling and simulation. This could be implemented in the company, in order to provide a more personalized response to the sponsors of clinical studies.

Bayesian PK/PD modeling and simulation is conducted at either individual or populational levels. It provides an understanding of the dosage required for different individuals with different needs. This modelling has also been greatly described in literature under "dose finding" and is mainly used in specific polulations such as in pacients with paediatrics and renal/hepetic impairments without having the need to expose them directly to the studied drug (1).

Phase I bioequivalence/bioavailability clinical trials are developed at BlueClinical Phase I unit, at Hospital da Prelada (Porto, Portugal). These studies are performed to support marketing authorizations (in Europe and US) of generic formulations, i.e. to demonstrate bioequivalence between Test and Reference products (2). BlueClinical does not work only with Phase I studies but also with Phase II. These studies are human trials in order to trully measure if the drug is safe for human use but also to assess its positive effects. The drug is moved to Phase III when it is considered safe and if the sponsor of the study believes it has potential. This generally involves a larger population and more clostly trials. These trials main focus is to prove the health authorities that the drug provides a positive effect. If the studies conducted perform well,the drug is approved and is then allowed to be marketed but a follow-up process is still conducted to test its longterm effects. In fact, these follow-ups are known as Phase IV studies.

The statistician clearly plays an important role throughout the drug development processin the pharmaceutical industry. Some of these responsibilities are listed below::

- Participate in the development plan envolving a drug study.
- Study design and protocol development. Randomization schemes.
- Data cleaning and database construction format.
- Analysis plan and program development for analysis.
- Report preparation. Produce tables and figures.
- Integrate clinical study results, safety and efficacy reports.

- Communication and New Drug Application (NDA) defense to the regulatory authority review panel.
- Publication support and consultation with other company personnel.

In recent decades, a variety of computational mathematical models have been applied in the different phases of a drug's development. These are frequently used to help compreehend the pharmacokinetic and pharmacodynamic of a given drug either by intravenous (IV) or oral administration in both humans and animals. In general terms, PK is often described as "what the body does to the drug" and PD as "what the drug does to the body". The individual PK models are often performed in early phases of the pharmacological studies, in order to describe the relationship betweeen the drug plasma concentration and time elapsed since administration. This defines the individual profile. However, individual analysis ignores differences in exposure and inter-invidivual responses and, consequently, produces imprecise estimates of mean responses and does not provide estimates of between-subject variability. This downside explains the reasoning behind the increasing application of the polulation focused approach in drug development. Population approaches can explain sources of PK variability within a polulation because it incorporates more than just concentration-time data. In addition, this model can integrate covirates information such as age, sex, weight, race, renal/hepatica function and concomitant medications, through different techniques such as nonlinear mixed effects models and Bayesian hierarchical models

Bayesian statistics, unlike traditional statistics, does not struggle with complex non-linear models used in biological systems (complex systems) because it is based on probabilistics where there is no distinction in the treatments of the unobserved model variables (parameteres and hidden variables) and observations (3). Computational limitations are the major downside factor in applying Bayesian methods as these can be quite expensive to compute as they solve high-dimensional integration problems (3). In the PK/PD models based on Bayesian interpretation, the prior knowledge results of previous experiments (or persoanal beliefs) about the event in question. For example, in some cases, it is necessary to describe compartments in the physiology of PK/PD models, which correspond to different organs or tissues in the body. These compartments descriptions along with the rate of blood flow provide a quantitative mechanistic framework by which scaled drug-specific parameters obtained by *in vitro* and *in vivo* extrapolation techniques, can be used to predict concentration-time profiles of new drugs both in the plasma and in the tissues, following a IV or oral administration. These models, allied with a Bayesian approach combined with a Markov chain Monte Carlo (MCMC) method can be used to simulate different scenarios and estimate the corresponding drug's parameters.

1.1. Objectives

The main objective of this study was to perform and compare Individual and Population analysis from a PK/PD model. Data was obtained from an antithrombotic case study, hence, safety (i.e. drug-induced adverse reactions) and efficacy of the tested drugs were assessed.

Specific objectives are:

- Model the time-average biomarker (Factor Xa) as a function of plasma drug (ME-2) concentrations;
- Model the drug (ME-2) plasma concentration, in a specific occasion, for a specific subject, as a function of time, dose and body weight;
- Model the relationship between neutrophil counts and drug exposure.

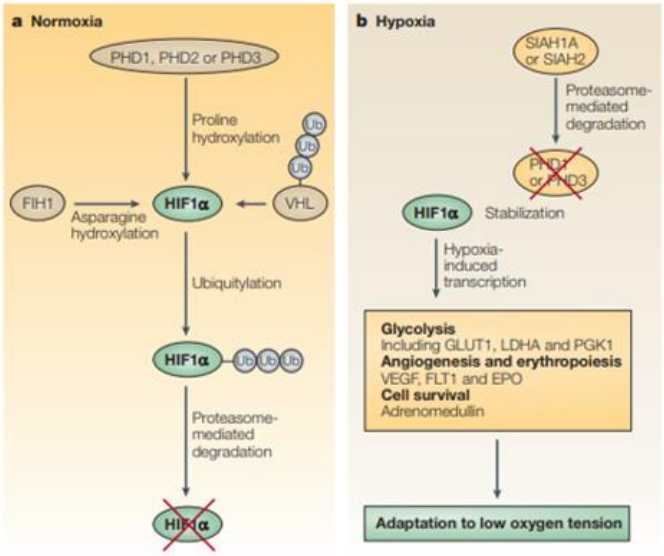
1.2. Report outline

The present report is divided into nine Chapters:

- The present chapter (Chapter 1) comprises a brief introduction, and the objectives.
- Chapter 2 contextualizes the epidemiology of vascular thrombosis, as well as existing therapies for the disease.
- Chapter 3 describes an overview of PK and PD concepts and specific endpoints;
- Chapter 4 describes PK/PD models.
- Chapter 5 reports Modeling and Simulation, where the Bayesian and MCMC approaches are involved.
- Chapter 6 refers to a specific phase I clinical trial study that explores the application of two models: a PK/PD model which relates a single exposure metric to a single continous PD outcome obtained from the simple non-linear regression and another model that represents a PK/PD model which relates observed drug concentrations to continous PD measurements obtained by the non-linear mixed-effects model.
- Chapter 7 reports the most complex model, the semi-mechanistic PK/PD model used to model the relationship between the side effect and the maximum effect. Present case relates neutrophil counts and drug exposure.
- Chapter 8 present the phase I & II Proof of Concept (PoC) to prevent post-operative (post-op) venous thromboembolism (VTE), and that explores population PK by two-compartmental model with first order absorption in a specific occasion for a specific subject as a function of time, dose and body weight.
- Chapter 9 concludes the developed work.

2. Vascular thrombosis and antithrombotic drugs

Vascular trombisis occurs when a clot is formed either inside a blood vessel or inside a cardiac chamber. (4,5). Thrombosis is often diagnosed as the first clinical manifestation of a tumour and the second leading cause of death of patients with cancer and third common cardiovascular disease after acute coronary syndrome and stroke (4,5). A significant correlation between the incidence of VTE events and the tumor aggressiveness and vice-versa has been demonstrated in literature (4,5). Also, the thrombotic events are related with atrial fibrillation patients in post-op (6). When the clot is formed and stuck into a vessel, it reduces the availability of nutrients and oxygen (O_2) to the cell around that vessel (6). O_2 serves as a primary regulator of this complex organ and thus, the cells try to adapt when in an O_2 deprivation environment (7).



EPO, erythropoietin; **FLT1,** fms-related tyrosine kinase 1 (a receptor for VEGF); **GLUT1**, glucose transporter 1; **LDHA**, lactate dehydrogenase A; **PGK1**, phosphoglycerate kinase 1; **VEGF**, vascular endothelial growth factor.

Figure 1: A schematic representation of the process of regulation of HIF1α levels by different condition of oxygen. **a)** Under normoxic conditions, HIF1α is degraded by the biding to ubiquitin. **b)** Under hypoxic conditions, HIF1α becomes on stability protein, and then can active some gens, which are involved in regulating glycolysis, angiogenesis, erythropoiesis and cell survival. [Sitkovsky (2005), Regulation of immune cells by local-tissue oxygen tension: HIF1α and adenosine receptors]

In an hypoxia situation, the lack of O_2 induces an adaptation response mediated by Hypoxia-Inducible Factors (HIFs). HIF1 is a heterodimeric transcription factor composed of a HIF1β subunit and a HIF1α subunit (oxygen-tension-regulated) (8,9) . HIF1α is the protein that is stabilized under hypoxic conditions and is degraded under normoxic conditions (8,9). Increased HIF1α protein stability and activity of the HIF1 complex, in turn, regulates the transcription of many hypoxia-responsive genes, including those that encode many glycolytic enzymes, inflammatory cytokines, and growth factors such as Vascular Endothelial Growth Factor (VEGF) (as shown in Figure 1) (8,9). Therefore, HIF1 α plays a significant

positive role in the supply of energy, in the inflammation process (migration of effectors of phagocytic cells including neutrophils and macrophages) and in the physiological process through which new blood vessels form from pre-existing vessels (angiogenesis) as well as in the thrombus natural resolution (9–11).

Thromboprophylaxis can reduce the prevalence of VTE significantly. Administration of chemical prophylaxis prevents or inhibits the clotting process by affecting one or more clotting factors in the blood (5,12). Depending on the inhibition target in the coagulation cascade, different anticoagulants are administred (5,12). This can be seen on Figure 2 (5,12).

Heparin is commonly used, by IV injection, in cardiopulmonary bypasses, catheterization, micro-vascular surgery (5,12) as well as in anti-inflammatory, anti-allergic and anti-cancer cases, among others (5,12). However, vitamin K antagonists, such as warfarin, are currently the most used oral anticoagulants despite having a narrow therapeutic window, variable PK and PD and extensive food and drug interactions, which requires regular coagulation monitoring and individual dose adjustments (13). Therefore, to overcome these issues other oral anticoagulants with sufficiently predictable PK/PD have been developed, such that they would not require regular monitoring or dosage adjustment during routine clinical use (13). Examples of those medicines are the oral agents targeted to inhibit thrombin or Factor Xa such as Edoxaban, Rivaroxaban and Apixaban (5).

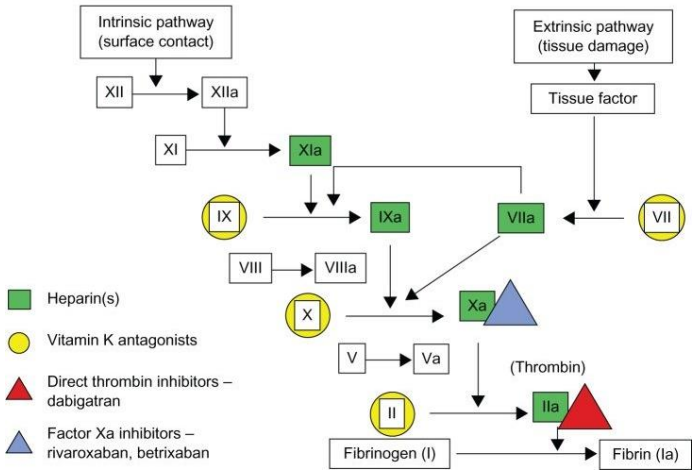


Figure 2: Summary of drugs interactions with blood factors of cascade of coagulation. [Ellis CR. Et all (2013), The clinical efficacy of dabigatran etexilate for preventing stroke in atrial fibrillation patients]

One oral agent that has been extensively tested in clinical trials is the 2-Methoxyestradiol (2ME2) (14,15). 2ME2 is an endogenous metabolite of estradiol existing at low levels in human plasma which has antiproliferative effects on endothelial cells and antiangiogenic activities (14,15). Phase I studies using pharmacological concentrations with a 2ME2 formulation showed that it was well tolerated with evidence of anti-cancer activity in patients with lower breast cancer procoagulant risk (14,15). That risk is reduced because the breast cancer cell lines express the progesterone receptor (PR) that mediates the induction of tissue factor, the cellular activator of the coagulation cascade. 2ME2 is shown to abrogate the induction of TF expression by limiting the transactivation potential of ligand-bound PR (16). Furthermore, 2ME2

inhibits angiogenesis, impeding the changes of the vascular network changes and subsequently the delivery of O_2 and nutrients to cells around that vessel leading to their death (7,16). Although the mechanism is still unclear, in 2017 an *in vitro* study showed that 2ME2 inhibits the nuclear accumulation expression of HIF1α causing downregulation of the transcription of important genes that code proteins involved in the angiogenesis and inflammatory cytokines. These cytokines regulate the response and migration of macrophages and neutrophils in normal response on situation of hypoxia (9,11). The authors also observed grade 4 reversible neutropenia on phase I due to higher doses of an 2ME2 analog (14).

However, on 2015 all in vivo clinical development of 2ME2 was suspended or discontinued, dude to its low bioavailability and extensive metabolism (17). Even tough, Selleck Chemicals (sellers of inhibitors) refers that solubility *in vivo* can be increased if solvents such as dimethyl sulfoxide and corn oil are added to the product (18).

The 2ME2 may have different chemical names, such as, 2-MEOE2, 2-ME2, Panzem, and others. Considering these different nomenclatures and based on the antithrombotic characteristics /advantages, we suppose that the fictional case available on Metrum Institute named of ME-2 drug could be the same of 2ME2 drug (6,19). This fictional case study ME-2 is a new oral drug developed that acts directly on Factor Xa inhibitor with indications on post-op prevention of VTE and prevention of thrombotic events. This report will describe two phases of clinical trials whose data was retrieved from Metrum Institute (license agreement in supplementary materials S1). Phase I takes into account safety, PK and biomarker PD. Inhibition of Factor Xa activity is the primary biomarker to be used for both efficacy and bleeding liability (6). For this data-driven report, we focused on the inhibition of Factor Xa activity and its relationship to PK by analysing Phase I data (6). For that, we modelled the PK and PD relationship in a couple of different ways to illustrate how simple nonlinear regression and nonlinear mixed effects regression describes PK/PD (Chapter 6) (6). This clinical development plan includes a Phase IIa multiple dose proof-of-concept (PoC) trial comparing one dose level of ME-2 to an active comparator (Enoxaparin) in patients undergoing total knee replacement (6). VTE's, bleeding events and PK are measured. In this trial some subjects were receiving higher ME-2 doses in phase I. Neutropenia was observed as an adverse event according to what was found in the literature (6). For this reason, data was analysed to assess whether there is sufficient probability of identifying a dose that is effective while still causing an acceptably small degree of neutropenia (6). To study this, the relationship between neutrophil counts and drug exposure was modelled (Chapter 7). It adopts the semi-mechanistic model proposed by Friberg and Karlsson , including the use of informative prior distributions for drug-independent parameters (6). Then, the combined Phase I and Phase IIa PK data using a population PK mode was analysed (Chapter 8) (6).

3. Pharmacokinetics and Pharmacodynamics

Drugs are substances intended to be used in the diagnosis, cure, treatment and prevention of diseases. They target a structure or a function of the body as a therapeutic effect (20).

Clinical safety and efficacy studies are usualy not performed on generic drug products (20). Since the formulation and manufacture of a drug product can impact the bioavailability and stability of the drug, the generic drug manufacturer has to demonstrate that the generic drug product is pharmaceutically equivalent (see PK Chapter 3.1), bioequivalent, and therapeutically equivalent (see PD Chapter 3.2) to the the equivalent listed referenc drug product (20). Drug product performance comparison for oral generic drug products is usually measured by *in vivo* bioequivalence studies in normal healthy adult subjects under fasted and fed conditions (20).

3.1. Basic Pharmacokinetic Concepts

Pharmacokinetics (PK) is the science of the Kinetics of drug Absorption, Distribution, Metabolism (biotransformation) and Elimination (ADME) (20). Figure 3, represents the dynamic relationship between the drug, the drug product, and the pharmacologic effect.

Absorption is the movement of a drug from its site of administration into the central compartment and the extent to which this occurs. The fraction of a dose of drug that reaches the bloodstreem is called bioavailability.

Several routes exist for the administration of drugs, such as oral, subcutaneous, IV, transdermals and others(20). In IV the entire dose is introduced directly into the bloodstream and has direct access to the systemic circulation (20), which means it has complete bioavailability. Nevertheless, oral dosing is the most most convenient and preferred route (20). However, the drug must cross the gastrointestinal tract (Gut) hence, the absorbed drug then passes through the liver, where metabolism and biliary excretion may occur before the drug enters the systemic circulation (first pass effect) (20). All these processes will, as consequence, decrease the plasma concentration of the drug (20). It is important to note that drug's plasma concentration (PK profile) influences its concentration on its target site and allows the identification of its therapeutic range (PD) (20).

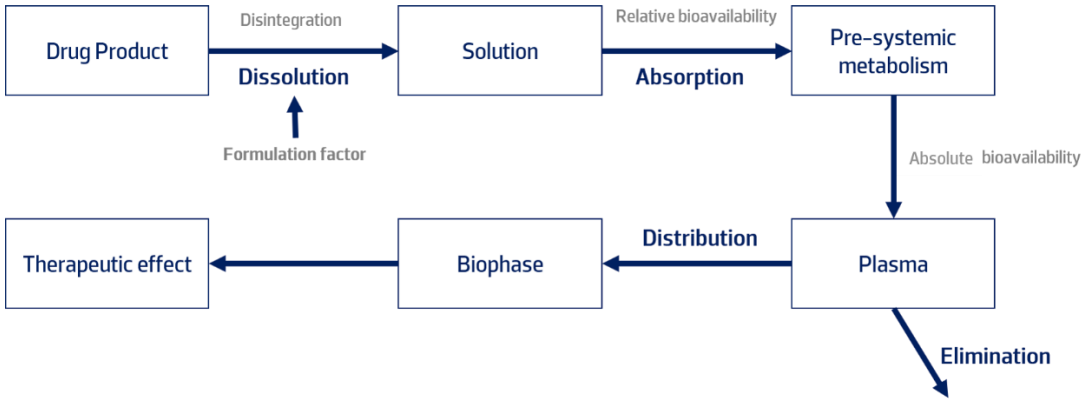


Figure 3: Schematic representation of dynamic relationship between the drug and the pharmacologic effect.

The distribution phase represents how the drug spreads through the body to the site of action(1,20,21). The Elimination phase also includes the metabolism (1,20,21). Metabolism describes a process where the initial compounds are transformed into anothers, called metabolites, which can be inactive or active (1,20,21). Drug Excretion is the removal of the intact drug. Polar drugs are excreted mainly by renal excretion. Other pathways envolve the excretion of drug throug the bile, sweat, saliva, milk and other body fluids (20).

During the development process, large numbers of patients are enrolled in clinical trials to determine efficacy and optimum dosing regimens as well as safety, among other information (20). The recommended dosage regimens in the approved labelling produce the desired pharmacologic response in the majority of the anticipated patient population (20). However, intra- and inter-individual variations will frequently result in either a subtherapeutic or a toxic response (20). For this reason, it is necessary to perform adjustments to the dose regimen (20). The discipline involved in this process of individually optimize dosing strategies based on the patient's disease state and patient-specific considerations is called Clinical pharmacokinetics (20).

Clinical PK is used to characterize the rate and extent (amount) of drug absorption to evaluate the safe and effective therapeutic management of drugs in an individual patient (20). For example, in cases where a single dose of a drug is administered orally to a patient, the therapeutic range can be obtained by the plasma concentration-time curve constructed by serial blood sample withdraw (Figure 4) (20). As seen in Figure 4, numerous parameters can be deduced from the plasma concentration-time curve (Table 1) (20,21). The concentration-time profile also gives information on other PK parameters, such as the distribution and elimination of the drug (20,21).

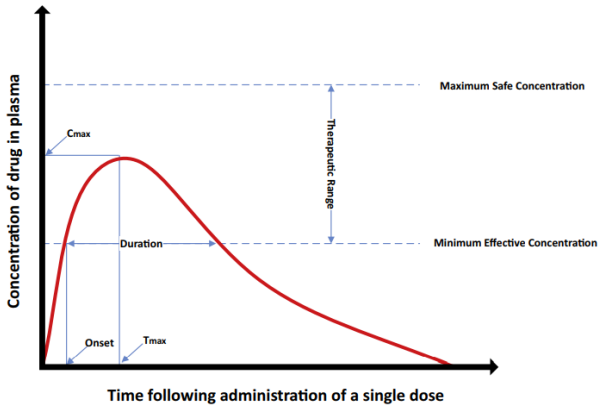


Figure 4: Typical schematic illustration of blood plasma concentration–time curve fowling administration of oral dose. Plasma level–time curve showing peak time (t_{max}) and plasma concentration (C_{max}) in relation to subject. [Mittal, B. et all (2017), Pharmacokinetics and Performulation. How to develop Robust Solid Oral Dosage Forms]

The theoretical aspect of PK, well as the experimental aspect, involves the development of PK models that predict drug disposition after drug administration (20,21). Several models exist to characterize a drug's PK, such as, compartmental models (Chapter 4.1), non-compartmental models and physiologicaly based PK models (20,21). The application of statistics is an integral part of PK studies to design and predict optimal dosing regimens for individuals or groups of patients (see Chapter 4) (20,21).

Table 1: Parameters deduced from plasma concentration-time Curve. [Mittal, B.(2017), Pharmacokinetics and Performulation. How to develop Robust Solid Oral Dosage Forms]

Parameter	Definition
C_{max}	Peak/ maximum plasma concentration.
T_{max}	Time of occurrence of the peak concentration.
MEC	Minimum effective concentration is the minimum plasma concentration needed to provide pharmacological effect is achieved.
MTC	Minimum Toxic Concentration is the concentration necessary to produce a toxic effect.
Therapeutic range	Range (between the MEC and the MTC) of plasma drug concentration between with the desired response is obtained avoiding toxic effects.
Onset	Time to achieve the MEC of drug.
Duration	Period during which the plasma concentration of drug exceeds the minimum effective concentration.
AUC	Area under the plasma concentration-time curve is the total amount of drug absorbed into the systemic circulation following the administration of a single dose (changes in AUC can modify the kinetics of distribution, metabolism, and excretion).

A drug's effect is often related to its concentration at the site of action, hence, monitoring this concentration could be useful (20,21). Nevertheless, the measurement of the concentration at the site of action is most of times impossible in a living organism, considering it represents a tissue or receptor site. Hence, measuring the concentration in body fluid (e.g. blood, urine) is the most convenient approach (20,21). Kinetic homogeneity is important to the foundation on which all therapeutic and toxic plasma drug concentrations are established, since it describes the predictable relationship between plasma drug concentration and concentration at the receptor site where a given drug produces its therapeutic effect (20,21). Changes in the plasma drug concentration reflect changes in drug concentrations at the receptor site, as well as, in PD (see Chapter 3.2) (20,21).

3.2. Basic Pharmacodynamic Concepts

Pharmacodynamics (PD) is the study of the biochemical and physiological effects of drugs on the body; it refers to the relationship between drug concentration at the site of action, as well the time course and intensity of therapeutic and adverse effects (20).

A PD endpoint is defined as an indicator of the pharmacological response for a therapeutic intervention, which can be quantitatively measured and evaluated (20). For example, for a long time, the Minimum Inhibitory Concentration (MIC) was the major PD endpoint for antibacterial drugs. Thus, the dosage was selected according to the plasma concentration of the drug that exceeded the MIC for as long as possible (Figure 5) (22).

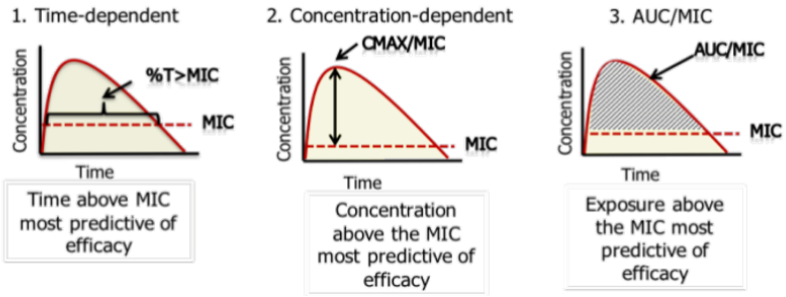


Figure 5 : Pharmacodynamic classification of antimicrobial agents. [Patricia J. Simner et all (2018), Understanding Pharmacokinetics and Pharmacodynamics].

Nevertheless, PD is intrinsically linked to PK. A relationship between the concentration of drug at the receptor site and the pharmacologic effect exists (20). If enough PK concentrations are tested, a maximum effect (E_{max}) can be determined (Figure 6).

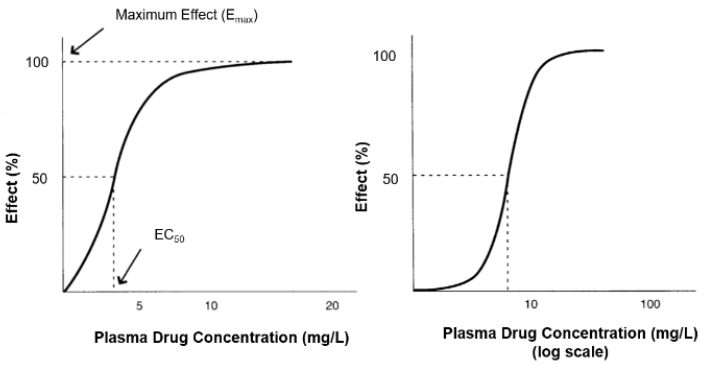


Figure 6: Relationship of drug concentration at the receptor to effect with different scales (as a percentage of maximal effect) [Lesson 1: Introduction to Pharmacokinetics and Pharmacodynamics [Internet]. Available from: https://www.ashp.org/-/media/store files/p2418-sample-chapter-1.pdf]

A way of evaluating a drug's potency is by comparing the concentration at which 50% of the maximum effect is achieved (EC_{50}) (Figure 7) (20).

When two drugs are tested in the same individual, the drug with a lower EC_{50} is considered the most potent (20,22). Meaning that the lower the dose of a drug, the more potent that drug is needed in achieving the same effect of the less potent drug (Figure 7) (20,22).

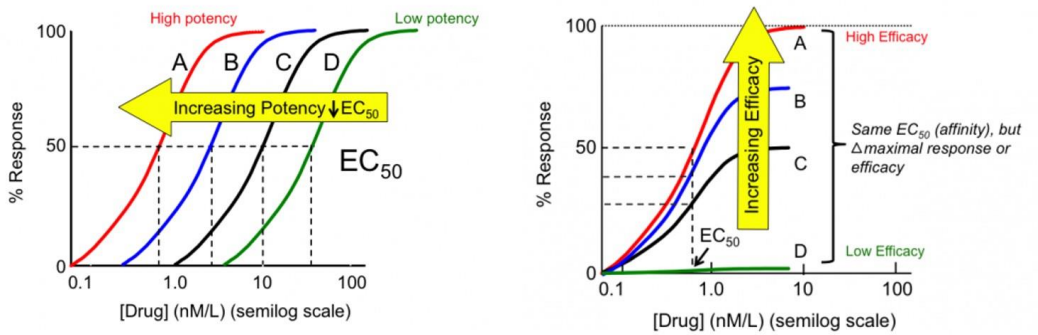


Figure 7: Schematic illustration of the dose-response curves of a differents of agonists (A, B, C, D). a) Illustrate the drug potency, where less value of EC_{50} represents the best of drug potency. b) Illustrate the drug efficacy, on when comparing differents drugs with same EC_{50}, the high efficacy is given to drug which the EC_{50} represents the 50% of response. [Basic Principles of Pharmacology [Internet]. Available from: http://tmedweb.tulane.edu/pharmwiki/doku.php/basic_principles_of_pharm]

Much like the MIC, EC_{50} can determine the duration of effect. This duration is determined by a complex set of factors, including the time that a drug is engaged on the receptor as well as intracellular signalling and gene regulation (20).

To assess the effect that a drug regimen is likely to have, the clinician should consider PK and PD factors (20). Both factors are important in determining a drug's effect (Chapter 4) (20). The effectiveness of some drugs can decrease with continuous use, which clinicians refer as tolerance (20). Tolerance can be caused

by PK or PD, and that affect the concentration/metabolism or the effectiveness (decrease of drug concentration at the receptor site results in a reduced effect with repeated exposure), respectively (20,23).

4. PK/PD Modeling

The Pharmacometrics is the science of developing and applying mathematical and statistical methods to characterize and predict a drug's PK, PD and biomarker-outcomes behavior (24,25). The PK/PD model is able to provide a description of the concentration–effect relationship and an estimate of pharmacological potency of the drug (24).

This methodology allows us to describe time dependence, discriminate inter-subject variability, and perform risk assessments at an individual level (24).

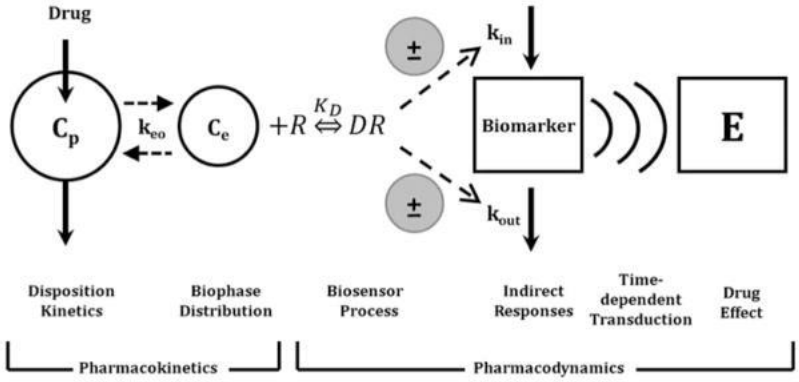


Figure 8: A schematic representation of time course of drug since entry in the systemic circulation until to provide the effect (pharmacodynamic models). C_p: Disposition Kinetics; C_e: Biophase Distribution; k_{eo}: Drug's flow; R: Receptor target (some time describes the receptor occupancy models); DR: Biding receptor-drug represent the Biosensor Process; The indirect responses can be the type of stimulate or to inhibit the production (k_{in}) or loss (k_{out}) of endogenous mediators; Time-dependent transduction represents the time lag between the plasma concentration and the effect observed; E: Drug effect. [Jusko WJ. Et all (1995), Convergence of direct and indirect pharmacodynamic response models. J Pharmacokinet Biopharm]

Good clinical trial designs and mathematical models (Figure 8) provide good precision and reproducible collected data (24,26). This data should include appropriate information regarding drug's concentration profile and its biomarker's outcome (24,26). Knowledge of a drug's behaviour combined with different structural models (as the ones described in Table 2), allows the estimation of unknown parameter values using nonlinear regression techniques (24,26).

Table 2: Principal mechanism of behaviour of drug and action associated PK/PD models

Models	Principal mechanism
Compartmental Models	Describes the drugs behaviour in the body. Useful to predict the time course of drug concentration in body.
Simple Direct Effect Models	Describes the effect-log concentration relationship. Useful to predict the time course of in vivo effects from plasma to drug measurements.
Biophase Distribution Models	Describes drug permeation to receptors in such site. Useful for delayed drug effects.
Indirect Response Models	Describes how drugs might induce their effects without a direct interaction drug-receptor. Useful to capture additional complexities related to specific drugs and biological systems.
Signal Transduction Models	Describes the time-dependent signal transduction to the final drug response. Useful for delayed drug effects dependent of secondary messengers.
Tolerance Models	Describes the expected pharmacological response after repeated or continuous drug exposure. Useful to capturing the functional adaption process.
More Complex Models	Describe more components of drug action, as was depicted in Figure 8, more than one model.

4.1. Compartmental Models

Compartmental systems are widely used to model PK and PD. Body is divided by one/several compartments (as needed), in order to describe the drug's behaviour in the body. Compartmental models are useful in predicting the time course of a drug's concentration in the body (Figure 9) (20,27).

As mentioned above, the body might be described by one-compartment (see Chapter 4.1.1) or a multiple-compartment (see Chapter 4.1.2) models (20,27). Each compartment represents a group of similar tissues or fluids, taking in consideration the drug's distribution (20,27). On multiple-compartment models, there is a central compartment, representing the highly blood perfused tissues, such as blood(plasma), heart, lungs, liver and kidneys. The central compartment communicates with less perfused compartments (peripheral compartments), such as fat tissue, muscle, tissue, and cerebrospinal fluid (20,27).

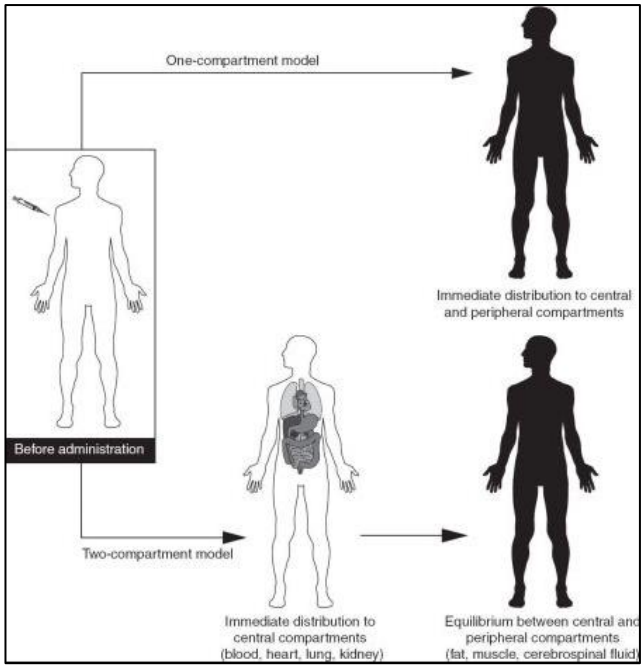


Figure 9: Schematics representation of compartmental models by IV bolus administration (administering a dose of drug over a very short time period) [Available from: https://i1.wp.com/obgynkey.com/wp-content/uploads/2017/06/image02837.jpeg?resize=451%2C466].

In an open model, the drug elimination is not represented by an excretory mechanism (20,27). Most PK models assume that elimination does not change over time, so the model is determined by how well it predicts drug concentration in fluids and tissues (20,27). The following parameters are commonly applied to one and two-compartment open models:

- The clearance of elimination (CL);
- The volume of distribution in the central compartment (V_1,);
- The absorption rate constant for oral administration (k_a);
- The elimination rate constant (k_e).

The models used in non-linear PK are largely based on Michaelis–Menten kinetics. For that reason, for those models, there are some changes on the equation below, replacing the k_e to k_m (the Michaelis–Menten elimination), and the V_1 is replaced by V_m (the maximum elimination rate).

4.1.1. One-compartmental models

The one-compartment model should be applied first, with the more complex models being applied afterwards (24,26). A visual representation is helpful to understand how the structure of the model works. In the Figure 10, the V_1 rectangle represents the one-compartment and the arrows indicate the drug's flow (24,26).

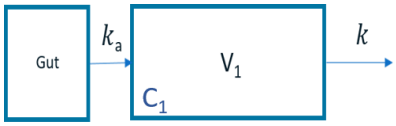


Figure 10: One-compartmental model with first order absorption from the gastrointestinal tract (Gut) compartment, parameterized in constant: the volume of distribution in the compartment (V_1), the absorption rate constant for oral administration (ka) and the elimination rate constant (k).

Following a dose administration, the drug distributes instantaneously to the entire body (body tissues and fluids) (24,26). This means that, for oral dosing, the amount of drug present on the Gut was absorbed into systemic circulation as is assumed that the drug is immediately distributed all body (24,26). A one-compartment model with a first-order kinetics, the remaining amount of the drug at time (t) after dose (D) can be calculated as (28):

Single dose (28):

$$C_1(t) = \frac{D}{V_1}\frac{k_a}{k_a - k}\left(e^{-k(t-t_D)} - e^{-k_a(t-t_D)}\right)$$

Multiple dose (28):

$$C_1(t) = \sum_{i=1}^{n} \frac{D_i}{V_1}\frac{k_a}{k_a - k}\left(e^{-k(t-t_{D_i})} - e^{-k_a(t-t_{D_i})}\right)$$

Steady state (28):

$$C_1(t) = \frac{D}{V_1}\frac{k_a}{k_a - k}\left(\frac{e^{-k(t-t_D)}}{1-e^{-k\tau}} - \frac{e^{-k_a(t-t_D)}}{1-e^{-k_a\tau}}\right)$$

where n is the number of doses and τ is the interval between two doses (28).

The model above represents the elimination rate constant (k), but when there is evidences or interest to study clearance (CL) parameter it is possible to replace the k above to (28):

$$k = \frac{CL}{V_1}$$

However, many drugs administrated by rapid IV injection (also known as IV *bolus*) demonstrate a plasma level time curve that does not decline as single exponential process (Figure 11) (24,26,29). That means, because some drugs do not distribute instantaneously to all parts of the body it is necessary to add more compartments (see Chapter 4.1.2 Multicompartmental models) (28).

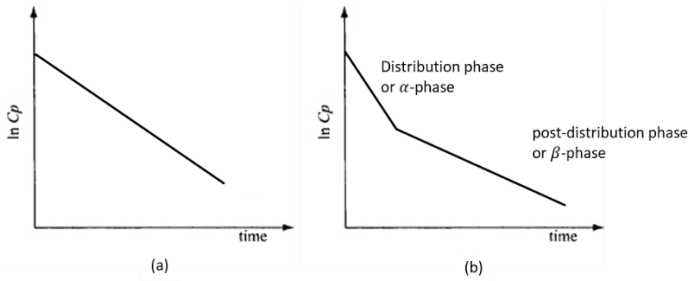


Figure 11: Schematic logarithmic scale of concentration of drug vs time profile after an IV *bolus* injection. a) one-comportment model; b) two-compartmental model

4.1.2. Multicompartmental models

The multicompartmental models help explain the drug's lower distribution rate and the relationship between the various factors is represented by a non-linear graph (as shown Figure 11) (20). That means that the drug does not distribute instantaneously to all parts of the body (20). It doesn't have a high perfuse rate even with IV bolus administration which distributes it faster to the blood stream (heart, liver, and so on) (20). There are two-compartmental models, three-compartmental models and son on. Adding more compartments may not necessarily improve the predictive value of the model (20). In fact, it is important to understand the distribution of drug to know how many compartments is needed to construct right model (20). In Figure 12, a two-compartmental model is shown where the drug movements of transfers between the central (V_1) and peripheral (V_2) (as explain on beginning of Chapter 4) by a first-order process (first-order kinetics is where a constant fraction of drug in the body is eliminated per unit of time); to maintain equilibrium. It also represents the elimination rate constant of drug in both compartments (20,28).

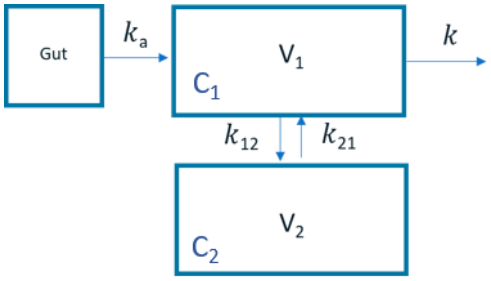


Figure 12: Two-compartmental model (C_1 and C_2) with first order absorption from the gastrointestinal tract (Gut) compartment, parameterized in constant of absorption ($\boldsymbol{k}_a$), transfer rate constant of drug from central compartment to peripheral compartment ($\boldsymbol{k}_{12}$), transfer rate constant of drug from peripheral compartment to central compartment ($\boldsymbol{k}_{21}$), and constant of elimination $\boldsymbol{k}$.

In this model, there are three more parameters, the volume of distribution of second compartment (V_2), the distribution rate constant from C_1 to C_2 (k_{12}), and the distribution rate constant from C_2 to C_1 (k_{21}) (28).

The second parameterisation terms are derived, such as the inter-compartmental clearance (Q) and V_2 given (28):

$$Q = k_{12} \times V_1$$

$$V_2 = \frac{k_{12}}{k_{21}} \times V_1$$

In the case of oral administration, at time t=0 the drug concentration in the C_1 and C_2 is zero, and the initial amount in the Gut (effective dose) equals to the multiplication of the administrated dose of the drug, the salt factor (fraction of administered dose that is made up of pure drug), and the bioavailability factor (fraction of dose that reaches the systemic circulation) (20,28). And then with time course, the amount of drug in Gut decreases with time, and drug concentrations in the central compartments increase (20,28). As Figure 11 represents, there is an initial phase of rapid decrease in plasma concentration from the central compartment (circulatory systems) into the peripheral compartments (body tissues) which is called alpha phase (rapid dissolution) (20,28). This phase ends when a pseudo-equilibrium of drug concentration is established between the central and peripheral compartments (20,28). Then, there is a phase of gradual decrease in plasma concentration that corresponds to the beta phase (slow elimination) (20,28). The decrease is primarily attributed to drug metabolism and excretion (20,28).

The α and β are "hybrid constants" and can be expressed by A and B respectively (28):(28)

$$A = \frac{k_a}{V} \frac{k_{21} - \alpha}{(k_a - \beta)(\beta - \alpha)} = \frac{k_a}{V_1} \frac{\frac{Q}{V_2} - \alpha}{(k_a - \beta)(\beta - \alpha)}$$

$$B = \frac{k_a}{V} \frac{k_{21} - \beta}{(k_a - \beta)(\alpha - \beta)} = \frac{k_a}{V_1} \frac{\frac{Q}{V_2} - \beta}{(k_a - \beta)(\alpha - \beta)}$$

The plasma concentration at any time t for a drug that enters the body by first-order absorption process and is distributed according to two-compartments model ($C_{tc}(t)$), according the α and β, is given by (28):

Single dose (28):

$$C_{tc}(t) = D(Ae^{-\alpha(t-t_D)} + Be^{-\beta(t-t_D)} - (A+B)e^{-k_a(t-t_D)})$$

Multiple dose (28):

$$C_{tc}(t) = \sum_{i=1}^{n} D_i \, (Ae^{-\alpha\left(t-t_{D_i}\right)} + Be^{-\beta\left(t-t_{D_i}\right)} - (A+B)e^{-k_a(t-t_{D_i})})$$

Steady state (28):

$$C_{tc}(t) = D(\frac{Ae^{-\alpha(t-t_D)}}{1-e^{-\alpha\tau}} + \frac{Be^{-\beta(t-t_D)}}{1-e^{-\alpha\tau}} - \frac{(A+B)e^{-k_a(t-t_D)})}{1-e^{-k_a\tau}}$$

where τ is the interval between two doses (28).

4.2.Simple Direct Effect Models

Overall, to characterize the effects/adverse effects of a drug, a simple direct effect model such as the simple E_{max} model can be applied as (26):

$$E = \frac{E_{max} \times C_p}{EC_{50} + C_p} \pm E_0$$

This equation incorporates the baseline effect (E_0) measurements of dynamic endpoints which are dependent of clinical trials, the maximum effect (E_{max}), and the drug concentration producing 50% of a maximal effect (EC_{50}) (6,26).

The equation becomes a model known as Hill's equation or Sigmoid E_{max} when it incorporates the Hill's coefficient (Y). This model assumes two things, first it assumes a drug's effects are proportional to the receptors occupancy and secondly, it assumes that plasma drug concentrations are in rapid equilibrium with the effect site (6,26). It is applied as (6,26):

$$E = \frac{(E_{max} \times C^Y)}{(EC_{50^Y} + C^Y)} + E_0$$

In the simplest way, the symbol γ is the sigmoidicity factor or steepness of the curve, if γ=1 hyperbolic curve, γ >1 for steeper curve and γ <1 for a smoother curve (Figure 13) (1,6). For the Hill coefficient bigger than 1, the concentration–effect relationship tends to result in quantal (all-or-none) response/effects. (26).

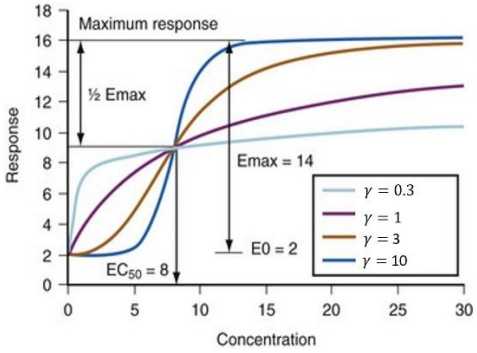


Figure 13: Schematic representation of sigmoidal curves generated by using the E_{max} equation to model the effect. Each curve corresponds to a different Hill coefficient (γ), which the higher γ value the higher the saturation will be. γ=1 is the hyperbolic curve; γ = 3 and γ = 10 are higher than 1, that denotes a steeper curve; γ = 0.3 is lower than 1 and that corresponds a smother curve. [Brian J. A. et all (2018) "Pharmacokinetics and Pharmacology of Drugs Used in Children"]

4.3. Signal Transduction Models

Sometimes, the effect is not observed at same time with the increase of drug plasm concentration (26,30). For those time-delays, the PD be explained by the drug–receptor binding time-dependent signal transduction (26,30). For a rapid receptor binding, the next equation describes the rate of change of the initial transit compartment ($Transit_1$) (26):

$$\frac{DTransit_1}{Dt} = \frac{1}{\tau}\left(\frac{E_{max} \times C_p}{EC_{50} + C_p} - Transit_1\right)$$

where in the E_{max} model denotes the drug–receptor interaction, and τ represents the mean transit time through this compartment (26,30). Subsequently more transit compartments might be added to describe to better describe the data, and consequently is proportional a delay in the onset of response and some delays in achieving the maximum effect (26). A typical example of transit compartment approach is the semi-mechanic model proposed by Friberg and Karlsson to describe myelosuppression process (Figure 14) (6,26). Transit-compartment model of myelosuppression includes a proliferating progenitor pool (Prol), three transit compartments ($Transit_i$), and a plasma neutrophil compartment corresponding to the absolute neutrophil count ($Circ$) (6,26). Drug effect (E_{Drug}) is driven by plasma drug concentration (C_p) and PD parameters (α) (6,26). An adaptive feedback function on the proliferation rate constant is governed by the ratio of initial neutrophils to current neutrophil count, raised to a power coefficient (γ) (6,26):

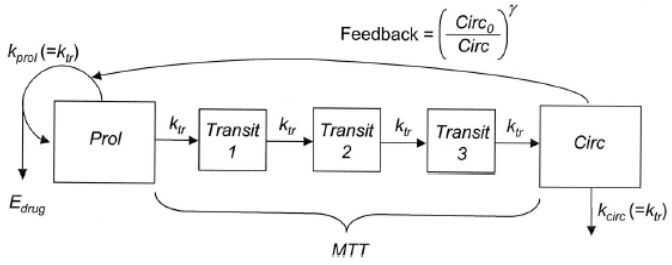


Figure 14: A schematic representation of the myelosuppression model by Friberg and Karlsson. [Gillespie B. (2010) MI250: Introduction to Bayesian PK-PD Modeling & Simulation.]

$$\frac{dProl}{dt} = k_{Prol}Prol(1 - E_{Drug})(\frac{Circ_0}{Circ})^{y} - k_{tr}Prol$$

$$\frac{dTransit_1}{dt} = k_{tr}Prol - k_{tr}Transit_1$$

$$\frac{dTransit_2}{dt} = k_{tr}Transit_1 - k_{tr}Transit_2$$

$$\frac{dTransit_3}{dt} = k_{tr}Transit_2 - k_{tr}Transit_3$$

$$\frac{dCric}{dt} = k_{tr}Transit_3 - k_{circ}Circ$$

$$E_{drug} = \alpha C_p$$

$$k_{prol} = k_{circ} = k_{tr}$$

$$MTT = \frac{n+1}{k_{tr}}$$

5. Modeling & Simulation

The Food and Drug Administration (FDA) has been using modeling and simulation to help make informed decisions on the development of new drug applications or to aid sponsors during the drug development process, to justify dose and dosing regimen (30). Yaning Wang defines Pharmacometrics as an "emerging science that quantifies drug, disease and trial information to aid efficient drug development and/or regulatory decisions" (25). From the clinical trial, the scientist is able to better understand the outcome of the combined exposure (PK) and response to the drug (PD) and thus evaluate the desired and undesired effects of the clinical trial, as well as the clinical needs of any given individual patient (25,30). The ability to integrate biological knowledge about the parameters across the development of these trials, allows for a better PK/PD modelling with appropriate statistics (Figure 15) (25,30). This statistics field which has the advantage of combining prior knowledge is Bayesian statistics (see Chapter 5.1) (25,30).

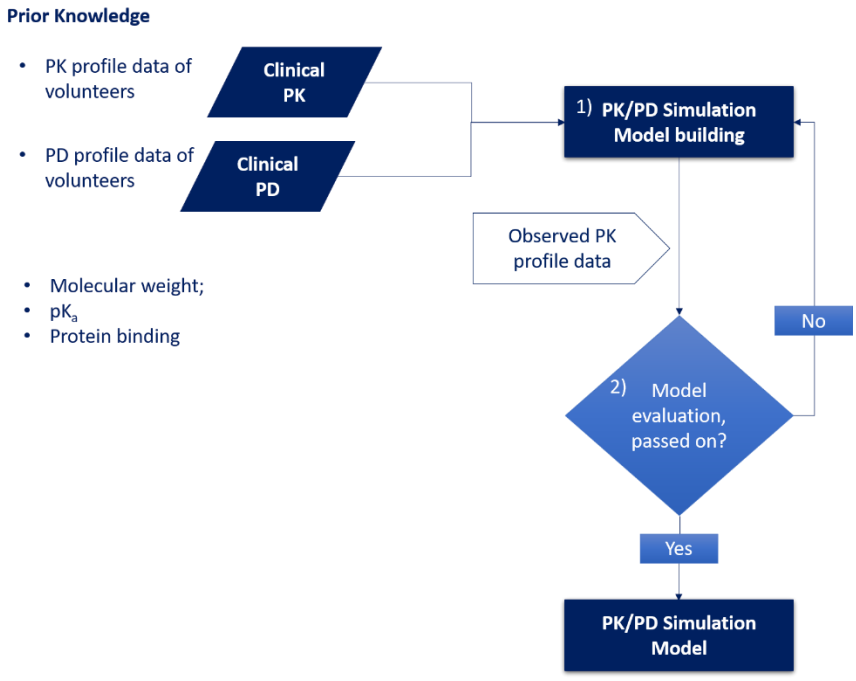


Figure 15: Flow-chart for model-based PK/PD and simulation. (1) Establishment of reference PK/PD model with prior information; (2) model evaluation.

5.1. Bayesian Approach

Bayesian statistics is a unique approach which allows the application of probabilistic models to statistical problems (30,31). It gives a solid mathematical mean of incorporating the prior beliefs and the information provided by observed data about a parameter, or a set of them, to produce new posterior beliefs (30,32). The Bayesian inference parameterizes the information about the parameter of interest, for instance, a drug's given parameter.

These parameters are represented by a vector θ of a probability (density) model $p(\gamma|\theta)$ giving rise to an observed data vector γ (3). If these parameters are treated as uncertain, and a 'prior' probability density $p(\theta)$ is allocated to them, the Bayes theorem gives the posterior density as (3):

$$p(\theta|\gamma) = \frac{p(\theta)p(\gamma|\theta)}{p(\theta)}$$

where $p(\gamma)$ is the marginal density for γ obtained by integrating over the prior (3). Since $p(\theta|\gamma)$ is regarded as a function of θ for fixed (observed) γ, we can rewrite this as (3):

$$p(\theta|\gamma) \propto p(\theta)p(\gamma|\theta)$$

so that the posterior is proportional to the prior, times the likelihood (3). The marginal likelihood can be done numerically through the approximation (for lower-order problems) but for most problems the Monte Carlo Simulation is used (see Chapter 5.2) (30,31). Overall, this equation means that the product of the likelihood of the observed data and the prior distribution is proportional to the probability of the posterior distribution, or that the distribution of the model parameters takes into account the observed data and prior knowledge (30,31).

One of the other differences between the Frequentist inference and the Bayesian inference, is the frequentist statistics tries to eliminate uncertainty by providing estimates while the Bayesian statistics tries to preserve and refine uncertainty by adjusting individual beliefs in light of new evidence (30,31).

In this report the empirical (sample) mean, standard deviation, the 'Naïve' standard error, the time-series standard error and the 2.5% and 97.5% empirical quantiles (95% CrI – credible interval) of the posterior samples were used to estimate parameters of interest. The 95% CrI is the Bayesian analogue of the 95% confidence interval in conventional statistics. In a 95% CrI, there's a 95% probability that the mean value of the parameter lies within this interval, while in 95% confidence intervals this means that if the same sampling method to select different samples is used and an interval estimate for each sample is computed, it is expected that the true population parameter falls within the interval estimates 95% of the time (33,34).

5.2. Monte Carlo Integration and Markov Chains

The Markov Chain Monte Carlo (MCMC) can solve an integral directly by computer simulation, solving the problem of evaluating the integrals of Bayesian analysis in computing the marginal likelihood and distribution, and then obtain better posteriors estimates (30,32). MCMC is a computer-driven method which combines two properties: Monte Carlo and Markov Chain (30,32). The Monte Carlo defines the estimates of distribution by drawing a large number of random samples from the distribution (32). It becomes easier to calculate the mean of this distribution instead of calculating the mean directly from the normal distribution's equations (32). The Markov Chain identifies how the sequential process was selected

by the random sampling generated (32). That is, each new random sample generated depends on the one before it, while new samples do not depend on any preceding it (32).

The Metropolis–Hastings algorithm is a MCMC method frequently used to obtain sequences of random samples (generally, for sampling from multi-dimensional distributions, especially when the number of dimensions is high), and to deal with the problem of autocorrelated samples that is inherent in MCMC methods. In Metropolis–Hastings algorithm, values are simulated, selected and then start to converge after a series of iterations, without any sample autocorrelation (thinning) (30). Autocorrelation is the correlation between the elements of a series and others from the same series separated from them by a given interval. In MCMC there isn't a default value of iterations on the burn-in phase which refers to the practice of discarding an initial portion of a Markov chain sample (30). When the parameters are strongly correlated, the Gibbs Sampler is usually applied, being a more complex approach than the Metropolis sampler, especially for Bayesian inference (30,32). However, Metropolis within Gibbs sampling removes the need to consider the multivariate proposals, where the key is that for each parameter the acceptation, the rejection or the proposal are taken parameter by parameter separately (30,32). Some studies, show that the Gibbs sampling approach produces the smallest bias and has the greatest precision on the within-subject imputation methods (30). The Metropolis–Hastings algorithms and Gibbs Sampler are random algorithms (that is, algorithms that make use of random numbers) and are an alternative to deterministic algorithms for inference (30,32).

5.3. Assessing Convergence

Accessing the convergence of MCMC is important to evaluate the precision of the posterior summary measures (33,34). This involves checking the stationarity (statistical properties of the process that do not change over time) of the chain and the accuracy of the posterior summary measures (33,34). Checking the convergence diagnostics can be done in a graphical or a statistical (formal) way (33,34). The graphical diagnostic consists in the inspection of the trace plots of the chain, which gives an informative impression of the stationarity and mixing rate of the chain for each parameter and the autocorrelation plots. It also shows the mixing rate of the chain and the dependency of the chain with its starting position (33,34). Another tool used in this report as a model selection criteria was the deviance information criterion (DIC) (33,34). The DIC is a generalization of the Akaike Information Criterion (AIC) (33,34). As is the case with AIC, smaller values of DIC indicate a better fitting model (33,34). There are others diagnostic tools, such as, Heidelberger and Welch (HW), Gelman–Rubin and Raftery And Lewis's (33).

The Heidelberger and Welch (HW) is a measure for assessing the stationarity which uses a statistical test to accept or reject the null hypothesis (31). The Gelman–Rubin is a measure for assessing convergence which compares the estimated between-chains and within-chain variances for each model parameter (33). The Raftery And Lewis's Diagnostic gives an estimate on the number of iterations required to

estimate a given level of precision in posterior samples, as well as estimating burn-in, to provide the posterior summaries (33).

5.4. Individual VS Population Analysis

As explained in the introduction chapter there are two analysis to PK/PD modelling, the Individual or Population analysis (see the advantages and disadvantages in Table 3).

The individual PK models are frequently performed in early phases of clinical pharmacology studies, in order to describe the relationship between the drug plasma concentration and time, defining the individual profile (35). Usually the Individual analysis of bioequivalence studies is characterized by the log ratio of the geometric means of two parameters: the AUC and the C_{max} (35) within subjects. In noncompartmental analysis, the AUC are frequently estimated by the trapezoidal (35).

Population PK is the study of variability in drug concentrations between individuals (36,37). It includes, at least, two-level of hierarchy, one level describes the estimates individual PK parameters, where individuals are processed simultaneously; and the other level describes how the estimates of the PK parameters differ between individuals (36,37).

Suppose that y_{ij}, denotes observed concentration for the j^{th} observation in subject i^{th} and t_{ij} the corresponding time. θ_i is vector of PK parameters for individual. At the first stage of the model, y_{ij} given θ_i and τ, the inverse of the residual error variance, is specified as (38):

$$p(y_{ij} \mid \theta_i, \tau) = N(f_{ij}, \tau^{-1}\epsilon_{ij})$$

where $y_{ij} \sim N(f_{ij}, \tau^{-1}\epsilon_{ij})$ given θ_i and τ, f_{ij} corresponds to the PK model evaluated at time t_{ij}, with the individual PK parameters (i.e. k_a, V, Cl) equal to θ_i. ϵ_{ij} is the residual error structure (38).

Then, the second stage of the model is specified as (38):

$$p(\theta_i \mid \mu, \Omega^{-1}) = N_p(\mu, \phi) \qquad \eta_i \sim N(0, \Omega)$$

where $N(,)$ represents the multivariate normal distribution, μ is the population PK behaviour, and Ω is the corresponding variance-covariance matrix representing the inter-subject variability (38).

The third stage of the hierarchical model can be defined by assigning prior densities to the parameter τ, μ and Ω (38):

$$p(\tau) = \Upsilon(\alpha, \beta)$$
$$p(\mu) = N_p(\eta, C)$$
$$p(\Omega^{-1}) = W_p(R^{-1}, \rho)$$

where, $\Upsilon\ (\alpha, \beta)$ is a distribution with parameters (α, β), η is the prior estimates of μ with variance-covariance matrix C, and W_p represents a p-dimensional Wishart distribution with mean R^{-1} and degrees of freedom ρ (38).

Overall, population PK/PD has two stages to describe the variability within the individuals and then interindividual variability (IIV) (variability in terms of patient characteristics, i.e. age, renal function or disease state) (36,37). Additionally, when drug concentration time profiles of the same patient are obtained on different occasions, there is often a high variability between the profiles, the inter-occasion variability. Non-linear mixed effects (NLME) modelling (see Chapter 4) has become increasingly applied for population PK but has only recently been described in the guidance of regulatory entities (FDA; European Medicines Agency (EMA)) (36,37). The NLME may determine the parameters for PD such as the E_{max} or EC_{50} (39). Population modelling is also used in phase I studies to help design sampling strategies for phase II–III studies and dose selection (40).

Table 3: Advantages and disadvantages of individual and populations modeling.

	Individual	Population
Advantages	User-friendly software	Linear and Nonlinear PK supported
	Model-independent	Descriptive and Predictive
	It defines complete individual PK profile	It allows systematic integration of data from more than one study
Disadvantages	Study design often limits analysis	Statistically complex
	Does not quantify the inter-subjects variability	It is often performed in late phases clinical trials

5.5. Software

In individual PK/PD analysis the most widely used software is the Window-Based Non-linear model fitting (WiNonlin) (41). WinNonlin is a tool tailored to the specific demands of PK data, being a standard for PK data analyses in the industry (41). It is user friendly, but it is not free, the same being the case for DoseMe and S-ADPT(40).

The NLME Modelling (NONMEM) is commonly used for modulating and simulating Population PK/PD analysis, but there are others, such as, WinBugs, MATLAB and MONOLIX (33,40). The NONMEM is the most popular statistical software used in population PK analysis, because it contains algorithms to estimate parameters in nonlinear mixed effects models (40). It also uses maximum likelihood to estimate the model's parameters obtained by integrating out the random effects, for example individual PK parameters (40). Those parameters are integrate by the NONMEM uses stochastic approximation

expectation maximization (SAEM) methods. (40). Bayesian methods have the advantage to provide exact estimates of the model parameters instead of approximating them (40).

The most popular and versatile Bayesian program is WinBUGS (33,40). It can import programs like Black Box, which has a function that has information regarding compartmental models (i.e. such as OneCptModel or TwoCptModel) (33,40). WinBUGS can also perform 3 stage hierarchical analyses instead of 2 stage hierarchical treatments (33,40).

Statistical Analysis Software (SAS) or R Studio allows the user to access raw data file, provide database management systems, statistical summaries, graphs and perform statistical analysis.(33). SAS is a standard software for clinical data analysis and reporting. It can read tables, listings and figures (TLFs) from different sources, and it manages to merge them in one clinical study report (CSR). Thus, this leads to improved reporting timelines. SAS needs a license, thus R Studio is a good alternative because it is a free and popular open source program and has been increasingly used in clinical trials studies.

In this report, R Studio and WinBUGS were used because they are suited for the task at hand and offer a complete and free environment for PK/PD analysis (33).

6. Case study 1: A pharmacokinetics and pharmacodynamics analysis of ME-2 drug in phase I clinical trial in healthy subjects

6.1. Introduction on case study 1

The activated blood coagulation Factor Xa inhibitors have begun to attract attention as new oral anticoagulants (42). Warfarin is, currently, the most used anticoagulant worldwide, but this has the disadvantage of having a narrow therapeutic window, variable PK and PD and extensive food and drug interactions, which requires regular coagulation monitoring and individual dose adjustments (42,43).

The Sponsor wants to submit ME-2 drug in the market as a new oral anticoagulant Factor Xa inhibitor. For that, as traditional clinical human's trials, this new oral drug has to be tested in Phase I Single Dose clinical trials to study its safety and efficacy. To measure that, it is necessary to trace the PK profile by plasma concentration of ME-2drug, and the PD profile by plasma concentration of Factor Xa (6). The Factor Xa activity will be measured as a biomarker as a proof of efficacy and bleeding liability of ME-2drug (6). For that, a nonlinear regression PK/PD model by the time-averaged Factor Xa inhibition and time-averaged ME-2 plasma concentrations were used. Then, a direct-action PK/PD model by time-matched Factor Xa inhibition and ME-2 plasma concentrations was added.

6.2. Methods on case study 1

Study design

The present dataset was obtained from a clinical trial phase I, parallel to a dose study with ten different single doses including the placebo-controlled.

Participants

Participants are 8 healthy adult volunteers per dose, with an age of 20-49 years. Subjects were included according to the inclusion criteria given by the protocol and excluded, even during the study, if there was a severe adverse effect. Written informed consent was obtained from each patient.

Interventions

On the dosing day, in groups of 8 subjects, one group received a dose of Placebo while the other-nine groups received one of nine different doses of ME-2: 1.25mg, 5mg, 10mg, 15mg, 20mg, 30mg, 40mg, 60mg or 80mg. The placebo control is used for assessment of safety/tolerability only. The venous blood samples were obtained from each healthy volunteer, where the blood was collected at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 hours after dose. For each blood sample collected we measured the PK profile (plasma concentrations of ME-2 drug) and the biomarker concentration (Factor Xa activity in plasma concentration).

Statistics

A one-compartment PK/PD model using a simple nonlinear regression and another using a nonlinear mixed-effects regression was implemented.

The simple nonlinear regression is used to modelling the time-averaged biomarker (Factor Xa) as a function of time-averaged plasma drug (ME-2) concentration in the i^{th} subject:

$$\bar{E}_{24,i} \sim N\left(\hat{E}_{24,i}, \sigma^2\right)$$

$$\hat{E}_{24,i} = \frac{E_{max}\bar{c}_{24,i}^{\gamma}}{EC_{50}^{\gamma} + \bar{c}_{24,i}^{\gamma}}$$

where $E_{24,i}$ denotes the drug effect (PD response) in 24hours per i^{th} subject, E_{max} is the maximal effect, $c_{24,i}$ denotes the drug concentration within 24 hours (the concentration-time curve from 0 to 24h), EC_{50} is the drug concentration at one-half of E_{max} (drug potency), γ denotes the *Hill* coefficient (determining the steepness the sigmoidal curve), and σ is the standard deviation.

Having as prior distribution information:

$$E_{max} \sim U(0, 100)$$

$$\log(EC_{50}) \sim N(0, 10^6)$$

$$\gamma \sim U(0, 10)$$

$$\sigma \sim U(0, 1000)$$

The nonlinear mixed-effects model is used to model the Sigmoid E_{max} curve related to the percentage of inhibition of Factor Xa activity to ME-2 plasma concentration on j^{th} occasion in the i^{th} subject:

$$E_{ij} \sim N\left(\hat{E}_{ij}, \sigma^2\right)$$

$$\hat{E}_{ij} = \frac{E_{max}c_{ij}^{\gamma}}{EC_{50}^{\gamma} + \bar{c}_{ij}^{\gamma}}$$

$$\log\left(EC_{50,j}\right) \sim N\left(log\left(\widehat{EC_{50}}\right), \omega_{EC_{50}}^2\right)$$

where E_{ij} denotes the drug effect (PD response) that follows a normal distribution with mean $\hat{E}_{ij}$ and σ the SD of PD response. E_{max} is the maximal effect, C_{ij} denotes the drug concentration, γ denotes the *Hill* coefficient (determining the steepness the sigmoidal curve), $EC_{50,j}$ is the drug concentration at one-half of E_{max}. ω is the variance of distribution of EC_{50} (interindividual random effect component).

Having as prior parameters distribution information:

$$E_{max} \sim U(0, 100)$$

$$\log\left(\widehat{EC_{50,J}}\right) \sim N(0, 10^6)$$

$$\gamma \sim U(0, 10)$$

$$\omega_{EC_{50}} \sim U(0, 10^5)$$
$$\sigma \sim U(0, 1000)$$

Each parameter of interest was estimated using the empirical (sample) mean, standard deviation, the 'Naïve' standard error, the time-series standard error and the 2.5% and 97.5% empirical quantiles (95% CrI) of the posterior samples. These samples are based on a simulation process implemented with two MCMC chains with 10.000 and 8.000 iterations each and a burn-in period of 5.000 and 6.000, respectively.

Model evaluation

The model evaluation was done by comparing visual predictive checks (VPC) derived from the distribution of observation and the distribution of prediction. That is, graphical comparison of observation and simulated prediction, which includes fixed and random effects, to assess the capabilities of the model to correctly describe the population trend and IIV. The graphical residual error was used to characterize the error model on the estimation of the parameters with enough precision, or not.

Software

The data assembly and graphical analysis was performed in R, Version 3.5.0, Diagnostic and goodness-of-fit (GOF) plots are generated in R using the lattice package. Individual and Population PK/PD modeling is performed using R and WinBUGS14, Version 1.4.3 using R2WinBUGS package to connect both tools, and Black Box.

6.3. Results on case study 1

Baseline

In the screening period, 80 healthy subjects were included to study. The weight's mean for the subjects is 72Kg where the minimum corresponds to 50Kg and the maximum is 98Kg. The age mean is 30 years where the younger subject is 20 years old and the oldest is 49 years old. There are 51.25 % females (41) and 48.75% males (39). 1280 samples for PK and PD analysis were collected and there were no missing values.

PK distributions are represented by time vs concentration for each dose of ME-2 Figure 16.

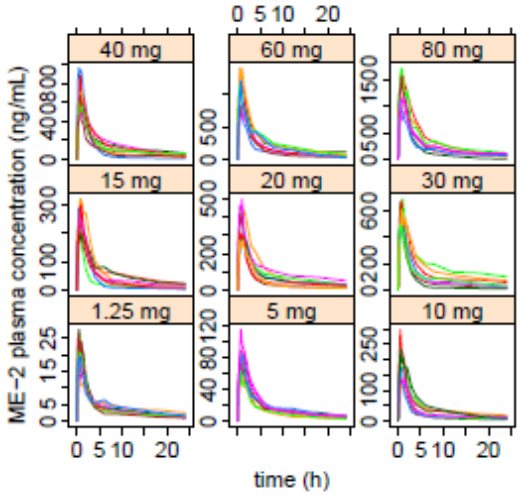


Figure 16: Baseline PK ME-2 drug by time. Each panel has the data for eight patients per doses of ME-2 drug.

PD is represented by concentration vs effect (Factor Xa inhibition), and for that purpose, Figure 17a shows the correlation of Fxa inhibition activity for each dosage by ME-02 and Figure 17b for all observations.

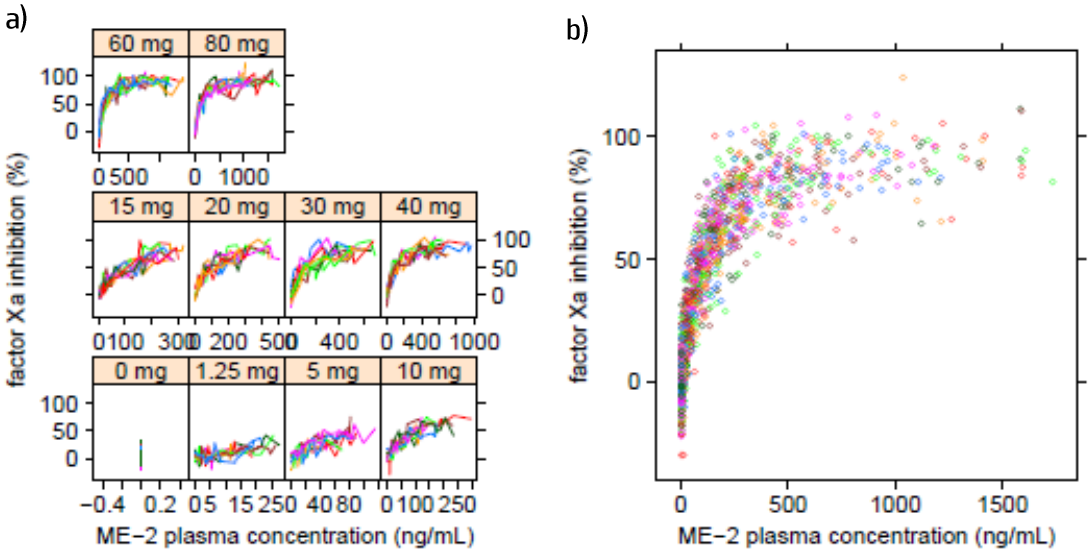


Figure 17: Correlation plot Factor Xa inhibition by ME-2 plasma concentration. a) Each panel has the data for eight subjects per doses of Placebo (0mg) and ME-2drug (1.25mg to 80mg). b) For all data subjects by colours.

The PK/PD relationship is represented by time vs effect, where Figure 18 shows the Fxa inhibition activity by time for each dose of ME-2.

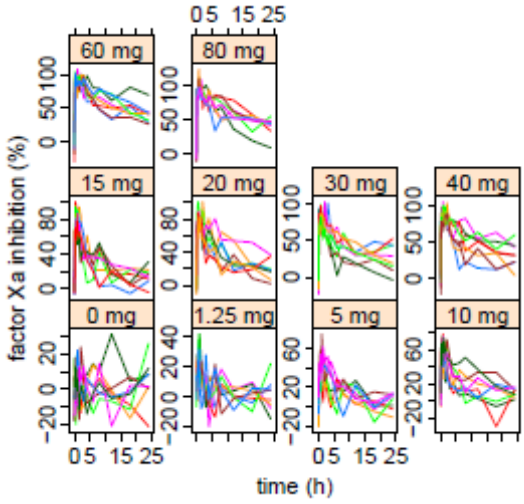


Figure 18: Baseline PK/PD of data observed. Each panel has the data for eight healthy subjects per doses of Placebo (0 mg) and ME-2 (1.25mg to 80 mg).

More details about the value of C_{max} and t_{max} of ME-2 drug and of Factor Xa inhibition activity for each drug are described in supplementary materials (S2). The basal value of Factor Xa inhibition activity in the placebo administration was 19.08 % (range: 8.15-26).

Modeling and Simulation

A nonlinear regression model was used to model the time average of concentration of ME-2 drug (ng/mL) and the time average of concentration of Factor Xa activity (%): each individual of the study has one observation obtained by the average of multiple observations. Priors information, such as the E_{max}, EC_{50}, Υ and σ, was incorporated into the model based on a Bayesian approach (2.5 to 97.5% CrI of distributions).

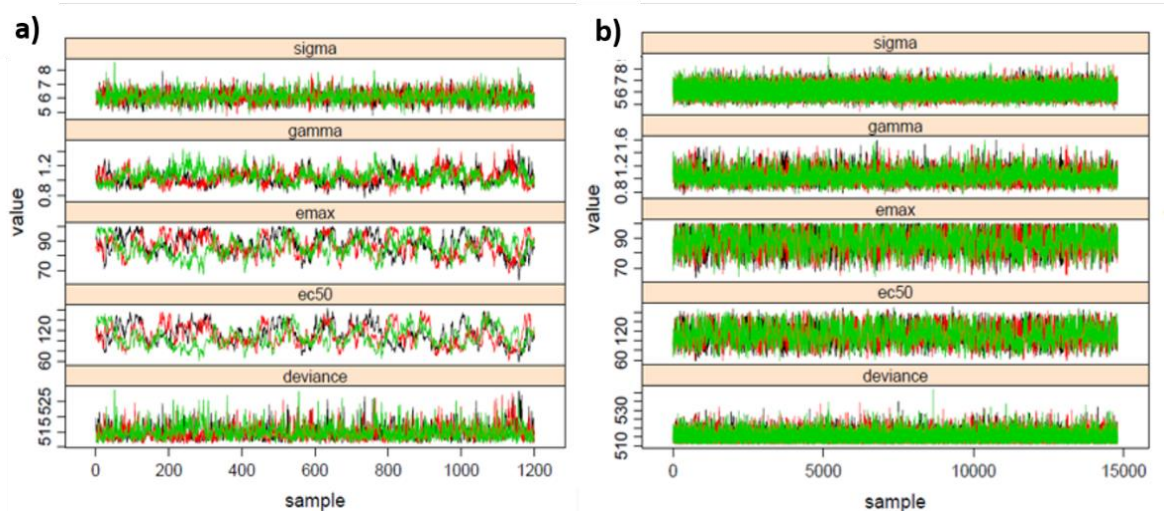


Figure 19: Trace plots for σ, Υ, E_{max}, EC_{50} and deviance informative cases. a) At 10.000 iterations, the first 5.000 were discard with a thinning parameter of 10; b) At 80.000 iteration, the first 6.000 were discard with a thinning parameter of 10. Each colour (red, green and black) of trace represents a chain of simulation.

Another model with a different number of iterations samples (Figure 19) was implemented to assess convergence and adequacy of sample sizes of MCMC simulation. The model was used with 10.000 iteration (Figure 19a) and did not show good convergence with the same happening at 80.000 iterations (Figure 19b).

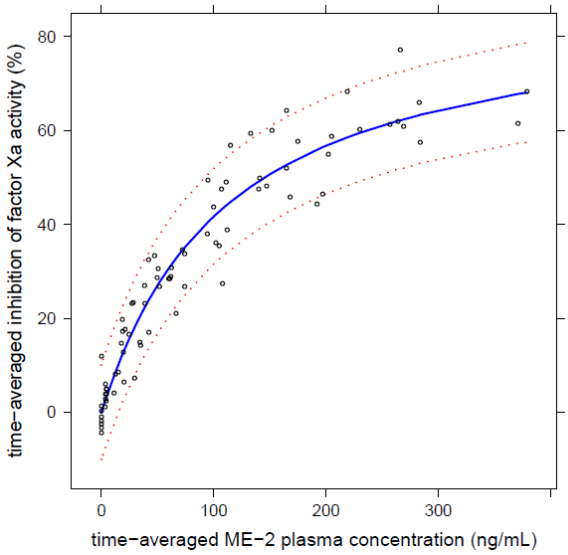
The parameters estimated in the final PK/PD model and the visual predictive checks (VPC) are represented in Table 4 and in the figure below (Figure 20 for individual predictions and Figure 21 for populational predictions), respectively.

Factor Xa during ME-2 administration and all parameters of the model were estimated with acceptable precision concerning the prior information included.

Table 4: Pharmacokinetic/pharmacodynamic prior distribution of parameters in healthy volunteers with nonlinear regression.

	Mean	**SD**	**Naive SE**	**Time-series SE**	**CrI [2.5% – 97.5%]**	**Effective N**
deviance	515	2.75	0.013	0.0249	512-522	12300
E_{max}	87.6	7.1	0.0337	0.168	73.7-99.2	1800
EC_{50}	112	20	0.0947	0.477	76.4 - 149	1760
Υ	1.04	0.102	0.000482	0.002	0.874-1.10	2580
σ	6.09	0.499	0.00237	0.00238	5.21-7.17	43900

Overall, this table demonstrates that the presented model adequately describes observed changes in the PK/PD model with 80.000 iterations, thus was considered as the final model. It was observed, in Figure 20, that the predicted posterior probability means of ME-2 score changes over time for different dose regimens using the final PK/PD model. Two different phases of response can be seen in the relationship between the time-averaged of ME-2 plasma concentration and the time-averaged of Factor Xa activity. In fact, in the beginning (in the Figure 20, from 0 ng/mL to nearly 112 ng/mL±20ng/mL) a quick proportional increase of plasma concentration of drug and inhibition of Factor Xa activity was observed. After that, it seems a breakpoint leads to a stagnation of the curve, which means that an increase in the drugs concentration is not proportional to the inhibition of the biomarker anymore.



o, observed values; ⎯, simulated median posterior; ····, 95% credible interval

Figure 20: Prediction Visual Predictive Check of PD based on developed PK/PD model of time-averaged biomarker and PK data ME-2drug. The interval prediction of time-average of ME-2drug dose and biomarker are represented the area between 95% CrI.

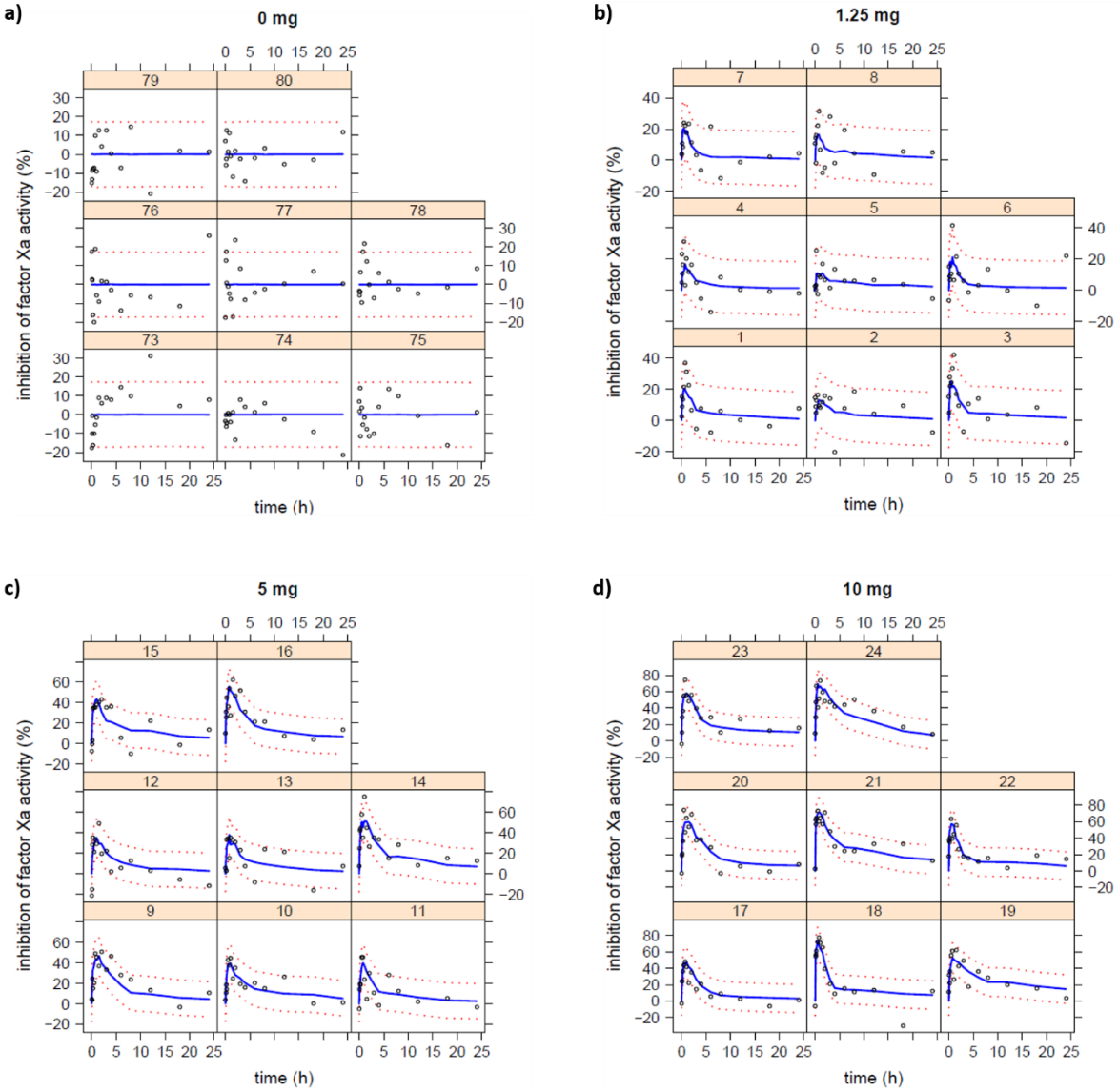
A nonlinear regression model was used, where each individual of the study has multiple observations per dose of ME-2 drug. Furthermore, the prior's information described before and the prior $\omega_{EC_{50}}$ was incorporated into the model based on a Bayesian approach to estimates the parameters of interest and its 95% CrI (2.5 to 97.5% CrI of distributions). Two models were tested with different number of iterations samples on Monte-Carlo simulations methods (the parameters estimates are shown in 2.5 to 97.5% IC distributions) to assess convergence and adequacy of sample sizes of MCMC simulation. In the 10.000 iterations model, the first 5.000 simulations were discarded, with a thinning parameter of 10. On the 50.000 iterations model, the first 4.000 was discard, with a thinning parameter of 10. The second model showed better convergence and a lower value of deviance information criterion (DIC first model = 9721, DIC second model = 9719). This metric is specifically to compare Bayesian models.

Table 5: Pharmacokinetic/pharmacodynamic prior distribution of parameters in healthy volunteers with nonlinear mixed effects regression.

	Mean	SD	Naive SE	Time-series SE	CrI [2.5% - 97.5%]	Effective N
deviance	9640	12.3	0.0741	0.101	9630-9670	14900
E_{max}	98.60	1.05	0.00634	0.0157	96.1-99.9	4480
EC_{50}	98.10	4.09	0.0246	0.0569	89.7-106	5180
Υ	0.99	0.0266	0.00016	0.00027	0.942-1.05	9750
σ	10.50	0.214	0.00129	0.0013	10.1-10.9	27100
$\omega_{EC_{50}}$	0.21	0.0286	0.000172	0.000294	0.158-0.269	9510

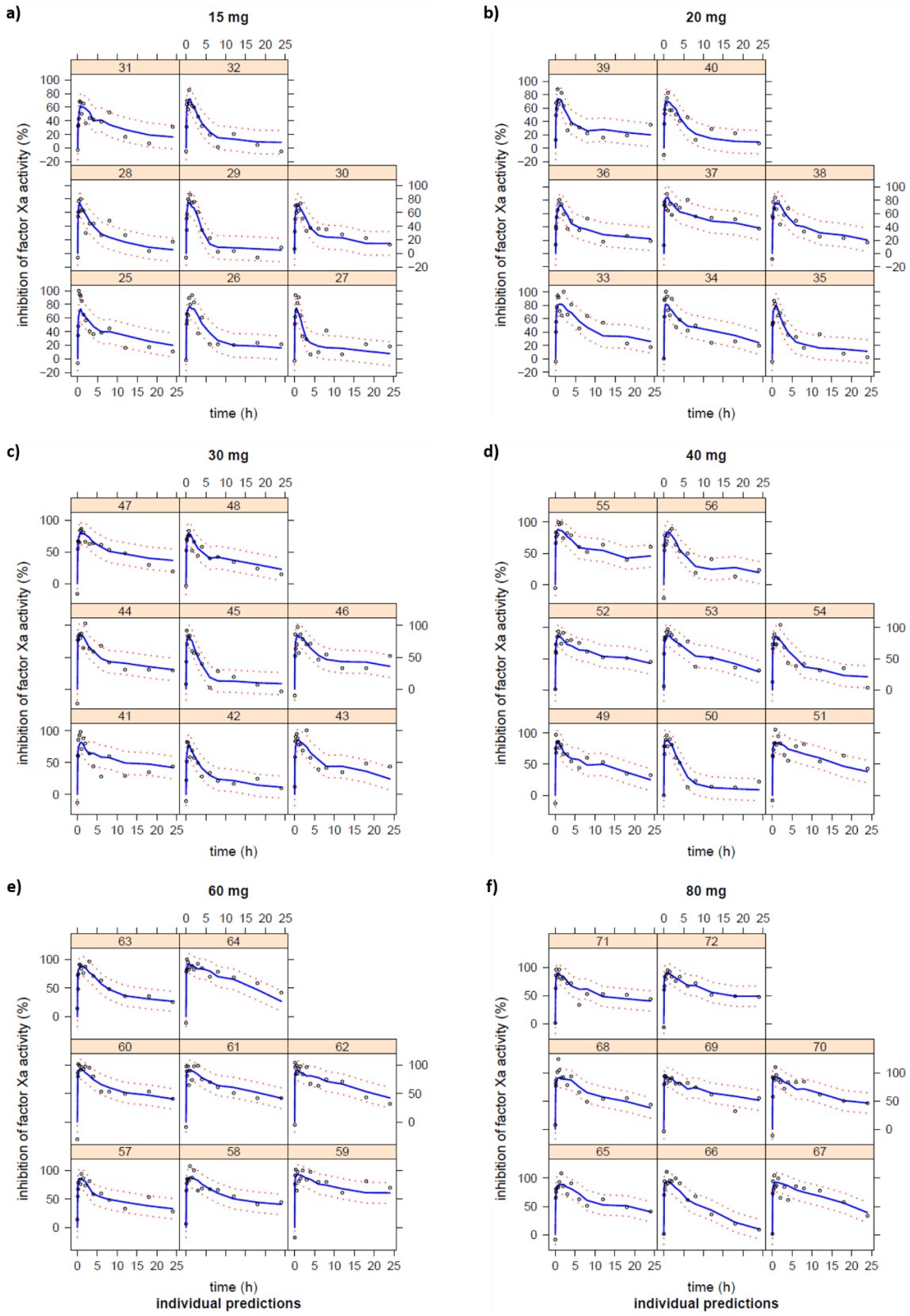
The parameters estimated for the final PK/PD model characterized in Table 5 and visual predictive checks (VPC) are represented in Figure 21 and Figure 22. Where Median of the predictions based depends of MCMC samples is a blue curve, dots represents the observation data, and the red dash line represents the 5% and 95% tails of the posterior prediction (credible interval 90%). In general, this table demonstrates that the presented model with the posterior distribution of parameters estimated (e.g. E_{max}, EC_{50}) with acceptable precision and with a reasonable random-effect.

The E_{max} estimated was 98.60 (95% CrI 96.1-99.9) and IIV was estimated at 0.21 (95% CrI 0.158-0.269) within these subjects. Again, all parameters of the model were estimated with acceptable precision concerning the prior information.



o, observed values; _, simulated median posterior; ····, 95% credible interval

Figure 21: PK/PD Individual Predictions the Inhibition of Factor Xa activity by time per doses of ME-2 drug. a) 0mg (Paracetamol); b) 1.25mg; c) 5mg; d) 10mg.



o, observed values; ⎯, simulated median posterior; ····, 95% credible interval

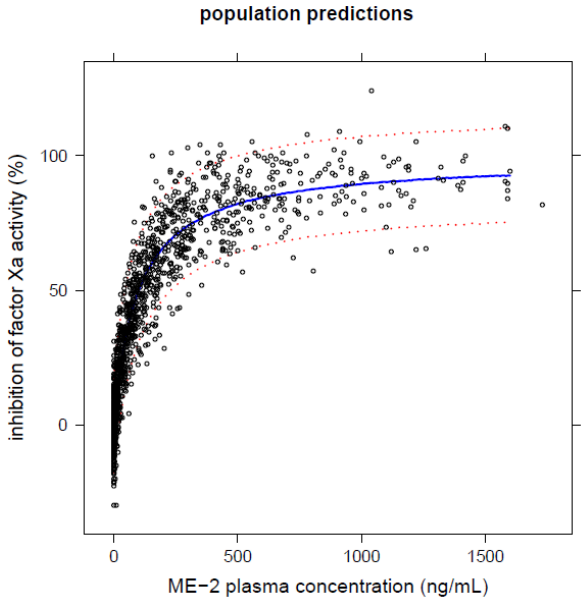
Figure 22: PK/PD Individual Predictions the Inhibition of Factor Xa activity by time per doses of ME-2 drug. a) 15mg; b) 20mg; c) 30mg; d) 40mg; e) 60mg; f) 80mg.

Residual based diagnostic plots of final model were examined to identify trends suggesting model misspecification. The figure in supplementary materials S3 shows that the data predicted is distributed according to the unity line, despite this only happening from 0 mg to 30 mg. Besides that, the graphic shows some uncertainty associated with each prediction of inhibition of Factor Xa activity. In fact, it was also observed that the individual and population model in relation to time suggests that the model is adequate (supplementary materials S4) (24).

Based on simulation, a graphic for PK/PD individual prediction subjects per doses can be seen in Figure 21 and Figure 22. It is possible to see that almost all cases approximately enclose the 90% of the prediction interval. For all doses it is possible to extract from the graphic the maximum concentration-effect peak and the time when it happens. Overall, the maximum concentration-effect peak occurs at the same time, between 0.5h to 1.5h. The E_{max} was lower than 70% in doses bellow 15mg (Figure 21), the 80% to 90% in 20mg (Figure 21) and 30mg (Figure 22), and higher than 90% above 40mg (Figure 22). Regarding AUC, it was observed that the lower areas of the graphics show a fast decrease of the median posterior predicted line after the maximum concentration-effect peak, that is, from 1.25mg until 20mg. Afterwards, from 30mg to 80mg dose of ME-2 drug shows a smooth descent of the mean posterior predict concentration line after the C_{max} point. However, almost all of the doses had a second peak after the maximum concentration-effect peak.

About PD (in supplementary materials figure S4), it was observed that the mean posterior prediction was straight line for the lower doses of ME-2 (1.25mg to 15mg) but with the higher doses of ME-2 (20mg to 80mg) it becomes a sigmoid. The percentage where inhibition of Factor Xa activitywas lower belongs to 1.25mg of ME-2 (nearly to 17%) and the opposite appears in the 80mg of ME-2 (100%, i.e. subject 70).

Lastly, the population PD from the final PK/PD model is represented in Figure 23 for all data. Where the interval prediction of ME-2drug dose and biomarker are represented the area between 2.5% and 97.5% of CI, where that area represents 95% probability to the predict value belong to follow that distribution. We observed a lot of density of plots/observations at the beginning of inhibition Factor Xa activity and ME-2 plasma concentration. The model shows that almost all dots of the observed data are enclosed in approximately 90% of the prediction interval. In this interval we observed a rapid increase of the median posterior prediction until to the EC_{50} of inhibition of Factor Xa than the increase of plasma concentration of ME-2 drug.



○, observed values; —, simulated median posterior; ····, 95% credible interval.

Figure 23: Population Prediction Visual Predictive Check of PD based on developed PK/PD model biomarker (inhibition of Factor Xa activity) and PK data of ME-2 plasma concentration.

After that, the increased plasma concentration of the drug is almost proportional to the inhibition activity of Factor Xa , that is, between 200ng/mL and 500ng/mL (EC_{50}-E_{max}). Then, more than 500ng/mL of drug plasma concentration does not correspond to an equal increase of inhibition of activity of the Factor Xa.

6.4. Discussion on case study 1

This study was performed with the motivation to understand the relationship between the PK ME-2 drug and the PD inhibition of Factor Xa. The relation between PK and PD was adequately described by the sigmoid E_{max} model. In the simple nonlinear regression, the effect site EC_{50} was of 112 ng/ml (95% CrI 76.4-149) and in the mixed effect model the EC_{50} was estimated to have a value of 98.10 ng/mL (95% CrI 89.7-106). The EC_{50} estimated was very similar in both models. The final PK/PD model was built by performing simulations on the same test conditions.

Based on PK/PD model outcomes, the simple nonlinear regression (Figure 20) shows the simulations of the predicted probability of ME-2 score changing over time for different doses, where this dose–response curve is a representation very similar to what really happens in the organism, since it takes into account aspects such saturation effect which leads to the side effect's appearance (44). The similar happens to the population predicted analyses (Figure 23) obtained from mixed effect's model, where the maximum effect is associated to a saturation of the drug in the organism (29). Before the saturation phase, the maximum

effect observed at the PD analysis was reached with 500ng/mL, which is the lowest plasma concentration value for the maximum effect. In fact, increasing the drug's concentration can have harmful side effects while not achieving better results. To achieve best range that explains the relationship between plasma concentration and the drug's effect, the EC_{50} and E_{max} are needed. As the concentration of 50% of the maximum effect was obtained at 200ng/mL, the optimal range was approximately between 200ng/mL and 500ng/mL (in supplementary materials Figure S5). The ME-2 doses which that range was reached for the PD analysis was 20mg and 30mg. However, we observed that the line obtained from lowest doses of ME-2drug (1.25mg to 15mg) was more linear than in an expected sigmoid model, never reaching the maximum effect.

Considering the PK/PD model, doses lower than 15mg do not exhibit a maximum effect higher than 60% on the inhibition of Factor Xa activity and the 20mg/30mg doses exhibit a maximum effect of 80% to 90% on the inhibition of Factor Xa. The percentage of coagulant inhibition is dependent on the objective to which the drug is used. In some cases, a higher percentage might be more adequate than a lower one, thus the concentration required has to be in line with the final aim for the effect. Regardless of the goal, the maximum concentration peak is observed between 0.5h and 1.5h.

In this study, we used a one-compartmental model that was the same model applied on a standard drug study (Enoxaparin) (45). However, in Figure 21 and Figure 22 a second peak was observed posterior of the C_{max} of the predicted curve for PK/PD model. This characteristic might represent that the drug does not distribute instantaneously to all parts of body. When this post-distribution phase is observed, a two-compartmental model is usually applied (45,46). Other studies report that the inclusion of some parameters of PK, such as clearance, volume of distribution and absorption rate, allow the study of these parameters influence on the risk of physiological haemorrhage (45–47). But in this case that information was not included because it was not available in the dataset.

Overall, ME-2 showed a dose-related inhibition Factor Xa activity effects in human subjects, but it is a simple case to compare to the most commonly used anticoagulants in the market (5,13,45). Sometimes, the database is not obtained from controlled tanning environments and difficulties in parameters estimation in the Hill equation (48) and Michaelis-Menten Equation (49) occur.

6.5. Conclusion on case study 1

In the present trial consisting of healthy individuals, a single dose of the ME-2 drug was shown to be efficient in inhibiting the Factor Xa biomarker and consequently proven to have antithrombic proprieties. Overall, the simulation obtained from ME-2 PK/PD profiles denotes that the maximum peak of concentration/effect happens within 30 minutes to 150 minutes. The PD profile denotes that the best range of plasma concentration and anticoagulant activity, was between 200 ng/mL (EC_{50}) and 500 ng/mL (E_{max}). The best dosage that describes the relationship between the effect and saturation of body

to the ME-2 drug was from 5 mg to 30 mg. Lastly, the PK/PD model was shown to be able to differentiate between the observed and the simulated data.

However, the model might be improved once it was observed a post-distribution phase by applying a two-compartmental model. In addition, if available, incorporating the PK parameters, such as clearance, volume of distribution and absorption rate, might allow for a better explanation of the drug's behaviour in body.

7. Case study 2: A Phase I study of ME-2 drug comparing the safety and efficacy versus drug-induced neutropenia

7.1. Introduction on case study 2

The previous study regarding phase I of the ME-2 drug shows its inhibitor activity of Factor Xa. Despite this, some cases of neutropenia were still observed in some subjects receiving higher doses of this drug. Neutropenia is a haematological disease defined by an absolute neutrophil count (ANC) below 1.5 x 10^9L. According to the National Cancer Institute's Common Toxicity Criteria, there are four grades of neutropenia (30):

- Grade 1: ANC ≥1.5 and <2.0 × 10^9 per litre;
- Grade 2: ANC ≥1.0 and <1.5 × 10^9 per litre;
- Grade 3: ANC ≥0.5 and <1.0 × 10^9 per litre;
- Grade 4: ANC <0.5 × 10^9per litre.

The aim of this study is to model the relationship between neutrophil counts and drug exposure, and then find the probability that identifies the effective doses of ME-2, with an acceptably small degree of neutropenia. With this in mind, informative prior distributions were also included for drug independent parameters.

7.2. Methods on case study 2

Study design

A Phase I multiple dose dataset was obtained from a parallel dose study with 5 different doses of ME-2 and placebo-controlled.

Participants

The study was conducted by administering the same dose to 4 different healthy volunteers per each dose for 7 days. This totals a number of 24 subjects tested. The demographics for the healthy subjects are between 20 to 45 years old and have no history of neutropenia. Subjects were included according to inclusion criteria given the protocol and excluded, even during the study, if there was a severe adverse effect. Written informed consent was obtained from each patient.

Interventions

The doses of oral drug administration are well defined in original study, comprised of five different doses of ME-2: 5mg, 10mg, 20mg, 40mg or 80mg, where the dosing administration was at the zero-hour mark, the second administration at 12h and the third at 168h. The placebo control's objective was the for assessment of safety/tolerability only.

The venous blood samples were obtained from each healthy volunteer, where the blood was collected at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 12.1, 12.2, 12.2, 12.5, 12.8, 13, 13.5, 14, 15, 16, 18, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 169, 170, 171, 172, 174, 176, 180, 186 and 192 hours after the first dose. For each blood sample collected we measured the PK (plasma concentrations of parent drug) and PD, which is measured by evaluating ANC daily for 12 days.

Statistics

The model used in this study was the semi-mechanistic model proposed by Friberg and Karlsson for drug-induced Myelosuppression (see chapter 4.3, Signal Transduction Models). The PK model is the two-compartment model.

The following PD parameters, which form the Stochastic model, for IIV are given by:

$$\log(MTT_j) \sim N\left(\log\left(\widehat{MTT}\right), \omega^2_{MTT}\right)$$

$$\log(Circ_{0j}) \sim N\left(\log\left(\widehat{Circ_0}\right), \omega^2_{Circ_0}\right)$$

$$\log(\alpha_j) \sim N(\log(\hat{\alpha}), \omega^2_{\alpha})$$

The normal logarithmic distribution for the residual variation in ANC is given by:

$$\log(ANC_{ij}) \sim N(Circ_{ij}, \sigma^2_{ANC})$$

The values reported in Table 6 relate to the prior distribution that is used to calculate the PD system parameters.

Table 6: Prior distribution information for PD system parameters to the semi-mechanistic model proposed by Friberg and Karlsson

	Mean	SD
$\boldsymbol{\log\left(\widehat{Circ_0}\right)}$	log(5.4)	0.2
$\boldsymbol{\log\left(\widehat{MTT}\right)}$	log(110)	0.16
$\boldsymbol{log(\gamma)}$	log(0.16)	0.16
$\boldsymbol{\frac{1}{\omega^2_{Circ_0}}}$	11	21
$\boldsymbol{\frac{1}{\omega^2_{MTT}}}$	37	61

$$\hat{\alpha} \sim U(0,1)$$

$$\omega_{\alpha} \sim U(0,5)$$

We estimated each parameter of interest using the empirical (sample) mean, standard deviation, the 'Naïve' standard error (the standard mean error, adjusted according to sample size), the time-series

standard error (which corrects the "naive" standard error for autocorrelations) and the 2.5% and 97.5% empirical quantiles (95% CrI) of the posterior samples. These samples are based on a simulation process implemented with two MCMC chains with 10.000 and 25.000 iterations each and a burn-in period of 5.000, for both.

Model evaluation

The model evaluation was done by comparing visual predictive checks (VPC) derived from the distribution's observation and the prediction's observations. This graphical comparison of the predicted observations and simulations prediction includes fixed and random effects that allow us to assess the capabilities of the model to correctly describe the population trend and IIV.

Software

The data assembly and graphical analysis is performed in R, Version 3.5.0, Diagnostic and goodness-of-fit (GOF) plots are generated in R using the lattice package. Individual and Population PK/PD modelling is performed using R and WinBUGS14, Version 1.4.3 using R2WinBUGS package to connect both tools, and Black Box.

7.3. Results on case study 2

Baseline

In the screening period, 24 healthy subjects were studied from which 50% were females (12) and 50% were males (12), ranging from 20 years old to 45 years old, and the bodyweight was contained between 56 Kg and 98 Kg (for more information about baseline by dosage see supplementary materials S6). The database for PK analysis was comprised of 2596 collected samples and there no missing values were found, and the same was observed for the PD analysis. PK distributions is represented by the following graphic for each dose of ME-2 by time (Figure 24).

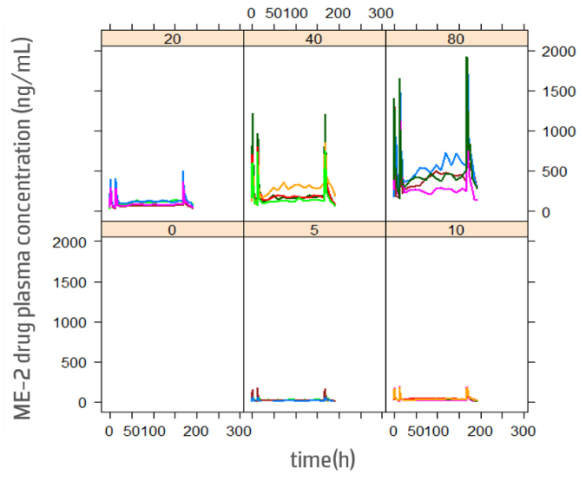


Figure 24: Baseline PK ME-2 drug in Phase I multiple dose. Plasma concentrations of ME-2 drug by time. Each panel has the data for eight patients per doses of ME-2 drug (0mg, 5mg, 10mg, 20mg, 40mg and 80mg denote in pink strip).

PK/PD distribution is represented in Figure 25 for each dose of ME-2.

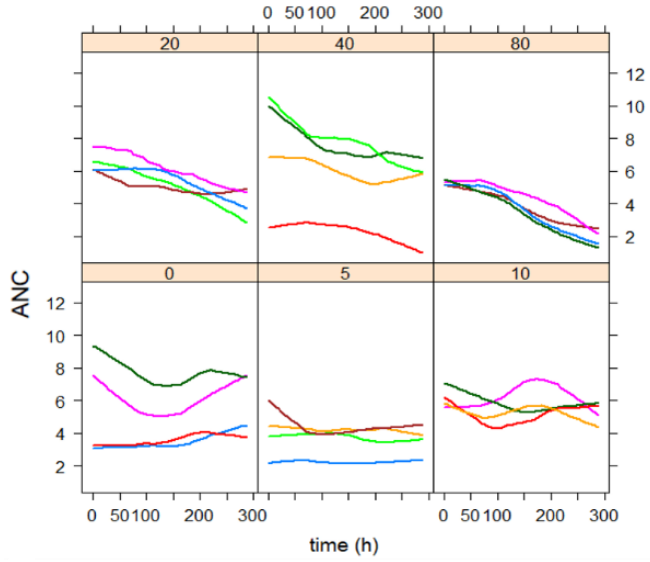


Figure 25: Baseline PK/PD of ME-2 drug in Phase I multiple dose., ANC by time. Each panel is data for eight patients per doses of ME-2 drug (0mg, 5mg, 10mg, 20mg, 40mg and 80mg denote in pink strip).

Modeling and Simulation

Bayesian analysis allowed the estimation of prior information of parameters used to model the semi-mechanistic model proposed by Friberg and Karlsson. Those parameters were $\widehat{Circ_0}$, $\widehat{MTT}$, γ, $\omega^2_{Circ_0}$ and ω^2_{MTT}, with 95% CrI. The posterior distribution was obtained and then two models were simulated by Monte-Carlo simulations.

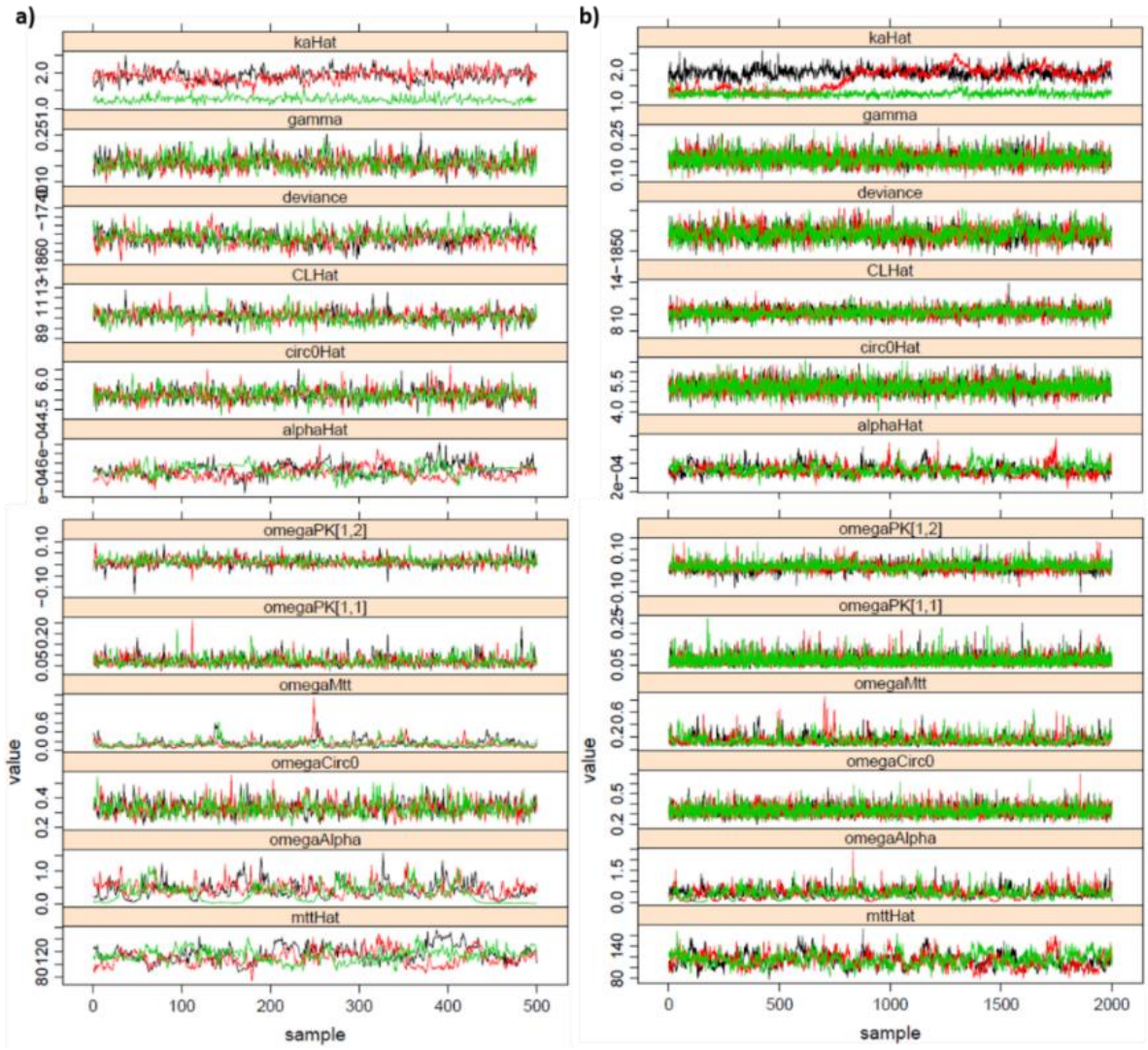


Figure 26: Trace of some parameters estimated which are ka, γ, CL, Circ, α, Ω, MTT a) At 10.000 iteration, the first 5.000 were discard with a thinning parameter of 10; b) At 25.000 iteration, the first 5.000 were discard with a thinning of 10. Each colour (red, green and black) of trace represents a chain of simulation.

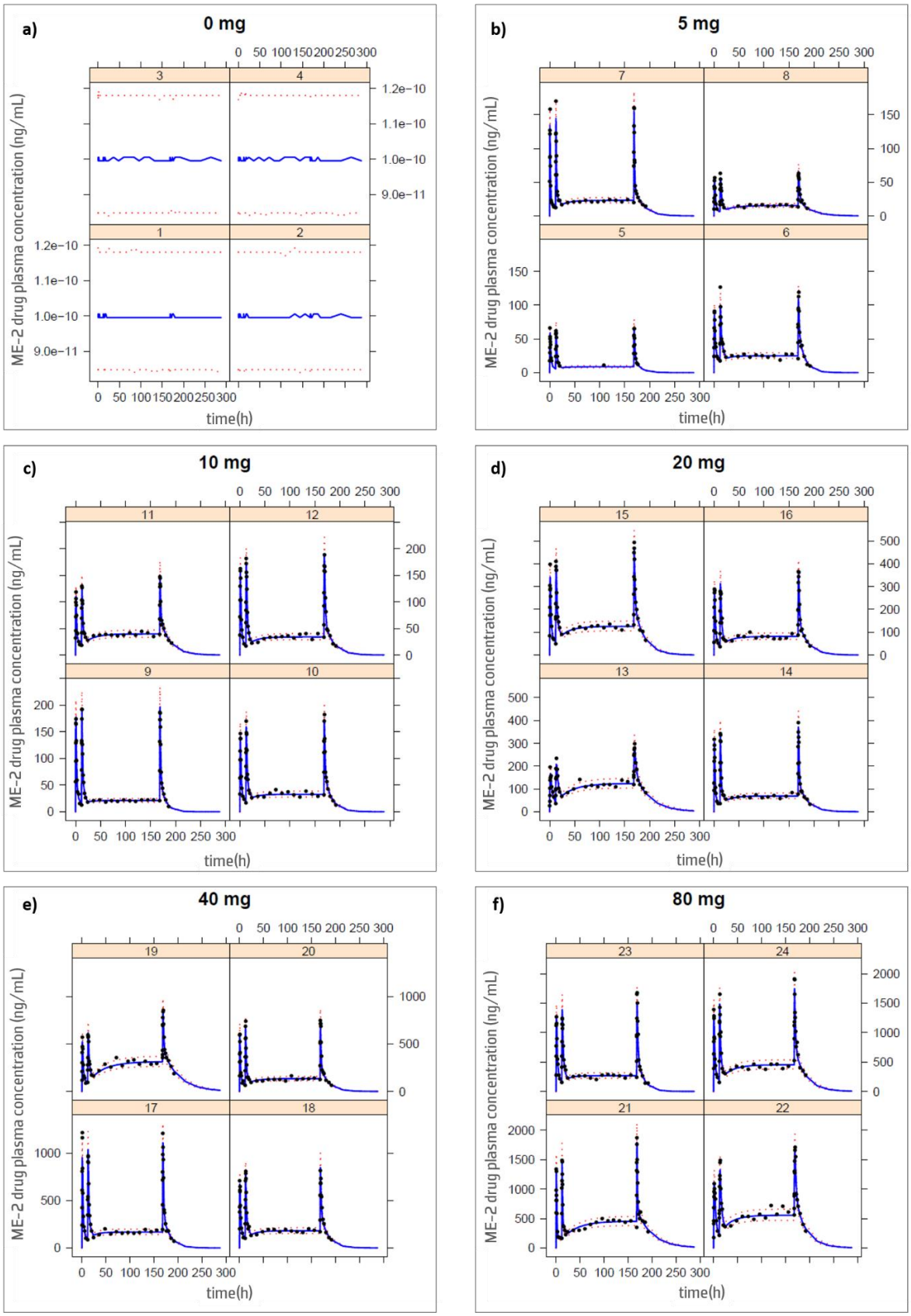
To compare the two models, the parameters generated were used to access the convergence and adequacy of the sample sizes by MCMC. At 10.000 iteration (Figure 26a) the chains did not show good convergence, while at 25.000 iteration (Figure 26b) the model shows better converge of chains. The final PK model selected was with 2500 iterations. The parameters estimated for the final PK model is represented in Table 7, and VPC is shown in Figure 27 and Figure 28.

The Table 7 demonstrated that the semi-mechanistic PK/PD model of neutropenia described the data adequately.

Table 7: Pharmacokinetic/pharmacodynamic prior distribution of parameters in healthy volunteers with semi-mechanic model

	Mean	SD	Naive SE	Time-series SE	CrI [2.5% – 97.5%]	Effective N
CL	10.30	0.611	0.008	9.12	9.86-11.5	2950
Q	16.4	1.4	0.018	0.09	13.80-19.10	1340
V_1	31.9	6.8	0.08	1.2	22.6-42.8	668
V_2	101	8.56	0.11	0.248	85.8-119	1580
k_a	1.63	0.354	0.005	1.14	1.28-2.22	585
σ_{PK}	0.101	0.0023	2.97e-05	3.27e-05	0.0964-0.105	5110
α	0.00031	7.58e-05	9.79e-07	4.3e-06	0.0002-0.0005	348
Circ	5.23	0.344	0.0044	0.0045	4.58-5.95	5840
MTT	114	12.7	0.164	0.799	91.5-141	264
γ	0.161	0.0257	0.0003	0.000432	0.116-0.217	3740

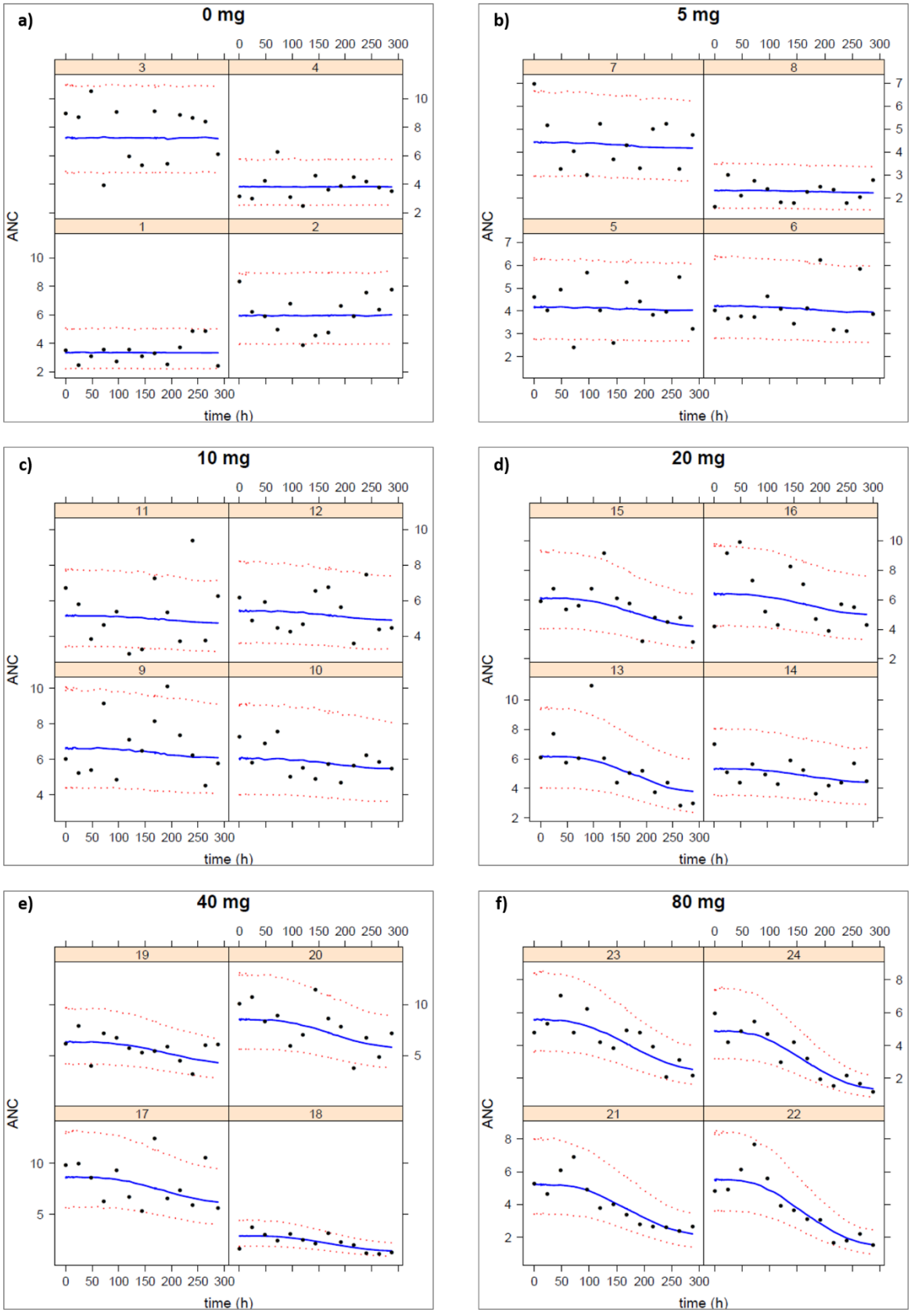
Based on simulation, a graphic for PK/PD individual prediction subjects per doses was obtained (Figure 27 and Figure 28). In Figure 27 it is possible to observe the C_{max} endpoint shown on the graphic. We observed that there was no peak with 0mg dosage, with 5 mg of ME-2 drug the maximum peak was very close to 150 mg at 200 hours, with 10 mg the maximum peak is very close to 200 mg at 180 hours, with 20 mg the maximum peak was observed to be very close to 500 mg at 180 hours, with 40 mg the C_{max} was observed at 800mg at 180hours, and lastly, with 80mg dosage the maximum peak was observed to be very close to 2000mg.



o, observed values; _, simulated median posterior; ····, 95% credible interval

Figure 27: VPC of Individual PK Predictions ME-2 per doses. a) 0 mg (paracetamol); b) 5mg ME-2; c) 10mg ME-2; d) 20mg ME-2; e) 70mg ME-2; f) 80mg ME-2.

Observing the PD individual simulation, in Figure 28, values higher than $2x10^9$ of ANC to 0 mg, 5 mg, 10 mg, and 20 mg were detected. For 40mg we observed one of four subjects with a value lower than $2x10^9$ per litre of ANC, meaning it had Grade 1 neutropenia. The remaining three subjects were observed to have a normal value of ANC (between $4x10^9$ and $7x10^9$ per litre). Lastly, for 80 mg it was observed that two subjects had values of ANC near $2x10^9$ per litre which means the subjects had normal ANC levels. The other two subjects had a value lower than $2x10^9$ per litre but higher than $1x10^9$ per litre, indicating Grade 1 neutropenia. Both the 40 mg and 80 mg dosages, were shown to reach a C_{max} of 500 mg/L.



o, observed values; —, simulated median posterior; ····, 95% credible interval

Figure 28: VPC of Individual ANC Predictions ME-2 per doses. a) 0 mg (paracetamol); b) 5mg ME-2; c) 10mg ME-2; d) 20mg ME-2; e) 70mg ME-2; f) 80mg ME-2.

7.4. Discussion on case study 2

Overall, 5 mg to 20 mg dosages demonstrated some stability in the ANC values from the beginning of the study until the end (> 2.0x109 per litre). Because of that, these doses were considered safety doses with no secondary effects attached.

On the other hand, 40 mg and 80 mg shows a quick decrease from the beginning to the end of the study, which indicates some instability in the subject's safety when exposed to these drug dosages. The dose of 40 mg shows that one out of four subjects can have Grade 1 neutropenia. However, this diagnostic may be influenced if that subject has a low initial value of ANC (although considered healthy because it is not less than 2x10-9) at the beginning of the study.

Though, it does not exclude the importance of the shape of the curve, that is, a certain decrease is observed from the beginning and end of the study. The population which took the multiple dose of 80mg has a 50% of probability to provide Grade 1 neutropenia.

Although in the literature, neutropenia was founded to be present when the plasma drug concentration was bigger than 500 mg/L (14). In this study we observed that same fact.

7.5. Conclusion on case study 2

Considering this study and the previous study (Chapter 6), the overall incidence of adverse reactions did appear to be dose dependent. This behaviour was also observed in this study where the drug's plasmatic concentration was bigger than 500mg/L. For that, it was concluded that the 20mg dosage seems to have better safety/efficacy ratio to proceed to the next phase of clinical trials. We suggest an improvement to this study analyses, with population modelling if there are differences between males and females.

8. Case study 3: A Population PK analysis by Phase I single dose, Phase II multiple dose &PhaseIIa proof-of-concept of studies of ME-2 drug to prevent of venous thromboembolism

8.1. Introduction on case study 3

ME-2 is an oral drug with a favourable pharmacokinetic profile in phase I single dose study, as described in chapter 6 (6). An antithrombotic profile with inhibitory Factor Xa activity was observed with maximum concentration-effect at 30 minutes and 150 minutes for the ME-2 drug (6).

However, we found that the distribution / elimination of the drug is not linear as we found a second peak after the maximum concentration-effect peak in the PK/PD model's concentration curve. That means, that after oral administration, the drug does not distribute instantaneously to all parts of body (see chapter multicompartmental models 4.1.2) (20). The one-compartmental model might not be enough to explain this feature and the drug's potential effective safe daily dose. For that reason, we applied a two-compartmental model to the drug for Phase I single dose, Phase I multiple dose and Phase IIa dataset. We also incorporated the PK parameters as, clearance, volume of distribution and absorption rate, to try explaining what happens with the drug's behaviour in a healthy subject body. The ME-2's drug study starts in the Phase I single dose study with the recommended starting dose and the maximum starting dose in healthy volunteers, as recommended by the FDA. The Phase I multiple dose was then applied to understand its safeness and its well tolerated dose. Afterwards, the Phase IIa multiple dose was conducted to focus on the drug's proof-of-concept (PoC), which consists on a comparison between the new drug with a reference drug.

In this study, the aim was to identify one dose regiment of ME-2 drug that has same efficacy when compared to the Enoxaparin drug but possibly safely reducing the risk of VTE in patients undergoing total knee replacement. We also aim to understand the difference between the Individual and Population analysis.

8.2. Methods on case study 3

Study design

This study was based on an accumulated ME-2 of the PK data, which is comprised of a combination of the Phase I single dose in healthy volunteers, the dataset obtained from a Phase I multiple dose study in healthy volunteers and the Phase IIa multiple dose study of 20mg ME-2 drug in healthy volunteers. The last study design was used to compare the effect of the 20mg of ME-2 doses to 30mg of Enoxaparin doses, but since we only have access to the ME-2 drug, we compared the 20 mg ME-2 doses to the standard dose drug (30 mg of Enoxaparin).

Participants

Phase I single dose study: 8 healthy volunteers per dose;

Phase I multiple dose study: 40 healthy volunteers.

Phase IIa single dose study: 20mg of ME-2 applied to 100 patients.

Healthy adults aging from 18 to 64 years old were included according to the inclusion criteria given by the protocol and excluded during the study if there was a severe adverse effect. Written informed consent was obtained from each patient.

Interventions

Phase I single dose, in groups of 8 subjects per doses of ME-2: 1.25mg, 5mg, 10mg, 15mg, 20mg, 30mg, 40mg, 60mg or 80mg. The venous blood samples were collected at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 hours after administering the dose for the PK assessments.

Phase I multiple dose, in groups of 8 subjects per doses of ME-2: 5mg, 10mg, 20mg, 40mg or 80mg where the dosing administration was at 0h, and after the first administration, at 12h and at 168h. The venous blood samples were obtained from each healthy volunteer, where the blood was collected at 0, 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 12.1, 12.2, 12.2, 12.5, 12.8, 13, 13.5, 14, 15, 16, 18, 20, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 169, 170, 171, 172, 174, 176, 180, 186 and 192 hours after the first dose. The PK was measured for each blood sample collected.

Phase IIa multiple dose, involves the treatment with of ME-2: 20mg. The first dosing administration was at 0h, and after the first administration, at 12h and at 168h. 3 to 6 sparse samples of venous blood were obtained for the PK assessment from each volunteer.

Statistical Analysis

For the structural PK model, a two-compartmental model was used to model the first order absorption to describe the ME-2 plasma concentration on the i^{th} occasion in the j^{th} subject as a function of time, dose and body weight. The normal logarithmic distribution for plasma concentration $\log(c_{ij})$ is given by:

$$\log(c_{ij}) \sim N(\log(\hat{c}_{ij}), \sigma^2)$$

Where $\hat{c}_{ij}$ represents the model function for two-compartments model as:

$$\hat{c}_{ij} = f_{2cpt}(t_{ij}, D_j, \tau_j, CL_j, Q_j, V_{1j}, V_{2j}, k_{aj})$$

This function takes into account the observation time (t), the sequences doses (D), the dose interval (τ), and the IIV (Ω) and is implemented by the parameters CL, Q, V_1, V_2, k_a. Those parameters are normally distributed when the exponent is equal to 0.75 and the allometric scaling bodyweight (bw) is integrated (centred around 70 kg). We get that:

$$\log(CL_j, Q_j, V_{1j}, V_{2j}, k_{aj}) \sim N\left(\log\left(\widehat{CL}\left(\frac{bw_j}{70}\right)^{0.75}, \hat{Q}\left(\frac{bw_j}{70}\right)^{0.75}, \widehat{V_1}\left(\frac{bw_j}{70}\right)^{0.75}, \widehat{V_2}\left(\frac{bw_j}{70}\right)^{0.75}, \widehat{k_a}\right), \Omega\right)$$

Where Ω is a matrix 5X5 with 5 degrees of freedom and others prior's distribution for the rest of the model parameters. These are given by:

$$log(\widehat{CL}) \sim N(0, 10^6)$$
$$log(\hat{Q}) \sim N(0, 10^6)$$
$$log(\widehat{V_1}) \sim N(0, 10^6)$$
$$log(\widehat{V_2}) \sim N(0, 10^6)$$
$$\log(\widehat{k_a} - \curlywedge_1) \sim N(0, 10^6)$$
$$\sigma \sim U(0, 100)$$
$$\Omega^{-1} \sim Wishart\left(5\begin{pmatrix} 0.05 & 0 & 0 & 0 & 0 \\ 0 & 0.05 & 0 & 0 & 0 \\ 0 & 0 & 0.05 & 0 & 0 \\ 0 & 0 & 0 & 0.05 & 0 \\ 0 & 0 & 0 & 0 & 0.05 \end{pmatrix}, 5\right)$$

Each parameter of interest was estimated using the empirical (sample) mean, the empirical (sample) standard deviation, the 'Naïve' standard error, the time-series standard error and the 2.5% and 97.5% empirical quantiles (95% CrI) of the posterior samples. These samples are based on a simulation process implemented with two MCMC chains with 10.000 and 50.000 iterations each and a burn-in period of 5.000 for both.

Proportionally, residual error structures were also explored. Visualizations of the post hoc Bayesian estimates versus the covariates were generated to identify possible covariate relationships.

Model evaluation

The model evaluation was done by comparing visual predictive checks (VPC) derived from the distribution of observations and the distribution of predictions. This graphical comparison of observations and simulated predictions includes fixed and random effects, to assess the capabilities of the model to correctly describe the population trend and IIV. The graphical residual error was used to characterize the error model on the estimation of the parameters with enough precision. The estimation is based on the goodness of fit plots (GOF) which consist of the weighted conditional predicted against the observed weighted conditional. These were used to assess model appropriateness, adequate model fit and to identify possible structural model misspecifications.

Software

The Population PK modelling was performed using R algorithm, Version 3.5.0, where calls and run the WinBUGS14, Version 1.4.3. The data assembly and graphical analysis were performed in R. The VPC and GOF plots were generated in R using the lattice package, and Black Box.

8.3. Results on case study 3

Baseline

This study included 212 subjects, 72 subjects were included in the Phase I single dose, 40 subjects in Phase I multiple dose and 100 subjects in Phase IIa multiple dose. In the first trial, 53%were females (38) and 47% were males (34), between 20 and 49 years old, with a mean of 30.48, and between 50kg to 98kg, with a mean of 71,76kg. In Phase I multiple dose, 40 subjects had 53% female (21) and 47% male representation (19), between 19 and 45 years old, with a mean of 30.7. The mean weight is 70.63 kg, where the where the minimum corresponds to 51 kg and the maximum is 100 kg. At last, 100 subjects in the Phase IIa multiple dose dataset 48% belong to the female group (48) and 52% to the male group (52). Here, the mean age is 40.44 years, between 26 years and 64 years. The mean weight is 81.15 kg, where the lightest had 51kg and the heaviest had 119kg. The baseline characteristics of all subjects per trials and dose are described in supplementary material S7.

The PK profile of ME-2 drug is represented in Figure 29 for each trial, the Phase I single dose trial in Figure 29a, the Phase I multiple dose trial in Figure 29b, and the Phase IIa multiple dose trial in Figure 29c.

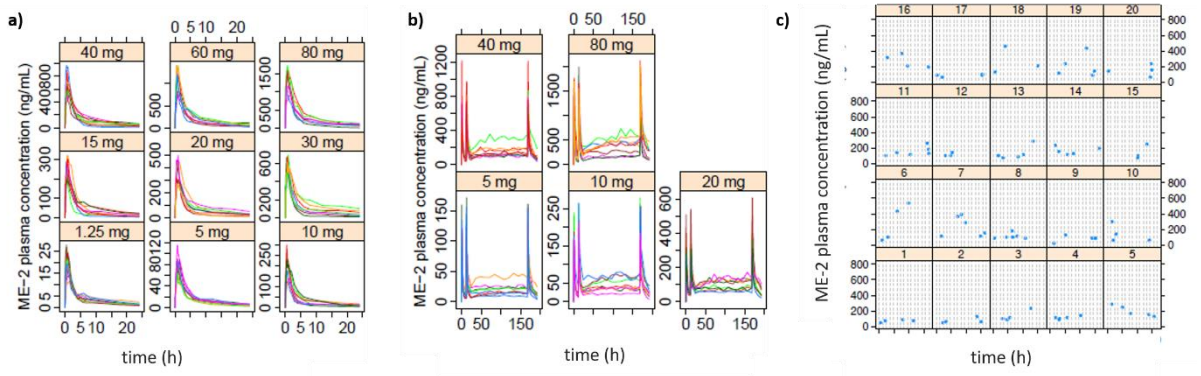


Figure 29: Baseline PK ME-2 drug by time: a) to Phase I single dose PK profile. Each panel has the data for eight patients per doses of ME-2 drug; b) to Phase I multiple dose PK profile. Each panel has the data for eight patients per doses of ME-2 drug; c) to Phase II PK profile of the first 25 subjects. Each panel has the data for one subject.

In the first study, a total of 1152 PK samples of ME-2 drug were collected. There had 64 missing values and 115 observation bellow the lower limit of quantification (BLQ) for 10 mg/L. These observations were excluded from the analysis resulting in a total of 973 PK observations above the BLQ used for modelling (supplementary material S7). In the second study, a total of 2.160 PK samples of ME-2 drug were collected. There were 35 BLQ observations (10 mg/L) and 39 sample were missing (see Table 8 for each peak of

ME-2drug or see only C_{max} and t_{max} in supplementary material S7). These 74 observations were excluded from the analysis resulting in a total of 2.086 PK observations above the BLQ used for modelling and simulation. (supplementary material S7).

Table 8: Baseline of PK parameters of Phase I multiple dose of the ME-2 drug.

ME-2	C_1	t_1	C_2	t_2	C_3	t_3
5	92.7	0.8	106,6	12.7	114.7	169.1
	(44.1-159)	(0.5-1.5)	(57-171)	(12.5-13)	(63.6-160)	(168.5-169.9)
10	170.6	0.8	203.8	12.8	207.75	168.8
	(119-239)	(0.5-1)	(130-264)	(12.75-13)	(147-279)	(168.5-169)
20	306.4	1	329	12.9	395.4	168.9
	(170-508)	(0.5-1.5)	(236-540)	(12.5-13.5)	(299-607)	(168.75-170)
40	705.25	0.91	755.9	12.78	824.12	168.81
	(489-1220)	(0.5-1.5)	(597-972)	(12.5-13)	(680-1210)	(168.5-169.5)
80	1342.38	0.97	1491.88	12.91	1748.25	168.91
	(719-1760)	(0.5-1.5)	(865-1980)	(12.5-14)	(986-2110)	(168.5-170)

C_1, average of maximum concentration of each subject after of first dosage.

C_2, average of maximum concentration of each subject after of second dosage.

C_3, average of maximum concentration of each subject after of third dosage.

t_{max}, average of time where maximum concentration was observed.

Concerning the third study, a total of 565 PK samples of ME-2 drug were collected. There were 100 samples with missing values, and these observations were excluded from the analysis resulting in a total of 465 PK observations above the BLQ used for model building (supplementary material S7). The average C_{max} was 248 mg, with the minimum value of 74.6 mg and the maximum value of 585mg. The average t_{max} was obtained at 96.23 hours (range: 14.47 hours and 159.56 hours).

Modeling and Simulation

The two-compartmental PK model, based on the prior information (parameters) from data Phase I single dose, Phase I multiple dose & Phase IIa multiple dose were modelled hierarchically with the use of Bayesian analysis. The parameters estimate to $CL, Q, V_1, V_2, k_a, \Omega$ with 95% CrI. The posterior distribution was obtained and then two models were simulated by Monte-Carlo simulations.

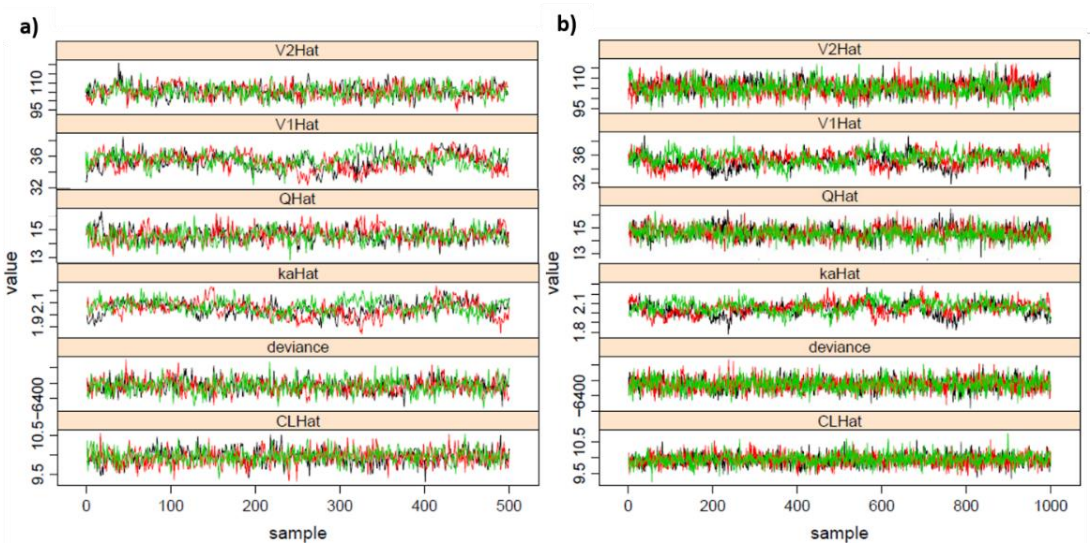


Figure 30: Trace of some parameters estimated which are V_2, V_1, Q, k_a, CL. a) At 10.000 iteration, the first 5.000 were discard with a thinning parameter of 10; b) At 15.000 iteration, the first 5.000 were discard with a thinning of 10. Each colour (red, green and black) of trace represents a chain of simulation.

To compare the simulated models, the generated parameters were used to assess convergence and adequacy of sample sizes by MCMC. At 10.000 iteration (Figure 30a) the chains did not show good convergence, while at 15.000 iteration (Figure 30b) better convergence values were observed. Based on these findings, the final PK model selected was the one with 15.000 iterations. The parameters estimated for the final PK model and VPC are represented in Table 9 and Figure 31 to Figure 38.

Table 9: Pharmacokinetic prior distribution of parameters in healthy volunteers with two-compartmental model.

	Mean	SD	Naive SE	Time-series SE	CrI [2.5% – 97.5%]	Effective N
CL	9.93	0.188	0.00344	0.00438	9.57-10.3	1910
Q	14.6	0.507	0.00925	0.0184	13.6-15.6	804
V_1	35.3	1	0.0183	0.0727	33.3-37.2	184
V_2	105	3.73	0.068	0.131	98-112	806
k_a	2.05	0.0669	0.00122	0.00526	1.92-2.18	175

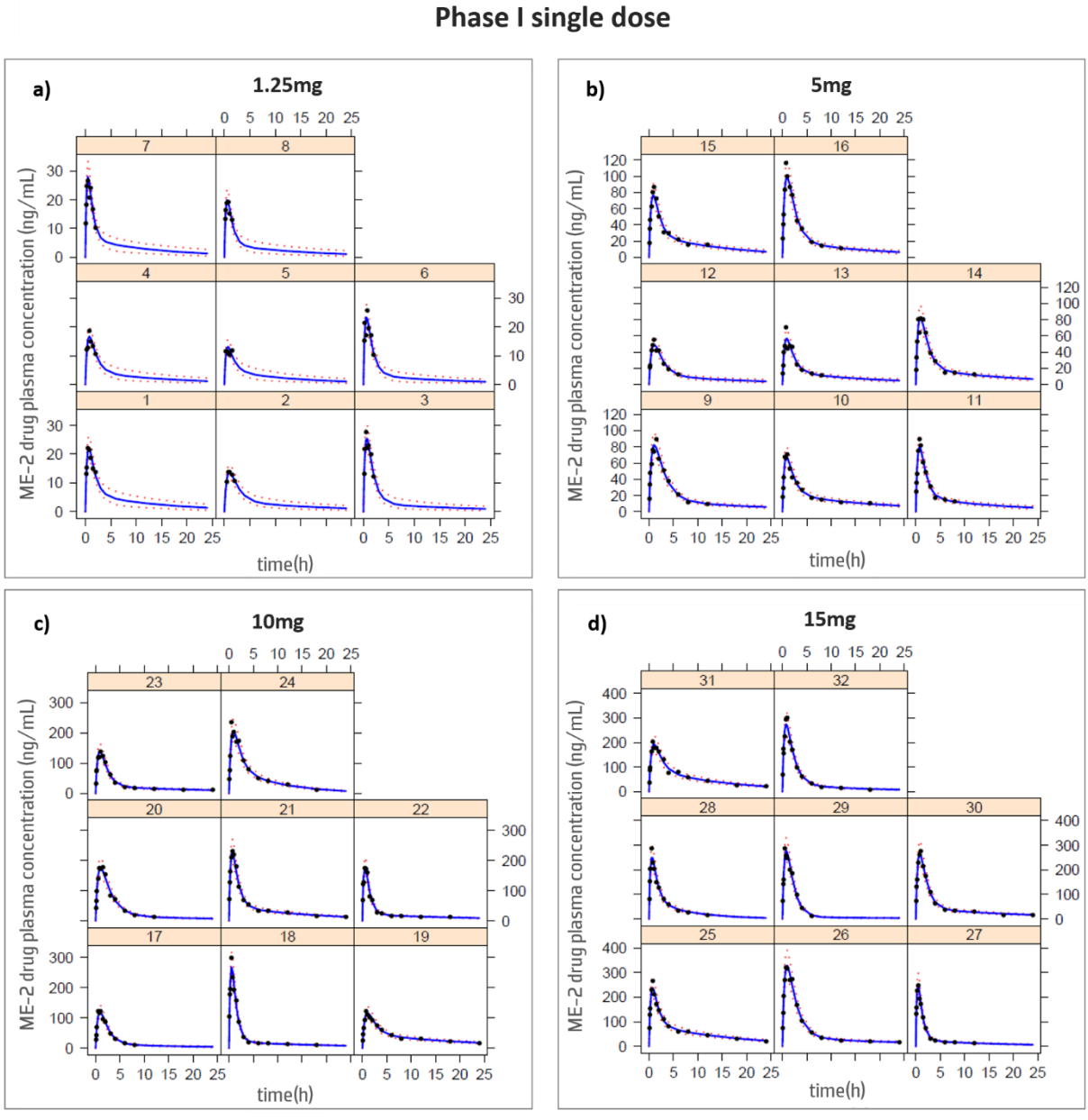
In general, this table demonstrates that the presented model had a posterior distribution of parameters estimated (e.g. CL, Q, V_1, V_2, and k_a) with acceptable precision.

The residual based diagnostic plots of the final model are represented in the supplementary materials S8, the figures revealed that the residuals fall on a blue straight line implying that the errors are normally distributed, and the random effect parameters are well distributed over the unit line. This suggests that the model is adequate.

Based on simulation, three graphics were obtained related to PK from individual subject's predictions per doses (Figure 31 to Figure 34) and other three more from population predictions per doses (Figure 35 to

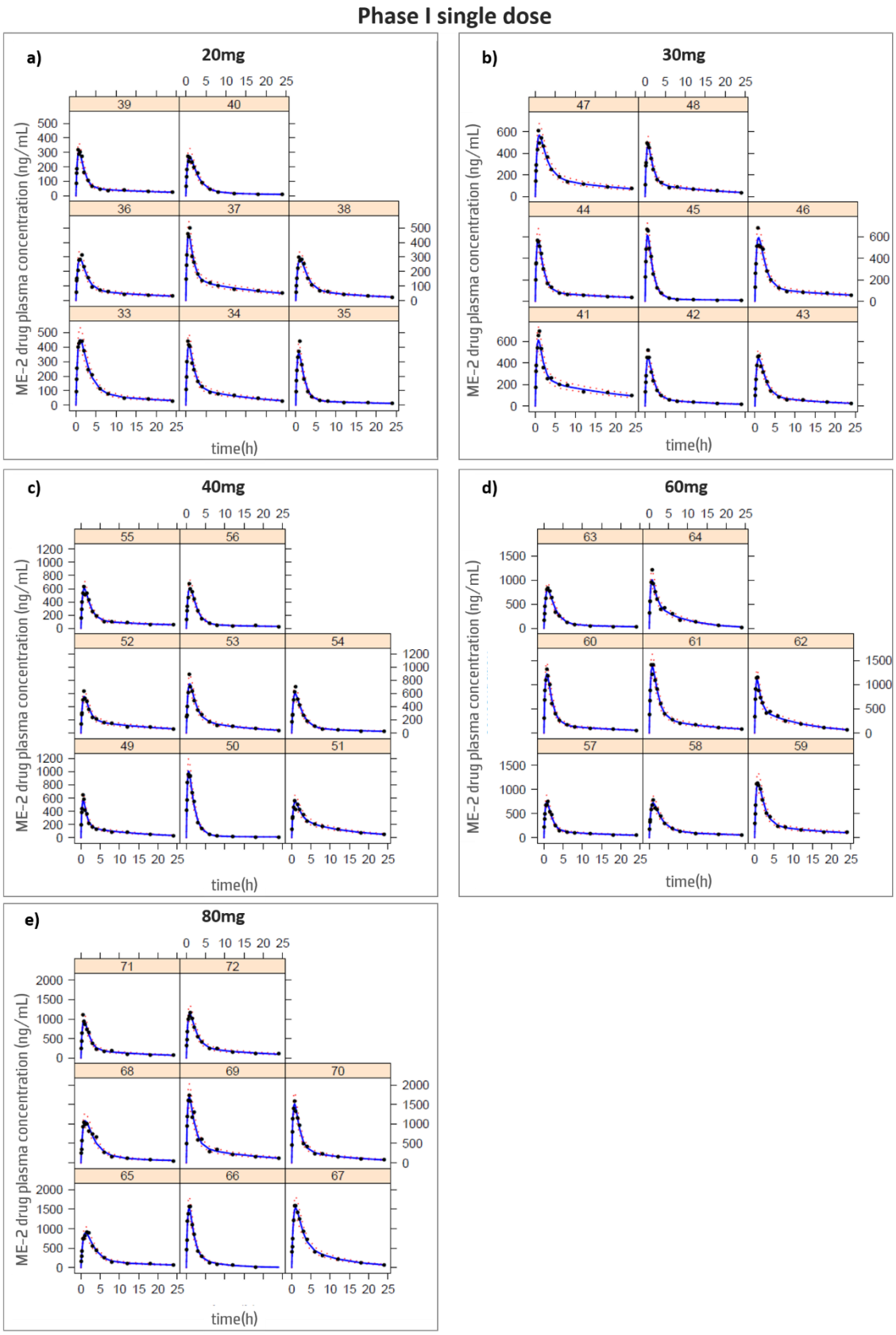
Figure 38). It is possible to extract C_{max} and t_{max} by observing the graphics created for each study regarding each dose of the drug.

In the Phase I single dose (Figure 31 and Figure 32), the C_{max} for 1.25mg, 5mg, 10 mg and 15mg was about 20 ng/mL, 80ng/mL, 150ng/mL and 300ng/mL, respectively. The C_{max} for 20mg, 30mg, 40mg, 60mg and 80mg was roughly 400ng/mL, 600ng/mL, 700ng/mL, 900ng/mL, 1200ng/mL. Overall, the t_{max} occurs between 0.5h to 1.5h. Regarding the shape of the concentration curve, a fast decrease of the median posterior predicting line was observed after the C_{max}.



o, observed values; —, simulated median posterior; ····, 95% credible interval

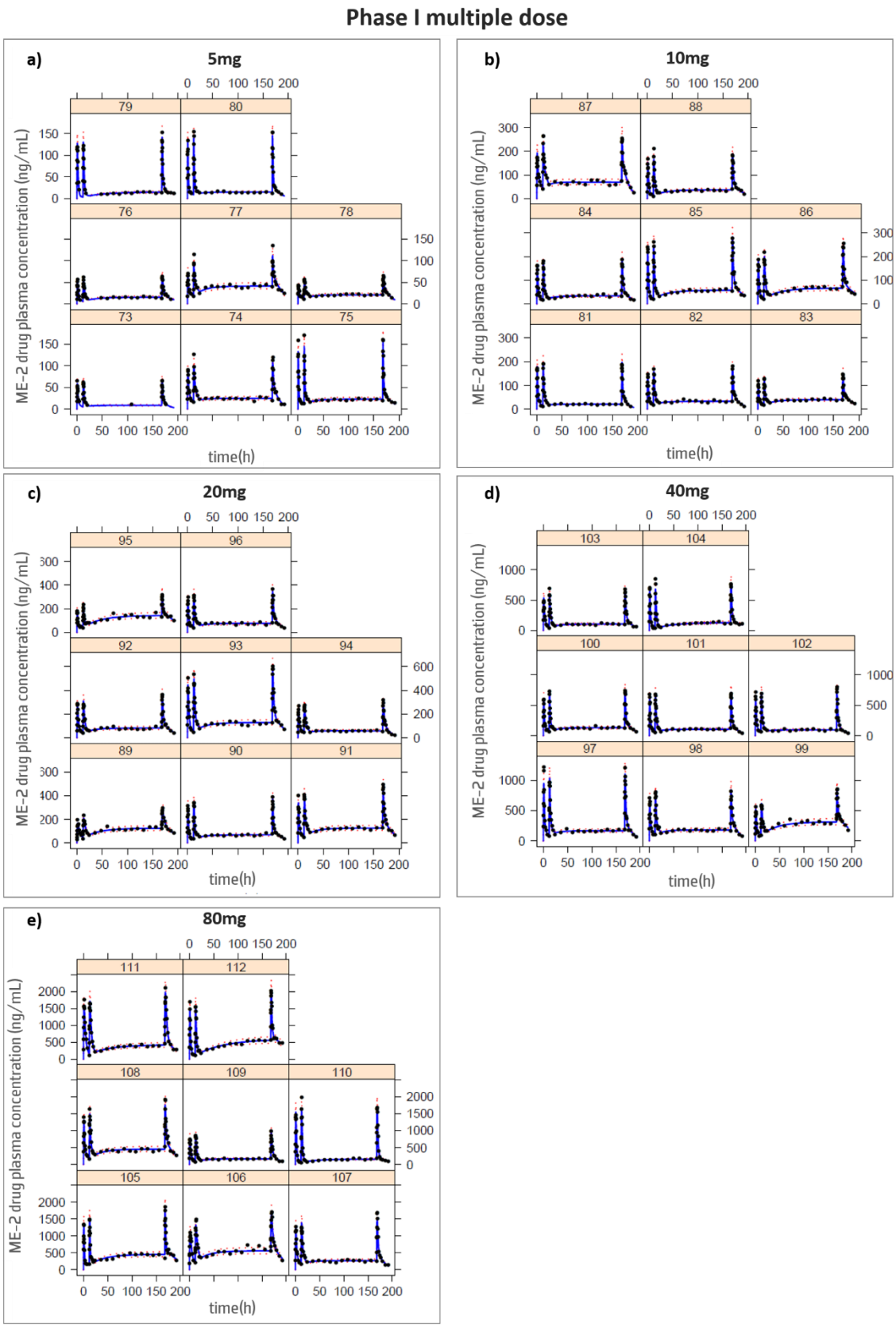
Figure 31: Phase I single dose Individual PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per doses of ME-2 drug. a) 1.25mg; b) 5mg; c) 10mg; d) 15mg.



o, observed values; _, simulated median posterior; ····, 95% credible interval

Figure 32: Phase I single dose Individual PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per doses of ME-2 drug. a) 20mg; b) 30mg; c) 40mg; d) 60mg; e) 80mg;

After Phase I multiple dose (Figure 33), the C_{max} after first, second and third administration of the 5mg, was 70ng/mL, 100ng/mL and 130ng/mL, respectively. For 10mg the first C_{max} was near 150ng/mL, second peak was 200ng/mL, and the third near 220ng/mL. Concerning 20mg the C_{max} after first, second and third administration dose was around 200ng/mL, 300ng/mL and 400ng/mL, respectively. For the 40mg, the first, second and third C_{max} after each dose was observed to be close to 550ng/mL, 650ng/mL and 800ng/mL, respectively. Lastly, 80mg has a C_{max} of 1100ng/mL, after the first dose, the second nears 1500ng/mL and at the third dose the C_{max} was around 2000ng/mL. Again, for each dose the t_{max} occurs between the 0.5h to 2h. Regarding the shape of the concentration curve, a fast decrease of the median posterior predicted line is observed after the C_{max}.

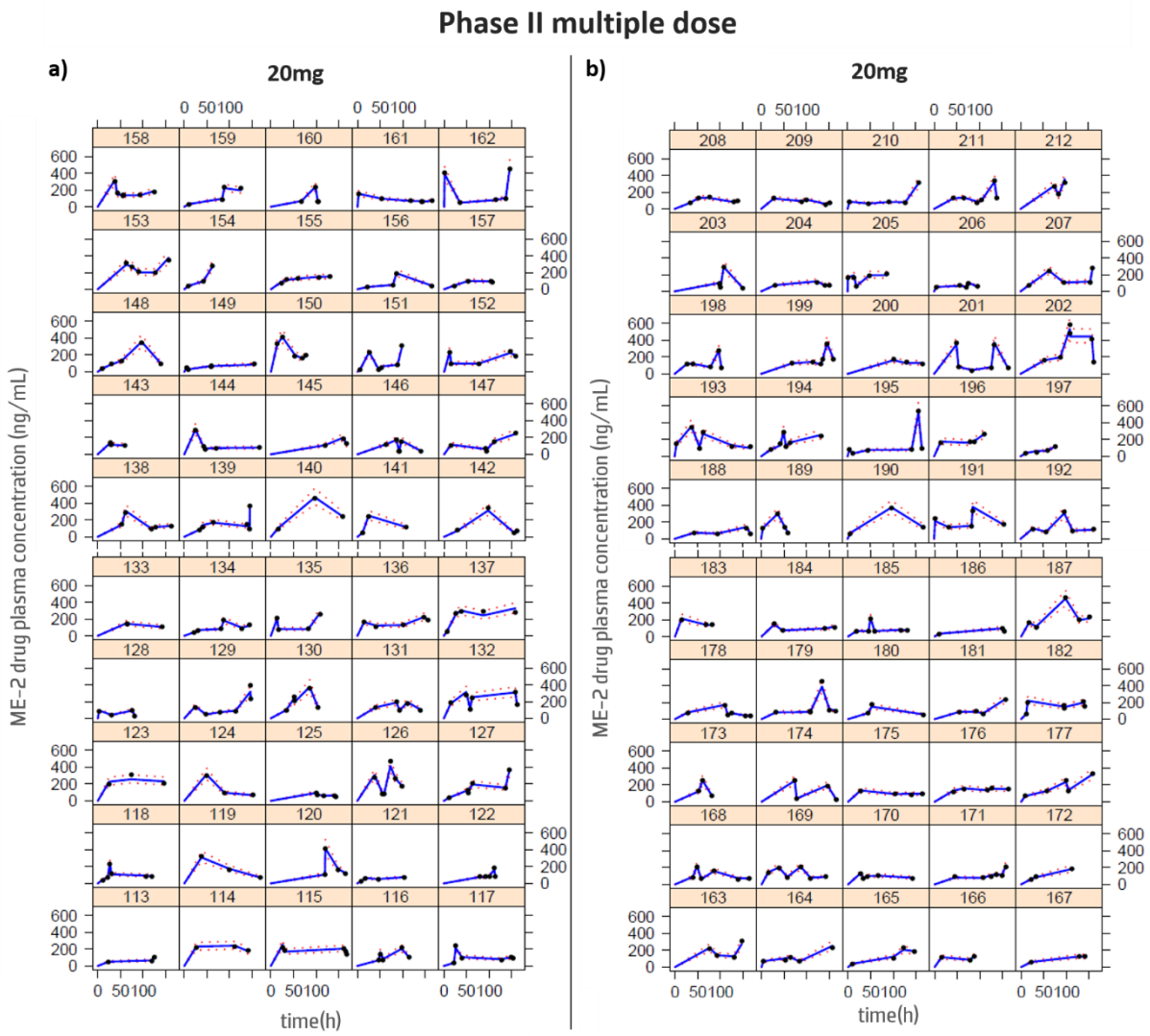


o, observed values; ⎯, simulated median posterior; ····, 95% credible interval

Figure 33: Phase I multiple dose Individual PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per doses of ME-2 drug. a) 5mg; b) 10mg; c) 20mg; d) 40mg; e) 80mg.

Overall, after multiple ME-2 doses no proportional increase between drugs and AUC was observed, and no substantial accumulation of ME-2 drug at steady state (day 7) was detected.

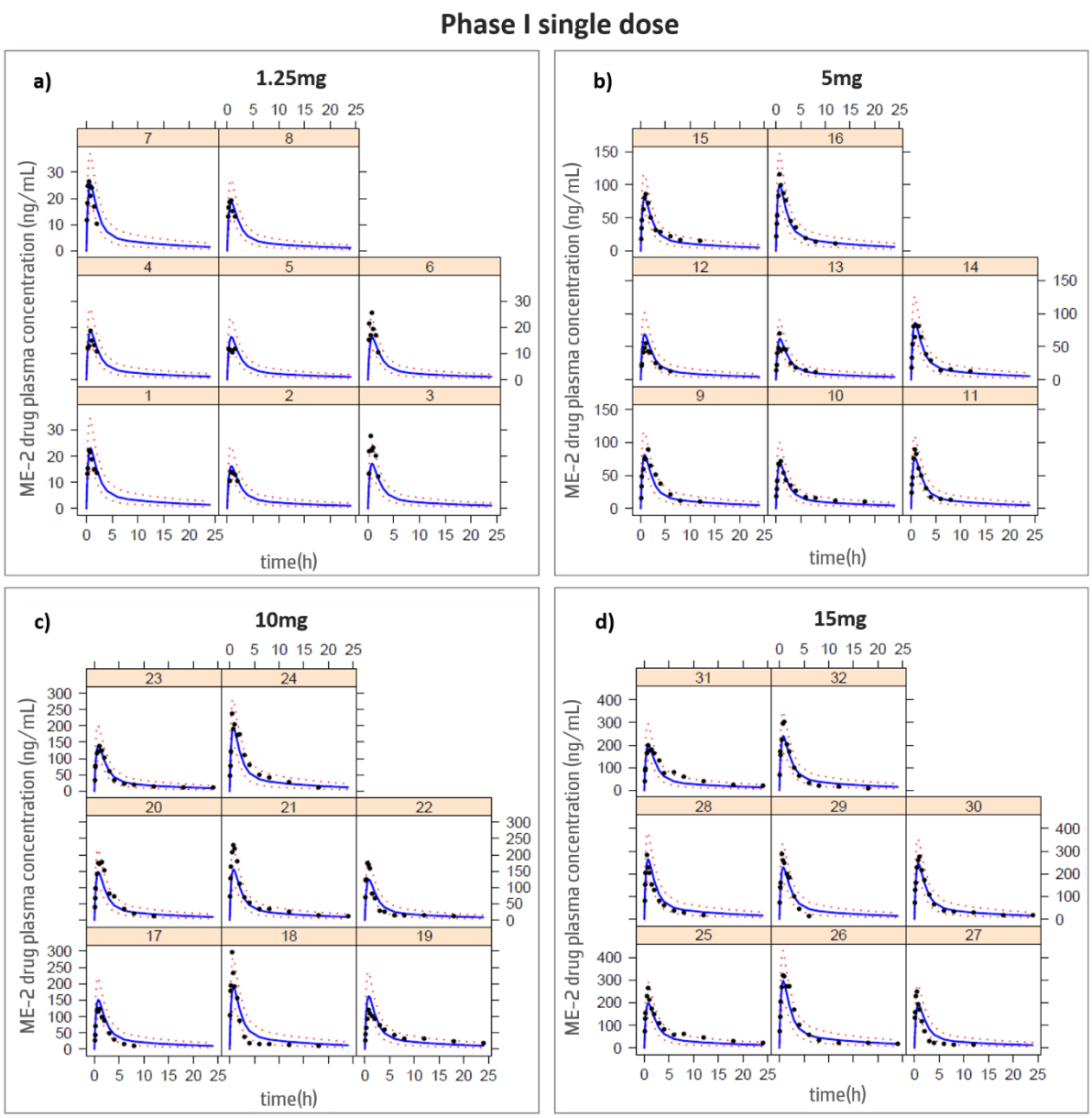
After Phase IIa multiple dose (Figure 34), it was observed that the C_{max} does not go beyond of 400ng/mL.



o, observed values; —, simulated median posterior; ····, 95% credible interval

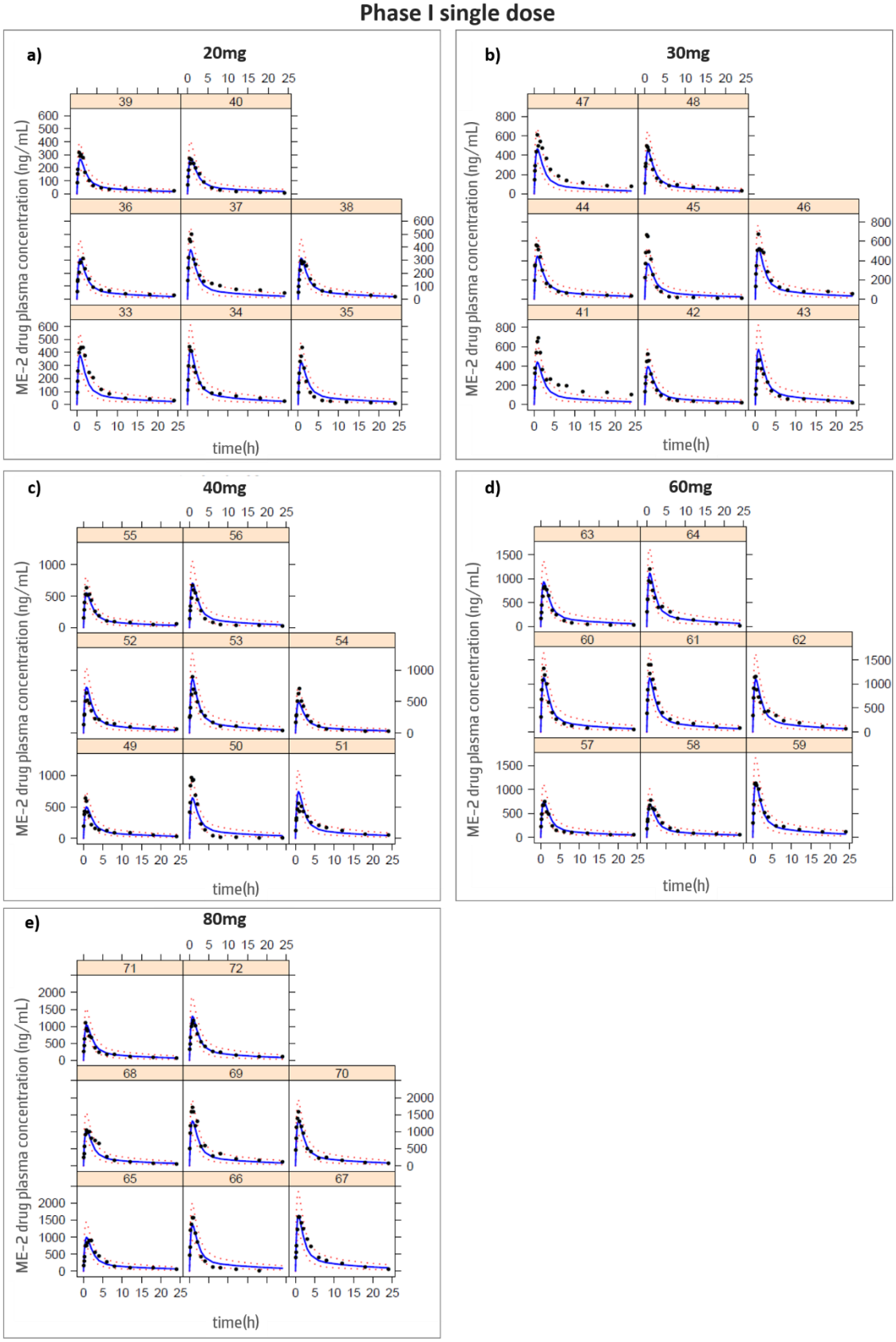
Figure 34: Phase IIa multiple dose Individual PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per 20mg of ME-2 drug. a) the subject number 113 to 162; b) the subject number 163 to 212.

The population analysis can be similar to the individual concerning C_{max} and t_{max} to Phase I single dose (Figure 35 and Figure 36), Phase I multiple dose (Figure 37) and Phase IIa (Figure 38).



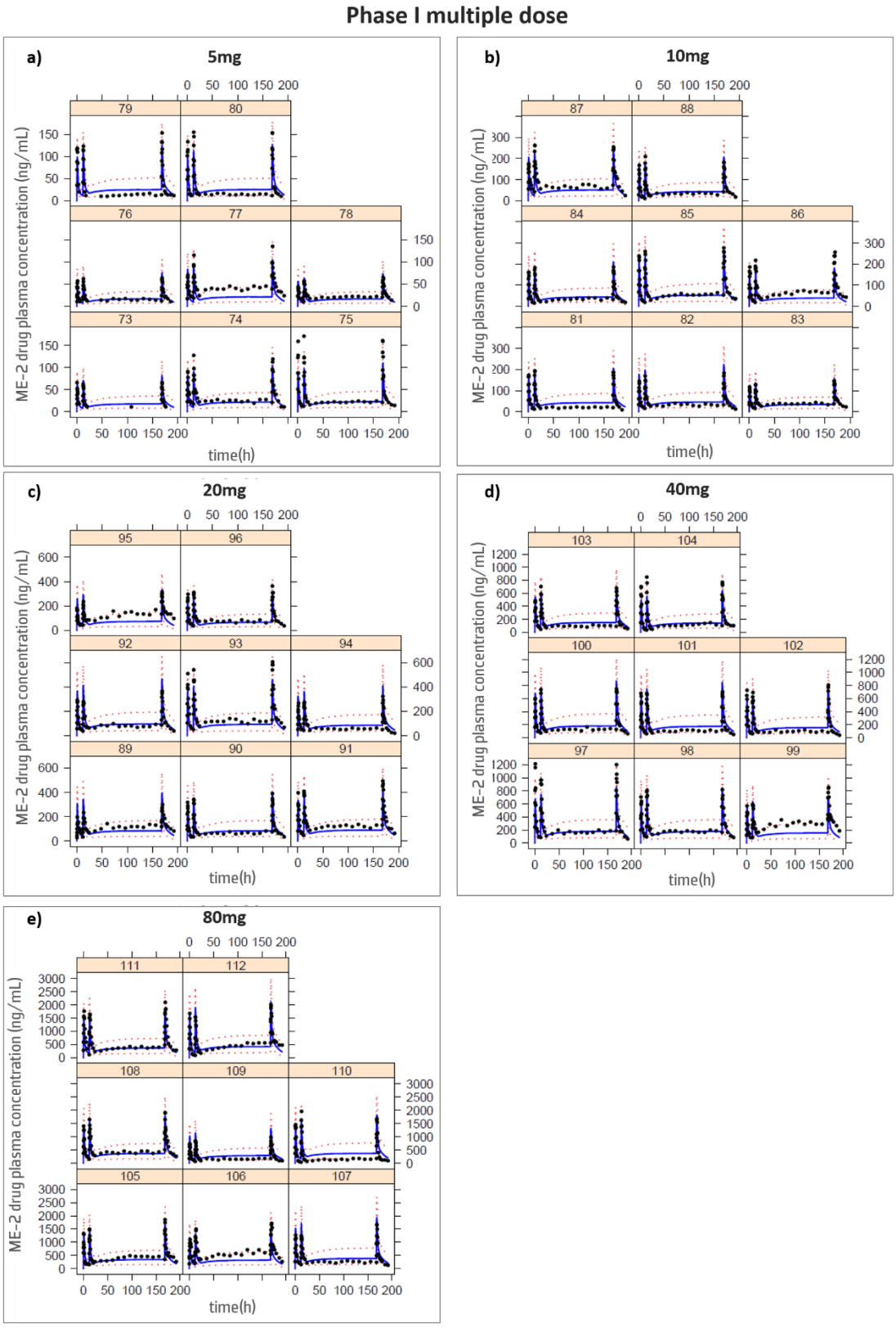
o, observed values; —, simulated median posterior; ····, 95% credible interval

Figure 35: Phase I single dose Populational PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per doses of ME-2 drug. a) 1.25mg; b) 5mg; c) 10mg; d) 15mg.



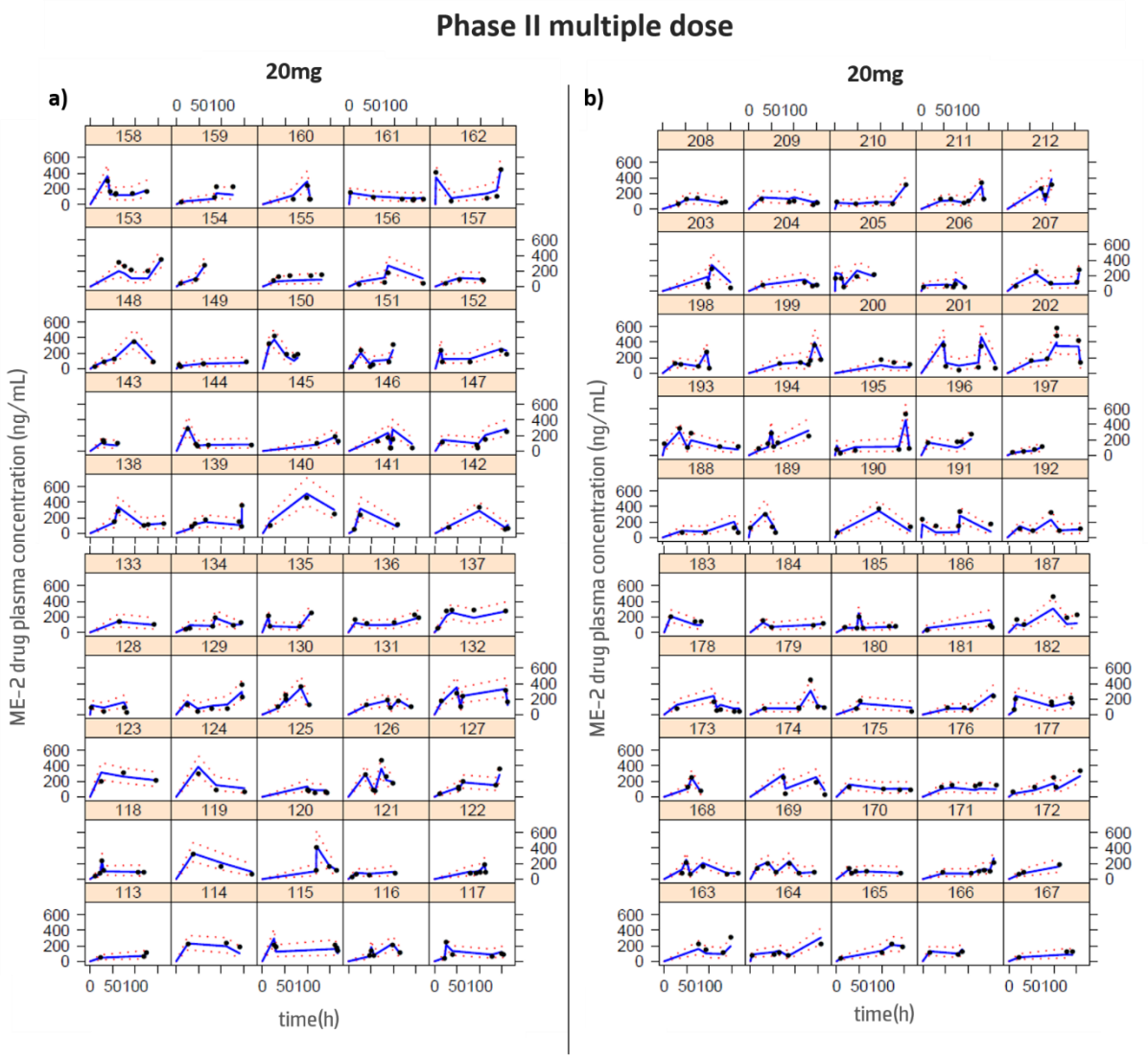
o, observed values; _, simulated median posterior; ····, 95% credible interval

Figure 36: Phase I single dose Populational PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per doses of ME-2 drug. a) 20mg; b) 30mg; c) 40mg; d) 60mg; e) 80mg.



o, observed values; _, simulated median posterior; ····, 95% credible interval

Figure 37: Phase I multiple dose Populational PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per doses of ME-2 drug. a) 5mg; b)10mg; c) 20mg; d) 40mg; e) 80mg.



o, observed values; —, simulated median posterior; ····, 95% credible interval

Figure 38: Phase IIa multiple dose Populational PK Predictions the ME-2 drug plasma concentration (ng/mL) by time per 20mg ME-2 drug. a) the subject number 113 to 162; b) the subject number 163 to 212.

8.4. Discussion on case study 3

This study demonstrated that the two-compartmental model was a good model to describe oral ME-2 in different phases of clinical trials. This means that the clinical ME-2 drug passed the Phase I single dose, Phase I multiple dose and Phase IIa with success. Contrary to some reviews, we show that clinical trial use of $2ME_2$ was cancelled because its plasma concentrations after oral administration was lower than the effective dose, meaning the drug has low bioavailability (14,15).

Also, this study demonstrated that oral ME-2 given once a daily might have the same efficacy which had been demonstrated on the previous studies PK/PD 20mg in chapter 6 (80% inhibition). In addition, this 20mg dose does not show adverse events associated with it, according to the study in chapter 7, even in

multiple doses. This dose of ME-2 drug in phase IIa is lower than the standard drug observed in literature, which shows a dose of Enoxaparin of 40mg (43). However, other studies have shown that to inhibit Factor Xa only 10mg of oral rivaroxaban was needed but the time where the C_{max} occurs was within 2.5 to 4 hours while the ME-2 drug was within 0.5 to 2 hours (50).

The individual analysis was very well fit, and the populational analysis was very similar to the individual. However, a wider gap was observed between the line of CrI.

8.5. Conclusion on case study 3

Considering this study, the 20mg dose regiment of ME-2 drug was defined has having a similar efficacy to Enoxaparin drug to where it also might safely reduce the risk of VTE in patients undergoing total knee replacement without hemorrhage.

Moreover, the population analysis had a bigger CrI width than the individual analysis, as was expected. This difference means that within-subject variability in all end points was less than between-subject variability. In fact, this does not mean that the Population model was incorrect because the CrI is estimated from the parameters and their variations.

9. Conclusion

In this report, individual and populational PK/PD models were built for an antithrombotic drug study. Unlike the populational posterior predictions, individual posterior predictions were very close the CrI, because the population analysis includes the inter-subjects' variations and the individual only includes intra-subjects' variations. The population analyses can support the optimization of individual dosing regimens by modelling PK/PD.

In the first case study, Chapter 6, the time-average biomarker as a function of plasma ME-2 drug concentrations a simple nonlinear regression was modelled. With this, it was possible to observe differences between the eight doses of ME-2 drug. This demonstrated a good profile between PK/PD, however, some side-effects were observed in some subjects, which meant that subjects had neutropenia.

In the second case study, Chapter 7, the semi-mechanistic model proposed by Friberg and Karlsson was used to model the drug's plasma concentration by absolute neutrophil count. With that model it was possible to evaluate the different stages of neutrophil maturation to understand the relationship between drug exposure and server-side effects. After that, in the third case study, a two-compartmental model was applied, a more complex model than the one used in the first case. This model showed a graphical improvement of the PK / PD profile. Moreover, the study was comprised of three clinical trials of ME-2drug: Phase I single dose; Phase I multiple dose; and Phase IIa. With this case study it was possible to use that PK model to understand individual and populational analysis according to the body weight.

We considered the best model to be the two-compartmental model to describe the PK/PD model (according to the body weight) and the best model to understand the side effect was considered to be the semi-mechanistic model proposed by Friberg and Karlsson.

The models' simulations were performed to replicate the same conditions of treatment and subjects. However, we suggest these simulations be applied to children as dose finding or another population with specific characteristics of the interest. There are ethical and physiological standards that apply only to this population (paediatric population), and one of those is that only a small number of blood samples can be collected. Furthermore, the nonlinear mixed effect model does not require frequent blood sampling as with the traditional analysis.

An appreciation of the statistical behaviour of the estimation tools and experimental designs used in PK/PD modelling have important economic, as well as drug labelling, therapeutic and scientific consequences.

9.1. Final Notes

During this period at BlueClinical, I had the possibility to take part in some clinical trials and understand the dynamics surrounding the company. It was undoubtedly a new area that I had never worked before and this internship allowed me to learn new skills and develop essential tools that will be useful in the future.

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S2: PK parameters of Phase I single dose of the ME-2 drug

Table S2: PK parameters of Phase I single dose of the ME-2 drug

ME-2	C_{max} mean (range)	t_{max} mean (range)	MI mean (range)	TMI mean (range)
0 mg	0 (0-0)	0 (0-24)	19.08 (8.15-31.1)	6.30 (0.167-24)
1.25 mg	20.73 (11.80-27.6)	0.75 (0.50-1.50)	31.61 (18.60-42.10)	1.56 (0.25-8.00)
5 mg	82.71 (55.80-116)	0.97 (0.75-1.50)	50.94 (35.80-75.30)	1.34 (0.75-2.00)
10 mg	188.13 (122-298)	0.81 (0.50-1.50)	68.59 (48.80-77.80)	0.78 (0.50-1.50)
15 mg	274.38 (202-322)	0.72 (0.50-1.00)	85.09 (67.90 – 99.80)	0.81 (0.25-1.5)
20 mg	379.50 (273-500)	0.84 (0.50-1.50)	89.38 (80.90-101)	0.88 (0.5-2.00)
30 mg	587.63 (466-693)	0.72 (0.50-1.00)	93.05 (82.70-103)	1.08 (0.17-3.0)
40 mg	714.63 (558-964)	0.72 (0.50-1.00)	97.83 (89.40-105)	1.02 (0.17-2.0)
60 mg	1075.75 (753-1410)	0.81 (0.50-1.00)	100.75 (93.50-108)	1.10 (0.08-3.0)
80 mg	1341.38 (911-1730)	0.84 (0.50-1.00)	105.75 (94.60-124)	0.81 (0.25-1.5)

C_{max}, average of maximum concentration of each subject (ng/mL);

t_{max}, average of time where maximum concentration was observed (hours).

MI, average of maximum inhibition of Factor Xa activity (%).

T_{MI}, average of time where maximum inhibition of Factor Xa activity was observed (hours).

S3: Residuals for Individual/Population Predictions of PK/PD Model of Phase I single dose of ME-2 Drug

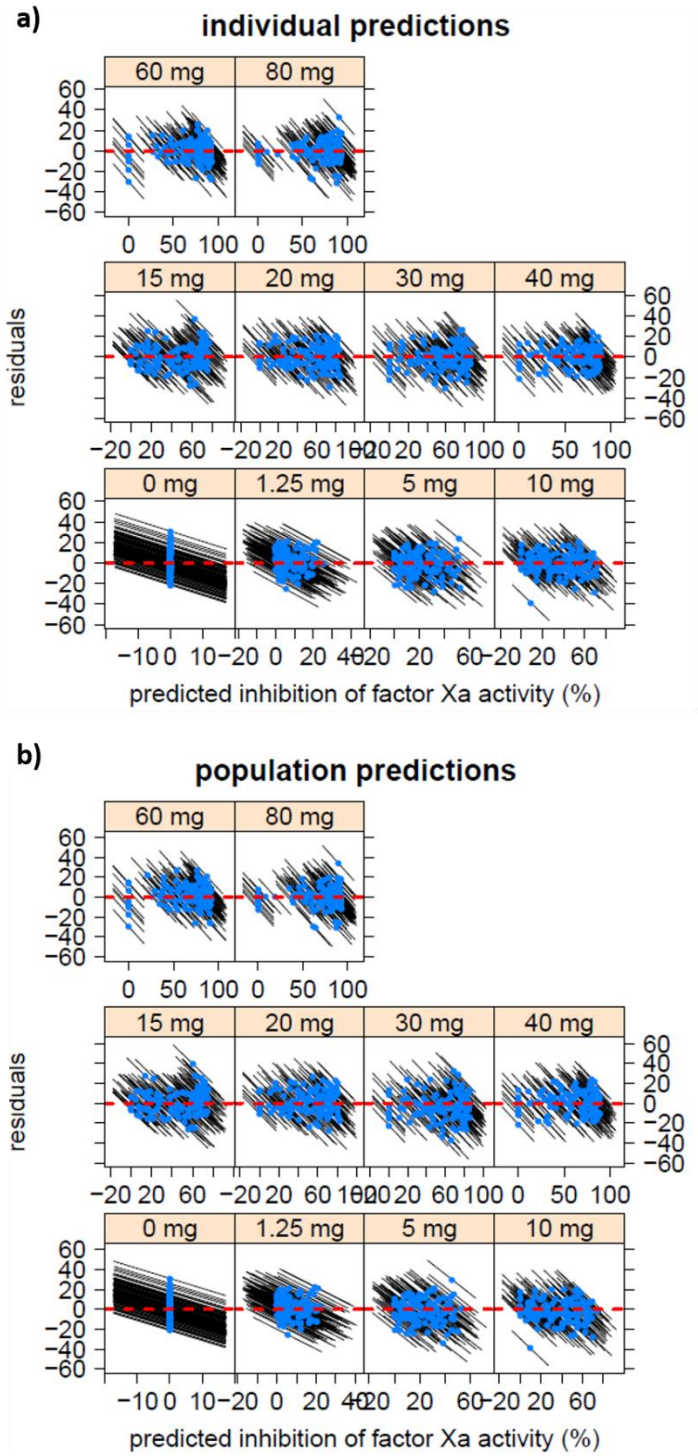


Figure S3: Goodness-of-fit plots for the predicted inhibition of factor Xa activity vs residuals. a) Individual predictions. b) Population predictions. Red dashed lines represent LOESS smoothing of data.

S4: Residuals for Individual/Population Predictions by time of PK/PD Model of Phase I single dose of ME-2 Drug

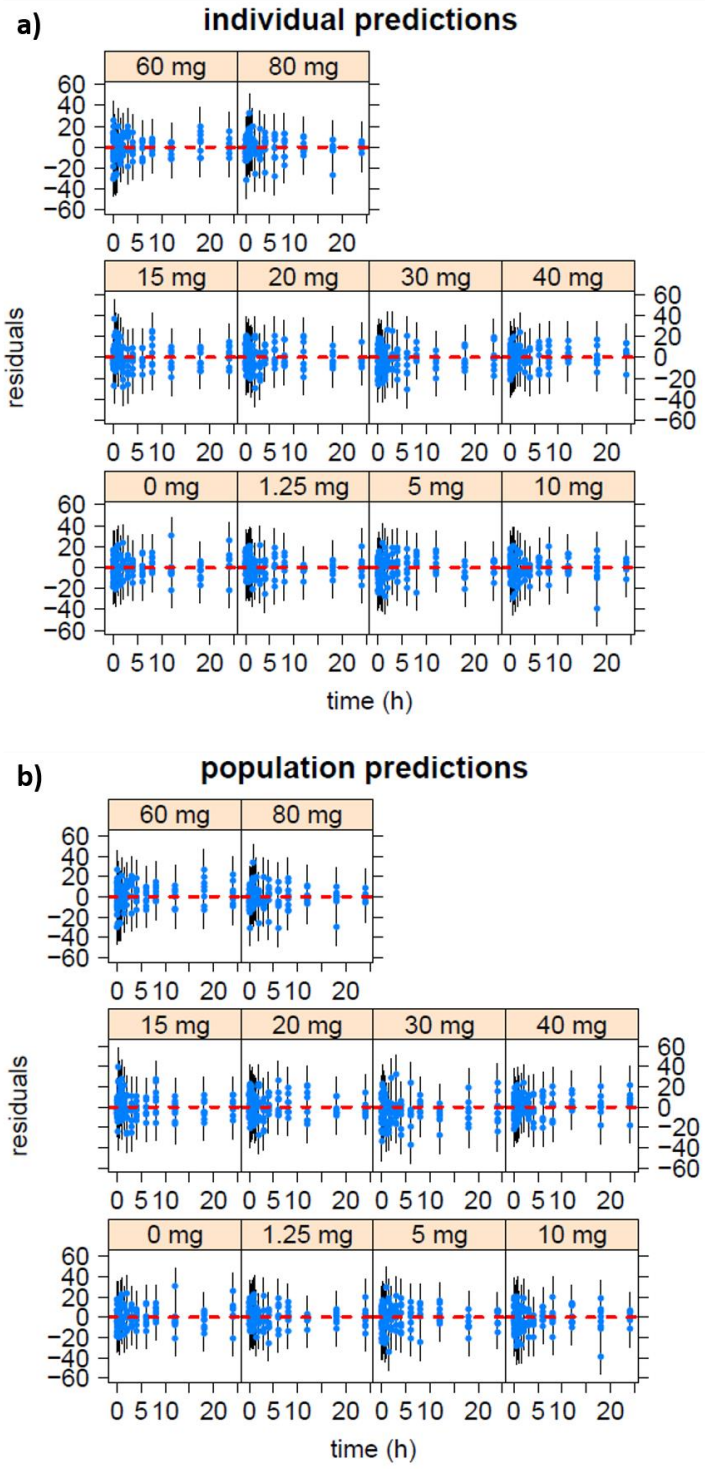
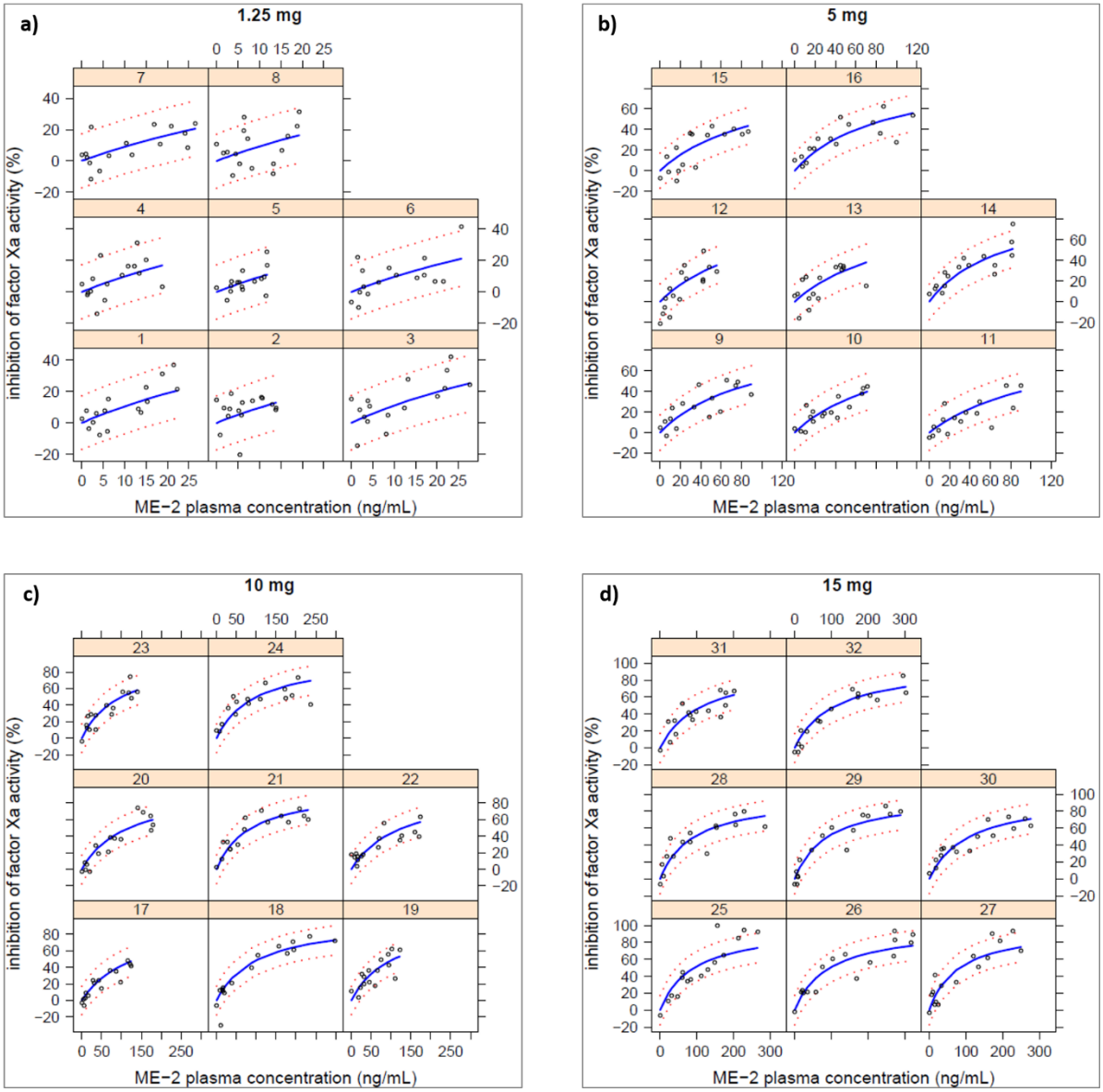


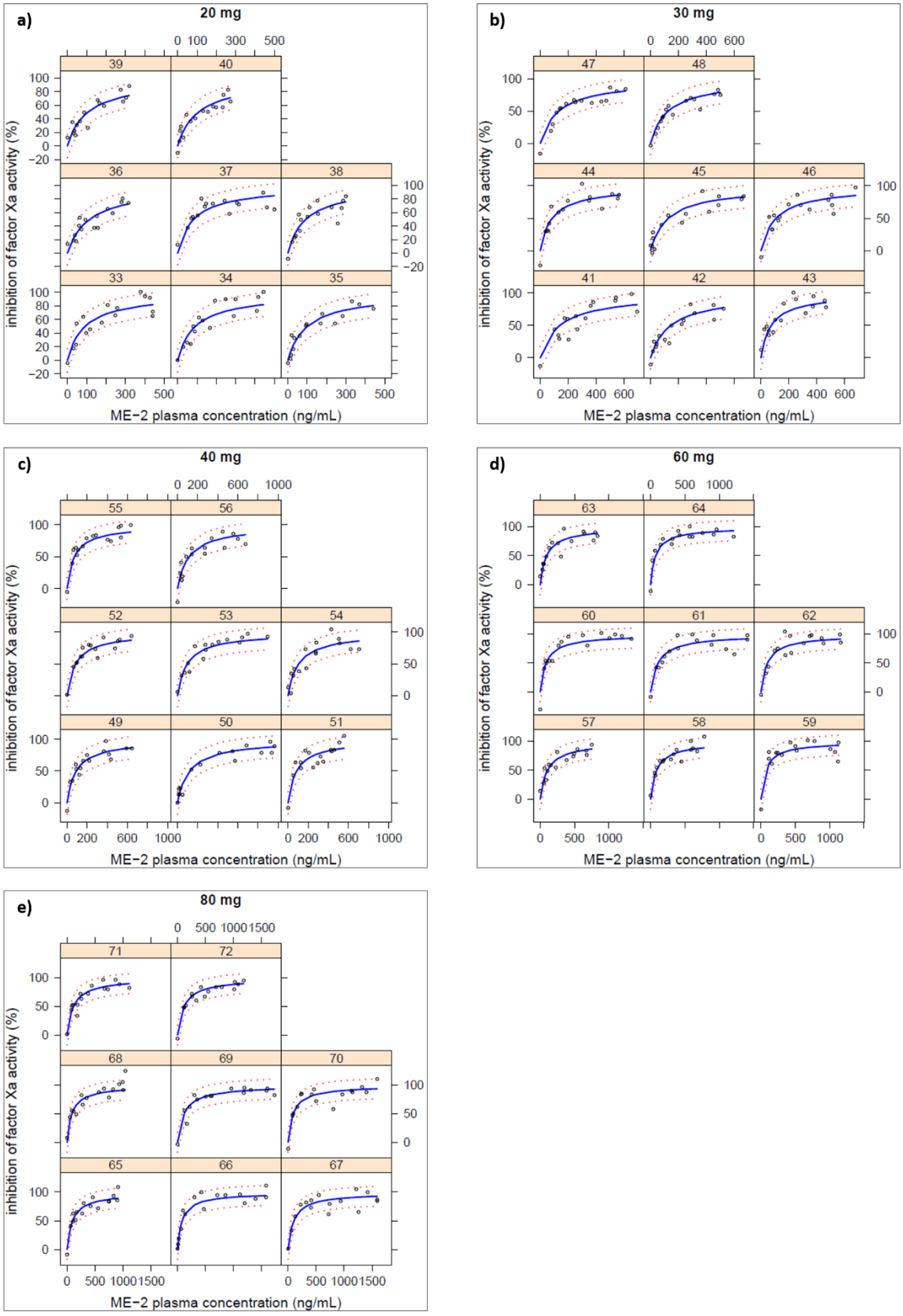
Figure S4: Goodness-of-fit plots for the residuals vs time after dose. a) Individual predictions. b) Population predictions. Red dashed lines represent the unity line y = 0.

S5: PD Individual Predictions of ME-2 plasma concentration to Inhibition of Factor Xa activity per doses



o, observed values; —, simulated median posterior; ····, 95% credible interval

Figure S5.1: PD Individual Predictions of ME-2 plasma concentration to Inhibition of Factor Xa activity per doses of ME-2. a) 1.25mg; b) 5mg; c) 10mg; d) 15mg.



o, observed values; _, simulated median posterior; ····, 95% credible interval

Figure S5.2: PD Individual Predictions of ME-2 plasma concentration to Inhibition of Factor Xa activity per doses of ME-2. a) 20mg; b) 30mg; c) 40mg; d) 60mg; e) 80mg.

S6: Baseline characteristics of all subjects Phase I multiple dose ME-2 drug

Table S6: Baseline characteristics of all subjects Phase I multiple dose ME-2 drug

Phase	ME-2	Age mean (range)	Female sex N (%)	bw mean (range)
Phase I multiple dose	0	33.00 (27-43)	1 (25)	69.50 (56-88)
	5	34.25 (26-45)	2 (50)	79.75 (63-98)
	10	29.00 (25-36)	2 (50)	72.25 (62-92)
	20	31.75 (26-40)	2 (50)	66.50 (59-71)
	40	33.5 (24-45)	3 (75)	68.50 (64-79)
	80	24.50 (20-31)	2 (50)	65.25 (57-75)

bw, bodyweight.

S7: Baseline characteristics of all subjects Phase I single dose, Phase I multiple dose and Phase IIa multiple dose ME-2 drug.

Table S7: Baseline characteristics of all subjects Phase I single dose, Phase I multiple dose and Phase IIa multiple dose ME-2 drug.

	ME-2	Age mean (range)	Female sex N (%)	bw mean (range)	C_{max} Mean (range)	t_{max} mean (range)
Phase I single dose	1.25	30.25 (21-37)	1 (10)	75.50 (53-87)	20.70 (11.80-27.3)	0.75 (0.50-1.50)
	5	31.13 (24-49)	6 (75)	72.75 (54-91)	82.71 (55.80-116)	0.97 (0.75-1.50)
	10	30.50 (20-41)	4 (50)	71.50 (56-90)	188.13 (122-298)	0.81 (0.50-1.50)
	15	28.63 (20-41)	3 (37.5)	73.00 (55-89)	274.38 (202-322)	0.72 (0.50-1.00)
	20	33.50 (22-44)	5 (62.5)	67.75 (51-85)	379.50 (273-500)	0.84 (0.50-1.50)
	30	29.25 (22-37)	5 (62.5)	74.00 (57-92)	587.63 (466-693)	0.72 (0.50-1.00)
	40	31.25 (24-38)	6 (75)	70.13 (50-92)	714.63 (558-964)	0.72 (0.50-1.00)
	60	29.50 (25-42)	5 (62.5)	68.75 (57-98)	1075.75 (753-1410)	0.81 (0.50-1.00)
	80	30.38 (25-38)	3 (37.5)	72.50 (53-91)	1341.38 (911-1730)	0.84 (0.50-1.00)
Phase I multiple dose	5	28.88 (20-45)	4 (50)	74.13 (53-100)	114.7 (63.6-160)	169.1 (168.5-169.9)
	10	30.25 (19-42)	4 (50)	67.25 (51-92)	207.75 (147-279)	168.8 (168.5-169)
	20	32.00 (24-42)	4 (50)	72.25 (59-98)	395.4 (299-607)	168.9 (168.75-170)
	40	33.63 (24-45)	3 (37.5)	74.13 (64-92)	824.12 (680-1210)	168.81 (168.5-169.5)
	80	29.13 (20-38)	6 (75)	65.38 (51-88)	1748.25 (986-2110)	168.91 (168.5-170)
Phase II	20	40.44 (26-64)	48 (48)	81.15 (51-119)	248 (74.6-585)	96.23 (14.47-159.56)

C_{max}, average of maximum concentration of each subject;

t_{max}, average of time where maximum concentration was observed.

S8: Residuals for PK Predictions Model of Phase I single dose, Phase I multple dose + Phase IIa of ME-2 Drug

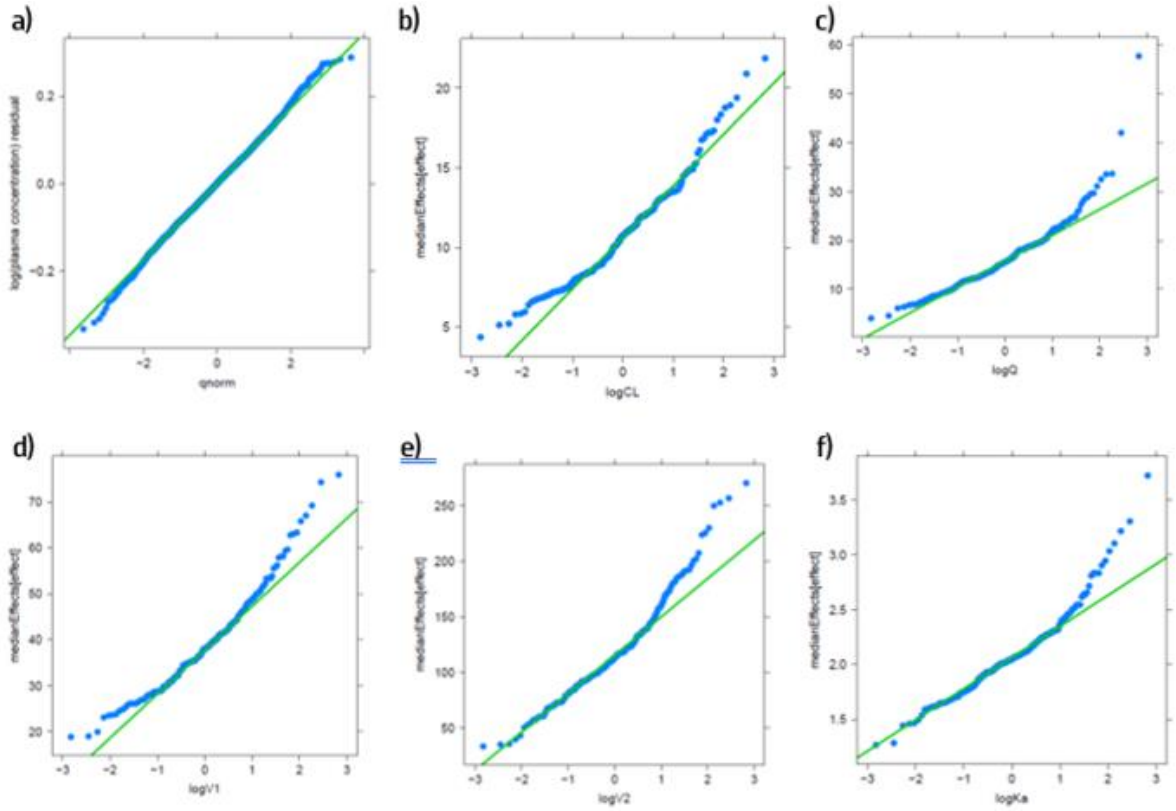


Figure S8: Goodness-of-fit plots for the final PK model. A) Residuals by standard normal quantile plots. b) CL vs. random effect plots; c) Q vs. random effect plots; d) V_1 vs. random effect plot; e) V_2 vs. random effect plot; f) k_a vs. random effect plot.