Quantifying Variability in drug disposition, response and public health outcomes: The implementation of pharmacometric based modeling and simulation approaches

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ABSTRACT: The aim of the dissertation was to identify the systematic contributors that modify the estimated population parameters and that explain sources of variability in drug exposure (Chapter 2-4), response, and clinical outcome (Chapter 5-7). The source of measurable variability evaluated in the thesis include patient characteristics in chapter 2-3, patient behavior in chapter 4 (e.g. dosing history), biological system in chapter 5-7, and inferior clinical practice in chapter 5-7. The dissertation was predominantly non-linear mixed effect modeling and Monte Carlo simulation methods in NONMEM® and R. Our results in chapter 2-4 showed that incorporating covariate information into population PK models identified substantial systematic contributors to the variability in drug exposure for both perphenazine and escitalopram. Race and smoking status in the past week were identified as two significant covariates affecting clearance of perphenazine. CYP 2C19 genotype, age, and weight strongly influenced the CL/F of escitalopram. The measurement error associated with an incorrect or incomplete dosing history affected the population PK parameter estimation of escitalopram in the non-linear mixed effect modeling process. Furthermore, our simulation results in chapter 5-7 showed that three intervention approaches may lead to lower cardiovascular risk

compared to current clinical practice strategy: 1) BP can be calibrated with respect to clinic visit times with consideration of PK/PD/dosing regimen. 2) BP-misclassification in current clinical practice is around 20~45% depends on clinic visit time. Optimal clinic visit time exists. In general, patients should avoid early morning and late afternoon visit which lead to the highest BP misclassification. 3) It is important to decrease patients' BP in a timely fashion. Initiating antihypertensive treatment with the higher tolerable dose as well as setting a lower goal BP of 120 mm Hg resulted in a significantly lower cardiovascular risk. In conclusion, the dissertation identified three potential interventions to be considered in the clinical practice or antihypertensive drug labeling for better BP management: BP calibration based on clinic visit time; patients should generally have post treatment clinic visit times between 11:00 AM ~ 3:00 PM; a high dose strategy for antihypertensive drug therapy.

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LIST OF ABBREVIATIONS BP: BLOOD PRESSURE

CATIE: the Clinical Antipsychotic Trials of Intervention Effectiveness

CI: Confidence intervals

CTS: Clinical Trial Simulation

CVD: Cardiovascular disease

CWRES: Conditional Weighted Residuals

EM: Extensive Metabolizer

FDA: Food and Drug Administration

GOF: Goodness-of-fit

IM: Intermediate Metabolizer

IPRED: Individual Predictions

MEMS: medication event monitoring system

PD: pharmacodynamics

PK: pharmacokinetics

PM: Pharmacometrics

PM: Poor Metabolizer

PI: Prediction intervals

PRED: Population predictions

PPC: Posterior predictive check

RES: Residuals

RM: Rapid Metabolizer

SE: Stander errors

SSRI: Selective Serotonin Reuptake Inhibitor

VPC: Visual predictive check

WRES: Weighted Residuals

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1.0 INTRODUCTION

1.1 OVERVIEW

Pharmacometrics (PM), as a rapidly evolving scientific discipline, has received increased attention from Food and Drug Administration (FDA), academia, and industry in recent years (1-7) Pharmacometrics requires knowledge across multiple disciplines including pharmacokinetics (PK), pharmacodynamics (PD), pharmacology, statistics, and computer science. The discipline has been described as Quantitative Pharmacology (QP) (8, 9). However, there is no consistent definition for the discipline. The most recent definition of the discipline is "the science of quantifying disease, drug, and trial characteristics with the goal of influencing drug development and regulatory and therapeutic decisions."(10). It also has been defined as "research focusing on non-linear mixed effects models, which describes response-time profiles observed in clinical trials with a focus on determining sources of variability within a studied population" (1) and "the science of developing and applying mathematical and statistical models to characterize, understand, and predict a drug's pharmacokinetics (PK), pharmacodynamics (PD), and biomarker-outcome behavior" (11). Pharmacometrics not only provides opportunities for model based drug development but also impacts drug approval, labeling decisions and has the potential to clarify clinical practice and public health issues.(2, 3)

Pharmacometrics uses two major techniques: modeling and simulation. Population modeling is a robust tool that estimates the central tendency and quantifies the potential sources of variability in drug exposure, efficacy measures, and adverse events in target populations. Patients in the target population may not have the same level of drug exposure or response given the same dose at the same time. The larger the variability in drug exposure and response in the intended population, the higher the risk of an efficacy and safety issue. An advantage of population modeling techniques is that sources of variability can be assessed and quantified. A portion of the variability in the drug

exposure and response may be explained by deterministic information, such as patient characteristics (e.g. age, gender, genotype, renal function et al.). The contribution to the variability that is explained by these factors is often defined as measurable variability. Some sources of measureable variability are unknown or unmeasured, and are lumped in the random effects of the mixed-effects model.

Simulation has been widely described as one of the most important applications of the population models (11-13). It has been used to interpolate situations between the original conditions where data were collected and then extended to predict new conditions, predicting the range of variability and uncertainty of relationship (12). The simulation approach has also has been increasingly used for the optimization of clinical trial designs by performing clinical trial simulation (CTS). Clinical trial simulations may also have a role in the public health area. The utility of clinical trial simulations applied to public health questions may include identifying potential issues associated with current clinical practice and the in-silico evaluation of the impact of potential clinical practice strategies on clinical outcomes. The models that have applicability here could include population PK/PD models, disease progress model, survival models etc.

As we discussed above that variability in drug exposure and response include measurable variability and true random variability. However, some sources of measureable variability are lumped in the random variability when an exact source of the systematic variability is not identified due to a lack of knowledge or measurement of deterministic information.

The major aim of this thesis is to use the modeling and simulation approaches to identify the systematic contributors that modify the estimated population parameters and that explain sources of variability in drug exposure (Chapter 2-4), response, and clinical outcome (Chapter 5-7). The source of measurable variability evaluated in the thesis include patient characteristics in chapter 2-3 (e.g. age, genotype et al.), patient behavior in chapter 4 (e.g. a patient's detailed dosing history), biological system in chapter 5-7 (e.g. natural rhythm of BP variability on a daily basis), and inferior clinical practice in

chapter 5-7 (e.g. follow up visit time of patients). Specifically, the thesis accomplishes the aime by addressing these following issues:

1) Chapter 2 and 3 aimed to identify the effect of patient characteristics on variability of drug disposition using highly sparse sampling measurement for both escitalopram and perphenazine.
2) Chapter 4 aimed to evaluate the effect of measurement error in dosing time on population PK parameter estimation using escitalopram as a model drug and the SPECTRUM data as a prototype clinical trial, specifically comparing patient reported time of last dose and medication event monitoring generated dosage histories.
3) Chapter 5 aimed to quantify the impact of cuff BP measurement error as well as ignoring the circadian rhythm of BP on measured treatment effect of antihypertensive agents using a current clinical practice paradigm.
4) Chapter 6 aimed to quantify the impact of ignoring the timing of patient clinic visit times and the cuff BP measurement error on the BP misclassification rate in the current clinical practice paradigm.
5) Chapter 7 aimed to quantify the risk of a cardiovascular disease related event associated with current clinical practice strategies as well as alternative strategies in patients with hypertension.

1.2 POPULATION ANALYSIS APPROACH

Biological and physiological systems are very complex and thus an ideal model may never be identified. This is made more challenging when one considers the interactions of the drug, physiologic and biological system. Despite these challenges, useful models can be developed based on the specific question that is being addressed in a particular system. The analysis approaches in population modeling include the naïve averaged data and pooled data approach, two stage approach, as well as non-linear mixed effect models. The naïve averaged data and pooled data approach is commonly used in preclinical pharmacokinetics or in getting initial estimates for more complicated non-linear mixed effect modeling analysis approach. The naïve pooled or average approaches have some significant drawbacks in that the actual shape of the response can be distorted by not

considering the within individual correlation and there is no information that can be gleaned regarding inter-individual variability in the response measure (11, 14). The two stage approach is a widely used approach due to its simplicity compared to non-linear mixed effect modeling. The first stage of this method is to estimate individual parameters separately without considering the correlation between individual data, and followed by the second stage where the population parameters are estimated by summarizing parameters across individuals. This carries forward the uncertainty in the within individual determinations along with the nature of selecting an appropriate summary statistic that reflects the nature of both the central tendency of the group/sample/population along with the variability of that group. The two stage method has been reported to overestimate random variability from some simulation studies (15). We will not discuss these approaches in greater detail here.

1.2.1 Non-linear mixed effect models

In pharmacometrics, we are frequently interested in developing mathematical models to describe the relationship of drug exposure over time, drug response over time, and disease progression over time as well as the relationship between drug exposure and drug response. These relationships are more likely to be nonlinear, hence involve in non-linear modeling. Non-linear mixed effect modeling approaches estimate the central tendency of these relationships as well as the random variability (i.e. inter-individual variability, inter occasion variability, inter-study variability, residual variability) simultaneously. Compared to the previously described methods, the approach can overcome the challenges from the data structure itself. These include issues such as correlation and imbalance of data, sparse data, various dosing history across different individuals (11, 16). Although other methods (e.g. Bayesian approach) can be used to for the type of problem, the frequent adaptation of the non-linear mixed effect modeling is the most commonly used analysis approach in pharmacometrics.

Nonlinear mixed effects modeling or nonlinear mixed effects modeling with full Bayesian MCMC uses maximum likelihood or other types of estimation to guide the identification of parameter values. The overall goal of the method is to identify the set of

parameters that maximizes the probability of the observed data given a specified model. The identification of parameter values can be achieve using multiple approaches including: 1) a parametric approach where the distribution of the random effect parameters is assumed a-priori to arise from a particular distribution (commonly normal or log-normal distribution); 2) a nonparametric approach requires assumption about the residual variance (structure and magnitude) and no assumptions are made about the distribution shape of other parameters; 3) a semi-parametric approach where the distribution of parameters are restricted over certain range but not all possible distributions. Each approach has unique advantages and disadvantages (11, 17, 18). The parametric approach is the most commonly used for population parameter estimation (11, 14-16, 19, 20).

1.3 MODEL DEVELOPMENT

Population modeling encompasses three elements. These are: 1) structural model that describes the central tendency of data; 2) the stochastic model which qualifies and quantifies the unexplained variability of parameters in models along particular hierarchies and; 3) the covariate model which describes the influence of patient characteristics on model parameters.

1.3.1 Structure model development

The structural model describes the central tendency of the observed data. Some general PK/PD models have been commonly used. PK models include the one compartment model, two compartment model, and three compartment model in combination with various elimination patterns (e.g. First order linear elimination, nonlinear elimination et al.) and absorption patterns (e.g. Zero order, first order, mixed first and zero order et al.). General PD models include the E_{max} model, the effect compartment model, the indirect response model etc (12, 21).

1.3.2 Stochastic model development

One of the foci of population modeling is to quantify the variability in the population model parameters. The stochastic model, that quantifies multiple levels of the variability of parameters in models, often includes inter-individual variability and (intra-individual) residual variability. Inter-individual variability is generally incorporated in the typical value for the population by an exponential, additive, or proportional function. Intra-individual variability may include inter-occasion variability and other residual variability. Residual variability is commonly modeled using additive, proportional, and combination of additive and proportional error structures. Misspecification of the stochastic model may lead to structural model misspecification, a biased estimation in population mean parameter, and poor simulation properties (11, 22, 23).

1.3.3 Covariate model development

As described previously, population modeling commonly focuses on explaining and understanding sources of variability in the observed data. A portion of the variability in the drug exposure and response can often be explained by available deterministic information, such as patients' characteristics (e.g. age, gender, genotype, renal function et al.). Covariate model development is the process of identifying and estimating the effects of covariates, hence potentially contributing to the right dose and labeling decision in particular for special population. Well developed covariate models also improve predictive performance of the model in simulation applications.

Covariate model development should focus on physiologically meaningful and clinically useful covariates. In addition, the data reduction step is a step to evaluate and examine covariate data before incorporating them into models. The concept was adopted from other fields and was first used in pharmacometrics by Dr. Marc Gastonguay (12, 24-26). The data reduction step helps to avoid false positive results, hence providing more interpretable covariate models (12). Each covariate should bring independent and unique information into model. Special attention should be taken when simultaneously including covariates that are highly correlated. Commonly used covariate screening methods include stepwise forward addition, stepwise backward elimination, stepwise forward

incorporation/backward elimination, and full model approaches (27). We will not discuss them in greater detail here (12, 28).

1.4 MODEL DIAGNOSTICS AND QUALIFICATION

No model will be a perfect reflection of a true system, therefore we are restricted to evaluation of the model predicated or conditioned on a particular purpose. Model qualification strategies are discussed below.

1.4.1 Model performance

Goodness-of-fit (GOF) is a basic internal evaluation of a model. GOF plots include population predictions (PRED) versus Observed data (also called dependent variable: DV) or time, residual (RES)/ weighted residual (WRES)/ conditional weighted residual (CWRES) versus PRED, individual predictions (IPRED) versus DV or time. These diagnostic plots provide information about the data set, bias in the structural model or stochastic models.

The visual predictive check (VPC) and posterior predictive check (PPC) are other ways to check the model performance. The PPC uses the Monte Carlo Simulation technique to simulate the model predicted posterior distribution of the data and compares the distribution of a simulated metric with the original metric from the observed data. PPC is one of most robust approaches for model qualification (12, 29-31). The visual predictive check also uses the Monte Carlo simulation technique to simulate the model output (e.g. concentration vs time, concentration vs effect, disease marker vs time et al.) and provides the basis for the creation of prediction intervals of the model that can be superimposed on the observed data. Visual predictive check assumes that uncertainty in estimated parameters is small relative to other sources of variability; hence the uncertainty in estimated parameters is not included in the simulation. Typically, one evaluates a 90% prediction interval and examines whether or not 90% of the data lie within this band.

1.4.2 Precision of parameter estimates

Precision of the estimated model parameters are reported as standard errors (SE) or confidence intervals (CI). The SE or CI of the estimated model parameters can be obtained from default method of the software (i.e. Fisher information matrix method in NONMEM) or from bootstrapping methods. Bootstrapping methods randomly resample from the entire dataset, and refit the developed model providing for the examination of the parameter estimation sensitivity to these perturbations. Hence bootstrapping is a more robust method to evaluate the precision of the estimated model parameters (32, 33).

1.4.3 Assumption checking

The randomization test (this can also be known as the permutation test – especially in other fields) is commonly used to evaluate the assumption about chi square χ^2 distribution of Δ -2logL between nested models in covariate model development (12). A randomization test for significant covariates in the final model can be conducted by creating multiple new data sets (usually > 1000) that are identical to original dataset except for re-randomization of the association of the covariate so that the Null Hypothesis will be true under randomization. The delta objective function values (OFV) from the original null OFVs can be calculated by running the final model with these new data sets. Thus, a critical statistic for a given alpha level that is unique to the original data set and model can be calibrated. The randomization test was used to evaluate significant covariates in our perphenazine paper.

Other model qualification methods also include sensitivity analysis, data splitting methods and cross validation methods. Sensitivity analysis is discussed later in this thesis.

1.5 CLINICAL TRIAL SIMULATION (CTS)

Simulation has been widely described as one of the most important applications of the nonlinear mixed effects of Bayesian population modeling approach (12). It has been widely used to interpolate situations between the original conditions where data were

collected and then extended to predict new conditions and predict the range of variability and uncertainty of predicted relationship(12). Model qualification techniques for simulation models have also been developed with a much higher standard of performance. These primarily utilize the posterior predictive check and VPC approaches to model qualification (12).

Deterministic and Monte Carlo Simulation are two types of simulation approaches. The former simulates from the fixed parameter and is interested in response in population mean or in a specific individual (12). Monte Carlo simulation incorporates random variability in population parameters and can also incorporate uncertainty in parameter estimates across the random and fixed effect elements of a model. The random variability can be from various probability distributions across multiple levels (12).

Clinical trial simulation (CTS) utilizes the Monte Carlo simulation approach to simulate clinical trials. Clinical trial simulation was recently defined as "the generation of a response for a virtual subject by approximating the trial design, human behavior, disease progress and drug behavior using mathematical models and numerical methods"(13). CTS has also has been increasingly used for optimization of clinical trial design. CTS allow one to predict and compare the clinical outcomes of competing trial designs, various human behaviors, and disease or drug related factors.

Models involved in CTS include: 1) input-output models: commonly referred to mathematical models of drug behavior and disease progress, such as population PK/PD models, disease progress models. 2) Covariate distribution models: defining virtual patients' characteristics and the correlation among these factors as well as how they occur in different patient or target subject groups. 3) Protocol deviations and execution models: patient adherence model, dropout model, censoring models etc.

As discussed previously, models are associated with uncertainty in parameter estimates. Sensitivity analysis provides for the evaluation of the impact of the posterior parameter distributions on simulated trial results (25, 26). Sensitivity analysis influences future study design and labeling decision makings by accounting for uncertainty of the

model itself. Sensitivity analysis includes local sensitivity analysis and global sensitivity analysis. Local sensitivity analysis tests certain parameter at fixed point estimates, while global sensitivity analysis simultaneously accounts for the uncertainty distributions in all parameters. Hence global sensitivity analysis a more robust method as the interaction of parameter elements is considered (25, 26, 34).

1.6 AIMS

The major aim of this thesis is to use the modeling and simulation approaches to identify the systematic contributors that modify the estimated population parameters and that explain sources of variability in drug exposure (Chapter 2-4), response, and clinical outcome (Chapter 5-7). The source of measurable variability evaluated in the thesis include patient characteristics in chapter 2-3 (e.g. age, genotype et al.), patient behavior in chapter 4 (i.e. a patient's detailed dosing history), biological system in chapter 5-7 (i.e. natural rhythm of BP variability on a daily basis), and inferior clinical practice in chapter 5-7 (i.e. follow up visit time of patients). Specifically, the thesis accomplishes the aim by addressing these following issues: 1) Chapter 2-3: The effect of patient characteristics on variability of drug disposition using highly sparse sampling measurement for both escitalopram and perphenazine. 2) Chapter 4: The effect of measurement error in dosing time on population PK parameter estimation using escitalopram as a model drug and the SPECTRUM data as a prototype clinical trial, specifically comparing patient reported time of last dose and medication event monitoring generated dosage histories. 3) Chapter 5: The impact of cuff BP measurement error as well as ignoring the circadian rhythm of BP on measured treatment effect of antihypertensive agents using a current clinical practice paradigm. 4) Chapter 6: The impact of ignoring the timing of patient clinic visit times and the cuff BP measurement error on the BP misclassification rate in the current clinical practice paradigm. 5) Chapter 7: The risk of a cardiovascular disease related event associated with current clinical practice strategies as well as alternative strategies in patients with hypertension.

Specific aims for each study are:

Chapter 1: Introduction

Chapter 2: Population pharmacokinetics of perphenazine in schizophrenia patients from CATIE: Impact of race and smoking

● To characterize PK for perphenazine in patients with schizophrenia using population analysis

● To identify and estimate potential covariates contributing to variability in perphenazine exposure using highly sparse sampling measurement and determine its clinical significance

● To examine the robustness of those comparisons using a randomization test approach

Chapter 3: Effect of age, weight, and CYP2C19 genotype on escitalopram exposure

● To describe PK of escitalopram in patients treated for major depression in a cross-national, US-Italian clinical trial

● To identify the potential contributors for the difference in susceptibility to toxicity in the Pisa versus the Pittsburgh based patients

Chapter 4: the effect of reporting methods for dosing times on the estimation of pharmacokinetic parameters of escitalopram

● To compare population PK models of escitalopram developed from dosage times recorded by a medication event monitoring system (MEMS) versus those ascertained from patient report of time of last dose.

Chapter 5: Use of Monte Carlo simulation approaches to evaluate the clinical implications of discordance between measured and true BP

● To evaluate the discordance in BP between the cuff measurements at casual clinic visits in current clinical practice and the true underlying BP.

● To identify the impact of the cuff BP measurement time on the discordance

Chapter 6: Evaluation of JNC VII BP treatment group misclassification with cuff BP measurement by trial simulation

● To quantify the BP misclassification rate using a casual clinic visit time in current clinical practice.

● To evaluate the influence of the BP measurement time and dosing time on the BP treatment group misclassification rate.

● To evaluate the influence of various levels of BP measurement error on the BP misclassification rate including the number of measurements per visit and last digit rounding effects.

Chapter 7: The impact of clinical practice strategies on the risk of cardiovascular disease (CVD) in patients with hypertension: a simulation study

● To evaluate CVD risk to patients that are treated using different clinical practice strategies for BP management in patients with hypertension using Monte Carlo Simulation approaches

2.0 SECOND CHAPTER

Population Pharmacokinetics of Perphenazine in Schizophrenia Patients from CATIE: Impact of Race and Smoking

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2.1 ABSTRACT

The goal of the study was to characterize population pharmacokinetics (PPK) for perphenazine in patients with schizophrenia from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). Patients (n = 156) received 8-32 mg of perphenazine daily for 14 to 600 days for a total of 421 plasma concentrations measurements. Nonlinear mixed-effects modeling was used to determine PPK characteristics of perphenazine. One- and two-compartment models with various random effect implementations and mixture distributions were evaluated. Objective function values and goodness of fit plots were used as model selection criteria. Age, weight, sex, race, smoking, and concomitant medications were evaluated as covariates. A one-compartmental linear model with proportional error best described the data. The population mean clearance and volume of distribution for perphenazine were 483 L/h and 18,200 L , respectively. Race and smoking status had significant impacts on perphenazine

clearance estimates. In addition, the estimated population mean clearance was 48% higher in nonsmoking African Americans than in nonsmoking other races (512 L/h versus 346 L/h). Active smokers eliminated perphenazine 159 L/h faster than nonsmokers in each race. Clearances for smoking African Americans versus smokers in other races were 671 L/h versus 505 L/h, respectively. **Keywords:** Perphenazine, Population Pharmacokinetics, Clinical Antipsychotic Trials for Intervention Effectiveness (CATIE), Race and Smoke, Schizophrenia

2.2 INTRODUCTION

Schizophrenia affects about 1.1 percent of the U.S. population age 18 and older each year (http://www.nimh.nih.gov/health/publications/schizophrenia/complete-publication.shtml). Current pharmacologic treatment for schizophrenia includes the first generation antipsychotics (FGA) and second generation antipsychotics (SGA). The NIMH funded Clinical Antipsychotic Trials for Intervention Effectiveness (CATIE) Schizophrenia Trial was the first systematically designed clinical trial that studied treatment selections for schizophrenia. The CATIE schizophrenia trial compared relative effectiveness of SGA to a typical representative of the FGA, perphenazine (35). Although SGAs are more commonly used clinically than FGAs, the results of the study showed that perphenazine, a conventional FGA, had similar efficacy to most of the SGAs including quetiapine, risperidone, and ziprasidone. Olanzapine appeared to have better efficacy than perphenazine, but it caused greater weight gain and glucose/lipid metabolism derangements (36, 37). In addition, the CATIE studies also found that perphenazine was more cost effective when compared with second-generation antipsychotics without significant differences in measured outcome (38). Similar to other phenothiazine antipsychotics, perphenazine is thought to produce its antipsychotic effect by binding to dopamine receptors.(39) After intravenous dosing, perphenazine is extensively metabolized by CYP2D6 in the liver (40). The total body clearance of perphenazine is around 100 L/h with a volume of distribution from 10 to 34 L/kg (41). The half-life of perphenazine is approximately 9.5 hrs with an oral bioavailability of 20% (41).

So far, limited data on the population pharmacokinetics (PPK) of perphenazine is available in the literature (42). PPK analysis is a robust tool for obtaining valuable pharmacokinetic (PK) information from large clinical trials under conditions of sparse concentration sampling (43, 44). The effect of potential covariates on drug exposure can also be evaluated in PPK analysis by incorporating patient specific information into the modeling process. Data from the CATIE schizophrenia trial provided the opportunity to determine PPK parameters for perphenazine in a typical schizophrenic patient population and evaluate the effect of potential covariates (i.e. age, body weight, sex, smoking status, and concomitant medications et al.) on perphenazine drug exposure.

The goal of this study was to characterize PPK for perphenazine in patients with schizophrenia from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) schizophrenia trial and to identify the potential contributors to variability in perphenazine exposure.

2.3 SUBJECTS AND METHODS

2.3.1 Subjects and Samples

The details of the study design of CATIE schizophrenia trial have been published elsewhere (36, 45). The study was conducted at multiple sites in the US. Patients diagnosed with schizophrenia were recruited into the study. Patients prescribed perphenazine (n = 156) received 8-32 mg of medication daily for 14 to 600 days. One blood sample per patient per visit was collected during patient clinical visits with up to six visits total. The last dose time before each blood sample was recorded as reported by the patient and the sample time noted exactly by the phlebotomist. This analysis excluded data that had undetectable perphenazine concentrations in a sample, unreported dose magnitude, or a missing time for the dose and/or blood sample. A total of 421 plasma concentration measurements meeting the aforementioned criteria were available for model development. The PK sampling for each patient was sparse with an average of 2.7 perphenazine concentration measurements per patient.

2.3.2 Patient characteristics

Patient demographics are summarized in Table 1. Patients in the study included 115 males and 41 females between the ages of 18-65. The average age of the subjects was 40 years with an average body weight of 88.9 kg. The patient population was predominantly Caucasian (65% (n=102)), with 29% (n=46) black/African American, 4% (n=6) Asian, and 1% (n=2) two/more races. In addition, 67% (n=104) of the subjects were active smokers.

2.3.3 Determination of Perphenazine Concentrations

Perphenazine was measured by reverse phase high performance liquid chromatography (HPLC) using electrochemical detection with settings of detector 1: +0.20V, detector 2: +0.73V, and the guard cell: +0.75V. This method was developed by the Clinical Pharmacology Lab at the University of Pittsburgh (46). Plasma was extracted using liquid - liquid extraction (ethyl acetate - n-hexane (4:2), v/v) and back extracted using 0.025M potassium phosphate; pH 2.4 separation was completed using a Nucleosil C18, 5 micron, 120 X 4.6mm I.D. with a flow rate of 1ml/min at room temperature. The assay was linear in the range from 0.5 - 25 ng/ml with inter-assay variability (C.V.) of 4.1-10.0% and reproducibility (C.V.) of 3.37-9.13%. The limit of quantitation for perphenazine was 0.5 ng/ml for this assay (46).

2.3.4 Population Pharmacokinetic Analysis

PPK analysis of perphenazine was performed using NONMEM® (version 5.1.1, Icon, Hanover, MD). The initial modeling focused on developing a base model where a model structure was identified without incorporating any covariates. One and two-compartment linear mammillary PK models with first-order absorption and elimination were evaluated using ADVAN2 TRANS2 and ADVAN4 TRANS4 during model development, respectively. Inter-individual variability was included in the base model as a random effect and to be log-normally distributed. The individual estimates of PK parameters ($P_j = P_{TV} \times e^{\eta_P}$) was a function of the both the population estimates (P_{TV}) and random variables (η_P). The parameter P_j was the estimated PK parameter value for the j^{th} individual, P_{TV} was the estimated value for the population, and η_P described the

difference between P_j and P_{TV}, which was assumed to be a normally distributed with a mean of zero and variance of ω_P^2 (47, 48). The absorption rate constant (K_a) was fixed to 1.6 hr^{-1} based on literature reported $t_{1/2}$ and t_{max} values (40, 41) because of difficulty directly estimating this parameter within this dataset. The residual error was comprised of, but not limited to, intra-individual variability, experimental errors, process noise, and model misspecifications, if any. Additive ($y_{ij} = \hat{y}_{ij} + \varepsilon_{ij}$), proportional ($y_{ij} = \hat{y}_{ij}(1+\varepsilon_{ij})$) and combined error structures ($y_{ij} = \hat{y}_{ij}(1+\varepsilon_{ij}) + \varepsilon_{ij}'$) were tested, where y_{ij} is the j^{th} observed perphenazine concentration in the i^{th} individual, $\hat{y}_{ij}$ is the corresponding model prediction, and ε_{ij} (or ε_{ij}') is a random variable assumed normally distributed with a mean of zero and a variance of σ_1^2 (or σ_2^2).

Patient demographic information, such as patient age, weight, race etc., may explain some of the variability in the perphenazine PK parameter estimates. In the study, both continuous covariates (e.g., age, weight) and discrete covariates (e.g., sex, race, smoking status, and concomitant medications) were tested. The effect of continuous covariates on PK clearance estimates was evaluated using the following model structures:

$$TVCL = \theta_{CL} * (Cov / Med_{\text{cov}})^{\theta_{Cov}} \text{ (centered power model)}$$

$$TVCL = \theta_{CL} * \exp(\theta_{Cov} * (Cov / Med_{Cov})) \text{ (centered exponential model)}$$

$$TVCL = \theta_{CL} + \theta_{Cov} * (Cov - Med_{Cov}) \text{ (linear model)}$$

$$TVCL = \theta_{CL} + \theta_{Cov} * Cov \text{ (linear model)}$$

$$CL = TVCL * e^{\eta_j}$$

where *TVCL* is the population estimate for clearance; Cov representes the subject specific value of continuous covariate; Med_{Cov} is the median value of the *Cov*. θ_{CL} and θ_{Cov} are estimated fixed effect parameters; η_j is the random variable describing the difference of the estimated CL for j^{th} subject from the typical population value.

Categorical variables were assigned to each of race (Black/African American=1, other races=2). The coding example of incorporating the effect of race on CL estimate was shown as following:

IF (RACE.EQ.1) TVCL=$\theta_{CL1;}$

IF (RACE.EQ.2) TVCL=θ_{CL2}

where θ_{CL1} *and* θ_{CL2} are typical values of CL for Black/African American and other races, respectively. The effect of discrete covariates such as sex, smoking status, and concomitant medication were tested using the following structures:

$$TVCL = \theta_{CL} + Cov * \theta_{Cov}$$

For male and female patients, sex was assigned to be 0 and 1, respectively. For smoking status, nonsmoker and smoker were assigned to be 0 and 1, respectively. If patients had a concomitantly administered medication, the *Cov* was assigned to be 1 for that medication, otherwise *Cov* was 0. θ_{Cov} was the estimated fixed effect parameter for the covariate. Other parameters (eg, *TVCL,* $\eta_{j,}$ *and* θ_{CL}) were the same as previously described.

A Bayesian approach (MAP or Empirical) conditioned on the population characteristics was used to estimate individual specific parameters. Both the base and final model were estimated using the first-order conditional estimation (FOCE) with interaction method. Continuous covariates (e.g. age, weight et al.) and discrete covariates (e.g. sex, race, smoking status, and concomitant medications et al.) were incorporated into each parameter by forward incorporation and backward elimination method. The covariate was retained in the model if the objective function value (OFV) decreased by 3.84 when adding one additional fixed effect parameter into model (χ^2 $p < 0.05$ $df = 1$). Goodness of fit plots were used as additional model selection criteria. These were generated using R® (version 2.6.2). Post-processing of NONMEM® outputs were performed using SPSS (version 14.0).

Bootstrapping and visual predictive check were used for model evaluation. Confidence intervals for the final parameter estimates were calculated from bootstrapping. A randomization test for significant covariates in the final model was performed using a method as implemented by Holford (49, 50). This was done by creating 1000 new data sets that were generated identically except for re-randomization of the association of the covariate so that the Null Hypothesis will be true under randomization. The delta OFVs from the original null OFVs were calculated by running

the final model with these 1000 randomized new data sets. The delta OFVs were sorted and the quantiles for the delta OFVs corresponding to original data set as well as P values were identified (significance criteria of $P<0.05$).

2.4 RESULTS

2.4.1 Population pharmacokinetic modeling

A one-compartment model with linear elimination and proportional error best described the perphenazine PK in this patient population. Race and smoking status during the last week were significant covariates affecting clearance. The process of final model development is summarized in Table 3. Diagnostic plots for the final model are listed in APPENDIX I. The final model was evaluated by bootstrapping as well as visual predictive check plots. Please refer APPENDIX II for predictive check plots. The randomization test showed that both race and smoking status were statistically significant factors that affected perphenazine. A histogram showing the delta OFVs for race and smoking status are presented in Figures 2a & Figure 2b, respectively.

The PK parameter estimates for the final model are listed in the Table 2. The population mean clearance and volume of distribution for perphenazine in the base model were 483 L/h and 18,200 L, respectively, without incorporating any covariates. Race and Smoking status were identified as two significant covariates for clearance of perphenazine. Patients who smoked in the past week eliminated the drug 159 L/h faster than nonsmokers, corresponding to a 33% increase in clearance compared to the whole population (483 L/h). Estimated population clearances for nonsmoking non-African Americans, smoking non-African Americans, nonsmoking African American, and smoking African Americans were 346 L/h, 505 L/h (346+159 L/h), 512 L/h, and 671 L/h (512+159 L/h), respectively. Estimated population mean clearance of perphenazine was 48% higher in nonsmoking African Americans than nonsmoker in other races. The combined effect of these two covariates is that smoking African Americans (671L/h) clear perphenazine 94% faster than non-smoking non-African Americans (346 L/h).

Post-processing of empirical Bayesian estimates for individual parameters are listed in Table 4. An independent-samples *t*-test showed a significant difference in the empirical Bayes estimated CL/F of perphenazine between African Americans (n=46) and other races (n=110) ($P<0.05$) (Figure 1a), active smokers (n=104) and non-smokers (n=52) ($p<0.01$) (Figure 1b), and smoking African Americans (n=38) and non-smoking non-African Americans (n=44) ($p<0.001$) (Figure 1c).

The analysis of the study showed that sex, age, weight were not significant covariates affecting the PK of perphenazine. The number of subjects on concomitant medications was less or equal to six for any medication and none of concomitant medications were identified as significant covariates for perphenazine clearance. (See APPENDIX III for detail)

2.5 DISCUSSION

In this study, we successfully captured the exposure characteristics for perphenazine in schizophrenic patients using a PPK approach. Before incorporating any significant covariates, the population mean clearance and volume of distribution for perphenazine were 483 L/h and 18,200 L, respectively. Estimated sub-population clearances for non-African American non-smokers, non-African American smokers, African American nonsmoker, and African American smokers were 346 L/h, 505 L/h, 512 L/h, and 671 L/h, respectively. Hansen and his colleague reported the clearance of perphenazine at approximately 100 L/h with the volume of distribution of 10 to 34 L/kg after intravenous administration. The bioavailability of perphenazine is around 20% (41), hence the oral clearance should be around 500 L/h with a oral volume of distribution of 3,500-11,900 L based on their study. The population kinetics study conducted by Jerling (42) showed that population mean value for oral clearance and volume of distribution was 520 L/h and 16,140 L. Hence, the population estimated PK parameters in this study using highly sparse sampling data are consistent with other literature reported values.

Smoking status in the most recent week was also identified as a statistically significant contributor to the variability in estimated perphenazine clearance in schizophrenia patients. Patients who smoked in the past week had a mean clearance of

748 L/h compared to 453 L/h for nonsmokers. An in-vitro metabolism study conducted by Olessen and his colleague (51) showed that besides CYP2D6, other enzymes such as CYP1A2, 3A4, and 2C19 are involved in the N-dealkylation of perphenazine. In addition, smoking has been identified as a potent inducer of hepatic CYP1A1, 1A2, and 2E1 (52). Hence, metabolism of CYP1A2 substrate can be induced in smokers (52). It is possible that perphenazine is metabolized by CYP1A2 clinically, which induced clearance of perphenazine at active smoker. The number of patients with schizophrenia who smoke is very high. (53, 54) One study reported the prevalence to be 88%(55), nearly three times the rate in the general population. Bigos and colleagues(47) has reported that 66% of patients with schizophrenia who attended CATIE olanzapine study were active smokers. In our study, 67% of schizophrenia patients were active smokers. Due to the fact that many patients with schizophrenia smoke, it is important to pay more attention to the effect of smoking on perphenazine pharmacokinetics.

Racial differences in clearance have been identified in a few antipsychotic agents which are CYP2D6 substrates, such as olanzapine(47), risperidone(56), and paroxetine(48). In these studies, African Americans cleared these drugs faster than Caucasians. In this study, the population mean clearance of perphenazine was 48% higher in African Americans than that in other races in patients with schizophrenia. Feng et al. found that race was a significant covariate for both paroxetine and risperidone clearance, but the race effect on clearance was no longer significant when CYP2D6 genotype was incorporated for paroxetine or when a 3-component mixture model for clearance was used for risperidone. Perphenazine is primarily metabolized by CYP2D6 located in the liver (40). Jerling and his colleague (42) also reported that CYP2D6 genotype was a significant covariate on estimated oral clearance of perphenazine. So it is possible that CYP2D6 genotype is confounding factor for the racial difference in perphenazine clearance. There is evidence supporting racial differences in CYP2D6 polymorphism expression frequencies between African Americans and Caucasians. Specifically, Caucasians showed approximately 3-fold higher frequencies for the non-functional allele CYP2D6*4 (57) compared to African Americans. These results suggest that observed race differences in clearance of perphenazine might due to racial

differences in CYP2D6 polymorphism frequencies between African Americans and others.

Racial differences in adherence to prescribed perphenazine regimen between African Americans and other races may be another possible contributor to differences in perphenazine clearance. Lower adherence to antipsychotic treatment has been reported by other studies.(58) Some examples have demonstrated that African Americans are less likely to be adherent to antihypertensive medications (59, 60) and inhaled corticosteroids (61) than Caucasians. Hence, both differences in the frequency of CYP2D6 polymorphism expression and adherence patterns to prescribed perphenazine regimen might contribute to racial differences in perphenazine clearance observed in this study. Further studies are required to identify the mechanisms contributing to the observed differences in perphenazine clearance across race. Racial information alone is not currently recommended for adjusting perphenazine dosage regimens in patients with schizophrenia.

To further explore the clinical significance of the difference in estimated clearance of perphenazine among these subpopulations, a response or tolerability analysis between these subpopulations should be performed. Due to lack of response data, we compared the administered dose across each of the subpopulations (Table 5). This evaluation was based on the assumption that physicians titrated the dose upward to achieve desired effects or titrated downward to avoid side effects and that these effects were concentration related. The average dose administered to active smokers was significantly higher than in patients who were not active smokers. In addition, the dose administered to African American was slightly higher than that to Caucasian patients, but this difference was not statistically significant. Therefore, until the mechanisms associated with the observed racial difference in perphenazine clearance are better understood, smoking status seems to be a more clinically significant factor for dose adjustment.

Similar to olanzapine (47) and risperidone (56, 62) in the CATIE trial, there were very large variabilities in perphenazine exposure. Jerling *et al.* (42) also reported wide variability for the PK of perphenazine. This wide variability in drug exposure, which may

result from sociologic (e.g. adherence etc.) or biological factors (CYP 2D6 polymorphism expression rates), poses a clinical challenge in schizophrenia treatment and may be one of the reasons for the high discontinuation rate observed in CATIE study. Another potential contributor to the large degree of exposure variability observed for perphenazine in this study relates to the nature of the dosage input information and assumptions. The PPK models were developed based on patient reported last dosing time along with the assumption of steady state and full adherence. In a study of escitalopram (63), it was shown that inaccurate dosing history and patient adherence information affected estimation of absorption rate, volume of distribution, but not the clearance parameters for escitalopram.

In conclusion, race and smoking status in the past week were identified as two significant covariates affecting clearance. The dosage regimen of perphenazine in these populations may need to be adjusted clinically based on patients smoking status.

2.6 TABLES

Table1: Patient demographics

Demographics	CATIE-Perphenazine
Sample size	156
Number of observations	421
Number of observations per subject	2.70
Age, mean years ± SD (range)	40.3 ± 10.6 (18-65)
Weight, mean kg ± SD (range)	88.9 ± 23.0 (49.4-195.0)
Gender, n (%)	
Male	115 (74%)
Female	41 (26%)
Race, n (%)	
Caucasian	102 (65%)
African American	46 (29%)
Asian	6 (4%)
Two or more races	2 (1%)
Smoking status, n (%)	
Active smoker	104 (67%)
Nonsmoker	52 (33%)

Table 2: Pharmacokinetic parameter estimates for perphenazine

Parameters	Parameter Estimates	95% CI
$CL_{AA\_nonsmoker}$ (l/h)	512	385~646.4
$Cl_{nonAA\_nonsmoker}$ (l/h)	346	270~434.7
Smoke on CL (l/h)	159	63.5~267.4
V (l)	19300	13865~65795
Ka (h^{-1}) (fixed)	1.6	N/A
ω_{cl} %	79.30%	68.1%~87.4%
ω_{v} %	78.50%	20%~122%
ω_{ka} %	336.10%	0.35%~518%
$\omega_{cl\text{-}v}$ %	100.00%	29% ~ 170%
σ_1 %	37.40%	33.3%~41.0%

CL, clearance; $CL_{AA\_nonsmoker}$, clearance for nonsmoking African American; $Cl_{nonAA\_nonsmoker}$, clearance for nonsmoking non-African American; Smoke on CL, effect of smoking status on clearance, which was assumed to be independent on race; V, volume of distribution; ω, coefficient of variation of inter-individual variability; σ_1, coefficient of variation of residual error (proportional); N/A, not available; 95% confidence interval (95%CI) was calculated from boots trapping step (re-sampling times=1000).

Table 3: Population Pharmacokinetic model development steps for perphenazine

Table 3. Population Pharmacokinetic model development steps for perphenazine

Covariate	Model	-2LL	D-2LL	P value
	One compartment model	**559.11**		
base model	Two compartment model	557.861	-1.249	> 0.05
1-1 (CL)				
Age (centered power model)	M1	558.589	-0.521	> 0.05
Weight (centered power model)	M2	557.037	-2.073	> 0.05
Sex	M3	558.349	-0.761	> 0.05
Race	**M4**	**553.352**	**-5.758**	**< 0.05**
Smoking status	**M5**	**545.87**	**-13.24**	**< 0.001**
1-2 (V)				
Age (centered power model)	M6	555.758	-3.352	> 0.05
Weight (centered power model)	M7	558.973	-0.137	> 0.05
Sex	M8	556.941	-2.169	> 0.05
2				
Smoking status(CL)+Race(CL)	**M9**	**540.681**	**-5.189**	**< 0.05**

*Abbreviations: -2LL, objective function value; Δ-2LL, difference in objective function values between two models, -2LL values in CL (Smoking status) and CL (Race) were compared with base model; -2LL values in CL (Smoking status, Race) was compared with model CL (Smoking Status). Δ-2LL of 3.84 units was considered significant (χ^2 df=1 $p<0.05$). The function structure of incorporating covariate was described in the methods section. Race: African American vs. other races; Smoke: subjects who smoked in the past week vs. subjects who did not smoke in the past week.

Table 4: Post processed perphenazine clearance by population

Population	Mean Clearance (L/hr)	Standard Deviation	P value
Race			<0.05
African American (n=46)	775.90	513.52	
Other Races (n=110)	597.05	477.98	
Smoking Status			<0.01
Active Smoker (n=104)	748.24	513.76	
Non-Smoker (n=52)	452.87	385.93	
Race & Smoking Status			<0.001
Smoking African American (n=38)	833.90	536.95	
Non-Smoking Non African American (n=44	444.23	406.64	

Table 5: Prescribed daily dose by population

Population	Mean of Prescribed Daily Dose (mg)	P value
Race		>0.05
African American (n=46)	25.04 (± 7.47)	
Other Races (n=110)	23.63 (± 7.77)	
Smoking Status		<0.05
Active Smoker (n=104)	25.33 (± 7.39)	
Non-Smoker (n=52)	21.62 (± 7.71)	
Race & Smoking Status		<0.05
Smoking African American (n=38)	25.55 (± 7.36)	
Non-Smoking Non African American (n=44)	21.27 (± 7.70)	

2.7 FIGURES

Figure 1: Perphenazine clearance by subpopulation

Figure 1a: Perphenazine clearance by smoking status

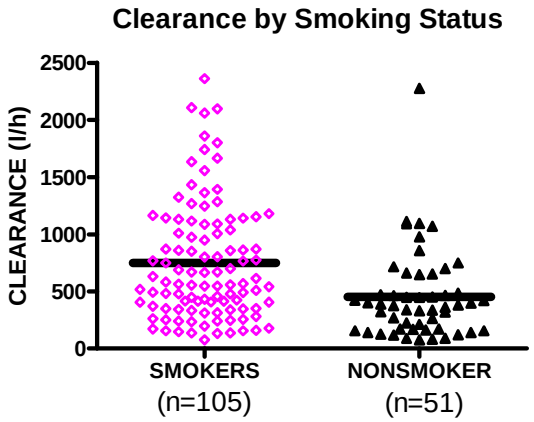


Figure 1b. Perphenazine clearance by race

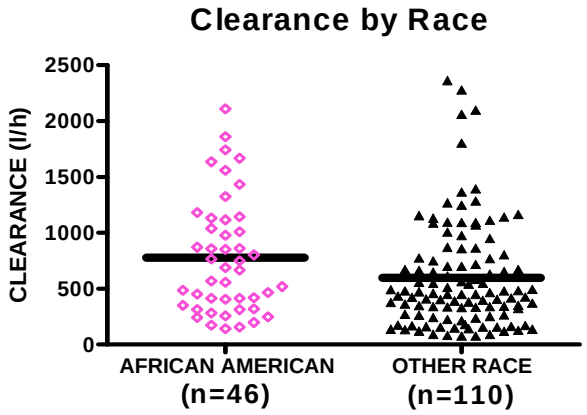


Figure 1c: Combined effect of smoking and race on perphenazine clearance

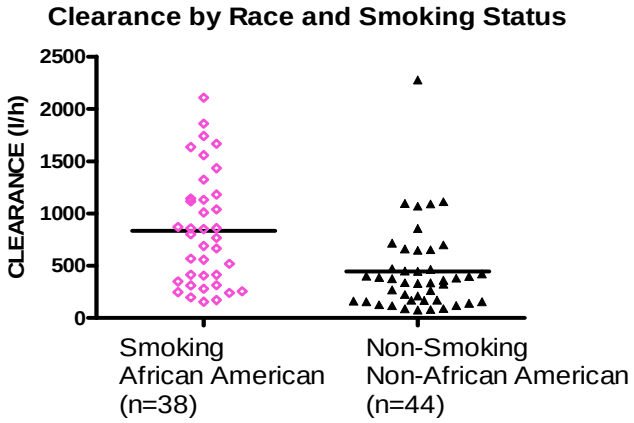
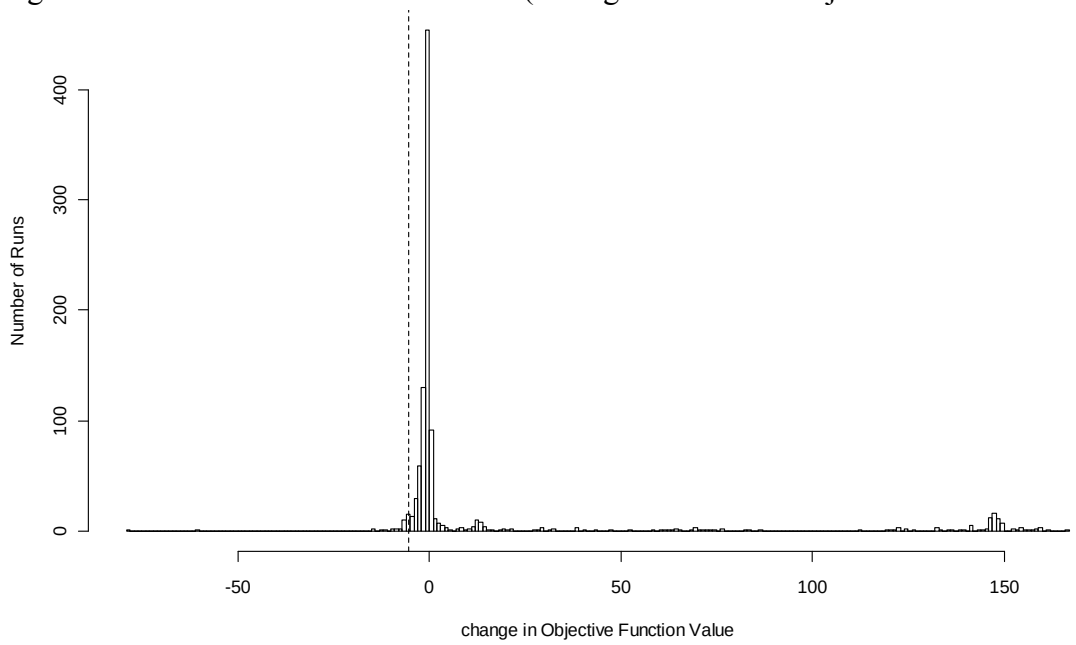


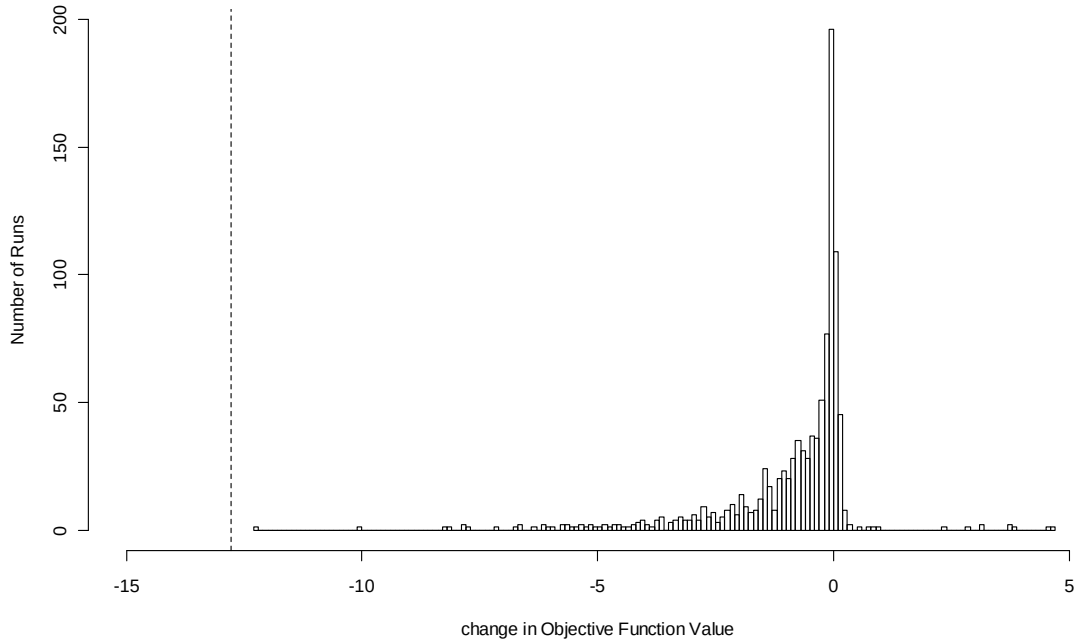
Figure 2: Randomization Test

Figure 2a: Randomization Test for Race (Histogram of Delta Objective Function Values)



Original Objective Function Value decreases was -5.189, P=0.031.

Figure 2b: Randomization Test for Smoking (Histogram of Delta Objective Function Values)

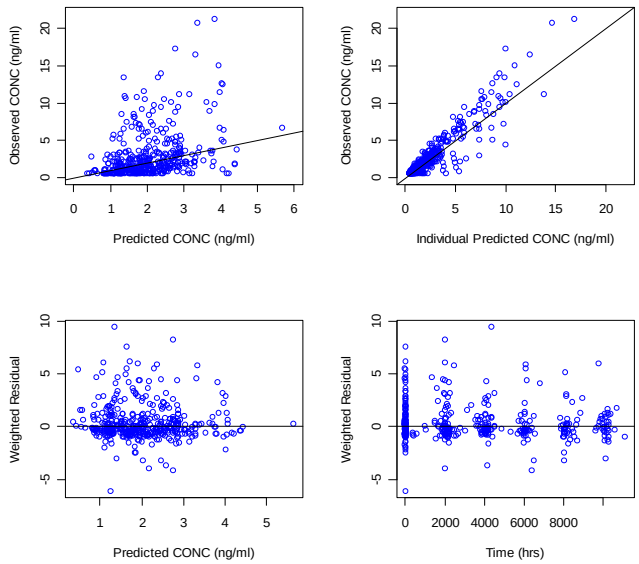


Original Objective Function Value decreases was -12.771, P=0.000

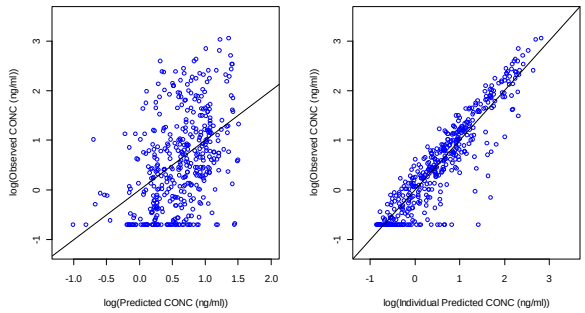
2.8 APPENDIX FOR CHAPTER TWO

I: Goodness of fit plots of the perphenazine final model

1) Goodness of fit plots of final PK model in normal scale

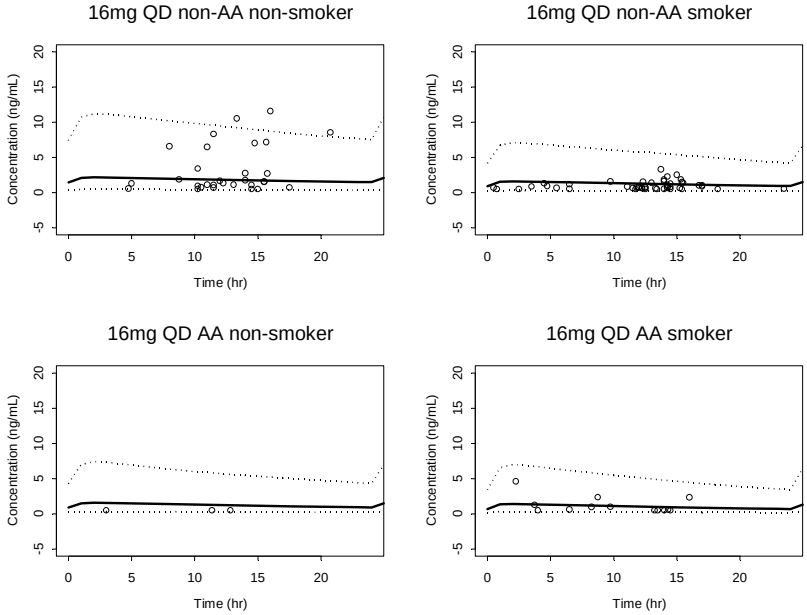


2) Goodness of fit plots of final PK model in nature log scale

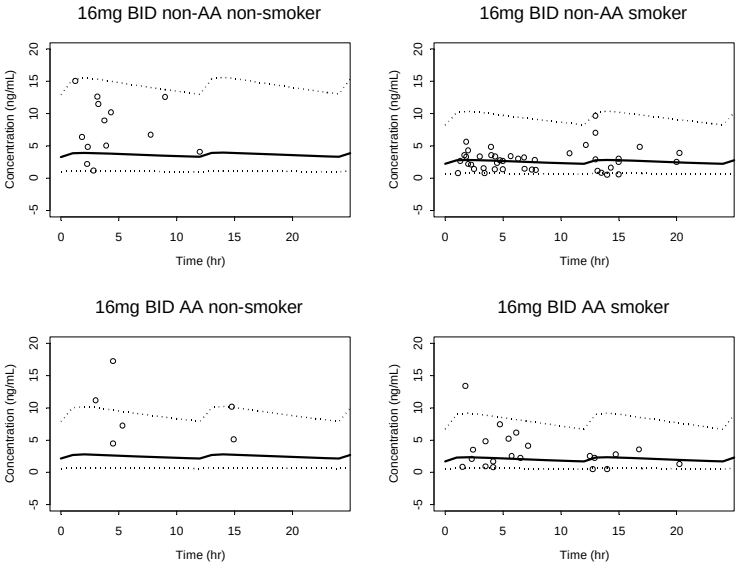


II: Predictive check plots for mostly administered dose levels (16mg QD/BID, 24mg QD) by race and smoking status in the study. Steady state of perphenazine concentration was assumed in the simulation. Solid lines were mean prediction. Dashed lines were 95% CI for the mean. Open points were observed perphenazine concentrations since most recent dosing time.

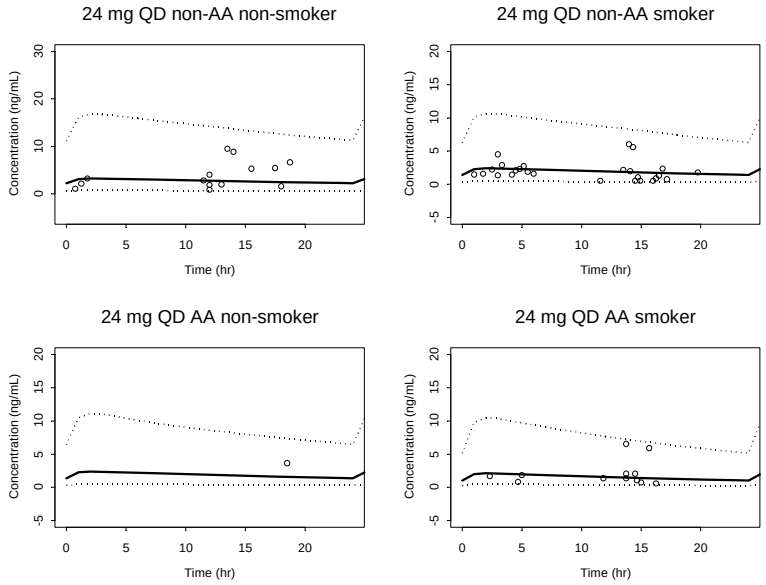
1) 16mg QD by race and smoking status



2) 16mg BID by race and smoking status



3) 24mg QD by race and smoking status



III: Effect of the concomitant medications on clearance of perphenazine

Covariate	**-2LL**	**D-2LL**	**P value**
Base model	**559.11**		
Base + Paroxetine (n=4)	556.421	-2.689	> 0.05
Base + Ranitidine (n=6)	555.85	-2.011	> 0.05
Base + Haloperidol (n=4)	556.127	-2.983	> 0.05

None of concomitant medications were identified as significant covariates for perphenazine clearance.

3.0 THIRD CHAPTER

Effect of age, weight and CYP2C19 genotype on escitalopram exposure

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3.1 ABSTRACT

Objective: The purpose of this study was to characterize escitalopram population pharmacokinetics (PK) in patients treated for major depression in a cross-national, U.S.-Italian clinical trial.

Methods: Data from the two sites participating in this trial, conducted at Pittsburgh (USA) and Pisa (Italy) were utilized. Patients received 5, 10, 15, or 20 mg of escitalopram daily for a minimum of 32 weeks. Nonlinear mixed-effects modeling (NONMEM) was used to model the PK characteristics of escitalopram . One and two compartment models with various random effect implementations were evaluated during model development. Objective function values (OFV) and goodness of fit plots were used as model selection criteria. CYP2C19 genotype, age, weight, BMI, sex, race, and clinical site were evaluated as possible covariates.

Results: 320 plasma concentrations from 105 Pittsburgh patients and 153 plasma concentrations from 67 Pisa patients were available for the PK model development. A one-compartmental model with linear elimination and proportional error best described the data. Apparent clearance (CL/F) and volume of distribution (V/F) for escitalopram without including any covariates in the patient population were 23.5 L/h and 884 L, respectively. CYP2C19 genotype, weight and age had a significant effect on CL/F, and patient BMI affected estimated V/F. Pisa, Italy patients had significantly lower clearances

than Pittsburgh patients that disappeared after controlling for patient CYP2C19 genotype, age, and weight. Post-processed individual empirical Bayes estimates on clearance for the 172 patients show that patients without allele CYP2C19*2 or *3 (n=82) cleared escitalopram 33.7% faster than patients with heterogeneous or homogeneous *2 or *3 (*17/*2, *17/*3, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3,n=46). CL/F significantly decreased with increasing patient age. Patients younger than 30 years (n=45) cleared escitalopram 20.7% and 42.7% faster than patients aged 30-50 years (n=84) and greater than 50 years of age (n=43), respectively.

Conclusions: CYP2C19 genotype, age, and weight strongly influenced the CL/F of escitalopram. Patients with heterogeneous or homogeneous CYP2C19*2 or *3 genotype had significantly lower clearances than patients with other genotypes. CL/F significantly decreased with either increasing age or decreasing body weight. These variables may affect patient tolerance of this antidepressant and, consistent with the NIH emphasis on personalized treatment, may provide important information in the effort to tailor treatments to patients' individual needs .

Keywords: Escitalopram, NONMEM, Pharmacokinetics, SPECTRUM study, CYP2C19 gene, pharmacogenetics

3.2 INTRODUCTION

Mood and anxiety disorders have been listed among the world's ten most disabling illnesses by the World Health Organization (64, 65). Escitalopram, the S-enantiomer of antidepressant citalopram, is one of the most commonly prescribed selective serotonin reuptake inhibitors (SSRI) (64). It selectively binds to the primary reuptake inhibitory site of the serotonin transporter to produce its activity against both depression(66-68) and anxiety disorders (64, 69, 70). Escitalopram is highly selective for the serotonin transporter and approximately 30-fold more potent than R-citalopram (71). Escitalopram has been shown to have efficacy and safety advantages over citalopram (72-75). After oral administration, maximum plasma concentrations are reached in about 4 hours. The half life of escitalopram is 27-32 hours. Therefore, it is commonly given once daily (76, 77).

The study "Depression: The Search for Treatment-relevant Phenotypes" was a clinical trial conducted to determine the mediators and moderators of treatment response in major depression(http://clinicaltrials.gov/ct/show/NCT00073697) (78). This study was conducted at two sites, Pittsburgh (USA) and Pisa (Italy). During the study, a significant number of Pisa participants experienced intolerable side effects at the starting dosages. This was not seen in the Pittsburgh patients. One explanation could be systematic differences in the disposition (pharmacokinetics) of escitalopram in the Pisa compared to the Pittsburgh patients. More specifically, if Pisa patients cleared the escitalopram more slowly than Pittsburgh patients, this difference would lead to higher drug exposure in Pisa patients at the same dosage level. The resulting higher concentrations may result in side effects. The sampling protocol in this study provides the basis for determining individual specific exposures that can then be evaluated across the two study sites.

Population pharmacokinetic (PPK) analysis is a robust tool for obtaining pharmacokinetic information, including inter-individual variability in exposure, from large clinical trials with sparse sampling (43, 44). The effect of potential covariates on drug exposure can also be evaluated using this approach. Data from this study provides the basis for determining the population PK characteristics of escitalopram in this patient

population. This includes the evaluation of the impact of patient specific factors on escitalopram disposition including the CYP2C19 genotype predicted metabolizer phenotype. A better understanding of the effect of such factors has the potential to play a key role in personalizing the treatment of depression. In clinical practice, first antidepressant prescriptions are more commonly *not* refilled than otherwise (79), most probably because many patients have difficulty tolerating the side effects of these medications. Yet, information about the effects of a small number of variables on clearance could lead to more patient-specific prescribing practices and, in turn, to much better treatment adherence.

Other population PK analyses using these types of data have been published for citalopram (80). In these reports, age and weight significantly affected the clearance (80). Limited information on the population pharmacokinetics of escitalopram is available in the literature (81-83). A 50% reduction in elimination rate of escitalopram in elderly patients (≥65 years old) compared to younger healthy volunteers has been reported (82) (www.cipralex.com/images/cipralex/smpc.pdf; www.lexapro.com). Sex had no clinically significant effect on escitalopram disposition healthy volunteers (77, 82). A population pharmacokinetic study in 24 patients with varying liver function showed a correlation of escitalopram clearance with CYP2C19 functional activity as measured by mephenytoin S/R excretion ratio. This study also demonstrated a relationship between body weight and the apparent volume of distribution for escitalopram (83). A classical pharmacokinetic study in healthy scandinavian subjects showed a 21% reduction reduction in the AUC_{0-12} (84). No systematic PPK analysis of escitalopram has been published in patients with major depression. This study provides the opportunity to do so and to evaluate how patient specific characteristics (such as CYP2C19 phenotype, race, age, weight/BMI, clinical trial location and sex) may affect the PK of escitalopram.

The goal of this study was to develop a robust population pharmacokinetic model describing escitalopram that evaluated patient-specific characteristics and the potential contribution of these factors to the observed variability in escitalopram exposure and potentially explain the difference in susceptibility to toxicity in the Pisa versus the Pittsburgh based patients.

3.3 SUBJECTS AND METHODS

3.3.1 Subjects and Concentration sampling

Escitalopram PK data were drawn from the study (http://clinicaltrials.gov/ct/show/NCT00073697, Depression: The search for treatment-relevant phenotypes) (78). This study was conducted at two treatment sites, Pittsburgh and Pisa, Italy. Participants were randomly assigned to a treatment sequence that began with interpersonal psychotherapy (IPT) or pharmacotherapy alone. Participants assigned to IPT who did not evidence a response at week 6 or a remission at week 12 had escitalopram added to their treatment. A total of 172 patients, aged 20-65 years old, were recruited in the study and randomly allocated to escitalopram alone or received escitalopram as an adjuctive treatment (105 patients from Pittsburgh and 67 patients from Pisa). All patients were in an episode of non-psychotic major depression defined by the DSM-IV diagnosis and were not receiving any other anti-depressant treatments. A daily dose of 5, 10, 15, or 20 mg of escitalopram was prescribed to patients for a minimum of 32 weeks. Blood samples (10 ml) for the determination of escitalopram drug concentrations were collected at weeks 4, 12, 24 and 36. 320 blood samples from 105 Pittsburgh subjects and 153 blood samples from 67 Pisa subjects were available for data analysis. The actual sample times and dates of all blood draws were recorded. Seventy-three of the Pittsburgh patients were also monitored using the Medication Event Monitoring System (MEMS) to provide dosage history timing information. All other patients provided a time of last dose.

3.3.2 Determination of Escitalopram Concentrations

Blood samples (10 ml) were collected by venipuncture using a tourniquet and a 21g needle placed into lavender top Vacutainer tubes containing 15 % EDTA. The blood was placed in a refrigerated tabletop centrifuge (5°C) and processed for 10 minutes at 1500g. The plasma layer was transferred into 5 ml polypropylene tubes and frozen at -70°C until analyzed.

The escitalopram concentration analysis method was developed by the Geriatric Psychopharmacology Laboratory at the University of Pittsburgh. Escitalopram was measured by reverse-phase high performance liquid chromatography (HPLC) using ultraviolet detection at a wavelength of 210 nm. Plasma was extracted using liquid-liquid extraction (ethyl acetate in heptane; 2:8, v/v) and back-extracted into 0.025 M potassium phosphate, pH 2.4. Separation was completed using a Nucleosil-100 C18 5 um HPLC column (Phenomemex, Torrance, CA), 120mm x 4.6 mm i.d. with a flow rate of 1.0 ml/minute. The assay was linear in the range of 2.5-500 ng/ml with an inter-assay variability (C.V.) of 2.9-3.93% for escitalopram.

3.3.3 Determination of Patient CYP2C19 Genotype

Genomic DNA was extracted from venous blood samples using the phenol chloroform method (n= 99), as well as the QIAamp 96 DNA Blood Kit (n = 125).

A total of four SNPs namely, CYP2C19*2 (rs4244285), *3(rs4986893), *17rs12248560) and a Tag SNP of *2, rs6583954 were genotyped across the CYP2C19 gene. Genotyping was performed by TaqMan assay for allelic discrimination using the Applied Biosystem Prism 7900HT instrument and analysed using the allelic discrimination end-point analysis mode of the Sequence Detection software package version 2.2 (SDS 2.2). Metabolizing status has been assigned according to the study by Rudberg et al(85).

Subjects were classified according to the methods described by Rudberg et al (85) specifically: Rapid metabolizers (RM) if they were homozygous for *17 allele; Extensive metabolizers (EM) if they were either homozygous for the wildtype allele *1/*1 or were *1/*17; Intermediate metabolizers (IM) if they carried any of *1/*2, *1/*3, *17/*2 or *17/*3 genotypes; Poor metabolizers (PM) if they were homozygous for either *2 or *3 alleles.

3.3.4 Population Pharmacokinetic Analysis

3.3.4.1 Base model development

Nonlinear mixed effects modeling for escitalopram PPK was performed using NONMEM® (version 5.1.1, Icon, Hanover, MD). A base model without any covariates was developed initially. One and two-compartment linear mammillary PK models with first order absorption and elimination was evaluated using ADVAN2 TRANS2 and ADVAN4 TRANS4 during model development, respectively. The base model also included a statistical model where the between subject variability (BSV) and within subject variability (WSV) was described. The BSV describes the unexplained random variability in individual values of structural model parameters. It was assumed that the BSV of the PK parameters was log-normally distributed. The relationship between a PK parameter (*P*) and its variance could therefore be expressed as shown below(47, 48):

$$P_j = P_{TV} \times e^{\eta_P}$$

Where, P_j was the value of PK parameter for the jth individual, P_{TV} was the typical value of *P* for the population, and η_P denoted the difference between P_j and P_{TV}, independently, which was identically distributed with a mean of zero and variance of ω_P^2.

The residual variability was comprised of, but not limited to, within subject variability, experimental errors, process noise and /or model misspecification. This variability was modeled using additive, proportional and combined error structures as described below(47, 48):

Additive error: $y_{ij} = \hat{y}_{ij} + \varepsilon_{ij}$

Proportional error: $y_{ij} = \hat{y}_{ij}(1 + \varepsilon_{ij})$

Combined additive and proportional error: $y_{ij} = \hat{y}_{ij}(1 + \varepsilon_{ij}) + \varepsilon_{ij}'$

Where y_{ij} was the jth observation in the ith individual, $\hat{y}_{ij}$ was the corresponding model prediction, and ε_{ij} (or ε_{ij}') was a normally distributed random error with a mean of zero and a variance of σ^2.

Both population characteristics and individual specific parameters were determined in this analysis. Model parameters for both the base model and the final model were estimated by the first-order conditional estimation (FOCE) with interaction method.

3.3.4.2 Final model development

The final model was developed by incorporating the effect of subject specific covariates on PK parameter estimates. Both continuous covariates (e.g., age, weight, and BMI) and discrete covariates (e.g., CYP2C19 genotype, clinical trial location, sex, and race) were tested.

The effect of continuous covariates (e.g., age, weight, and BMI) on PK parameter estimates was tested using three possible model structures. The following example illustrates the implementation of these model structures for continuous covariate on CL:

$TVCL = \theta_{CL} * (Cov / Med_{\text{cov}})^{\theta_{Cov}}$ (Centered power model);

or $TVCL = \theta_{CL} * \exp(\theta_{Cov} * (Cov / Med_{Cov}))$ (Centered exponential model);

or $TVCL = \theta_{CL} + \theta_{Cov} * (Cov - Med_{Cov})$ (linear model);

$CL = TVCL * e^{\eta_j}$

TVCL was the typical value of CL in the population; η_j was the random effect describing the difference of the jth subject from the typical population value; Cov represents the subject specific value of the continuous covariate; Med_{Cov} was the median of *Cov*. θ_{CL} and θ_{Cov} were estimated fixed effect parameters.

The effect of a discrete binary covariate (clinical trial location and sex) on PK parameter estimates was tested as well; the following example illustrates an example of the effect of sex on CL(47, 48):

$TVCL = \theta_{CL} + Sex * \theta_{Sex}$

$CL = TVCL * e^{\eta_j}$

For male patients, sex was equal to 0, while for female patients sex was equal to 1. θ_{Sex} was an estimated fixed effect parameter for covariate sex, representing the numerical differences in the typical CL value between females and males. Other parameters (eg, *TVCL,* η_j, θ_{CL}, *and* θ_{Sex}) are described previously.

Categorical variables (2C19 genotype and race) with more than two categories were assigned to each of subgroup (i.e. RM/EM=1, IM/PM=2, missing=3). An example of incorporating effect of genotype on CL estimate was shown in the following expressions: (47, 48):

IF (GENE.EQ.1) $TVCL=\theta_{CL1}$

IF (GENE.EQ.2) $TVCL=\theta_{CL2}$

IF (GENE.EQ.3) $TVCL=\theta_{CL3}$

$$CL = TVCL * e^{\eta_j}$$

Where θ_{CL1}, θ_{CL2}, and θ_{CL3} were typical values of CL for 2C19 RM/EM, IM/PM, and missing subpopulations, respectively.

The possible relationship between individual Bayesian estimates for each parameter and the covariates was initially assessed by a graphical method using R® (version 2.6.2). Continuous covariates (e.g., age, weight, and BMI) and discrete covariates (e.g., CYP2C19 genotype, clinical trial location, sex, and race) were incorporated into each parameter in a stepwise fashion. The covariate was retained in the model if the objective function value (OFV) was decreased by 3.84 when adding one additional fixed effect parameter into model (χ^2 $p < 0.05$ df = 1). Goodness of fit plots were used as additional model selection criteria. Additional post-processing of NONMEM® outputs were performed using SPSS (version 14.0).

3.4 RESULTS

3.4.1 Patient characteristics

Patient demographics are summarized in Table 1. The average age of the subjects was 39.52 years with an average of body weight of 76.25 kg and average BMI of 27. The majority of subjects were white (n=161, 93%) with minority black/African American (n=8, 5%) and Asian subjects (n=3, 2%). The histogram distribution plot of the escitalopram sampling times after most recent dose is shown in Figure 1a. These sampling times were distributed in a broad time range. The distribution of sampling times provide more information to estimate population PK parameters of escitalopram compared to many population studies which only have trough sample available (86). The mean time after dose for concentration sampling the Pittsburgh patients was 11.99 hours and in the Pisa patients was 11.76 hours. Both groups had a large standard deviation around these times, specifically 11.4h for Pittsburgh and 11.8h for Pisa. Histograms of patient age, weight, and BMI are shown in Figure 1 panels b-d.

All markers were in Hardy-Weinberg equilibrium as confirmed by Pedstats software1. DNA samples from 128 patients out of 172 were available for genotyping. Allele *3 has not been detected in our samples. According to the methods described by Rudberg et al.(85), we identified 5 RM (4 Pittsburgh vs 1 Pisa), 77 EM (54 Pittsburgh vs 23 Pisa), 43 IM (28 Pittsburgh vs 15 Pisa), and 3 PM (3 Pittsburgh vs 0 Pisa). CYP2C19 frequency for RM, EM, IM, and PM were 3.9%, 60.2%, 33.6%, and 2.3%, respectively, among all detected samples. The frequency of RM, EM, IM, and PM were 4.5%, 60.7%, 31.5%, 3.4% for the Pittsburgh site and 2.6%, 59.0%, 38.5%, 0% for the Pisa site, respectively. No DNA samples were available for genotyping for 44 patients out of 172 total. Among these 44 patients, 16 were from Pittsburgh and 28 were from Pisa.

3.4.2 Population pharmacokinetic modeling

A one-compartment model with linear elimination and proportional residual error best described the escitalopram pharmacokinetics in this patient population. Oral clearance (CL/F) and volume of distribution (V/F) in the patient population for escitalopram were 23.5 L/h and 884 L in base model without incorporating any patient specific covariates, respectively. Patient CYP2C19 genotype, age, and weight had a significant impact on CL/F estimates, and patient BMI significantly affects V/F estimates.

Clinical trial location was a significant covariate for CL/F in the initial univariate forward analysis. However, the effect of clinical site in CL/F disappeared after controlling for patient CYP2C19 genotype, age, and weight effects on CL/F. The process of final model development is summarized in Table 2. Figure 2 shows diagnostic plots for the final model. The scatter plots of the observed versus predicted population concentrations and observed versus predicted individual concentrations were distributed symmetrically around the line of unity. The weighted residuals were distributed symmetrically around zero. No systematic shift in residuals was evident from the plots of weighted residual versus predicted population concentrations and weighted residual versus time after dose.

Estimates for the full set of population PK parameters along with the standard errors for final model are listed in Table 3. Patient genotype was initially included as a model covariate on CL/F five categories: RM (n=5) vs EM (n=77) vs IM (n=43) vs PM (n=3) vs missing (n=44). This was followed with a three category analysis by pooling RM and EM into one subpopulation (n=83), and IM and PM into one subpopulation(n=46). Both models showed that the CYP2C19 genotype is a significant covariate affecting escitalopram clearance estimates. However, breaking genotype into five categories did not improve model fit compared to the three category model. Estimated population CL/F (arising from the posterior mode of the marginal likelihood distribution for this parameter) for CYP2C19 RM/EM (n=83) and IM/PM (n=46) and the individuals missing genetic information (n=44) were 26 , 19.8 , and 21.5 L/Hr, respectively.

CL/F significantly decreased in a centered power function model as patient age increased. The age relationships are shown as: $CL/F = CL_0*(age/40)^{-0.336}$ *L/hr.* The clearance also increased with increasing weight with the following relationship:
$CL/F=CL_0*(Wgt/76)^{0.333}$.

Post-processed individual empirical Bayes estimates on CL/F for the 172 patients in different subpopulations are summarized in Table 4. RM/EM cleared escitalopram

33.7% faster than IM/PM subpopulation with mean CL/F (± SD) values of 29.73 ± 13.13 and 22.23 ± 11.04, respectively ($P<0.005$). A one way ANOVA revealed a significant difference ($P<0.005$) in the empirical Bayesian estimated CL/F of escitalopram across three age groups (20~30, 30~50, 50~65 years of age with mean CL/F (± SD) values of 31.03 (±14.88) L/h, 25.71(±11.31) L/h, and 21.74 (±9.89), respectively). Patients younger than 30 years (n=45) cleared escitalopram 20.7% and 42.7% faster than patients aged 30-50 years (n=84) and 51 years of age or older (n=43), respectively. Pittsburgh patients (n=105) cleared escitalopram 28% ($P<0.001$) faster than patients from Pisa, Italy (n=67) with mean CL/F (± SD) values of 28.55 ± 13.54 and 22.28 ± 9.32, respectively ($P<0.001$).The scatter plots of the empirical Bayes estimates for CL/F (n=172) by 2C19 genotype, clinical trial location, age, weight are shown in figure 3. Patient BMI, Sex, and Race were not significant covariates affecting CL/F.

Estimated V/F increased in a power function relationship ($V/F=V_0*(BMI/27)^{1.11}$) with patient BMI with estimated exponent of 1.11. Patient body weight, age, and clinical location did not significantly affect V/F estimates.

3.5 DISCUSSION

In this study, we successfully captured population and individual level exposure information for escitalopram in patients with major depression using sparsely sampled data. This study showed that apparent clearance of escitalopram varies nearly 10-fold in patients (n=172) with major depression, ranging from 6.24 to 67.10 L/h. CYP2C19 genotype, age, and weight were identified as significant contributors to the variability in escitalopram clearance in this patient population. This extends previous findings that showed a correlation with functional capacity of the CYP2C19 enzyme using Mephenytoin S/R enantiomer excretion ratios as a correlate of population clearance and weight as a correlate of population weight (83). This study also establishes a population pharmacokinetic model that incorporates data for many more subjects (n=172) than previous models (n=24) (83).

Recently, Rudberg et al (85) showed the impact of the 2C19*17 polymorphisms on escitalopram concentrations in Norwegian psychiatric patients. In these patients, individuals homozygous for the CYP2C19 *17/*17 alleles showed a 42% reduction in observed concentrations. In our study, however, no difference in clearance between CYP2C19 *17/*17 alleles (RM,n=5) and *17/*1, *1/*1 alleles (EM, n=77), and no difference between heterozygous *2 allele (IM, n=43) and homozygous *2 allele (PM, n=4) were identified. This may be attributed to the small number of patient with *17/*17 and homozygous *2/*2 alleles. However, this study showed that RM/EM cleared escitalopram 33.7% faster than IM/PM subpopulation with mean CL/F (± SD) values of 29.73 ± 13.13 and 22.23 ± 11.04, respectively ($P<0.005$).

A significantly lower escitalopram elimination rate (50%) in elderly patients (≥65 years old) compared to younger healthy volunteers was reported previously (82) (www.cipralex.com/images/cipralex/smpc.pdf; www.lexapro.com). This finding is confirmed in our study and extended as a continuous relationship across age. This is consistent with reports that CYP 2C19 activity decreases with increasing age (87) and is now more specifically quantified in the case of escitalopram. The CL/F significantly decreased in a centered power function model as patient age increased. Patients younger than 30 years cleared escitalopram 20.7%, and 42.7%faster than patients 30~50, and >50 years, respectively. Hence, the dose of escitalopram may need to be adjusted clinically based on patient age, especially for those over 50. This change in clearance and therefore exposure may be of particular concern in patients with panic symptoms as these individuals may be more sensitive to concentration-related side effects. A concentration related amygdala activation with acute administration of citalopram may be related to panic and anxiety symptomatology (88).

Elderly patients are at highest risk of completed suicides in the first month of treatment when therapy is not fully tailored and excessive exposure may occur (89). Long-term excessive exposure in the elderly may lead to an increased risk of hyponatremia (90), GI bleed secondary to platelet-related effects (91, 92), an increased risk of falls and fragility fractures (93) as well as bradycardia (94).

Clinical trial location was a significant covariate in the initial univariate forward analysis (Table 2). There may be several contributors to this systematic difference in elimination across site. In our study, correlation between clinical trial location (Pittsburgh vs Pisa) and 2C19 genotype was not significant. The frequencies of RM/EM and IM/PM were 65.2%, 34.8% for Pittsburgh site and 61.5%, 38.5% for Pisa site, respectively. Hence, patients from Pittsburgh are virtually indistinguishable from Pisa patients on the basis of genotype frequencies.

However, the weights and BMI values were significantly different (approximately 14 kg heavier and 3.3 BMI points larger in Pittsburgh) for the two sites. When genotype, age, and weight were incorporated as a covariate affecting clearance, the site factor no longer had a significant impact on clearance (the inclusion of clinical site after incorporating genotype, age, and weight together only improved the model fit by 3.32 objective function points ($p>0.05$)). This is in contrast to earlier analyses where genotype information was not available and weight was not a significant covariate on clearance (data not shown). Post-processed individual empirical Bayes estimates on CL/F, which includes the effect of genotype, age, and weight differences between locations, shows that Pittsburgh patients cleared escitalopram 28% faster than patients from Pisa, Italy. The resulting difference in exposure for a given dose may partially explain the difference in tolerability for escitalopram between Pittsburgh and Pisa patients. In this case, at a given dose, patients in Pisa were more likely to experience a higher concentration exposure. However, the difference was accounted for by differences in CYP2C19 genotype, age, and body weight. Therefore, the dosage regimen for a patient may need to be adjusted on the basis of genotype predicted phenotype, age and weight.

Sex has not been reported to exert clinically significant effect on PK parameters of escitalopram in healthy volunteers (77, 82). This was confirmed in the population PK analysis described herein. Similarly, race did not have a statistically significant effect on the PK parameter estimation. However, 93% of patients in this study were white, 5% were Black/African American and 2% were Asian. It is possible that the relatively small percentage of African Americans and Asians in this study prevented the detection of any systematic difference across race.

In conclusion, apparent clearance (CL/F) and volume of distribution (V/F) for escitalopram in the patient population were 23.5 L/h and 884 L, respectively. This is consistent with preliminary population analyses reported by Areberg (83). CYP 2C19 genotype, age, and weight strongly influenced the CL/F of escitalopram. Patients with CYP2C19 RM/EM cleared escitalopram significantly faster than those with 2C19 IM/PM and older patients had a significantly lower apparent clearance compared with younger patients. Patients with higher body weights cleared escitalopram faster compared to those with lower body weights. Incorporating age weight and genotype into the population PK model accounted for the majority of the variability in escitalopram exposure in this study. Therefore, establishing a patient's metabolizer genotype and incorporating age, weight and BMI into this assessment can better guide therapeutic decision-making with respect to the dosing strategy for escitalopram and potentially minimize excessively high exposure to this SSRI. What is of particular note for community practice is that two of these variables (age and weight) are routinely collected and require no specialized equipment or laboratory tests. Thus, physicians can readily take these variables into account when determining appropriate starting dose and upward titration schedules.

3.6 TABLES

Table1: Patient demographics

Demographics	Pittsburgh Patients	Pisa Patients	All patients
Number of Subjects	105	67	172
Number of Observations	320	153	473
Number of Observations for each subject	3.048	2.2836	2.75
CYP2C19 genotype			
Rapid metabolizers (RM, *17/*17)	4	1	5
Extensive Metabolizers (EM, *17/*1,*1/*1)	54	23	77
Intermediate Metabolizers (IM, *1/*2, *1/*3, *17/*2, *17/*3)	28	15	43
Poor Metabolizers (PM, *2/*2, *3/*3, *2/*3)	3	0	3
Missing	16	28	44
Age, Mean Years ± SD (range)	38.84 ± 12.05 (20.4-64.67)	40.58 ± 11.20 (21-65)	39.52 ± 11.73 (20.41 - 65)
Weight, mean kg ± SD (range)	81.6 ± 20 (31.9 - 139.7)	67.8 ± 15.2 (40-116)	76.25 ± 19.45 (31.9-139.7)
BMI, mean lbs/in^2 ± SD (range)	28.20 ± 6.78 (15.55 - 48.26)	24.94 ± 4.52 (16.63-37.41)	26.93 ± 6.20 (15.55 - 48.26)
Sex, n (%)			
Male	47 (45)	7 (10)	54 (31)
Female	58 (55)	60 (90)	118 (69)
Race, n (%)			
White	94 (89)	67 (100)	161 (93)
Black/African American	8 (8)	0 (0)	8 (5)
Asian	3 (3)	0 (0)	3 (2)

Table 2: Population pharmacokinetic model development for escitalopram

Covariate		Model	-2LL	Δ -2LL	P value
1		Base model	2729.78		
Univariate Forward Selection					
1~1 (CL)	CYP2C19 Genotype	M1	2721.40	-8.38	< **0.05**
	Age (Centered Power Model)	M2	2724.24	-5.54	< **0.05**
	Weight (Centered Power Model)	M3	2725.15	-4.64	< **0.05**
	BMI (Centered Power Model)	M4	2729.30	-0.49	> 0.05
	Sex	M5	2748.44	18.66	> 0.05
	Race	M6	2726.60	-3.18	> 0.05
	Site (Pittsburgh vs. Pisa)	M7	2719.78	-10.01	< **0.05**
1~2 (V)	Weight (Centered Power Model)	M8	2729.08	-0.70	> 0.05
	BMI (Centered Power Model)	M9	2725.93	-3.85	< **0.05**
	Age (exponential model)	M10	2729.69	-0.09	> 0.05
	Site (Pittsburgh vs. Pisa)	M11	2728.42	-1.36	> 0.05
Stepwise Backward Elimination					
2~1	CL(Genotype+Age+Wgt)+V(BMI)	M12	2707.11		
	Eliminate Weight from CL	M13	2711.23	4.13	< **0.05**
	Eliminate Age from CL	M14	2712.83	5.72	< **0.05**
	Eliminate Genotype from CL	M15	2715.02	7.91	< **0.05**
	Eliminate BMI from V	M17	2712.65	5.54	< **0.05**
3~1	CL(Genotype+Age+Wgt+Site)+V(BMI)	M18	2703.79	-3.32	> 0.05
Final model	***CL(Genotype+Age+Wgt)+V(BMI)***	***M12***	***2707.11***		

Table 3: Escitalopram pharmacokinetic parameter from final model

Parameters	Final Model Estimates	SE%
CL for 2C19 Rapid and Extensive (L/Hr)	26	7.20%
CL for 2C19 IM and PM (L/Hr)	19.8	8.50%
CL for 2C19 missing (L/Hr)	21.5	7.80%
Age on Clearance	$CL_1=CL_0*(Age/40)^{-0.336}$	42.00%
Weight on Clearance	$CL_2=CL_1*(Wgt/76)^{0.333}$	54.10%
V (L)	947	10.20%
BMI on V	$V*(BMI/27)^{1.11}$	49.50%
Ka (hr^{-1})	0.8	N/A
ω_{cl} %	48.5%	15.10%
ω_v %	62.0%	40.30%
ω_{Ka} %	78.9%	87.00%
$\omega_{cl,v}$ %	9.4%	N/A
$\omega_{cl,Ka}$ %	47.8%	N/A
$\omega_{V,Ka}$ %	81.3%	N/A
σ_1 %	28.9%	8.80%

CL, clearance; V, volume of distribution; SE, standard error; ω, coefficient of variation of inter-individual variability; σ, coefficient of variation of residual error

Table 4: Post-processed individual empirical Bayes estimates on clearance

Population	Mean Clearance (L/Hr)	Standard Deviation (L/Hr)	P value
Genotype information			<0.005
Rapid and Extensive (n=82)	29.73	13.13	
IM and PM (n=46)	22.23	11.04	
Missing (n=44)	23.41	10.65	
Age			<0.005
< 30 years old (n=45)	31.03	14.88	
30~50 years old (n=84)	25.71	11.31	
50~65 years old (n=43)	21.74	9.89	
Clinical Trial Location			<0.001
Pittsburgh patients (n=105)	28.55	13.54	
Pisa patients (n=67)	22.28	9.32	

3.7 FIGURES

Figure 1a: Frequency histogram showing the distribution of the sampling time after most recent doses (hrs)
Figure 1b: Frequency histogram of patient age
Figure 1c: Frequency histogram of patient body weight
Figure 1d: Frequency histogram of patient BMI

Figure 1a

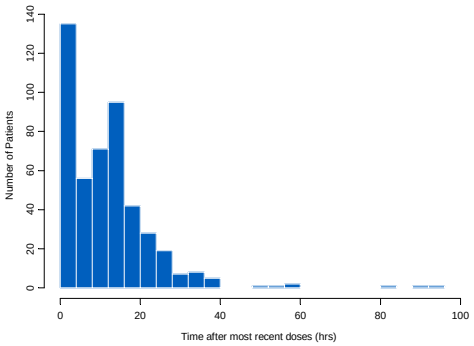


Figure 1b

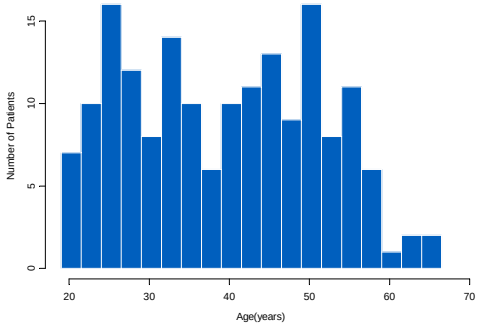


Figure 1c

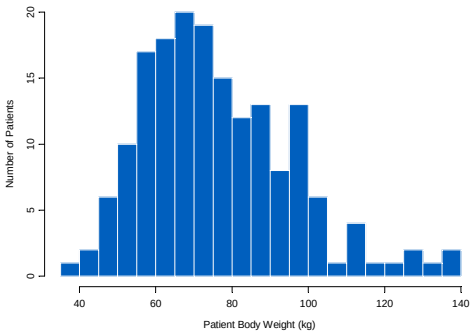


Figure 1d

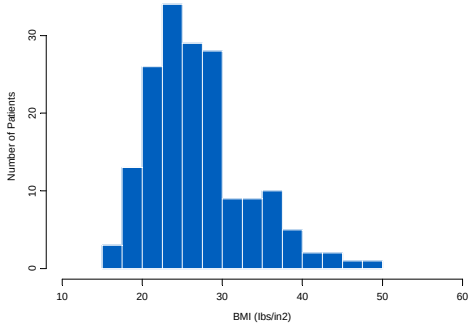


Figure 2: Diagnostic plots of final PK model. (A) Population predicted vs observed concentrations (B) Individual predicted vs observed concentrations (C) Weighted residuals versus concentration (D) weighted residuals versus time.

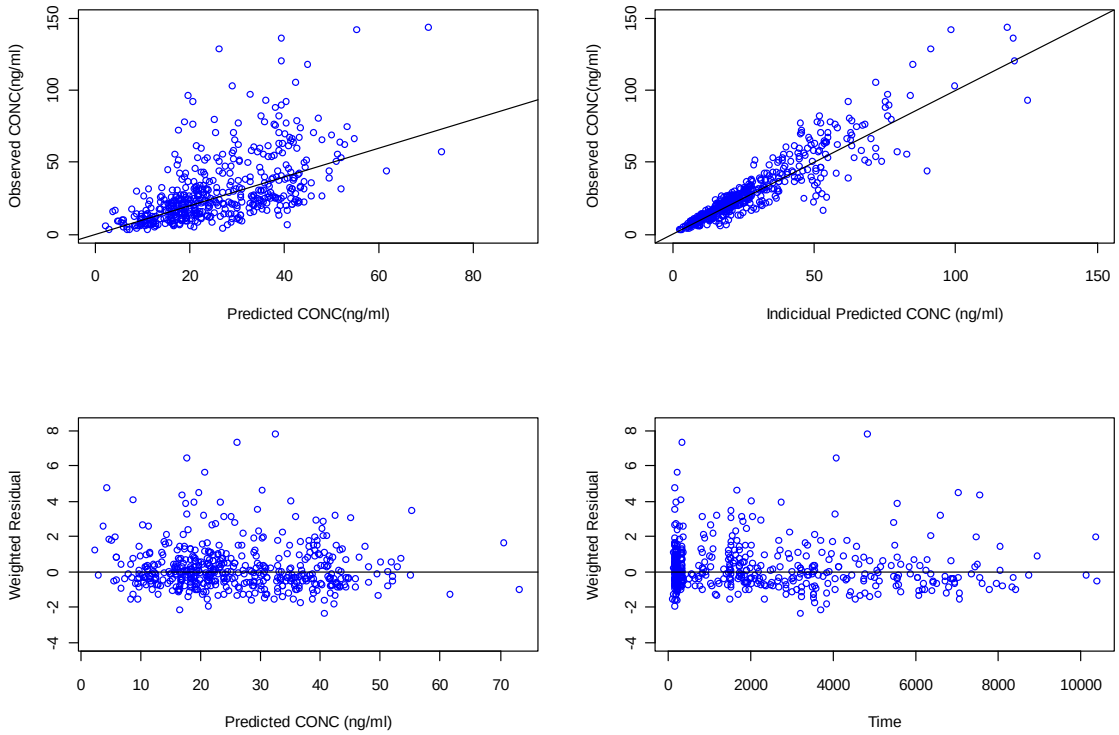
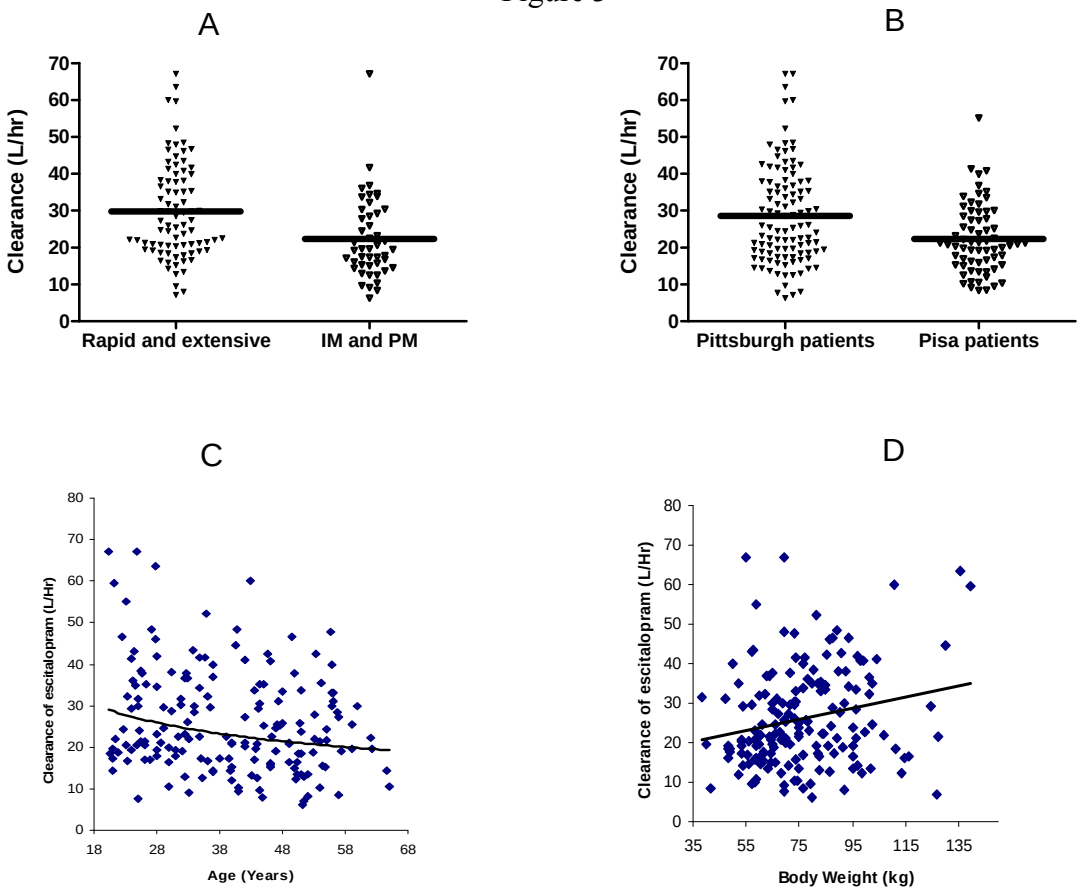


Figure 3: Escitalopram clearance by (A)CYP2C19 genotype, (B) clinical trial location, (C) age and (D) weight

Figure 3



4.0 FOURTH CHAPTER

The effect of reporting methods for dosing times on the estimation of pharmacokinetic parameters of escitalopram

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4.1 ABSTRACT

The objective of this study was to compare population pharmacokinetic (PPK) models of escitalopram developed from dosage times recorded by a medication event monitoring system (MEMS) versus the reported times from patients with diagnosed depression. 73 patients were prescribed doses of 10, 15 or 20 mg of escitalopram daily. Sparse blood samples were collected at weeks 4, 12, 24 and 36 with 185 blood samples obtained from the 73 patients. NONMEM was used to develop a PPK model based on dosing records obtained from MEMS prior to each blood sample time. A separate PPK analysis using NONMEM was performed for the same population using the patient reported last dosing time and assuming a steady state condition as the model input. Objective function values (OFV) and goodness of fit plots were used as model selection criteria. The absolute mean difference in the last dosing time between MEMS and patient reported times was 4.48 ± 10.12 hrs. A one compartment model with first-order absorption and elimination was sufficient for describing the data. Estimated oral clearance (CL/F) to escitalopram was statistically insensitive to reported dosing methods, (MEMS vs. patient reported: 25.5 (7.0%) vs. 26.9 (6.6%) L/hr). However, different dosing report methods resulted in significantly different estimates on the volume of distribution (V/F) (MEMS vs. Patient reported: 1000 (17.3%) vs. 767 (17.5%) L) and the absorption rate constant Ka (MEMS vs. Patient reported: 0.74 (45.7%) vs. 0.51 (35.4%) hr^{-1}) for escitalopram. Furthermore, the parameters estimated from the MEMS method were similar to literature reported values for V/F (~1100L) and Ka (~ 0.8-0.9 hr^{-1}) arising from traditional PK approaches.

Keywords: medication event monitoring system (MEMS), population pharmacokinetics, escitalopram, selective serotonin reuptake inhibitors (SSRI), antidepressant

4.2 INTRODUCTION

Population pharmacokinetic (PPK) analysis is a robust tool for obtaining valuable pharmacokinetic information from large clinical trials, where protocols may be limited to sparse drug concentration sampling (43). However, it is difficult to obtain accurate dosing times from these clinical trials, as most dosages are not taken under direct observation. This is especially true for outpatients with chronic disease (95-97). Population PK model input comprised of the patient reported last dosing time and a steady state assumption is the most commonly used approach in PPK analysis with sparse sampling (95-98). Unfortunately, a significant drawback to this approach is the steady state assumption which neglects partial adherence of outpatients to prescribed medication regimens, dosing frequency, and the timing of doses (96, 97). In fact, only an average of 43-78% adherence has been reported for outpatients with chronic treatment (99). As such, the assumption of a steady state condition during PPK modeling may not be appropriate for data sets containing patients with partial adherence.

One method for obtaining accurate outpatient dosing records is the medication event monitoring system (MEMS), an electronic system which has been commercially available since 1989 (99). MEMS detects medication bottle opening and closing over time and records these events (95). It is one of the most effective indirect methods for accurately determining the patient adherence (95, 97, 99, 100). MEMS generated information provides an alternative method for model development, providing detailed dosing records and allowing the assumption of a steady state condition to be avoided (95, 98). This approach has been successfully employed for PPK analysis with a lopinavir study providing for the recreation of the entire concentration versus time profile over the entire treatment duration (98).

Escitalopram, the S-enantiomer of antidepressant citalopram, is a commonly prescribed selective serotonin reuptake inhibitors (SSRI) (74, 101). It selectively binds to the primary reuptake inhibitory site of the serotonin transporter to produce its antidepressant effects (68, 71). Escitalopram has activity against both depression (66-68, 72, 101) and anxiety disorders (69, 70, 101). After oral administration, maximum plasma

concentrations of escitalopram are reached in about 4 hours (74, 76, 77) with a half-life of 27-32 hours (74, 76, 77). Therefore, it is commonly given as a once daily dose (76, 77).

The goal of this study was to compare the PPK parameter estimates using input profiles based on dosage history times recorded by the MEMS versus input profiles based on the last patient reported dosage time and assuming a steady state condition. Two separate PPK models for escitalopram were developed based on these separate sets of recorded dosing times with a subsequent comparison of PPK model parameters. In addition, due to difficulties commonly encountered during model development for orally administered drug, two separate model parameterizations were evaluated involving either a fixed or estimated oral compartment absorption rate (Ka).

4.3 SUBJECTS AND METHODS

4.3.1 Subjects and Sampling

Escitalopram PK data was obtained from the Pittsburgh patients in a large randomized clinical trial (Clinical Trials Gov Identifier: NCT00073697) conducted at two international treatment sites, including the University of Pittsburgh and the University of Pisa, Italy (http://clinicaltrials.gov/ct/show/NCT00073697). In summary, 73 patients, aged 18-66 years old were recruited. The patients were in an episode of non-psychotic major depression defined by the DSM-IV diagnosis and were not receiving any other anti-depressant treatments. A daily dose of 10, 15, or 20 mg of escitalopram was prescribed to patients over 69-441 days. Blood samples (10 ml) for the determination of escitalopram drug concentrations were collected at weeks 4, 12, 24 and 36, and a total of 185 samples were available for the data analysis. The actual sample times and dates of all blood draws were recorded along with the date and time of the patient reported time for the last dose. Concurrently, the entire dosing history was monitored using the MEMS system.

4.3.2 Determination of escitalopram Concentrations

Blood samples (10 ml) were collected by venipuncture using a tourniquet and a 21g needle into lavender top Vacutainer tubes containing 15 % EDTA. The blood was placed in a refrigerated tabletop centrifuge (5°C) and processed for 10 minutes at 1500g. The plasma layer was transferred into 5 ml polypropylene tubes and frozen at -70°C until analyzed.

Escitalopram was measured by reverse-phase high performance liquid chromatography (HPLC) using ultraviolet detection at a wavelength of 210 nm. This method was developed by the Geriatric Psychopharmacology Laboratory at the University of Pittsburgh. Plasma is extracted using liquid-liquid extraction (ethyl acetate in heptane; 2:8, v/v) and back-extracted into 0.025 M potassium phosphate, pH 2.4. Separation is completed using a Nucleosil-100 C18 5 um HPLC column, 120mm x 4.6 mm i.d. with a flow rate of 1.0 ml/minute. The assay is linear in the range of 2.5-500 ng/ml with an inter-assay variability (C.V.) of 2.9-3.93% and accuracy (C.V.) of 1.8-3.9%. The limit of quantitation for escitalopram 2.5 ng/ml for this assay.

4.3.3 Data Analysis

Dosing discrepancies between MEMS recorded and patient reported last dosing time were calculated as the MEMS recorded time minus the patient reported time as shown in the Equation 1:

Time interval = MEMS recorded last dose time – Patient reported last dose time (Equation 1).

Positive values indicate the MEMS recorded times were later than patient reported times, while a negative value reflects that the patient reported times were later. The absolute time interval between the two recording methods was also calculated by taking the absolute value of Equation 1.

Nonlinear mixed-effects modeling was used to develop PPK models in NONMEM® (version 5.1.1). The PPK models consisted of a PK structure model and a statistical model where between subject and within subject variability were described. To consider multiple dosing effects on each observed escitalopram concentration, the PPK

model was developed based on the ten day dosing history recorded by MEMS prior to each observed concentration. The ten day dosing records involved in the analysis were longer than five times the literature reported half-life (27-32 hr) of escitalopram, and doses given before these dosing records should not affect the measured escitalopram concentration (76, 77). A PPK analysis using NONMEM was also evaluated in the same population using the patient reported last dosing time with the assumption of a steady state condition. One and two-compartment linear mammillary PK models with first-order absorption and elimination were evaluated during model development. Model parameters were estimated using the first-order conditional estimation (FOCE) with interaction method. Objective function values and goodness of fit plots were used as model selection criteria. Diagnostic plots and post-processing of NONMEM® outputs were performed using R® (version 2.6.2) and SPSS® (version 14.0).

4.4 RESULTS

4.4.1 Population reported last dosing time

185 blood samples from 73 patients were available for data analysis with study statistics summarized in Table 1. A discrepancy in the last dosing time between the two reported dosing methods was observed. The mean and standard deviation of the absolute time intervals in the last dosing time between the two different reported dosing methods was 4.48 ± 10.12 hrs. However, the time intervals (MEMS reported last dosing time minus the patient reported) were almost symmetrically distributed with a median value and standard deviation of 0.133 ± 11.073 hrs. A histogram plot of time intervals calculated from the clinical trial is shown in Figure 1.

4.4.2 Population pharmacokinetics analysis

The proportion of blood samples collected during selected time intervals based on MEMS or patient reported last dosing times are shown in Table 2. Within the first four hours after the most recent dose, a total of 36.76% (68 observations) and 29.19% (54 observations) of the blood samples were collected based on the MEMS and patients

reported dosing times, respectively. A frequency histogram further illustrates this sampling distribution for escitalopram concentration measurements after the most recent dose for MEMS (Figure 2a) and patient self reported dosage times (Figure 2b), respectively.

Although PPK with sparse sampling are typically modeled with a fixed Ka value, initial modeling attempts focused on estimating Ka in addition to the other PK parameters. Numerical convergence was achieved when modeling concentration data based on either patient reported last dosing time as the model input with a steady state condition assumption or MEMS dosing history as the model input. A one compartment model with first order absorption and elimination accurately described the data. Goodness of fit plots for models developed from the MEMS records (Figure 3) and from patient reported last dosing time (figure 4) demonstrate that the models adequately described the data. The scatter plots of the observed versus predicted population concentrations and observed versus predicted individual concentrations were distributed symmetrically around the line of unity. The weighted residuals were also distributed symmetrically around zero with certain variance. Estimates for the full set of population PK parameters along with the standard errors from both models are listed in Table 2. Estimated oral clearance, volume of distribution, and absorption rate for escitalopram were 25.5L/hr (SE: 7.0%), 1000 L (SE: 17.3%), and 0.74 hr^{-1}(SE: 45.7%), respectively, for the model developed from MEMS records, and 26.9 L/hr (SE: 6.6%) , 767 L (SE: 17.5%) , and 0.511 hr^{-1} (SE: 35.4%) for the model developed from the patient reported last dose times. Population clearance, volume of distribution and absorption differed by 5%, 23.3%, and 31% using the MEMS dosage inputs versus the patient reported last dosing time and a steady state assumption. Parameters estimated from MEMS methods were similar to the literature reported volume of distribution (~1100 L) and absorption rate (~0.8 - 0.9 hr^{-1}) following oral administration (76, 77).

Post-processing results for individual parameter estimates are listed in Table 3. A paired t-test showed that there was no significant difference in the estimated oral clearance of escitalopram between models in these 73 patients for the empirical Bayes estimates of the clearance parameter. However, different reported dosing methods

resulted in significantly different model estimates for the volume of distribution ($P<0.001$) and absorption rate ($P<0.001$) for escitalopram at the level of the individual empirical Bayes estimates.

The population PK model was also assessed by fixing Ka to the literature reported value of 0.8 hr^{-1} (76, 77). Goodness of fit plots for models developed from the MEMS records and patient reported dose time are presented in Figure 5 and Figure 6, respectively. PPK parameters estimates along with their standard errors are listed in Table 4. Post-processing of individual parameter estimates from NONMEM output is shown in the Table 5. As before, there was no significant difference in estimated clearance using different dosing reporting methods during model development. However, estimates on volume of distribution were still sensitive ($P<0.001$) to the reported dosing methods even with the Ka value fixed to the literature value in both models.

4.5 DISCUSSION

In this study, we successfully performed a PPK analysis for orally administered escitalopram using two different reported dosing methods as the input to the model. The inputs considered were either the MEMS generated dosing histories or the patient reported last dosing time. This approach is analogous to that described by Vrijens *et al.* during a PPK analysis of the drug lopinavir (98).

It is well known that accurately estimating the Ka value is a challenge in PPK analysis, especially when data sampling is sparse(62, 80). In this study, however, 36.76% (68 observations) and 29.19% (54 observations) of the blood samples were collected before the maximal concentration following the most recent MEMS recorded and patient reported dose, respectively. Hence, initial modeling attempts included the estimation of a Ka value in addition to the other PK parameters. Numerical convergence was achieved for both models. There were 5%, 23.3%, and 31% differences in the estimated population clearance, volume of distribution, and absorption rate, respectively, between the two models. Also, the estimated oral clearance of escitalopram was

statistically insensitive to model inputs based on either the MEMS (25.5 L/hr) or patient reported dosing times (26.9 L/hr). Different reported dosing methods resulted in significantly different estimates for the volume of distribution and absorption rate constant for escitalopram both at the population level (MEMS vs. patient reported: V/F 1000 L vs. 767 L; Ka 0.74 vs. 0.51 hr^{-1}) and at post-processed individual level (MEMS vs. patient reported: V/F 1005.31 L vs. 764.98 L; Ka 0.76 vs. 0.51 hr^{-1}). Furthermore, estimates from the MEMS model were closer to the literature reported V/F (~1100L) and Ka (~0.8-0.9 hr^{-1}) (76, 77). Knowing the dosing history from the MEMS, Ka was more accurately predicted even when concentration data were sparse, and the estimated Ka population value from MEMS model was almost equivalent to that reported in the literature (76, 77). Hence, it is likely that inaccurate patient reported dosing times in addition to sparse sampling are the primary difficulties for obtaining accurate Ka values from study data.

A study conducted by Vrijens *et al*. (98) attempted to model liponavir concentration data using these two reported dosing report methods. These results showed that model convergence was not achieved using patient reported last dosing time assuming steady state conditions for the model input. In contrast, numerical convergence was achieved when the MEMS dosing history was used as the model input. In our study, numerical convergence was achieved for both reported dosing history and model structures, and clearance could be reasonably estimated in all cases This was likely due to the longer half life of the escitalopram (27 ~ 32 hours) compared to shorter half life of lopinavir (5 ~ 6 hours).(102) As a few hour deviation in recorded dosing time may have a relatively small effect on clearance estimation for a drug with a longer half-life. PPK parameters were correctly predicted previously for a longer half-life drug, citalopram ($t_{1/2}$: 30hr), using patient reported last dosing time with a steady state assumption modeling approach (80). In contrast, both a higher bias and imprecision in predicted clearance were reported when utilizing a similar analysis for the shorter half-life drug, risperidone ($t_{1/2}$: 6-7 hrs) (62). In this study, the absolute time interval in the reported last dosing time between the two patient dosing histories was 4.48 hrs, which only accounted for 13~16% of half-life of the escitalopram.

The investigators anticipated that the findings would be consistent with Vrijens et al (98) with respect to convergence problems and biased PK parameter estimates. It was surprising that the results contradicted their findings with adequate convergence of the model and little bias in the clearance estimation at both the individual and population level for escitalopram given the patient reported time of last dose. This may be accounted for in differences in study design, patient population or the long half-life of escitalopram. In particular, it may be that the patients, knowing they were being monitored, provided more accurate times of last dose information than otherwise would have been collected (103).

In this study, the use of MEMS dosage histories versus the patient reported time of last dose, helped with the determination of absorption (Ka) and distribution (Vd), but clearance determination was virtually unaffected. Despite this, patterns of drug exposure may be critical in evaluating response, non-response, and toxicity. These patterns cannot be generated or recreated using the time of last dose information. Exposures calculated in this manner may not only have incorrect PK parameter values but only provide an average exposure over a period of time. This pattern of exposure and response issue is not addressed in this study. The necessity of MEMS monitoring may be determined by the drug half-life and the nature of the concentration effect relationship and the sensitivity of this relationship to patterns of exposure that are not captured in the PPK analysis using reported time of last dose.

4.6 TABLES

Table 1. Patient democratic information

Demographics	Mean ± SD
Number of Subjects	73
Number of Observations	185
Age (years)	39.47 ± 11.35
Weight (lb)	81.83 ± 43.81
Sex	Male: 32 Female: 41
Race	Caucasian: 67 African american: 2 Asian: 1 American Indian & African American: 1 American Indian & Caucasian: 1 Unknown :1

Table 2. Sampling time distribution following the last dosing time based on either MEMS records or patient reported times.

Sampling time after the most recent dose (hr)	Cumulative percent samplings during the elapsed time	
	MEMS	Patient reported
4	36.76%	29.19%
8	48.65%	43.24%
12	60.00%	57.84%
16	74.59%	72.43%
20	84.86%	85.95%
28	91.89%	94.05%
100	100.00%	100.00%

Table 3. PPK model parameters without fixing Ka using either MEMS or patient reported dosing records as the input.

Parameters	MEMS records	Patient reported dosing time
OFV	1029.148	1031.989
CL (L/hr) (SE %)	25.5 (7.0%)	26.9 (6.6%)
V (L) (SE %)	1000 (17.3%)	767 (17.5%)
Ka (hr^{-1}) (SE %)	0.74 (45.7%)	0.511 (35.4%)
ω_{cl} % (SE %)	53.5% (14.7%)	48.3% (26.7%)
ω_{v} % (SE %)	64.3% (43.7%)	18.7% (517.1%)
ω_{Ka} % (SE %)	88.9% (106.8%)	62.6% (225.8%)
σ_1 % (SE %)	15.2% (105.2%)	23.7% (19.9%)
σ_2 (SE %)	3.61 (108.5%)	2.92 (72.0%)

Table 4. Individual empirical Bayes estimates on PK parameters without fixing Ka value using either MEMS or patient reported dosing records as the input.

Estimated Parameters	N	MEMS records mean ± SD	Patients reported dosing time mean ± SD	P value
CL (L/hr)	73	27.84 ± 12.42	29.18 ± 12.38	0.080
V (L)	73	1005.31 ± 348.53	764.98 ± 24.12	< 0.001
Ka (hr^{-1})	73	0.76 ± 0.23	0.51 ± 0.08	< 0.001

Table 5. PPK model parameters with a fixed Ka using either MEMS or patient reported dosing records as the input.

Parameters	MEMS records	Patient reported dosing time
OFV	1029.190	1033.881
CL (L/hr) (SE %)	25.5 (7.1%)	27 (6.4%)
V (L) (SE %)	1020 (15.5%)	855 (15.3%)
Ka (hr^{-1}) (SE %)	0.8 (0.0%)	0.8 (0.0%)
ω_{cl} % (SE %)	53.6% (14.7%)	47.4% (26.2%)
ω_{v} % (SE %)	64.5% (44.7%)	0% (fixed)
ω_{Ka} % (SE %)	93.1% (64.8%)	97.5 % (153.7%)
σ_1 % (SE %)	15.5% (115.8%)	23.9% (19.7%)
σ_2 (SE %)	3.55 (118.3%)	3.02 (65.7%)

Table 6. Individual empirical Bayes estimates on PK parameters with a fixed Ka value using either MEMS or patient reported dosing records as the input.

Estimated Parameters	N	MEMS records Mean ± SD	Patients reported dosing time Mean ± SD	P value
CL (L/hr)	73	27.88 ± 12.42	29.15 ± 12.00	0.095
V (L)	73	1017.45 ± 353.63	855.28 ± 0.00	< 0.0001
Ka (hr^{-1})	73	0.81 ± 0.26	0.78 ± 0.20	0.227

4.7 FIGURES

Figure 1: Time interval (hrs) between the last dose time recorded by MEMS and the patients (median ± SD: 0.133 ± 11.073hrs).

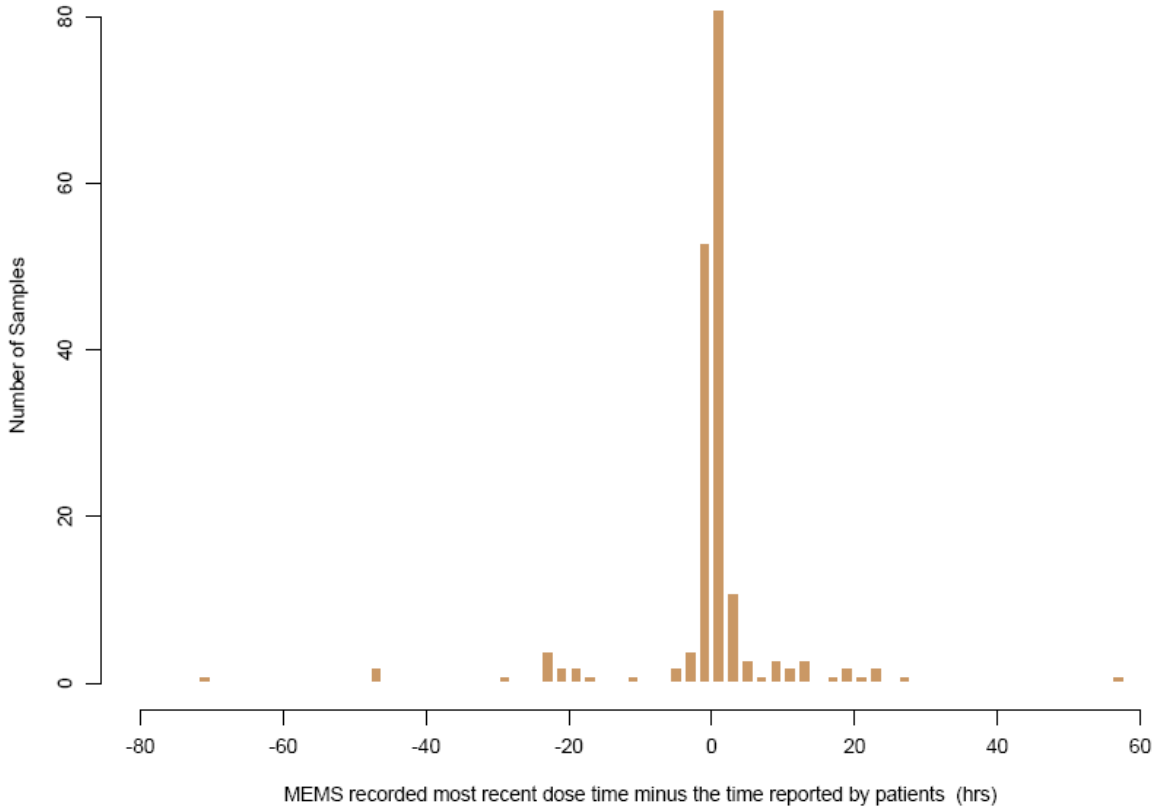


Figure 2: Frequency histogram showing the sampling distribution for escitalopram sampling measurements based on a) MEMS records and b) patient reported dosing times. The x-axis is broken into 4-hour bins, and the y-axis is the number of blood samplings during that time range.

Figure 2a.

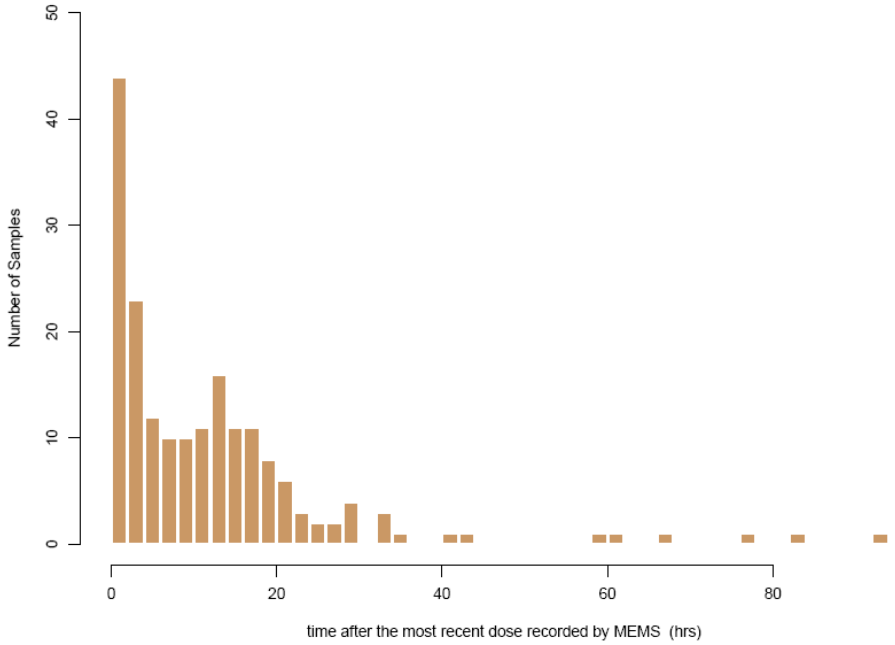


Figure 2b.

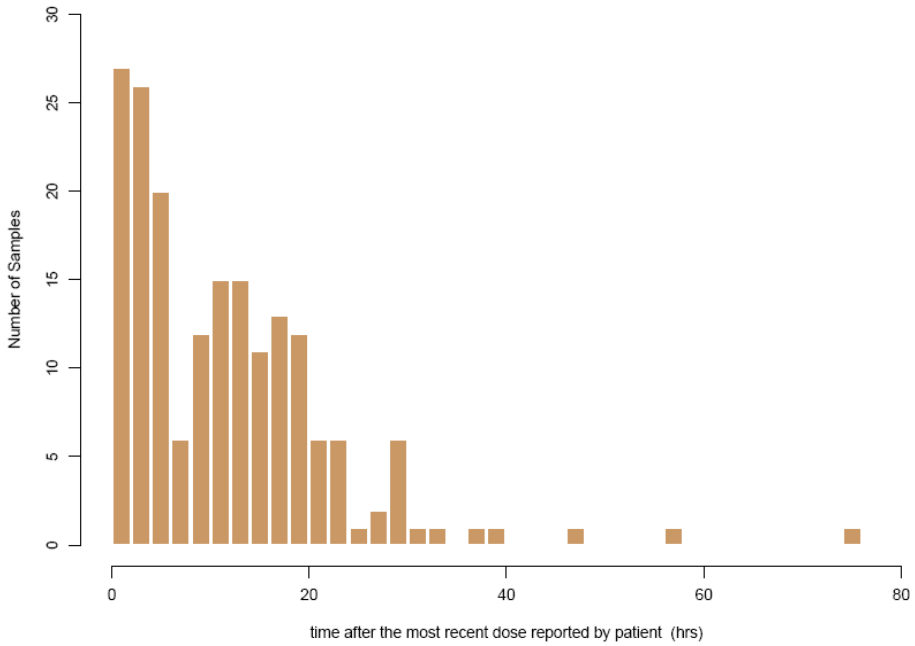


Figure 3. Goodness of fit plots for the PPK model using MEMS recorded dosing time as the model input and estimating Ka in addition to the other PK parameters

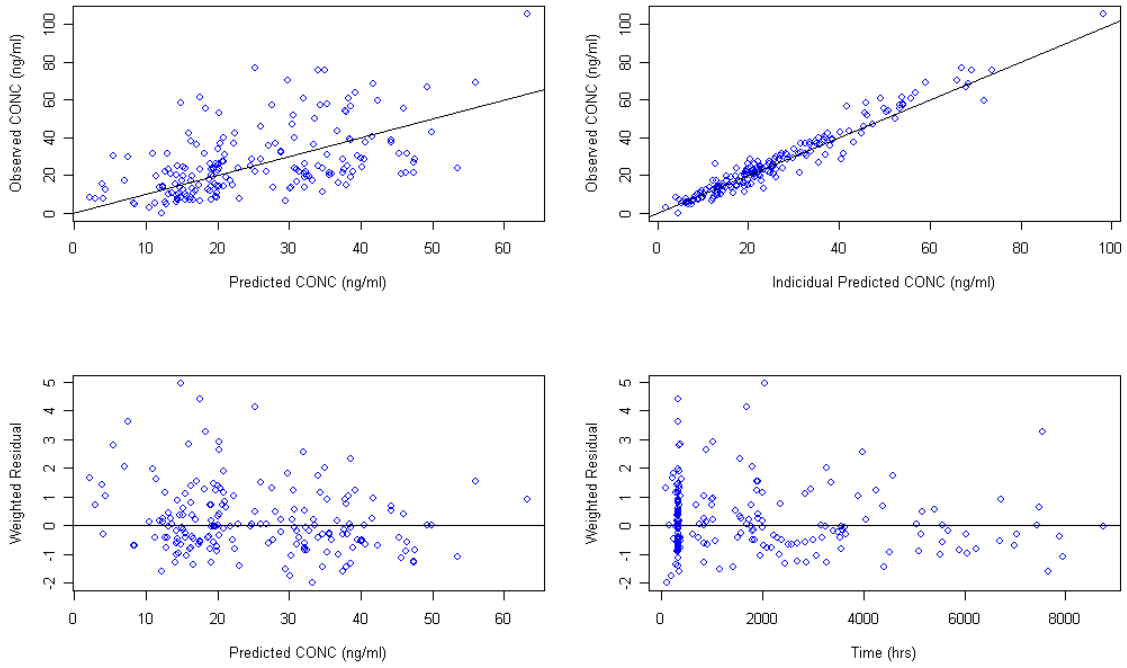


Figure 4. Goodness of fit plots for the PPK model using the patient reported last dosing time as the model input and estimating Ka in addition to the other PK parameters

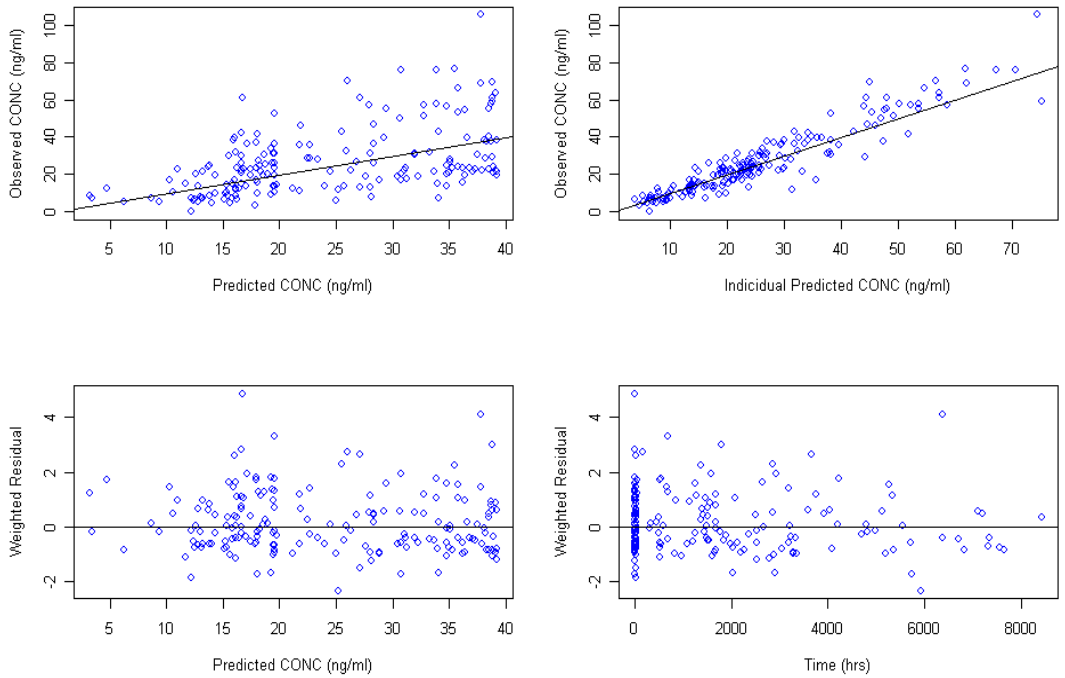


Figure 5. Goodness of fit plots for the PPK model using MEMS recorded dosing time as the model input and Ka fixed to 0.8 hr^{-1}

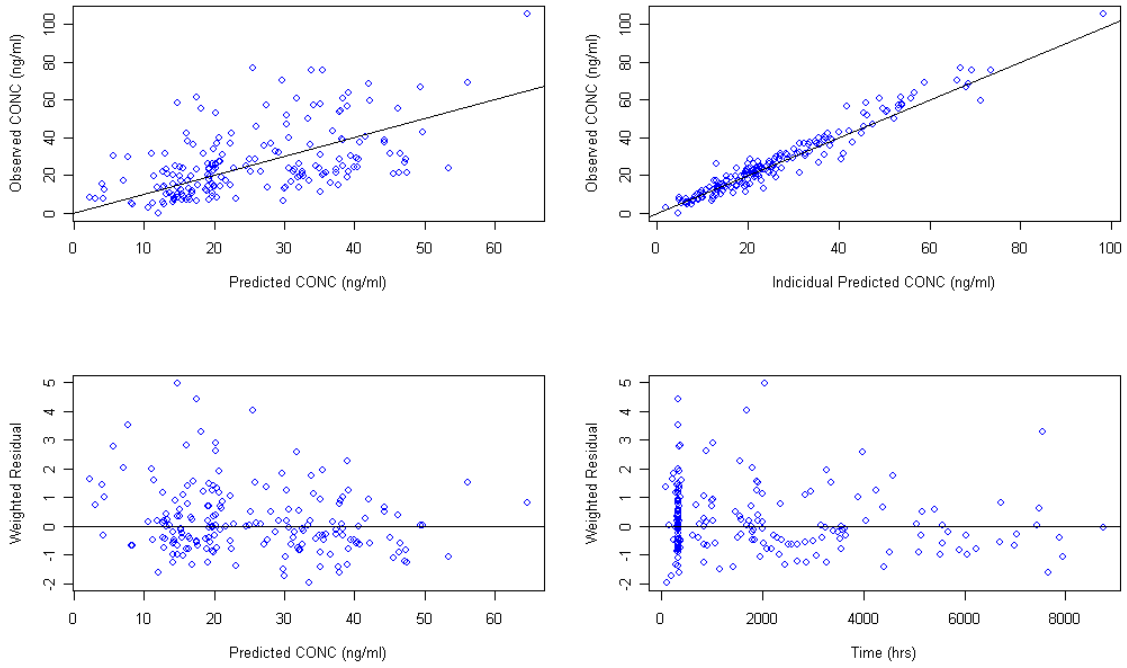
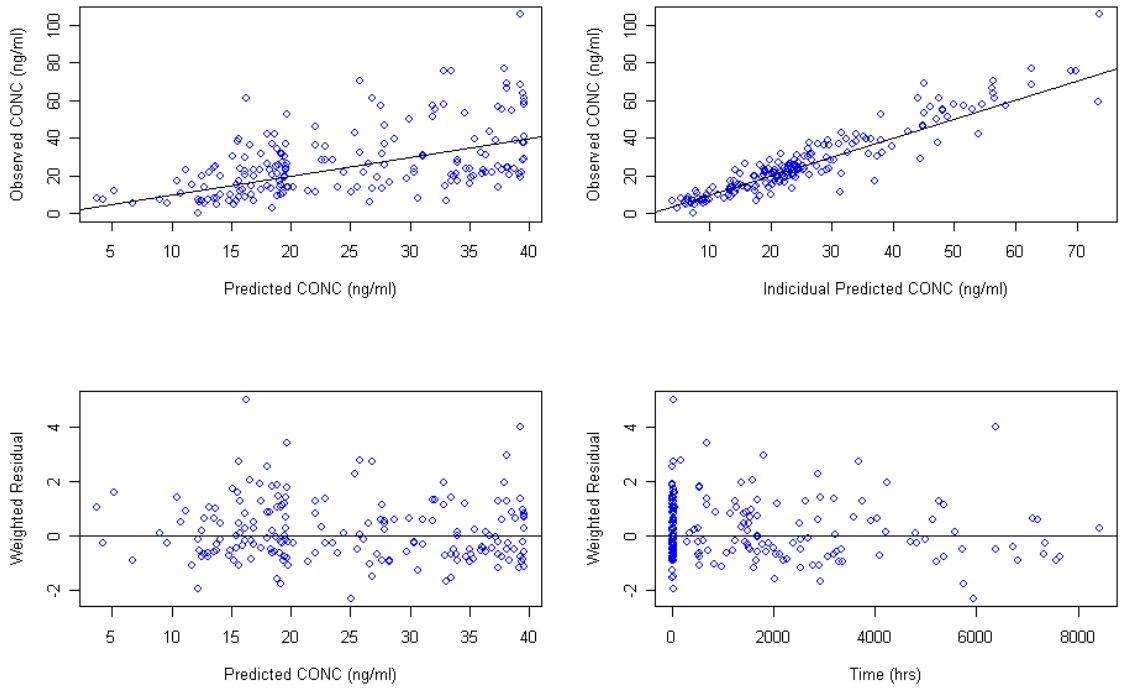


Figure 6. Goodness of fit plots for the PPK model using the patient reported last dosing time as the model input and Ka fixed to 0.8 hr^{-1}



5.0 FIFTH CHAPTER

Use of Monte Carlo Simulation Approaches to Evaluate the Clinical Implications of Discordance between Measure and True Blood Pressure

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5.1 ABSTRACT

Treatment decisions for hypertension using sphygmomanometer in current clinical practice do not account for the timing of BP measurement. The study aimed to evaluate the clinical implications of discordance between measure and true BP in current clinic practice and to propose a BP calibration system to decrease the impact of timing of BP measurement on the discordance. A clinical trial simulation case study was performed using in-silico Monte Carlo Simulation. The time-course of BP without and with treatment effect of antihypertensive were simulated from baseline BP model as well as pharmacokinetics and pharmacodynamics models. Virtual subjects' characteristics were from FDA internal database. Baseline BP model was qualified by virtual predictive check and global sensitivity analysis. Our results showed that the discordance between measure and true treatment effect of antihypertensive was over 5 and 10 mm Hg in 57.4% and 26.3% of patients in a typical clinic visit, respectively. Cuff BP measurement time need to be adjusted based on baseline clinic visit time as well as dosing regimen specific PK/PD considerations to better identify true treatment effect. BP could be calibrated based on patients' baseline and after treatment visit times to decrease the discordance in case timing of measurement is adjustable. **Keywords:** Hypertension, Sphygmomanometer, Circadian Rhythm, Modeling and Simulation, Blood Pressure Calibration

5.2 INTRODUCTION

Despite the fact that over 70 antihypertensive agents from various classes are available in the market and millions of Americans are treated for hypertension, the cardiovascular morbidity and mortality associated with hypertension remains a leading cause of overall morbidity and mortality (104, 105). The National Health and Nutrition Examination Survey (NHANES) found that only 36.8% and 50.1% percent of hypertensive patients have their blood pressure (BP) well-controlled (below 140/90 mmHg) in 2003-2004 and 2007-2008, respectively, in the United States (104, 105). Multiple epidemiologic studies have demonstrated that this lack of response has significant public health ramifications with respect to cardiovascular related morbidity and mortality (106). Understanding the reasons for poor BP control is an important public health issue.

The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) has suggested that improper BP measurement, non-adherence to antihypertensive dosing regimen, inadequate doses, and inappropriate combination therapy may contribute to the lack of response (106, 107).

BP measured using sphygmomanometer (cuff BP) during routine clinic visits are most commonly used for identifying hypertension and making treatment decision in current clinic practice. It is widely known that cuff BP measurement is associated with significant noise (108-118). The cuff BP measurement is affected by white-coat effect and alert reaction. This measurement approach is also potentially affected by many factors including type of measurement device, cuff size, patient position, and training level of personnel (108-118). This problem has been studied in detail and various guidelines have been developed to address the issue (119, 120). In the current manuscript we are not addressing this issue, however, we are cognizant of the contribution of this error towards BP measurement and account for this in our research.

One of the important aspects of improper BP measurement that is not widely studied is the timing of the BP measurement. It is widely known that inherent circadian rhythm of BP exists on a daily basis (121, 122). Morning BP surge and BP declines from

day to night are also well known due to availability on the observations of 24-h ambulatory BP monitoring (ABPM) (123). Hence, BP is a time specific measurement. In addition, it has been shown (121) that time course of BP reduction upon ingestion of certain antihypertensive agents changes with actual dosing history of patients. Hence, actual dosing time may be an additional confounder to clock-time dependent BP readings.

Treatment decisions are made based on cuff BP measurement during routine clinic visits in current clinical practice. This does not account for the confounding factors such as measurement time with respect to the circadian rhythm of BP, as well as dose administration time, and cuff measurement errors. Thus, the widespread use of cuff BP measurement to detect elevated BP and manage ongoing hypertension without controlling for these confounding factors may contribute to inadequate clinical management.

In this paper we utilize in-silico Monte Carlo Simulation methods to characterize the impact of the above mentioned factors. Monto Carlo Simulation has been widely used to predict potential trial results by incorporating trial design and numerical models, which may include drug behavior model, human execution behavior model, and disease progress model etc.(13). In the simulation, we define the routine clinic visits without controlling for the above mentioned confounding factors as casual clinic visits. Both true BP and cuff measurements at either casual or specified clinic visits were simulated in the study. The first aim is to evaluate the discordance in BP between the cuff measurements at casual clinic visits in the current clinical practice and the true underlying BP. The second aim is to identify the impact of the cuff BP measurement time on the discordance. For both the aims, we explored the impact of cuff measurement errors.

5.3 METHODS

5.3.1 Virtual Subjects characteristics and sample size

The mean of 24-h systolic BP of virtual subjects were necessary to anchor the simulation of 24-h BP profile of virtual subjects for the evaluation of the study aims. The Virtual

subjects' characteristics were obtained from the internal database of Food and Drug Administration (FDA). The mean of 24-h systolic BP of virtual subjects were adapted from ABPM measurements of 3840 patients with essential hypertension by pooling information of several New Drug Applications (Figure 1).

5.3.2 Simulation of baseline BP profile

The population baseline BP model developed by Hempel and colleagues (121) was qualified and adopted for the simulation of long term baseline BP. A brief description of the model is presented here:

A 24-h ABPM data on multiple occasions were available to support the modeling of circadian changes in hypertensive patients in the Hempel's paper (121). The inter-individual variability was estimated for the rhythm-adjusted 24-h mean, amplitude of the cosine terms, and clock time. The inter-occasion variability was estimated for the rhythm-adjusted 24-h mean and clock time. Baseline BP profiles were described using a function with two cosine terms:

$$Bsl(t) = \kappa_{1d} + \theta_1 \cdot \exp(\eta_1) \cdot \left[1 + \sum_{i=1}^{2} \theta_{2i} \cdot (1 + \eta_2) \cdot \cos\left(\frac{i \cdot \pi \cdot (t + \eta_3 + \kappa_{2d})}{12} - \theta_{2i+1}\right) \right] \quad Equation \quad 1,$$

where θ denotes the fixed-effect parameters and κ and η denote the random-effect parameters. In equation 1, Bsl(t) is BP as a function of time, t is clock time, θ_1, is rhythm-adjusted 24-h mean, η_1 is inter-individual variability on baseline, κ_{1d} is inter-occasion variability in rhythm-adjusted 24-h mean, d indicates different study days, θ_{2i} is amplitude of the cosine terms, η_2 is inter-individual variability in the amplitudes, θ_{2i+1} is parameter for phase shift of the cosine terms, η_3 is inter-individual variability on clock time, and κ_{2d} is inter-occasion variability on clock time.

The baseline model was qualified by visual predictive check using time stamped two days ABPM data from 225 subjects derived from hospital before anchoring our simulation in the study. In addition, global sensitivity analysis was performed to evaluate impact of uncertainty distribution in the parameter estimates of the baseline model on the results of our analysis. Uncertainty distribution for the variance-covariance matrix of the

parameter estimate in baseline BP model were assumed to be multivariate normal distributions based on standard errors (SE%) associated with the estimates of the population parameters. Around 1000 sets of population parameters were simulated from these distributions. These 1000 sets of population parameters were used to for 1000 replicate clinical trials, therefore 1000 sets of analysis endpoints were obtained. Finally, analysis endpoints from these 1000 clinical trials were plotted versus the uncertainty in each of the model parameters over 95%CI range to evaluate the impact of uncertainty of parameter estimates on our results. Uncertainly in cuff measurement error was also incorporated in global sensitivity analysis. The Standard deviation of BP measurement error associated with devices and personnel was assumed to be a uniform distribution between 3 and 7 mm Hg (25, 26).

The estimated values of the baseline BP model parameters were adapted from the original paper except of θ_1, η_1 two parameters. As we mentioned above (in Virtual Subjects characteristics and sample size section), the rhythm adjusted 24-h mean BP of virtual subjects was directly sampled from internal database of FDA to anchor the simulations to represent distribution of rhythm adjusted 24-h mean in the US population.

A continuous one month (720 h) baseline BP (Figure 2a) was simulated using Monte Carlo Simulation implemented in R® (version 2.9.1).

5.3.3 Simulation of BP profile with one month antihypertensive treatment

The time-course of treatment effect described for moxonidine by Hemple and his colleges was adapted for the Monte Carlo Simulation of antihypertensive treatment effect (121). A brief description of the pharmacokinetic/pharmacodynamic (PK/PD) model of moxonidine is described below:

The baseline BP model described in the above section was extended to incorporate the effect of moxonidine (121). The drug effect on BP was described as a function of moxonidine concentrations in the effect compartment to account for the delay in the drug effect. Inter-individual variability for the maximum effect and the concentrations required for 50% of the maximum effect were estimated.

A moxonidine dose of 0.3 mg once daily (8:00 AM) was used as initial dose regimen for all virtual subjects. Perfect adherence was assumed in the simulation. True BP profiles with moxonidine treatment were simulated by superimposing the moxonidine response to the baseline BP profiles generated in the above section (Figure 2).

5.3.4 Simulation strategy for cuff BP measurements

Casual clinic visit times of virtual subjects for cuff measurements after one month moxonidine treatment were assumed to follow a uniform distribution during the office working hours (8:00 AM~6:00 PM). For every virtual subject, two casual clinic visit times to assess cuff measurement were simulated on day 30 of moxonidine treatment. True point BP values at each of the corresponding clinic visit times were captured from the simulated long term true BP profile. A placebo effect was randomly generated for the virtual subjects with a mean reduction in BP of 4 mm Hg and a standard deviation of 2 mm Hg (124). Cuff BP measurement error was assumed to be normally distributed with a mean of zero and standard deviation of 5 mm Hg based on literature survey (108, 110, 113, 116, 117). The observed cuff BP measurement at each casual clinic visit was generated by combining the true point BP and randomly generated cuff BP measurement errors (Figure 3).

In addition to casual clinic visits, an office hours (8:00 AM ~ 6:00 PM) simulation for cuff measurements under the same dosing regimen for all virtual subjects was performed to explore an optimal clock time for the cuff measurement with respect to an 8:00 AM dosing time. Cuff measurements at these visits were simulated in accordance to the method described above.

5.3.5 Evaluation of the discordance between measured BP and its true value

True BP decreases (ΔBP) and cuff measured ΔBP at clinic visit times from baseline in virtual patients after one month of moxonidine treatment were compared. True BPs of each virtual subject at both baseline and after treatment were defined as the mean values

of BP profiles during office hours (8:00 AM ~ 6:00 PM) before and after treatment, respectively. True ΔBPs were defined as the difference between the true mean BP after one month treatment and initial baseline for each subject during the same office hours (8:00 AM ~ 6:00 PM).

The cuff measured ΔBP at casual clinic visit time was calculated by subtracting the observed cuff BP at casual clinic visit time from that in the initial baseline for each virtual patient. The ability of the cuff measures to reflect the true ΔBP from baseline were evaluated at both the population and individual level. Population means of cuff measured ΔBP at casual clinic visit time among 3840 virtual subjects were compared to the true ΔBP to evaluate the accuracy of cuff BP measures in identifying drug response at population level. Paired comparison between cuff measured ΔBP at casual clinic visit time and true ΔBP was also performed within the same individual. The percentage of subjects who had a difference greater than 5 and 10 mm Hg were calculated.

Optimal clinic visit times for cuff BP measurements were also identified by comparing cuff measured ΔBP at specified visit times (8:00 AM ~ 6:00 PM) to the true ΔBP as described above.

5.3.6 BP calibration with respect to clinic visit times at both baseline and after treatment

Whether the BP could be calibrated with respect to clinic visit times before and after treatment if patients were not able to visit clinic at optimized clinic visit time was evaluated. The population mean BP profile at baseline and with moxonidine treatment during office hours (8:00 AM ~ 6:00 PM) was calculated. The population mean of the calculated BP decreases from baseline at various clinic visit times after moxonidine treatment were generated.

A similar analysis was performed for other anti-hypertensives (i.e. drugs which have delayed effect or exhibit concentrations at steady state much higher than their EC_{50}) that do not change the BP circadian rhythm.

5.3.7 Analysis platform

NONMEM® (Version VI, University of California at San Francisco, CA) was used to simulate moxonidine concentrations in the plasma and effect compartments for virtual subjects. Simulation of the BP profiles, graphics and post-processing of NONMEM® outputs, visual predictive evaluation, and global sensitivity analysis were performed in R® (version 2.9.1). 1000 replicates of the monte carlo simulation was performed to generate prediction intervals (PI) of the simulation endpoints accounting for the uncertainty in parameter estimates from baseline BP profile model.

5.4 RESULTS

A demographic of the virtual subjects and the distribution of their systolic BP were shown in Table 1 and Figure 1 respectively. The mean systolic BP was assumed to represent the baseline of rhythm adjusted 24-h mean for virtual subjects. The mean systolic BP at baseline in this sub-population was 144.3 mm.

Visual predictive check performed in the study using actual ABPM data from 225 subjects showed that baseline BP model normalized to that from Hempel and his colleagues (121) were able to reasonably describe circadian rhythm of BP profile over clock times. (Refer to appendix for additional information).

A 30 day 24-h time course of systolic BP accounting for circadian patterns, inter-occasion and inter-individual variability were simulated for 3840 virtual subjects using the baseline model and the moxonidine PK/PD model. The time course for population mean (with 95% PI) of the baseline and antihypertensive treatment effect are shown in Figure 2. In the simulations, 0.3 mg moxonidine once daily dosing regimen administered at 8:00 AM was followed. It can be seen, the BP decreased significantly from baseline after drug ingestion, and gradually returned to baseline before the next dose was taken.

The true mean treatment effect (± SD) in the population with moxonidine after 30 days treatment for this population was 11.8 ± 10.9 mm Hg (Table 2). The mean treatment effect (± SD) as assessed by cuff measures at randomly selected clinic visit times (8:00 AM to 6:00 PM) were 11.72 ± 12.4 without cuff measurement error and 11.60 ± 14.3 with cuff measurement error (error ~ N(0,5 mm Hg)) respectively in the population as shown in Table 2.

Paired comparison of cuff measured ΔBP to true values within the same individual indicated that the difference between true ΔBP and measured ΔBP was over 5 and 10 mm Hg in 57.4% and 26.3% of patients with the cuff measurement error and in 28.8% and 9.2% of patients without cuff measurement error (circadian rhythm only), respectively (table2).

In addition, BP measured at two different clinic visit times on the same day (Random visit 1 VS Random visit 2) was not consistent within the same individual. Approximately 26.8% (95% PI: 23.4%~31.9%) and 57.1% (95% PI: 54.5%~60.8%) of the patients deviated for the ±10 mm Hg and ±5 mm Hg categories respectively (not shown in table).

The same baseline and after treatment visit were specified to the same clock time for virtual subjects during office hours (from 8:00 AM to 6:00 PM). The discordance between the true ΔBP and the cuff measured ΔBP at the specified clinic visit times at both population and individual levels is shown in table 3. Population mean in cuff measured ΔBP from baseline systematically over- or under-estimated the true ΔBP depending on the clock time of cuff BP measurement.

When the true treatment effect was 11.8 ± 10.9 mm Hg, the effect with cuff BP measurements ranged from 6.1 ± 13.9 to 13.2 ± 13.2 mm Hg. Cuff BP measured at 10:00 AM or 4:00 PM most correctly evaluated mean response of moxonidine at the population level with 8AM once daily dosing regimen.

The ability of the cuff measured ΔBP at specified clinic visit time (8:00 AM to 6:00 PM) to reflect the true ΔBP at the individual level varied with the time of cuff BP measurement as well (Table 3). In general, cuff measured ΔBP deviated from the true value significantly within the same individual. Cuff measurement at certain clock time did perform better than other times. Specifically, morning time before 9:00 AM was identified to be the worst time frame to evaluate actual ΔBP at the level of an individual patient for monxonidine with 8AM once daily dose of 0.3 mg. However, significant bias from actual ΔBP was still observed for BP measured during the other time frame, which was more than 10 mm Hg in 18% of patients and 5 mm Hg in 51% of patients.

The potential for BP calibration based on patients' clinic visit times at both baseline and with 0.3 mg 8:00 AM once daily dose of moxonidine treatment are shown in Figures 5a and 5b. The population mean of systolic BP at each specified clock time visit for both baseline and after 0.3 mg moxonidine treatment are summarized in Figure 5a. Figure 5b illustrates BP calibration at four representative baseline measurement times with a fixed dosing time of 8:00 AM. Given an 8:00 AM baseline BP measurement, 12:00 PM after treatment visit may best measure true ΔBP (11.8 mm Hg) from baseline. If 9:00 AM is the after treatment visit time, a measured cuff BP at 9:00 AM could be calibrated to 4:00 PM measurement by adding 3 mm Hg. Antihypertensives that exhibit concentrations at steady state that are much higher than their and have a smaller effect on the circadian rhythm of the BP profile, the BP measurements at various baseline and after treatment visit times can be calibrated as shown in Figure 6.

Global sensitivity analysis showed that our analysis endpoints were robust across the uncertainty in all parameters reported in Hempel's paper except of amplitude parameter for first cosine term and the random effect parameter for the inter-individual variability on clock time (h). For example, percent of patients whose measured BP deviated from true values (ie: for the ±10 mm Hg categories: 13.7% ~ 8% without measurement error and 30% ~ 24% with measurement error) were negatively correlated to amplitude parameter for the first cosine term (95%CI: -0.087 ~ -0.048). The inter-individual variability on clock time (variance 95%CI: 3.59~28.47 h) were positively correlated to percent of patients whose measured BP deviated from true values (ie: for the

±10 mm Hg categories: 7% ~15% without measurement error and 24.5% ~ 30% with measurement error). Hence improved estimates on these two population parameters may improve precision of the analysis endpoint. Global sensitivity analysis also showed that percent of patients whose measured BP deviated from true values (for the ±10 mm Hg categories: 15.5% ~ 38.3% with measurement error) is highly associated with the standard deviation of BP measurement error (3 ~ 7 mm Hg) (refer to appendix for additional information).

5.5 DISCUSSION

The simulation results indicate that cuff measured ΔBP systematically over or under-estimates the actual ΔBP upon administration of moxonidine 0.3 mg once daily for one month in virtual patients with hypertension. This deviation varies depending on the time of day when the BP is measured relative to the dosage administration time. The deviation of cuff measured ΔBP from true ΔBP has been demonstrated in three ways. First, with casual clinic visit times, the difference between true ΔBP and measured ΔBP was over 5 and 10 mm Hg in 56.9% and 26.3% of patients, respectively, using a paired comparison within the same individual. Second, measurement of BP at specific clinic visit times (10:00 AM ~ 4:00 PM) resulted in better estimation of the true ΔBP from baseline. However, significant bias from actual ΔBP was still observed for the BP measured ΔBP during this time frame. The deviation was more than 10 mm Hg in 18% of patients and 5 mm Hg in 50% of patients (table3). Finally, the results indicate that BP could be calibrated based on patients' clinic visit times at both baseline and after treatment. The calibration scale varies with respect to PK/PD properties of the antihypertensive.

A fundamental question in clinical practice is whether cuff measured ΔBP can correctly evaluate true ΔBP at the level of an individual patient. The analysis conducted in this paper demonstrated that cuff measured ΔBP deviates from the true value significantly at the individual level and it is sensitive to the time of cuff BP measurement. An optimal time frame for baseline and time after dosage administration exists that improves the accuracy of the cuff measured ΔBP. Optimizing the clinic visit time based

on dosage administration time and baseline improved the accuracy of the cuff measured ΔBP.

In clinical practice, clinic visits during optimal time frame may not be feasible for many patients. The analysis suggests a useful BP calibration scale for physicians to decrease the effect of clinic visit times by considering internal circadian rhythm of patient specific BP profile before and after treatment.

The BP reduction upon ingestion of an antihypertensive agent depends on the PK/PD properties of that agent. The optimal time frame and BP calibration scales for cuff SBP measurement in this study may not be applicable to all antihypertensives and may vary with PK/PD of the medication and dosing regimen prescribed. Clinical visit time selection and BP calibration must account for the dosing times and the PK/PD properties of the antihypertensive(s) used.

Changing to an optimal time frame improved accuracy of cuff measured ΔBP to a limited extent. It did not, however, completely correct the measurement bias. These issues may lead to misclassification of the patient's hypertension, and therefore incorrect treatment decision making at the individual patient level.

The population baseline BP model developed by Hempel and colleagues (121) was qualified and adopted for the simulation of long term baseline BP in our simulation. Several other models were also available in the literature to describe the daily based variability of BP. Some models focused on the BP deference between daytime and nighttime BP such as the square wave fit model (125), some model focused on the transit phase between day time and night-time BP changes such as double logistic model (123, 126, 127). The limitation of these models is the lack of description of the important features regarding BP fluctuations. In addition, between individual variability as well as inter-occasion variability within individuals are rarely estimated. Cosinor models and fourier analysis with different number of harmonics etc. (121, 122, 128) focused on describing the 24-h BP curve. With more than one cosine term, cosinor and fourier models share a similar description to circadian BP changes. The baseline BP profiles

were described in this work use a function with two cosine terms. This model quantifies the time-course of the baseline BP in hypertensive patients accounting for variability between and within patients. The advantage of the model is the estimation of the inter-individual variability for the rhythm-adjusted 24-h mean, amplitude of the cosine terms, and clock time as well as the inter-occasion variability for the rhythm-adjusted 24-h mean and clock time. Visual predictive checks performed in the study using actual ABPM data from 225 subjects showed that the baseline BP model were able to reasonably describe circadian rhythm of BP profile over clock times (refer to appendix for additional information).

In this study, the mean BP during office hours (8:00 AM ~ 6:00 PM) was selected as the true BP for the virtual patients' BP. The time window selected reasonably accounts for the time of the BP measurement that drives the treatment decision making in current clinic practice. This BP measurement at a given time (during the office-hours) is assumed to represent the individual's average. In addition, cardiovascular risk models published were developed based on BP measurement during the day time window. This allows the extension of the simulations to explore potential public health outcomes.

In conclusion, cuff BP measurement time may need to be adjusted based on baseline clinic visit time as well as dosing regimen specific PK/PD considerations to better identify true ΔBP in each virtual subject in current clinic practice. BP could be calibrated based on patients' baseline and after treatment visit times to better appreciate circadian rhythm of BP profile. Changing clinic visit time decreases the measurement bias from internal circadian rhythm of BP, hence improves accuracy of cuff measured ΔBP to certain extent. It does not, however, completely correct the measurement bias due to existence of random cuff measurement error from other recourse such as device, cuff size, and training levels of personnel.

5.6 TABLES

Table 1. Summary of extracted data from FDA internal database

	N (%)	24-h mean of SBP mean (range)	AGE mean (range)
Male	2121 (55.2%)	144.3 (120.2 ~ 199.7)	56 (21~84)
Female	1719 (44.8%)	144.2 (120.2 ~ 201.1)	57 (23~86)
All	3840	144.3 (120.2~201.1)	56 (21 ~ 86)

Table 2. Difference in BP decreases from baseline: True ΔBP VS. Cuff measured ΔBP at random clinic visit times

-		Population mean ΔBP	Percent of subjects with measured ΔBP deviating from the true	
		Mean ± SD (mm Hg)	Deviation = Measured ΔBP - True ΔBP	
			|Deviation| >= 10 mm Hg	|Deviation| >= 5 mm Hg
True ΔBP from baseline		11.76 ± 10.9	-	-
Cuff measured ΔBP from baseline at random visit time	With measurement error	11.72 ± 12.4	26.3% (22.2%~32.2%)	56.9% (53.5%~61.3%)
	Without measurement error	11.60 ± 14.3	9.2% (4.5% ~ 17.6%)	28.4% (18.2%~39.6%)

Table 3. Difference in BP decreases from baseline: True ΔBP VS. Cuff measured ΔBP at specified clinic visit times

-		Population mean ΔBP	Percent of subjects with measured ΔBP deviating from the true	
			Deviation = Measured ΔBP - True ΔBP	
		Mean ± SD (mm Hg)	|Deviation| >= 10 mm Hg	|Deviation| >= 5 mm Hg
True ΔBP from baseline		11.8 ± 10.9		
Cuff measured ΔBP from baseline at certain clock time	8:00 AM	6.1 ± 13.9	41.2% (38.8%~44.3%)	67.5% (65.9%~69.8%)
	9:00 AM	10.5 ± 13.5	23.2% (20.7%~26.7%)	54.6% (52.3%~57.5%)
	10:00 AM	12.2 ± 13.6	19.8% (18.0%~22.8%)	51.8% (49.8%~54.4%)
	11:00 AM	12.9 ± 13.2	19.3% (17.6%~22.2%)	51.3% (49.3%~54.2%)
	12:00 PM	13.2 ± 13.2	19.0% (17.4%~21.7%)	51.1% (49.3%~54.1%)
	1:00 PM	13.0 ± 13.3	18.6% (17.2%~22.0%)	50.7% (49.0%~53.3%)
	2:00 PM	12.6 ± 13.3	18.2% (16.6%~21.6%)	50.6% (48.5%~52.9%)
	3:00 PM	12.3 ± 13.2	18.4% (16.5%~21.4%)	50.4% (48.4%~53.4%)
	4:00 PM	11.9 ± 13.2	18.6% (16.8%~22.0%)	50.8% (49.0%~53.9%)
	5:00 PM	11.2 ± 13.3	19.8% (17.9%~23.2%)	51.7% (49.9%~54.6%)
	6:00 PM	10.8. ± 13.4	21.9% (19.5%~25.6%)	53.5% (51.2%~56.1%)

5.7 FIGURES

Figure 1: Histogram of 24-h mean systolic blood pressure for virtual subjects (n=3840)

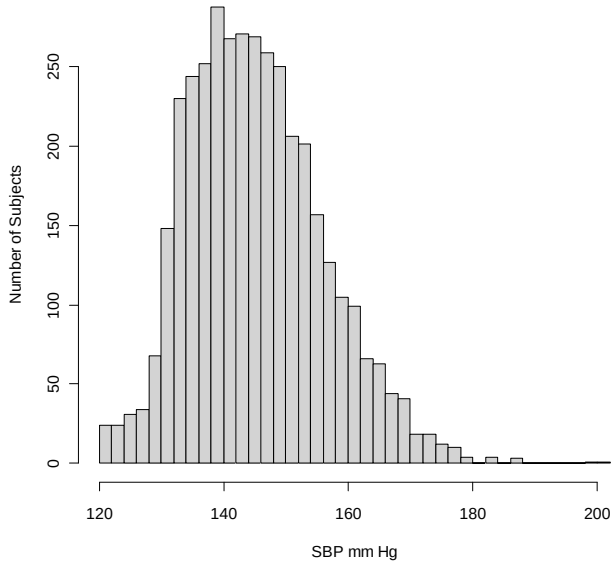
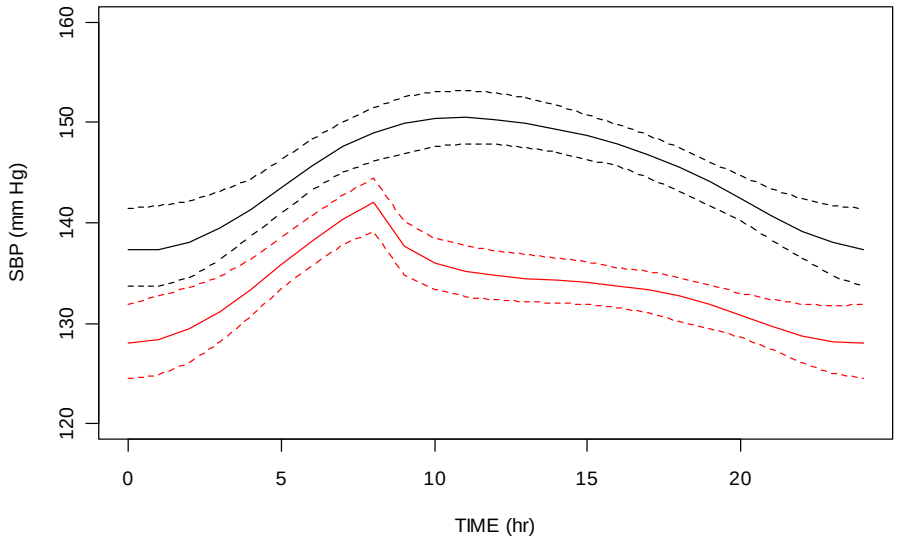
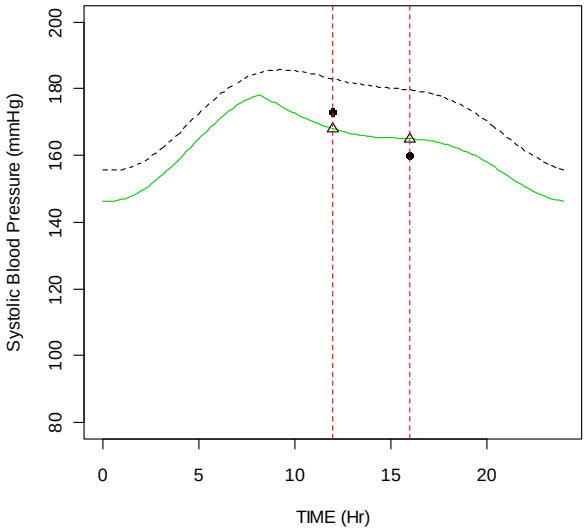


Figure 2: Simulated 24-h BP profiles for baseline and with treatment of 0.3 mg QD moxonidine



Black line is the population mean BP at baseline. Black dashed line is its 95% prediction interval. Red line is the population mean BP after one month treatment with moxonidine. Red dashed line is its 95% prediction interval.

Figure 3: Scenario of observed cuff BP measurement generation (0.3 mg 8:00 AM QD moxonidine)



Black dashed lines are baseline BP before taking moxonidine. Green solid lines represent SBP after 0.3mg QD moxonidine. Dashed vertical lines represent two randomly selected clinic visit time between 8:00 AM to 5 PM. Open triangle are true SBP at randomly selected clinic visit time. Black solid dots represent observed cuff BP measurement at patients' clinic visit time.

Figure 4a: Population mean of true BP at specified clinic visit time for both baseline and day 30 of moxonidine treatment.
Figure 4b: BP calibration at specified clinic visit time for moxonidine.

Figure 4a.

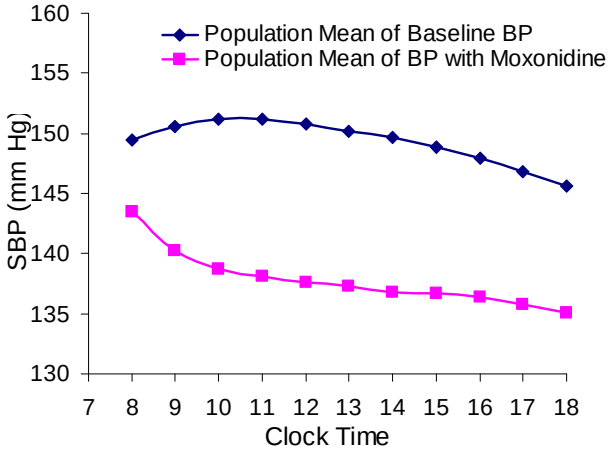


Figure 4b.

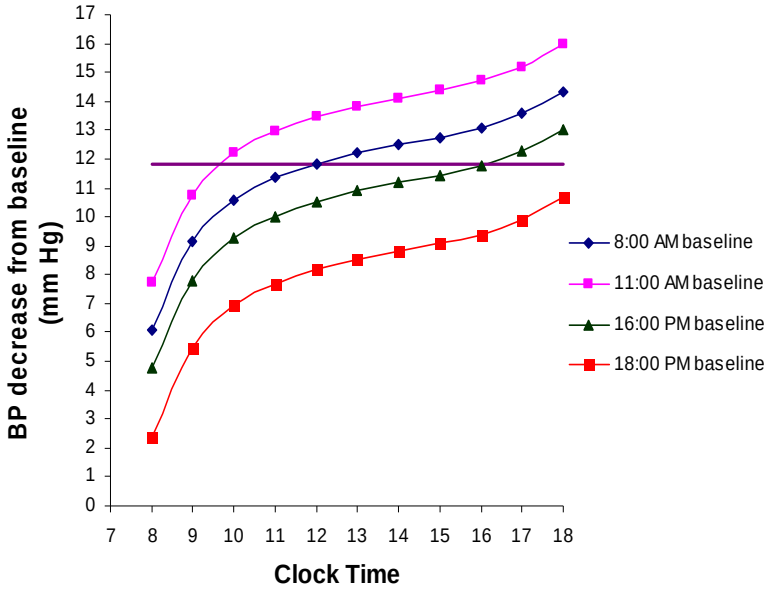


Figure 5a: Population mean of true BP at specified clinic visit time for both baseline and day 30 of treatment for anti-hypertensives which do not change the circadian rhythm of BP).
Figure 5b: BP calibration at specified clinic visit time for anti-hypertensvies which do not change the circadian rhythm of BP

Figure 5a.

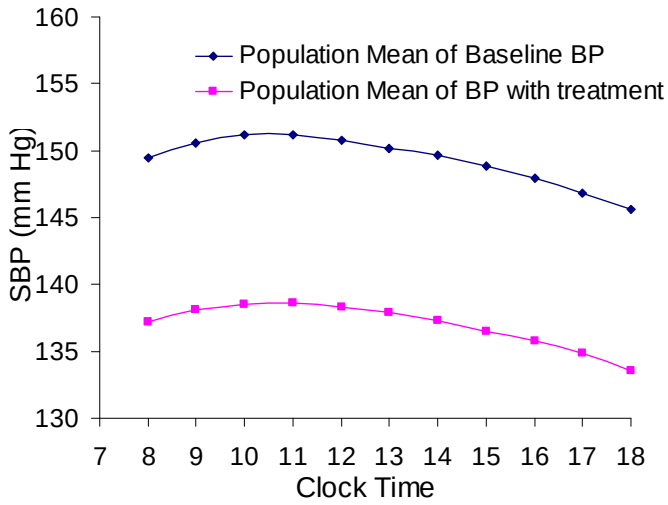
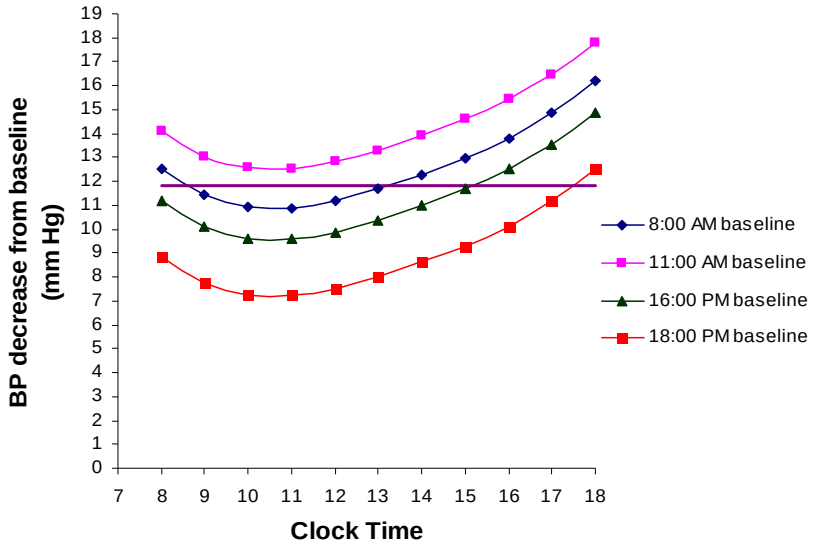


Figure 5b.



2.8 APPENDIX FOR CHAPTER FIVE

Figure 1: Visual predictive check for baseline model with observed ABPM data.

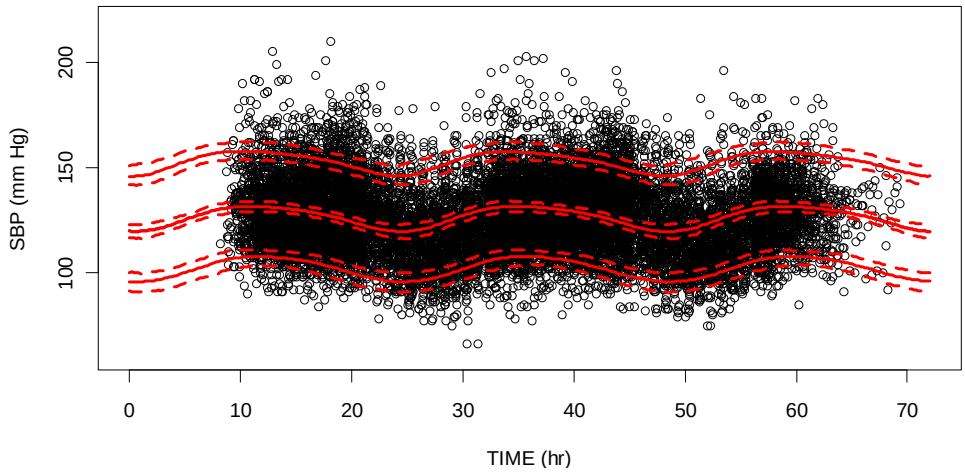


Figure 2: Global sensitivity analysis

Figure 2a: Fixed effect parameters vs. Percent of patients with |deviation| > 10 mm Hg

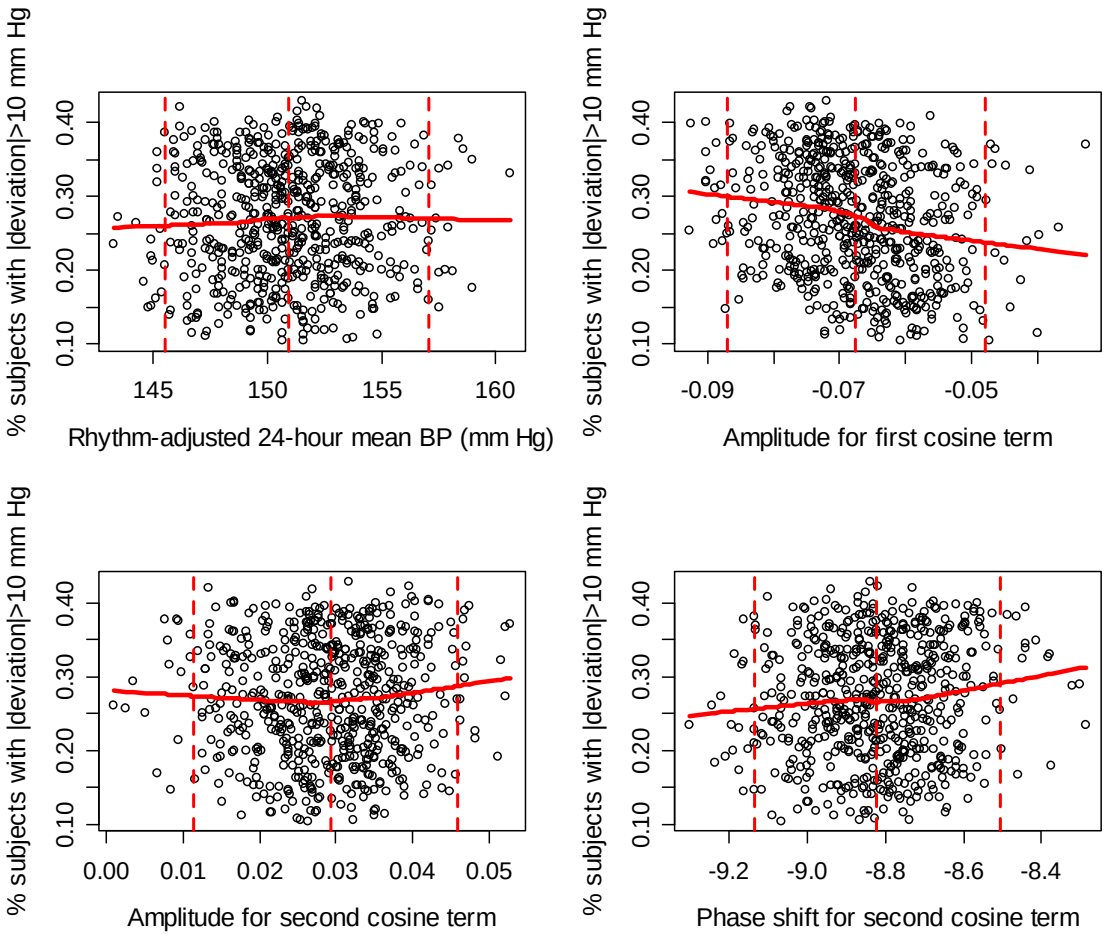


Figure 2b: Inter-individual variability vs. Percent of patients with |deviation| > 10 mm Hg

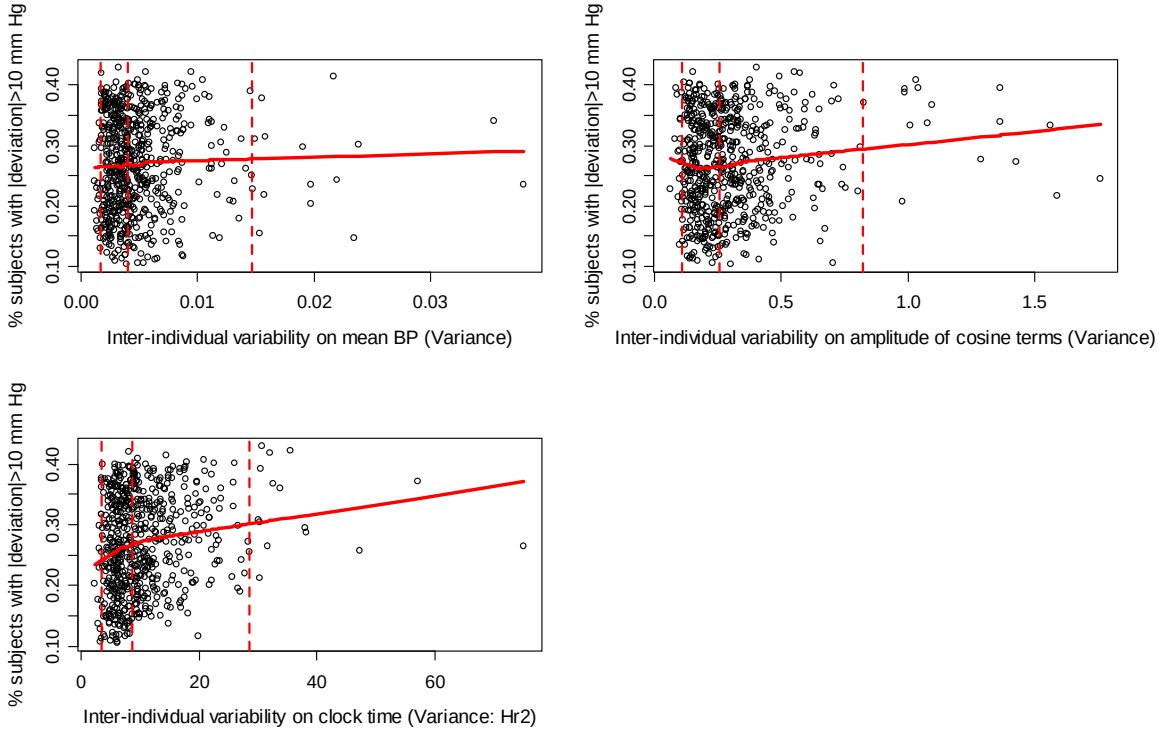


Figure 2c: Inter-occasion variability vs. Percent of patients with |deviation| > 10 mm Hg

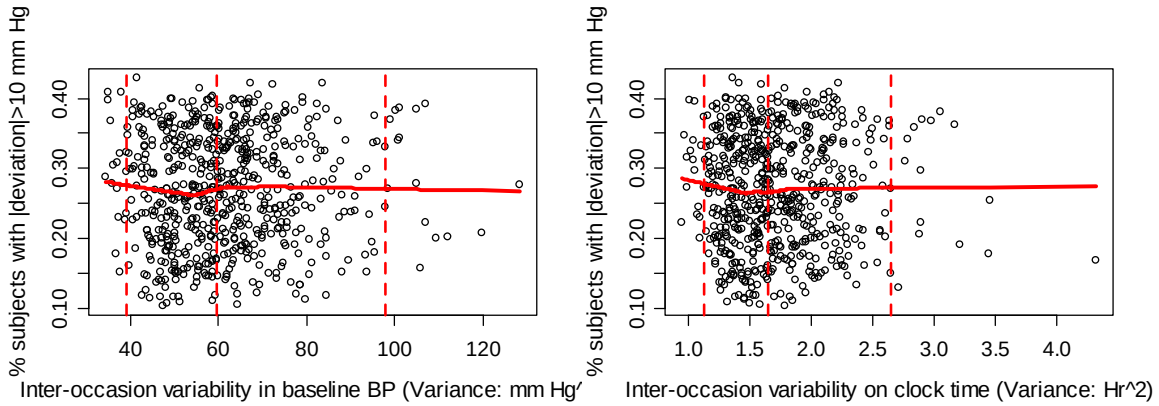


Figure 2d: Standard Deviation of BP measurement error vs. Percent of patients with |deviation| > 10 mm Hg

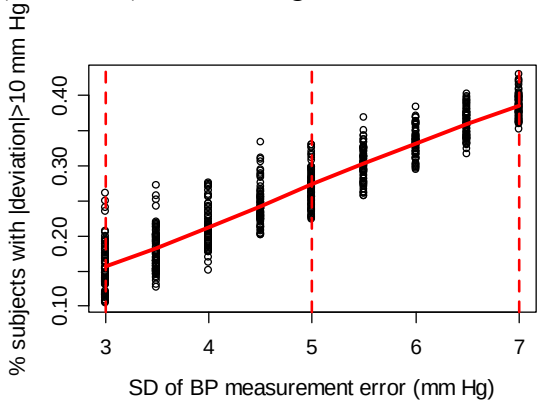


Table 1: Uncertainty distribution in the parameter estimates of the baseline model on analysis endpoints.

Population Parameters	95% CI	|dev| > 10 mm Hg (without measurement error)	|dev| > 10 mm Hg (with measurement error)
24-h mean BP (mm Hg)	145.5 ~ 157.1	9.5% ~ 11.3%	26% ~ 27.5%
Amplitude, first cosine term	**(-)0.087~ (-) 0.048**	**8% ~ 13.7%**	**24% ~ 30%**
amplitude, second cosine term	0.011 ~ 0.046	10% ~ 12.5%	26% ~ 29%
phase shift, second cosine term	(-)9.13 ~ (-)8.50	9.5% ~ 12%	26% ~ 29%
inter-individual variability in mean BP (variance)	0.002~ 0.015	10% ~ 11.5%	26.5% ~ 27.8%
inter-individual variability on amplitude of 1st and 2nd cosine term (variance)	0.11 ~ 0.82	9.5% ~ 13%	26% ~ 29.5%
inter-individual variability on clock time (h) (variance: hr^2)	**3.59 ~ 28.47**	**7% ~15%**	**24.5% ~ 30%**
inter-occasion variability in baseline (variance: mm Hg^2)	39.24 ~ 97.91	10.50%	27%
inter-occasion variability on clock time (h) (variance: hr^2)	1.12 ~ 2.64	10.50%	27%
SD of measurement error (mm Hg)	3 ~ 7		15.5% ~ 38.3%

6.0 SIXTH CHAPTER

Quantifying Blood Pressure Misclassification Resulting from Cuff Blood Pressure Measurements: A Clinical Trial Simulation Case Study

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6.1 INTRODUCTION

Hypertension is a major risk factor for cardiovascular disease (106). The recent National Health and Nutrition Examination Survey (NHANES 2007-2008) found that the control rate of hypertension in Americans increased to 50% compared to 37% in 2003-2004. However, half of adults in the US with hypertension still do not have their blood pressure (BP) adequately controlled (104, 105) .

Inappropriate treatment decisions, such as inadequate dosing and inappropriate combination therapy, may contribute to the poor BP control rate as suggested in the Seventh Report of the Joint National Committee (JNC 7)(107). To address the ongoing hypertension crisis in American adults the JNC 7 has updated BP classification thresholds and corresponding drug therapies for proper BP management.

BP measurements obtained using a sphygmomanometer are commonly used for BP classification, and by association, identifying hypertension and making dosing decisions in current clinical practice as suggested in JNC guidelines (107). Compounding these dosing decisions is that BP measurements associated with using a sphygmomanometer are prone to measurement errors due to the device and intra-subject variability (119, 120). Some studies have reported the measurement error induced BP

misclassification (110-112). However, one of the important confounding factors not considered in these BP misclassification studies is the impact of BP measurement timing on BP classification. Daily fluctuations in BP may be difficult to distinguish from hypertension when measurements are obtained at different times throughout the day (122). In addition, BP reduction upon ingestion of an antihypertensive agent depends on the concentration of the antihypertensive agent. The concentration, in turn, depends on the patient dosing history. This relationship between dosing and clinic visit time is not accounted for in current clinical practice or the JNC guidelines. This introduces an assumption that cuff BP measurements are estimates of true mean BP (110). Biological variability combined with anti-hypertensive drug response notwithstanding the contribution of BP changes secondary to the time of day when BP is measured. Therefore, the timing of a clinic visit may affect the classification of a measured BP. This, in turn, affects the treatment decision undertaken for the patient.

Despite the availability of BP measurement guidelines (119, 120), many studies have suggested that these guidelines are not fully translated into clinical practice (113-116). BP treatment decisions are often based on a single measurement (115). The observers' 'rounding' to 0 or 5 as the last digit for a BP measurement is another source of measurement error (113). However, the quantitative implication of these practices on BP misclassification (e.g. incorrect treatment decisions) has not been adequately studied.

In our previous study, we evaluated the discordance between sphygmomanometer measured BP versus the true mean BP. This previous study accounted for confounding factors such as internal circadian rhythm of BP, antihypertensive treatment effect on the BP profile, clinic visit times as well as BP measurement error. The potential for BP calibration across different clinic times to capture true treatment effect of anti-hypertensive(s) using these factors was also explored (129). However, the impact of BP measurement discordance on BP misclassification is unknown. If patients' true BP is near a treatment decision making threshold, this may have a greater sensitivity to misclassification based on the measurement error. This sensitivity to misclassification may result in a suboptimal treatment decision, such as under-treatment for high risk patients or providing unnecessary medicine to false positive hypertensive patients.

In this study, we extended the previous analysis to incorporate BP treatment misclassification rate. We aimed to evaluate a general BP misclassification rate using a casual clinic visit time similar to that in current clinical practice. Second, we evaluated the influence of the BP measurement time and dosing time on the BP misclassification rate. This provided the basis for examining whether or not there was an optimal clinic visit time accounting for PK/PD characteristics. Finally, we evaluated the influence of various levels of BP measurement error on the BP misclassification rate including number of measurements per visit and rounding the last digit from a BP measurement.

6.2 METHODS

6.2.1 Virtual Subjects characteristics and sample size

The Virtual subjects' characteristics were obtained from the internal database of Food and Drug Administration (FDA). The mean 24-h systolic BP of each virtual subject was required to anchor the simulation of 24-h BP profile of virtual subjects for the evaluation of the study aims. The mean of 24-h systolic BP of virtual subjects were adapted from the ambulatory blood pressure monitoring (ABPM) measurements of 3840 patients with essential hypertension by pooling information from several New Drug Applications.

The analysis results were also evaluated by sampling systolic BP distribution from the NHANES III Adult Database (http://www.cdc.gov/nchs/tutorials/Nhanes/Downloads/intro_III.htm). Subjects with a systolic BP greater than 140 mm Hg were considered hypertensive. The systolic BPs from the NHANES database comprised the mean of three point estimates of BP. We utilized these BPs assuming they reflected the rhythm adjusted 24-h mean BP of virtual subjects to anchor the simulations of continuous BP profile.

6.2.2 Simulation of baseline BP profile and BP profile with one month antihypertensive treatment

The population baseline BP model developed by Hempel and colleagues was qualified and adopted for the simulation of long term baseline BP (121, 129). The detailed description of the model was presented in Hempel's paper and our previous paper (121, 129). Briefly, the baseline model in Hempel et al. was developed to describe BP circadian rhythm using 24-h ABPM data on multiple occasions. Circadian BP was described using two cosine terms with inter-individual variability for the rhythm-adjusted 24-h mean, amplitude of the cosine terms, and clock time, and inter-occasion variability for the rhythm-adjusted 24-h mean and clock time. The estimated values of the baseline BP model parameters were adapted from the original paper with the exception of rhythm adjusted population mean BP (θ_1) and it inter-individual variability (η_1). The rhythm adjusted 24-h mean BPs of virtual subjects was directly sampled from internal database of FDA and the NHANES database to anchor the simulations and provide a representative distribution of rhythm adjusted 24-h mean in the US population.

The BP model was previously qualified using observed ambulatory blood pressure monitoring (ABPM).(129) A global sensitivity analysis was performed after generating our analysis endpoints to quantitatively evaluate the impact of uncertainty in parameters distribution estimates from the baseline model on the results of our analysis using the methods described previously (25, 26, 34, 129).

Two types of antihypertensive agents were simulated in our study: 1) a type I antihypertensive agents that induce a change in the shape of the BP circadian rhythm (e.g., Moxonidine, clonidine) and 2) a type II antihypertensive agent that shifts the baseline BP circadian rhythm with no changes to the shape (e.g. drugs with delayed effect or with an EC50 much lower than drug concentrations at steady state).

A continuous BP profile with treatment effects for these two types of antihypertensive agents were simulated as follows:

1) Moxonidine was used as a prototype for the first type of antihypertensive agents. The time-course of treatment effect described for moxonidine by Hemple (121) was adapted for the Monte Carlo simulation of an antihypertensive treatment effect. One

month of continuous true BP profiles with a 0.3 mg QD dose of moxonidine treatment were simulated by superimposing the moxonidine response on simulated baseline BP profiles as described above. Various dosing times (e.g., 8:00 AM, 12:00 PM, and 8:00 PM) were also explored to evaluate the effect of dosing time on the analysis results.

2) For agents that do not change circadian rhythm of BP, the BP profile was shifted down relative to the baseline BP profile. The drug response was assumed to follow normal distribution ~ N(11.8 mm Hg, 10.9 mm Hg) (a similar treatment effect to the 0.3 mg (QD) moxonidine combined with the placebo effect).

The simulated duration of treatment for both antihypertensive treatments was one month (720 hr). Perfect adherence was assumed in the both simulations.

6.2.3 Simulation strategy for BP measurements

Casual visit times were defined as the random clinic visit time during office hours and assumed to follow a uniform distribution (8:00 AM~6:00 PM). For each virtual subject, two casual clinic visit times to assess cuff measurements were simulated on day 30 of treatment. The cuff BP measurements were simulated as described previously (129). True point BP values at each of the corresponding clinic visit times were captured from the simulated BP profile. The cuff BP measurement error was assumed to be normally distributed with a mean of zero and standard deviation of 5 mm Hg (113, 124). The observed cuff BP measurement at each casual clinic visit was generated by combining the true BP value at clinic visit time with randomly generated cuff BP measurement errors.

In addition to casual clinic visits, clinic visit times were also constrained to a specific clock time during office hours (8:00 AM ~ 6:00 PM) for all virtual subjects. This was done to explore whether there was an optimal clinic visit time for the cuff measurement. Cuff measurements at these visits were simulated as described above.

6.2.4 Evaluation of BP misclassification

The true BP for each virtual subject was defined as the mean value of the BP profiles during office hours (8:00 AM ~ 6:00 PM). Both the true and casual cuff BP measurements were categorized into four stages based on the JNC 7 guidelines. These stages were: i) normal (BP<120/80); ii) pre-hypertension (120/80-139/89); iii) stage 1 hypertension (140/90-159/99); and iv) stage 2 hypertension (≥160/100). A binary outcome was assigned based on thief the categorical assignment from the virtual observed response was consistent with true JNC hypertension category (i.e., whether there was a correct category assignment). The percentage of patients with a misclassified JNC hypertension category was calculated for each clinic visit. The percent of patients with BP classifications that were inconsistent with two casual clinic visit times on the same day was also evaluated. Misclassification rates based on clinic visits at specified clock times were evaluated to identify whether there was the optimal clinic visit time. The misclassification rate of the ABPM method was also calculated by comparing the mean ABPM measurement to the true mean BP with the method described above.

The sensitivity of the analysis results to various dosing times, personal preferences for rounding to 0 or 5 as the last digit of measured BP, and taking one additional measurement at each casual clinic visit was also evaluated.

6.2.5 Analysis platform

NONMEM® (Version VI, University of California at San Francisco, CA) was used to simulate moxonidine concentrations in the plasma and effect compartments for virtual subjects. Simulation of the blood pressure profiles, graphics, post-processing of NONMEM® outputs, model qualification, and global sensitivity analysis was performed in R® (version 2.9.1). The 95% PI for the analysis results was estimated by simulating 1000 replicates accounting for posterior parameter distribution of the baseline BP model.

6.3 RESULTS

The mean (range) of the 24-h mean systolic BP for virtual subjects (n=3840) was 144.3 (120.2–201.1 mm Hg). A histogram of the systolic BP distribution has been shown in the previous paper (129). The average age of the virtual subjects was 56 years (21~86 years) with 55% of male and 45% of female.

A 30 day, 24-h time course of systolic BP accounting for circadian patterns, inter-occasion and inter-individual variability were simulated for 3840 virtual subjects using the baseline model and the moxonidine PK/PD model. In these simulations, a dosage regimen of 0.3 mg moxonidine once daily orally was used with administration times of 8:00 AM, 12:00 PM, and 8:00 PM.

Approximately, 24% of patients' BP was misclassified based on their true BPs using an 8:00 AM 0.3 mg once daily dosing regimen of moxonidine. The percent of misclassification varies with different dosing regimens for anti-hypertensive agents based on how the circadian rhythm of the BP profile is affected. The percentage of patients with BP misclassifications was 32.0% and 23.5% using 12:00 PM and 8:00 PM dosing times respectively. This is shown in table 1. Anti-hypertensives that did not change the circadian rhythm of the BP profile demonstrated a misclassification rate of 22.8%, similar to the morning or evening dose of moxonidine (Table 1).

For the simulations, if virtual subjects went to the clinic at two different times within the same day, the treatment decisions were not always consistent. As shown at Table 2, the percentage of patients with an inconsistent BP classification was 33.5%, 40.2%, 32.0%, and 30.9%, respectively, for moxonidine with an 8:00 AM, 12:00 PM, 8:00 PM QD dosing regimen, and type II anti-hypertensives.

That percent of patients with BP misclassification from cuff measure varies with different clock times is observed for both types of anti-hypertensive agents (Figure 1). The impact of clinic visit time on the BP misclassification depends on the type of agent (type I vs type II) as well as the dosing time (moxonidine 8:00 AM vs 12:00 PM vs 8:00 PM QD dosing). The medications that changed the shape of the BP profiles within the time window (8:00 AM – 6:00 PM) had the greatest effect on misclassification. Cuff

measurements at specific clock times performed better than randomly selected times. Early morning (8:00 AM – 10:00 AM) or late afternoon (2:00 PM – 6:00 PM) was identified as the time intervals most likely to result in BP misclassification. Approximately 40% of patients were misclassified to an incorrect BP treatment group with a 8:00 AM clinic visit using an 8:00 AM QD dosing regimen or 6:00 PM clinic visit using a 12:00 PM QD dosing regimen. The best clinic visit time frame was between 12:00 PM – 2:00 PM for moxonidine in conjunction with an 8:00 AM or 8:00 PM QD dosing regimen for both type I and type II antihypertensive agents. Twenty percent of patients, however, were still misclassified to an incorrect BP group. The moxonidine 12:00 PM QD dosing regimen had highest percentage misclassification. The best clinic visit time for this dosing regimen was 1:00 PM where the percent of patients with BP misclassification was around 22% (Figure 1).

The percentage of patients with BP misclassifications decreased to 20.2% if two BP measurements were taken per casual clinic visit time (separated by 12 minutes) with an 8:00 AM QD dosing of moxonidine (Table 3). One additional BP measurement at a casual clinic visit corrected the misclassification in 4% of patients. If the last digits of the measured BP was rounded to a 0 or a 5, the misclassification rate of the measured BP was increased to 26.1% for one BP measurement per casual visit and 20.8% for two BP measurements per casual visit. The terminal digit rounding with one measurement resulted in an increase in BP misclassification of 2 % (24.4% vs. 26.1%). BP misclassification increases for both cases, but the increase was not statistically significant. The relationship between percent of patients with BP misclassification using one or two measurements with or without rounding error for specified clinic visit times is shown in Figure 2.

The entire analysis was repeated using systolic BP distribution obtained from NHANES adult data. Mean (range) of the 24-h mean systolic BP for virtual subjects (n=3840) at baseline was 155.9 mm Hg (140~238 mm Hg). The results were similar (not shown) and not statistically different from that presented above from FDA database.

As shown in Figure 3, a global sensitivity analysis found that BP misclassification rate (19.5%–38.7%) was linearly correlated to the standard deviation of BP measurement error (3–10 mm Hg). Global sensitivity analysis also showed that the BP misclassification rates in our analysis were robust across the uncertainty in all parameters reported in Hempel's paper except for the amplitude of the first cosine term and the random effects parameter for the inter-individual variability on clock time (h) (refer to appendix for additional information). The percentage of patients with BP misclassification (26%–23%) were negatively correlated with the amplitude of the first cosine term (95%CI: -0.087 – -0.048). The inter-individual variability on clock time (variance 95%CI: 3.59–28.47 h) was positively correlated with the percent of patients with BP misclassification (23%–26.5%). Therefore, robust estimates of these two population parameters are important in understanding the BP misclassification rate.

6.4 DISCUSSION

In this study we extended a prior analysis to BP treatment group misclassification accounting for confounding factors such as internal circadian rhythm of BP, antihypertensive treatment effect on the BP profile, clinic visit times, and BP measurement error. The simulation results indicate that on average 25% of patients were misclassified to an incorrect BP category for a casual clinic visit (random visit time within office hours from 8:00 AM to 6:00 PM). The BP misclassification depends on PK/PD characteristics (type of anti-hypertensive) as well as dosing time of the type I drug (Table 1).

Clinic visit time (i.e. between 8:00 AM to 6:00 PM) was found to impact BP misclassification rate, ranging from 20%–40% depending on the time of day of the measurement (Figure 1). Previously estimated BP misclassification rates at a casual clinic visit time were a mathematical average of the BP misclassifications at each clock time from 8:00 AM to 6:00 PM.

The BP misclassification rates between 11:00 AM to 3:00 PM were lower than that in other time frames for both types of drugs. The BP misclassification can be as high as 40% at early morning (8:00 AM – 10:00 AM) or late afternoon (16:00 PM – 18:00 PM) visits depending on PK/PD characteristics as well as dosing regimen. However, the BP misclassification rate remained around 19% even at optimal clinic visit times. This is because even after accounting for intrinsic factors (e.g. circadian rhythms) contributing to BP measurement error, there is still bias due to cuff measurement error arising from the device, cuff size, and training levels of personnel.

Two types of antihypertensive agents were tested in this study: 1) type I: agents that change the circadian rhythm and 2) type II: agents that shifts the entire BP profile downward. It is worth noting that the dosing time does not change the BP misclassification rate for type II anti-hypertensive drugs as administration does not change the circadian rhythm of BP. These drugs could have delayed effect or their concentrations at steady state are much higher than their own EC50 (typically seen in ACEs inhibitors and ARBs.)

In this study, we tested our results in two populations: hypertensive patients from NDAs in the internal database of FDA and NHANES Adult Database (BPs >=140 mm Hg). Baseline BP distributions from the two population likely represent the US adult population to the best of our knowledge. Our simulation showed that our analysis results from FDA data base could be extended to the NHANES population.

BP misclassification increases by 5% with only one BP measurement is obtained compared to two measurements per clinic visit. The BP misclassification rate with two measurements may be slightly under-estimated. In our study, we considered the measurement error from all sources as a random error. However, Cuff measurement error includes the device error from lack of calibration, impropriate cuff size, and observer's error from uncertainty in interpreting the korotkoff sounds, (108, 113, 130) position of the patients etc. This device error may be a systematic error if both measurements were taken by the same sphygmomanometer device, although it may be considered as a

random error for generalization of results. Ignoring the correlation between the two measurements may underestimate the BP misclassification rate.

In conclusion, it is likely that a significant percentage of hypertensive patients have been misclassified to an incorrect BP treatment category as a result of issues relating to current clinical practice and cuff BP measurement. The BP misclassification depends on the time of day when the BP is measured, PK/PD properties of antihypertensive, and dosing regimen. In general, early morning (8:00 AM – 10:00 AM) or late afternoon (16:00 PM – 18:00 PM) was identified to be the worst time frame for clinic visit and provided highest BP misclassification rate for all scenarios. One additional measurement per clinic visit would decrease the BP misclassification rate especially when last digit preference to 0 or 5 for BP measurements exists.

6.5 TABLES

Table 1. Percent of patients with BP misclassification: Cuff measured BP at casual clinic visit times. BP measurement error was assumed as normally distributed with mean zero and standard error of 5 mm Hg.

	Percent of patients with BP misclassification (measured BP vs. true BP)			
	Drugs altering the BP profile: moxonidine			Drugs that do not alter the BP profile
	8:00 AM QD	12:00 PM QD	8:00 PM QD	
Random Visit vs. True Value	24.4% (22.5% – 27.3%)	32.0% (29.3%– 34.8%)	23.5% (20.9% – 27.1%)	22.8% (19.8%– 26.0%)

Table 2. Percent of patients with inconsistent BP classification: Cuff measured BPs at two casual clinic visit times within the same day. BP measurement error was assumed as normally distributed with mean zero and standard error of 5 mm Hg.

	Percent of patients with inconsistent BP classification (Casual visit 1 vs. Casual visit 2)			
	Drugs altering the BP profile: moxonidine			Drugs that do not alter the BP profile
	8:00 AM QD dose	12:00 PM QD dose	8:00 PM QD dose	
Random Visit 1 vs. Random Visit 2	33.5% (30.9% – 36.0%)	40.2% (37.7% – 42.8%)	32.0% (29.4% – 35.6%)	30.9% (28.0%–34.7%)

Table 3. The impact of one versus two BP measurements with and without rounding error on BP misclassification. Moxonidine doses were taken at 8:00 AM QD and office visits were randomly selected during clinical office hours (i.e. 8 AM – 6 PM).

	Percent of patients with BP misclassification (95%PI)	
	Without Rounding Error	With Rounding Error
One Measure	24.4% (22.5% – 27.3%)	26.1% (24.2%-28.9%)
Two Measures	20.2% (17.6%-23.5%)	20.8% (18.5%-24.1%)

6.6 FIGURES

Figure 1. Percent of patients whose BP was misclassified based on cuff measurements at specified clock time visit. Moxonidine doses were administered at 8:00 AM QD for all patients.

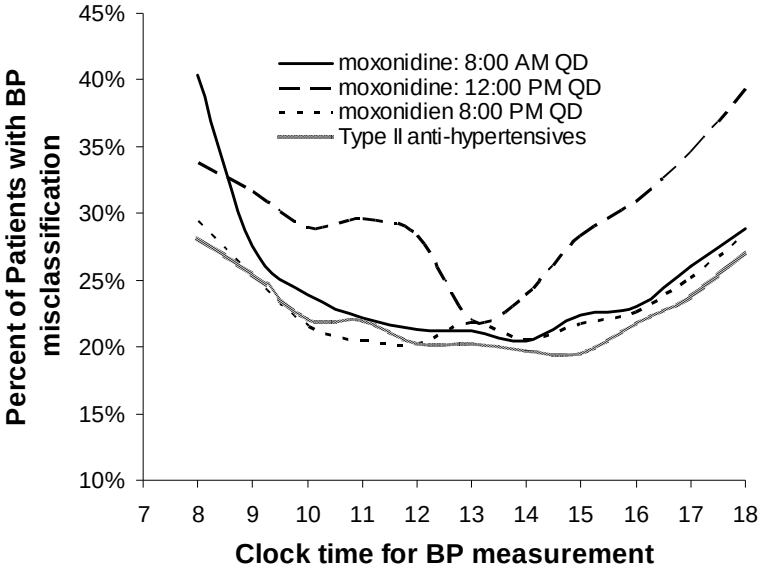


Figure 2. Percent of patients with BP misclassification based on single vs multiple BP measurements and measurement rounding error for different clock time visits. Moxonidine doses were administered at 8:00 AM QD for all patients.

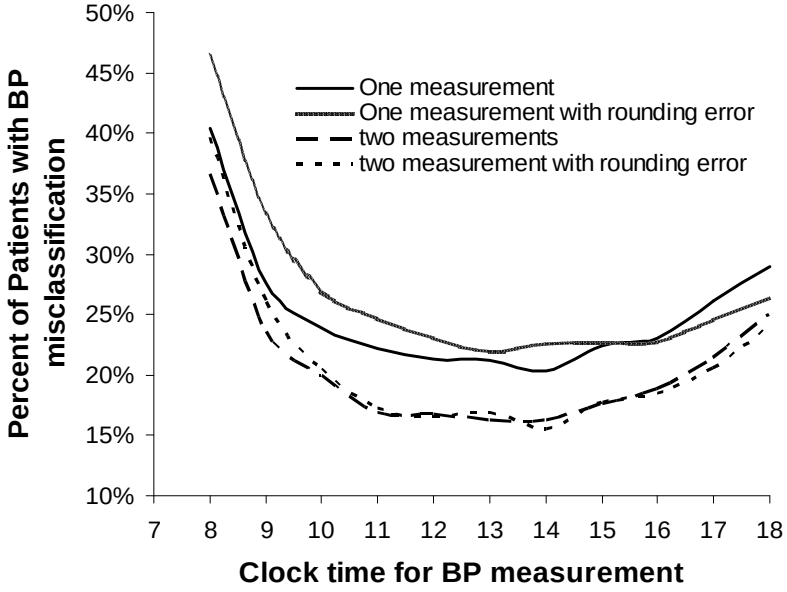
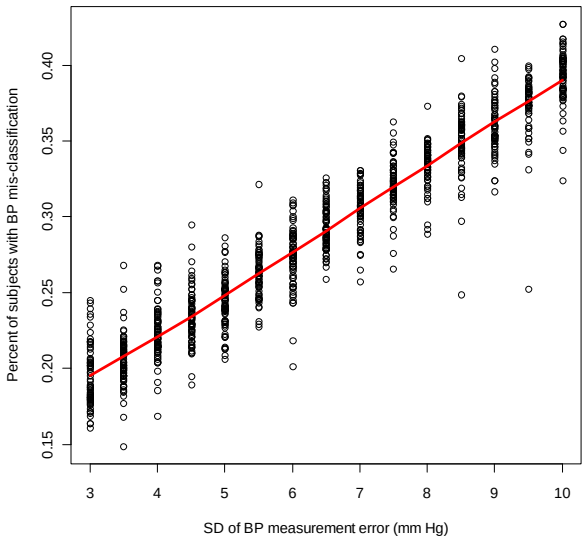


Figure 3. Fraction of patients with BP misclassifications based on BP cuff measurement error. Moxonidine doses were administered at 8:00 AM QD for all patients.



6.7 APPENDIX FOR CHAPTER SIX

Figure 1: Global sensitivity analysis

Figure 1a: Fixed effect parameters vs. Percent of patients with BP misclassification

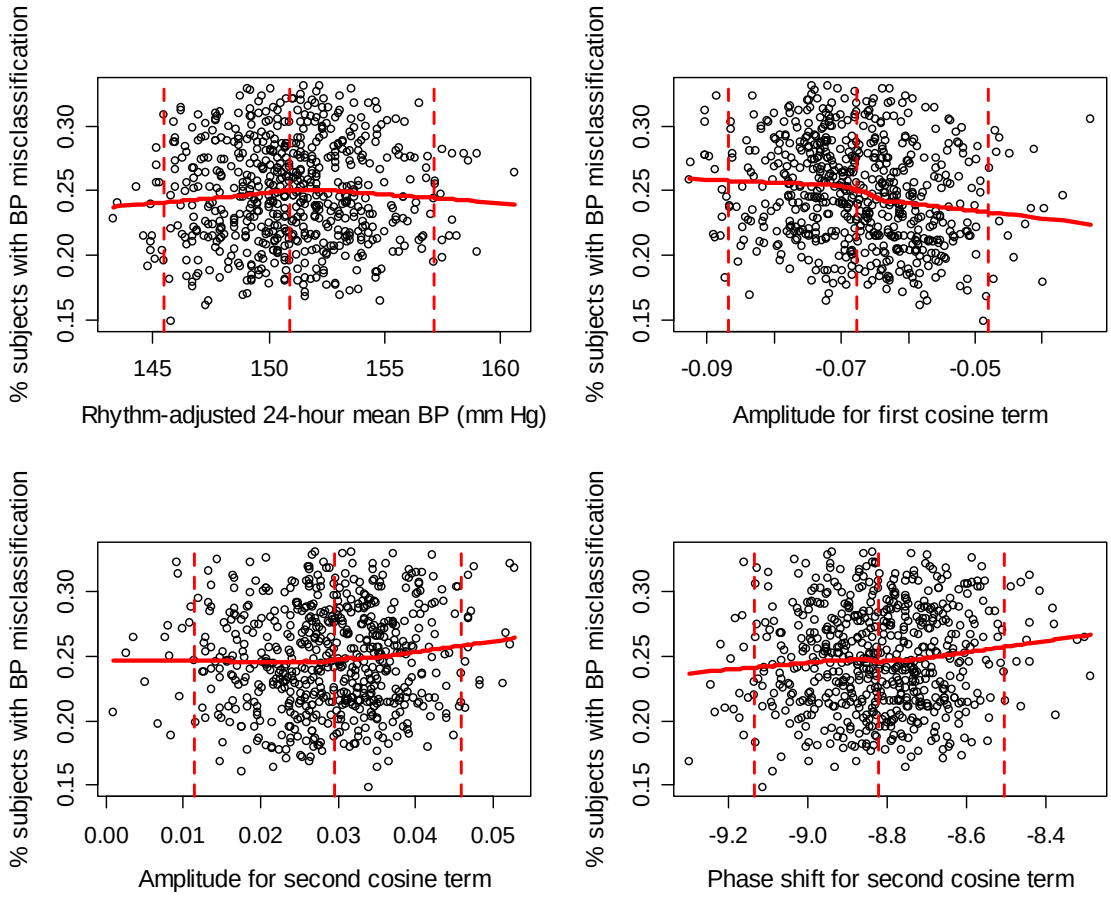


Figure 1b: Inter-individual variability vs. Percent of patients with BP misclassification

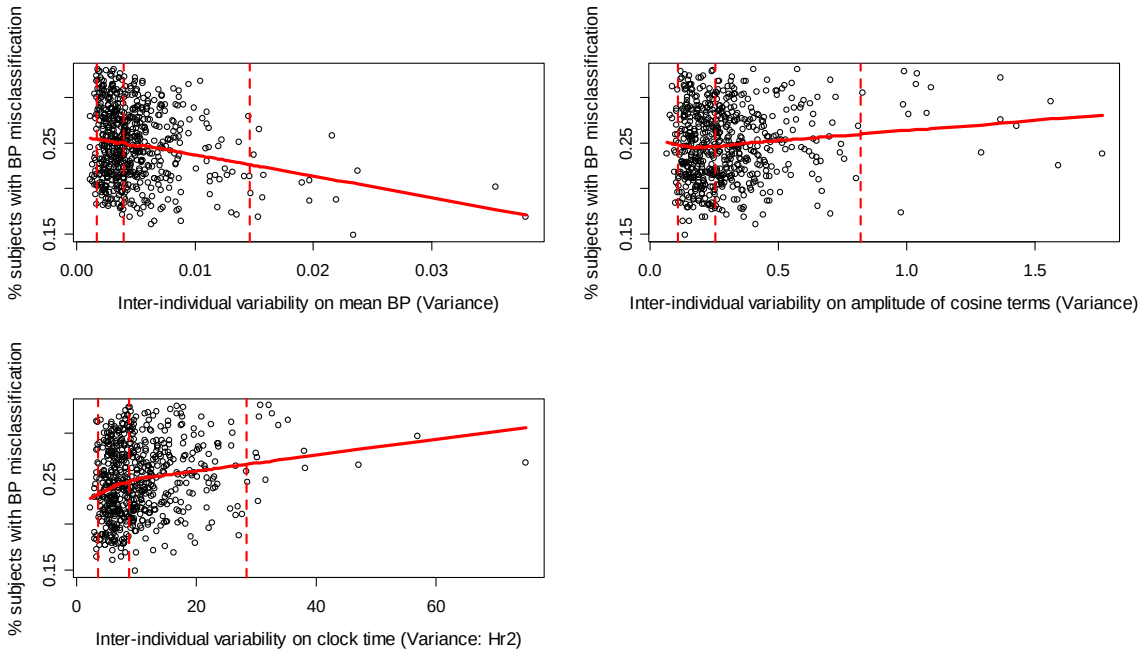


Figure 1c: Inter-occasion variability vs. Percent of patients with BP misclassification

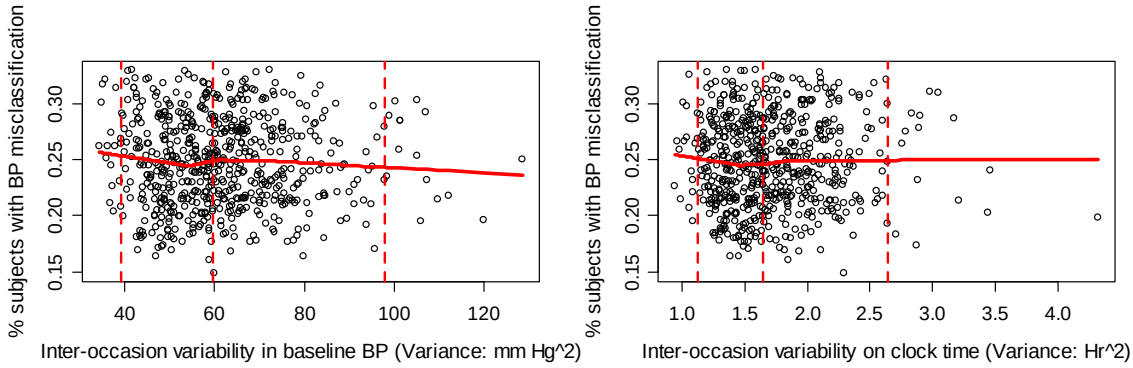
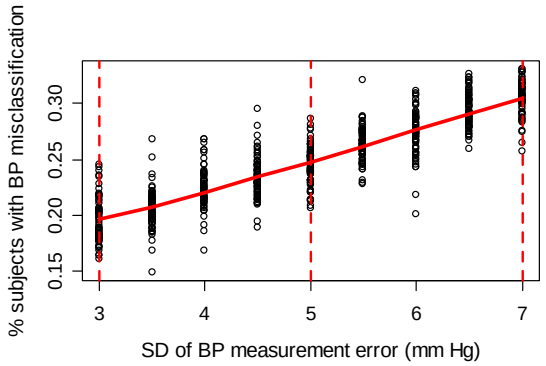


Figure 1d: Standard Deviation of BP measurement error vs. Percent of patients with BP misclassification



7.0 SEVENTH CHAPTER

Impact of various clinical practice strategies on cardiovascular risk for the treatment of hypertension: a clinical trial simulation study

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7.1 INTRODUCTION

Cardiovascular disease (CVD) events associated with elevated blood pressures remain a leading cause of overall mobility and mortality (131, 132). It is well known that elevated blood pressure contributes significantly to the risk of cardiovascular disease (CVD) (106, 107, 133). It has been shown that BP reduction significantly decreases the risk of CVD (107, 134). Therefore, the goal of controlling BP in patients with hypertension is to lower their CVD risk. However, the proportion of patients with BP under control is still below the Healthy People 2010 Goal of 50%.

The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) guideline suggests a target BP of less than 140/90 mmHg. The JNC 7 guideline suggests titrating dose upward to the highest tolerable dose before adding other class of drugs to achieve the BP goal and the use of combination anti-hypertensive therapy when BP is more than 20 mm Hg above this goal BP. However, the JNC 7 provides little detail to guide a specific clinical practice strategy, specifically regarding dose titration strategy (107, 135). The Institute for Clinical Systems Improvement 2008 guideline titled "Hypertension diagnosis and treatment" suggested that "The lowest recommended dose of the chosen drug should be used initially. If tolerated, the dose can be increased or additional medications added to achieve goal blood pressure." (136). Similarly, the 2003 European Society of

Hypertension/European Society of Cardiology (ESH/ESC) guidelines suggested initiating with low dose for both single and combination therapy.

However, studies have suggested that more than two third of patients with hypertension will need more than two antihypertensive drugs to reach a goal BP of < 140/90 mmHg (137). Initiating treatment with a low dose of antihypertensive will likely delay the time to reach the goal BP while dosages are titrated upward and a second class of antihypertensive agent are added to the regimen. In addition, there are at least two other factors that can further delay the time to achieve the BP target. These include the measurement error associated with clinic based cuff BP measurements and neglecting the circadian nature of BP within individuals over the course of a day. Both of these factors can lead to a significant errors in classifying patient risk level, leading to an incorrect treatment decision in 20–40% of clinic visits (138).

A specific scenario could occur as follows: a patient has a clinic visit measurement of BP <140/90 that underestimates their true BP of >140/90. In this case, it is very likely that patients will not be titrated to a higher dose level or have an additional antihypertensive included in their dosing regimen at the clinic visit. This will expose the patient to an increased risk of a cardiovascular event until the next follow up visit, which may not occur until 3- to 6-months later. In addition, follow up visits may not be guaranteed for each patient for personal or economic reasons. In cases where additional clinic visits do not occur for these false negative patients, one could posit that this proportion of patients may never achieve an appropriate goal BP. Hence, the strategy of initiating treatment from low dose may lower the risk for side effects of the antihypertensive, such as orthostatic hypotension. This increase in safety may potentially be offset by increased CVD risk in the time necessary to properly control a patient's BP.

It has been widely discussed that increased risk of CVD associated with elevated BP exists even below the current goal BP of 140/90. The CV risk doubles with BP elevations of 20/10 mm Hg in systolic/diastolic BP starting from 115/75 mm Hg for patients 40–70 years (134). A study conducted by Vasan and his colleague (139) showed that individuals with a high normal BP have a risk-factor adjusted hazard ratio of 2.5 in

women and 1.6 in men for a CVD event. It also has been reported that non-hypertensive subjects with a systolic BP of 130-139 mm Hg (or a diastolic BP 85-89 mm Hg) have an increased risk of CVD (134, 139). Hence, there is a potential benefit for patients with hypertension if a lower goal BP than the current one of 140/90 mm Hg is targeted. However, the impact of a clinical practice strategy targeting a lower BP on risk of CVD patients with hypertension has not been studied.

Our previous study evaluated BP measurement discordance and BP treatment group misclassification rates associated with the current clinical practice. In this study, we extend our previous studies to CVD event risk using the survival model proposed by Framingham *et al.* (106).

The study aimed to identify a better strategy that further decreases the CV risk in US population compared to the current clinical practice. The clinical outcomes of various clinical practice strategies, including initiating hypertensive treatment with high dose and setting a lower goal BP of 120 mm Hg was estimated . In addition, we compared the risk of CVD in patients with hypertension across the various proposed clinical strategies.

7.2 METHODS

7.2.1 Virtual Subjects characteristics and sample size

Mean systolic BP over 24-h from virtual subjects was used to anchor the simulated 24-h BP profiles of virtual subjects for the current study. The virtual subjects' characteristics (n=877) included 24-h mean systolic BP, age, sex, total cholesterol, HDL cholesterol, smoking status, diabetes status, and treatment for hypertension. These characteristics were obtained from the internal database of Food and Drug Administration (FDA) by pooling information of several New Drug Applications without disturbing the relationship between the risk factors within individuals.

The same analysis was repeated by sampling systolic BPs from the population distribution along with other CV risk factor covariates (age, sex, total cholesterol, HDL cholesterol, smoking status, diabetes status, and treatment for hypertension) from the NHANES III Adult Database (http://www.cdc.gov/nchs/tutorials/Nhanes/Downloads/intro_III.htm). Subjects with a systolic BP higher than 140 mm Hg (>130 mm Hg for patients with diabetes) were considered hypertensive. These systolic BPs from NHANES database were calculated as the mean of three BP measurements. We utilized these BPs are the rhythm adjusted 24-h mean BP of virtual subjects to anchor the simulations of continuous BP profile.

7.2.2 Simulation of the baseline BP profile

The population baseline BP model developed by Hempel and colleagues (107, 121) was qualified and adopted for the simulation of long term baseline BP. The detailed description of the model was presented previously (107, 121, 129, 138). Briefly, the baseline BP model was developed to describe BP circadian rhythm using 24-h ABPM data over multiple occasions. Daily BP fluctuations were described using a function with two cosine terms including inter-individual variability for the rhythm-adjusted 24-h mean, amplitude of the cosine terms, and clock time and inter-occasion variability for the rhythm-adjusted 24-h mean and clock time. The published parameter values for the baseline BP model parameters were adapted from the original paper with the exception of rhythm adjusted population mean BP (θ_1) and it inter-individual variability (η_1). The rhythm adjusted 24-h mean BPs for virtual subjects was directly sampled from internal database of FDA and the NHANES database.

A global sensitivity analysis was performed after generating our analysis endpoints to quantitatively evaluate impact of uncertainty distribution in the parameter estimates of the baseline model on the results of our analysis(25, 26, 34).

7.2.3 Simulation of the BP profile with antihypertensive treatment

Patients were eligible for virtual treatment with up to four antihypertensive drugs A, B, C, and D simultaneously. To simplify the simulation, three dose levels were assumed for each drug: low, medium, and high. The dose response relationship of the four drugs A, B, C, and D with respect to various dose levels are described in Table 1 (140-142). It was assumed that A, B, C, and D were from different classes of antihypertensive agents, hence having different mechanisms of action. Therefore, the drug responses were considered to be additive when combination therapy was used.

7.2.4 Cox model for estimation of general CV risk

A cardiovascular risk model published in 2008 by Framingham *et al.* was adopted for CV risk calculation (106). The general formula of 10 year CV risk is that $p = 1 - S_0(t)^{\exp(\sum_i^p \beta_i X_i - \sum_1^p \beta_i \overline{X_i})}$, where $S_0(t)$ is the baseline survival at follow-up time t (here t=10 years;), $ß_i$ is the estimated regression coefficient (log hazard ratio), X_i is the log-transformed value of the i_{th} risk factor, X_i (bar) is the corresponding mean, and p denotes the number of risk factors.

The CV risk for patients was calculated at time zero and after six month of treatment with different simulated clinical practice strategies. The CV risk of patients after 6 month of treatments was compared among these strategies. Parameter values of the regression coefficients were directly adopted from the publication (106).

7.2.5 Clinical practice strategies for BP control

Virtual patients were treated for hypertension using the four strategies detailed below. Differences in CV risk for virtual patients with different clinical practice strategies were evaluated using Monte Carlo Simulation. The duration of the virtual clinical study was six months; the CV risk for each patient was calculated before the study as well as six months post treatment:

Strategy I: The goal BP was 140 mm Hg (130 mm Hg for patients with diabetes). Virtual patients were initially started on the lowest dose of a single antihypertensive or

the lowest dose of combination therapy if their baseline BP was >20 mm Hg above the target BP (≥ 160 mm Hg or ≥150 mm Hg for patients with diabetes). Patients returned for follow-up and dose/medication adjustments at monthly intervals. Dose and medications were adjusted based on cuff measurement at a casual clinic visit during office hours. Each drug has three dose levels: low, medium, and high. Dose of initial drugs were titrated upward to the highest dose before adding other class of drugs to achieve the BP goal. The maximum numbers of drugs for a patient were four drugs from different classes. If a patient's measured BP at any follow up visit reached goal BP, the dose of antihypertensive(s) was assumed to be adequate and maintained for 6 months. A systolic BP <90 mm Hg was selected as the cut off value for unacceptable hypotension. If this threshold was reached, the dose of the antihypertensive agent(s) was decreased. The dosage was further decreased when measured BP at subsequent follow up visits were <90 mm Hg. The total simulated time period for treatment was 6 months.

Strategy II: Virtual patients were initially started on the highest dose of single antihypertensive therapy if their baseline BP was <160 mm Hg and on the highest dose of combination therapy if their baseline BP was ≥ 160 mm Hg. The dosage was also adjusted based on monthly cuff measurement at a casual clinic visit during office hours. A drug from another class at the highest tolerable dose was added if measured BP at the follow up clinic visit was still above the target BP of 140 mm Hg.

Strategy III: Equivalent to strategy I but with a target BP of 120 mm Hg instead of 140 mm Hg.

Strategy IV: Equivalent to strategy II but with a target BP of 120 mm Hg instead of 140 mm Hg.

Cuff BPs measured at casual clinic visit times (random time between 8:00~6:00 PM) were used for virtual treatment decision making. The cuff BP values were generated as previously described [Jin]. The true point BP value at each of the corresponding clinic visit time was captured from the simulated BP profile. The cuff BP measurement error was assumed to be normally distributed with a mean of zero and standard deviation of 5

mm Hg (113-115, 117). The observed cuff BP measurement at each casual clinic visit was generated by combining the true BP value at clinic visit time with a randomly generated cuff BP measurement error.

7.2.6 Analysis platform

NONMEM® (Version VI, University of California at San Francisco, CA) was used to simulate moxonidine concentrations in the plasma and effect compartments for virtual subjects. Simulation of the blood pressure profiles, graphics and post-processing of NONMEM® outputs, and global sensitivity analysis was performed in R® (version 2.9.1). The 95% prediction intervals (PI) for the analysis results were determined by running 1000 simulation replicates accounting for the posterior parameter distributions (uncertainty) in the model of baseline BP profile.

7.3 RESULTS

A total of 877 virtual subjects were included in this clinical trial simulation. Demographics of the virtual subjects are shown in Table 1. The mean systolic BP was assumed to represent the baseline of the rhythm adjusted 24-h BP mean for virtual subjects.

The estimated 10 year CV risk at baseline was 18.1%. The estimated 10 year CV risk after 6 months of treatment decreased to 14.9%, 13.3%, 14.2%, and 12.1% for strategies I, II, III, and IV, respectively. The absolute risk reductions (ARR) from baseline after 6 months of treatment with strategies I, II, III, and IV were 3.2%, 4.8%, 3.9%, and 6.0%, respectively. The impact of the ARR on the 10 year cardiovascular event risk corresponds to preventing 1 CV event for every 31, 20, 26, and 17 patients using current clinical practice strategies, strategy II (high dose strategy), strategy III (low dose strategy targeting 120 mm Hg), and strategy IV (high dose strategy targeting 140 mm Hg), respectively.

Implementing these strategies in 10000 hypertensive patients over one year would result in a reduction of 32 CVD events with the current clinical practice strategy, 48 with the high dose strategy, 39 with the low dose strategy and 120 mm Hg goal BP, and 60 with high dose strategy and 120 mm Hg goal BP. These results are illustrated in Figure 1.

Therefore, initiating treatment with the high dose strategy would result in a reduction of 50% more CVD events compared to that with current clinical practice. The 120 mm Hg as target BP strategy would lead to a reduction of 22% more CV events and the high dose strategy with the 120mmHg target BP would result in 87.5% more CV events compared to that with current clinical practice.

Global sensitivity analysis showed that the posterior parameter distributions reported in Hempel's paper had no systematic impact on our estimated clinical outcomes. Estimated risks of CVD given various clinical practice strategies were robust across the uncertainty in all parameters reported.

7.4 DISCUSSION

In this study, results from previous BP discordance and BP misclassification studies (138) are extended to evaluate the impact on the CV risk. The simulation results indicate that different clinical practice strategies result in different CVD risk for patients with hypertension. Initiating treatment with the highest tolerable antihypertensive dose or setting the target BP to 120 mm Hg would lower CVD risk for hypertensive patients better than the current clinical practice strategy.

The high dose strategy, in particular, showed the potential benefits for aggressively treating elevated BP. Patients might eventually reach the same adequate dose level or number of antihypertensive agents for both the low dose strategy (current clinical practice strategy) and high dose strategy as long as the goal BP was the same and the correct dosage adjustment was made at each follow up visit. Nevertheless, our results showed that the delayed time for patients to achieve a target blood pressure level resulted

in significant additional CV risk. Treating 10,000 hypertensive patients for one year would result in a reduction of 32 CVD events with current clinical practice strategy and 48 with the high dose strategy. Therefore, initiating treatment with a high dose of the antihypertensive agents is predicted to be 50% more effective at reducing CVD events a year compared to current clinical practice. This result emphasizes the importance of rapidly achieving a goal BP. In addition, high dose strategies would likely decrease the number of follow up visits and reduce the work load in clinical practice. This strategy has greater potential for antihypertensive drugs that have a high degree of tolerability at higher dosages and that do not exhibit serious dose related adverse drug events, such as angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARB). However, particular caution in dosing should be followed for patients with high risk of orthostatic hypotension, such as patients with diabetes, autonomic dysfunction, and some geriatrics (107).

This study demonstrates the beneficial effect of setting a lower target BP 120 mm Hg on CVD risk. Treating 10,000 hypertensive patients for one year would result in a reduction of 32 CVD events with current clinical practice strategy, 48 with high dose strategy, 39 with low dose strategy and 120 mm Hg goal BP, and 60 with low dose strategy and 120 mm Hg goal BP. Hence setting a lower goal BP alone led to an additional 22% (39 vs. 32) and 25% (60 vs 48) reduction in CVD events a year for strategy III (low dose targeting 120 mm Hg) and strategy IV (high dose targeting 120 mm Hg), respectively. Setting a lower BP target would likely have greater benefit for patients who achieve the target BP of 140 mm Hg with one or two antihypertensive agents as these patients have the potential to increase their dose or include additional agents to further decrease their BP.

A CVD risk model that predicts future CVD events has received increasing attention recently (133). Several CVD risk functions have been developed (106, 143-147). We adopted the CVD risk function proposed by the Framingham Heart Study (106). The CVD risk model not only estimates the general risk of experiencing all CVD events but also the risk of individual components such as stroke, coronary heart disease, cerebrovascular, peripheral arterial disease, and heart failure etc. The goal of this study

was to evaluate the effect of clinical practice strategies on risk of all CVD events, hence the general risk function was used in the simulation.

In conclusion, achieving a goal BP in a timely fashion by initiating hypertension treatment with highest tolerable dose for both monotherapy and combination therapy demonstrates potential for significant reduction in CVD event risk. This strategy should be considered for antihypertensive agents that have neither significant safety issues nor dose related side effects. Finally, setting target BP to 120 mm Hg would provide an additional reduction in CVD risk in patients with hypertension.

7.5 TABLES

Table 1: Summary statistics for virtual subject risk factors used in the simulation

Characteristics	All (n=877)	Women (n=330)	Men (n-=547)
Systolic BP, 24-h mean (range), mm Hg	151.2 (130.6~187.5)	151.5 (130.7~187.5)	151.1 (130.6~187.4)
Age, mean (range),y	55.9 (23~82)	56.3 (28~80)	55.8 (23~82)
Total-C, mean (range), mg/dL	204.1 (80.0~340.3)	210.0 (104.4~340.3)	200.6 (80.0 ~ 328.7)
HDL-C, mean (range), mg/dL	53.9 (22.8~135.3)	58.1 (22.8~119.9)	51.4 (22.8 ~ 135.3)
Smoking, n (%)	15.6%	11.5%	18.1%
Diabetes, n (%)	14.4%	14.8%	14.1%

Table 2. Dose response relationship of antihypertensive drugs assumed in our simulation

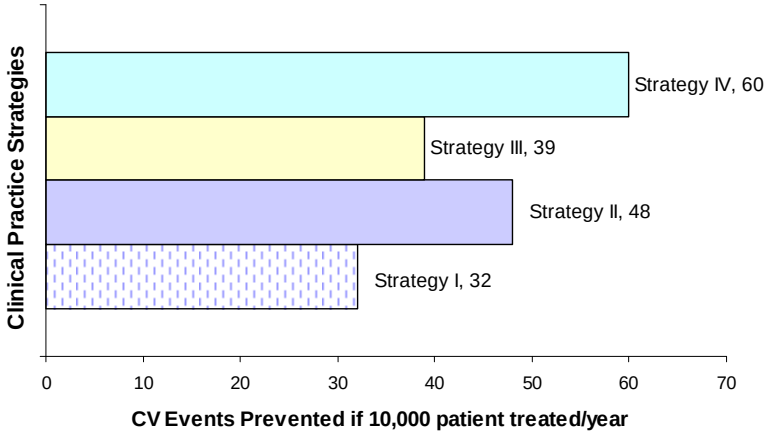
	Drug response in mm Hg (ΔBP)			
	First drug: A	B	C	D
Low dose	6 ± 12	5 ± 12	5 ± 12	5 ± 12
Med dose (additional response from low dose)	3 ± 2	2 ± 2	2 ± 12	2 ± 2
High dose (additional response from Medium dose)	2 ± 2	2 ± 2	2 ± 12	2 ± 2

Table 3. CV risk at baseline and after six months of treatment using four different BP maintenance strategies.

N=877 subjects	CVD risk (95% Prediction Interval)	Absolute Risk Reduction (ARR)	Number Needed to Treat (NNT)
Baseline	18.1% (17.6%~18.6%)		
Strategy I: Low Dose Strategy	14.9% (14.6%~15.3%)	3.2%	31
Strategy II: High Dose Strategy	13.3% (12.9%~13.6%)	4.8%	21
Strategy III: Low Dose Strategy with goal BP of 120 mm Hg	14.2% (13.7%~14.6%)	3.9%	26
Strategy II: High Dose Strategy with goal BP of 120 mm Hg	12.1% (11.7%~12.5%)	6.0%	17

7.6 FIGURES

Figure 1. CV events prevented using four different BP maintenance strategies



7.7 APPENDIX FOR CHAPTER SEVEN

Figure 1: Global sensitivity analysis

Figure 1a: Fixed effect parameters vs. Cardiovascular Disease (CV) Risk

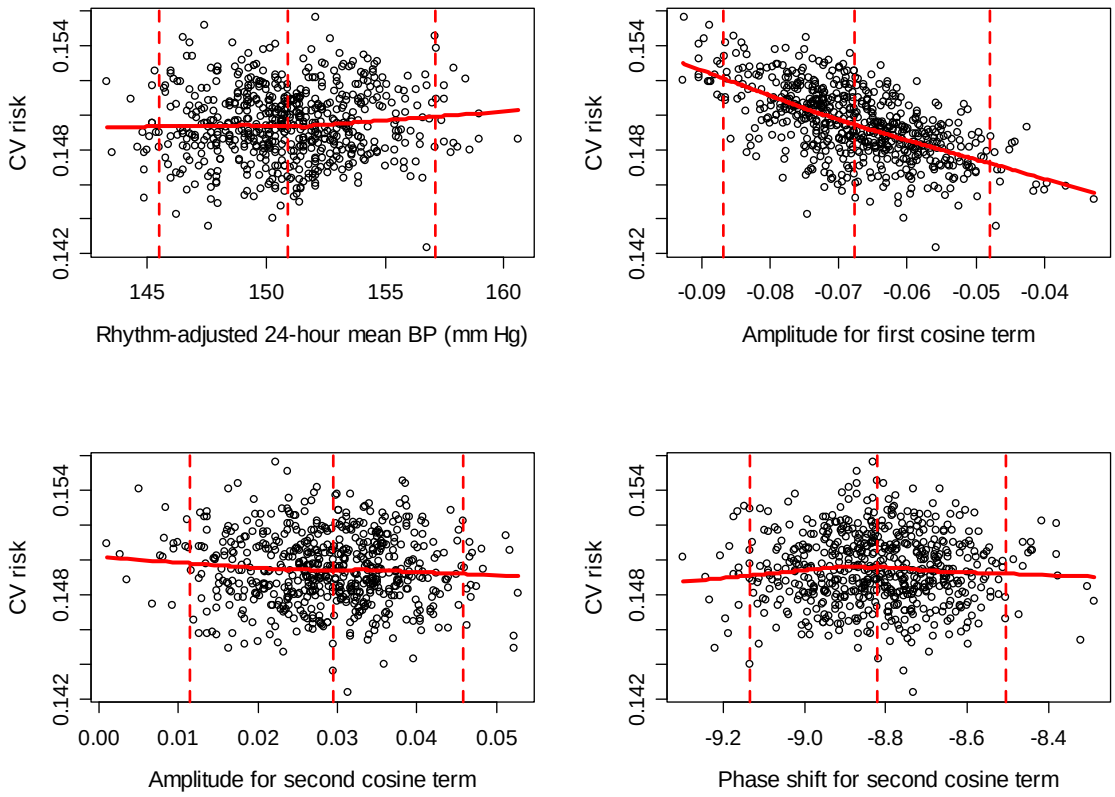


Figure 1b: Inter-individual variability vs. Cardiovascular Disease (CV) Risk

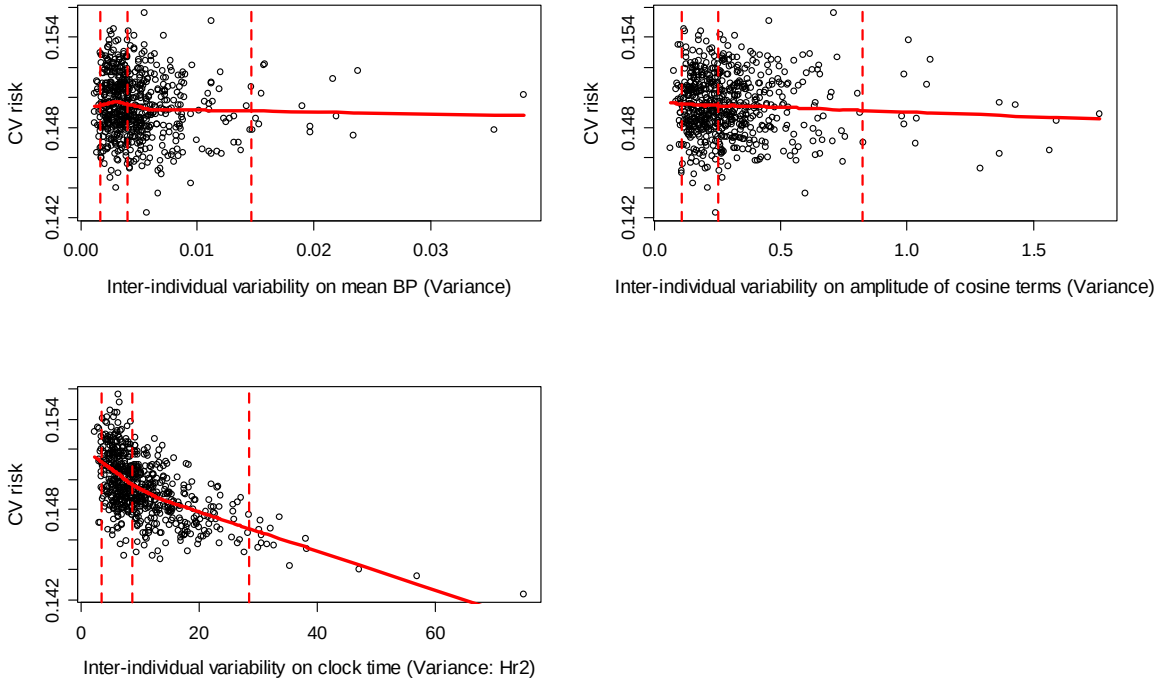


Figure 1c: Inter-occasion variability vs. Cardiovascular Disease (CV) Risk

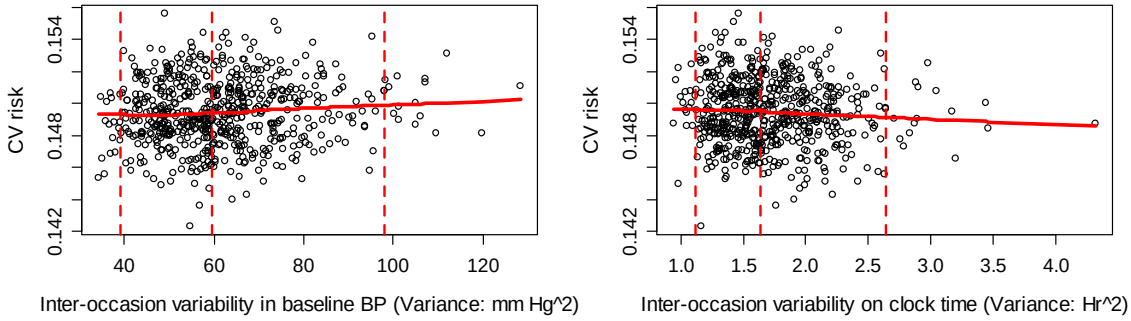
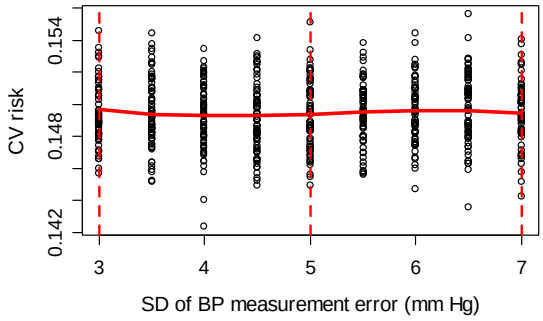


Figure 1d: Standard Deviation of BP measurement error vs. Cardiovascular Disease (CV) Risk



8.0 CONCLUSIONS

The major aim of this thesis is to use the modeling and simulation approach to identify systematic contributors to variability in estimated population parameters and the sources of variability in drug exposure, response, clinical practice and patient outcome. The thesis aimed to apply pharmacometrics to the public health area identifying potential issues associated with current clinical practice and patient outcome. This approach was also used to evaluate the impact of various alternative clinical practice strategies on clinical outcomes.

Population PK models were developed for both perphenazine and escitalopram. Our results showed that incorporating covariate information into population PK models identified substantial systematic contributors to variability in drug exposure for both of these drugs Race and smoking status in the past week were identified as two significant covariates affecting clearance of perphenazine. The data for perphenazine were very sparse, so the covariate relationships were confirmed using a randomization test procedure to reduce the possibility of an excessive type I error rate. The dosage regimen of perphenazine in these populations may need to be adjusted clinically based on patients smoking status. Perphenazine is primarily metabolized by CYP2D6 located in the liver. Therefore,t is possible that CYP2D6 genotype is confounding factor for the racial difference in perphenazine clearance. Further studies are required to identify the mechanisms contributing to the observed differences in perphenazine clearance across race. Racial information alone is not currently recommended for adjusting perphenazine dosage regimens in patients with schizophrenia.

CYP 2C19 genotype, age, and weight strongly influenced the CL/F of escitalopram. Patients with CYP2C19 RM/EM (*17 /*17, *1/*1, or *1/*17) cleared escitalopram significantly faster than those with CYP2C19 IM/PM (heterozygous or homozygous *2 or *3) genotype. Older patients taking escitalopram had a significantly lower apparent clearance compared with younger patients. Patients with higher body weights cleared escitalopram faster compared to those with lower body weights. Incorporating age, weight, and genotype into the population PK model accounted for

exposure differences between Pisa (Italy) patients and patients from Pittsburgh. Establishing a patient's metabolizer genotype and incorporating age, weight and BMI into this assessment can better guide therapeutic decision-making with respect to the dosing strategy for escitalopram and potentially minimize excessively high exposures to this SSRI. Off particular note for community practice is that two of these variables (age and weight) are routinely collected and require no specialized equipment or laboratory test. Thus, physicians can readily take these variables into account when determining appropriate starting doses and dosage titration schedules.

A population PK analysis was performed for orally administered escitalopram using two different reported dosing methods as the the dosage history input to the model. Our results showed that the measurement error associated with an incorrect or incomplete dosing history affected the population PK parameter estimation in the non-linear mixed effect modeling process. The dosing report methods considered were either the MEMS generated dosing histories or the patient reported last dosing time. Our results suggested that the necessity of MEMS monitoring may be determined by the drug half-life, the nature of the concentration effect relationship, and the sensitivity of this relationship to patterns of exposure. These patterns of exposure are not captured in the population PK analysis using reported time of last dose. The use of MEMS dosage histories versus the patient reported time of last dose, stabilized the estimation of absorption (K_a) and distribution (V_d), but clearance determination was virtually unaffected. Despite this, patterns of drug exposure may be critical in evaluating response, non-response, and toxicity. These patterns cannot be generated or recreated using the time of last dose information. Exposures calculated in this manner also provide an average exposure over a period of time. The pattern of the exposure and the response to treatment are not addressed in this study.

The measurement error associated with typical clinic based cuff BP measurements had significant effect on estimated drug response, hence clinical outcomes. Significant contributors to this variability included the cuff BP measurement errors and the fact that the circadian changes in BP were not accounted for. These effects may be addressed with the following strategies:

1) Cuff BP measurement time may need to be adjusted based on baseline clinic visit time as well as dosing regimen specific PK/PD considerations to better identify the true ΔBP in each virtual subject using the current clinical practice paradigm. The BP could be calibrated based on patients' baseline and treatment visit times to better correct for the circadian rhythm of BP profile. Optimizing the clinic visit time decreases the measurement bias contributed by the circadian rhythm of BP, improving the accuracy of the cuff measured ΔBP to certain extent. It does not, however, completely correct the measurement bias due to existence of random cuff measurement error. This cuff error may be contributed by the device, cuff size, and training levels of personnel.

2) It is likely that a significant percentage of hypertensive patients have been misclassified to an incorrect BP treatment category as a result of issues relating to circadian variability in BP and the cuff measurement error and how this is amplified by current clinical practice strategies. The BP misclassification rate in the current clinical practice is around 20~40% depending on the time of day when the BP is measured, PK/PD properties of antihypertensive, and the dosing regimen. In general, early morning (8:00 AM – 10:00 AM) or late afternoon (16:00 PM ~ 18:00 PM) were identified to be the worst time frames for clinic visits and provided the highest BP misclassification rate. One additional measurement per clinic visit would decrease the BP misclassification rate by 4% (absolute decrease). This effect was exacerbated when the effect of a last digit preference to round the blood pressure measurement to the nearest 0 or 5 for BP measurements exists.

3) Achieving a goal BP in a timely fashion by initiating hypertension treatment with highest tolerable dose for both mono-therapy or combination therapy shows the potential for significant reduction in CVD event risk. This strategy should be considered for antihypertensive agents that have neither significant safety issues nor dose related side effects. Setting the goal BP to 120 mm Hg would provide an additional reduction in risk of CVD for patients with hypertension.

Our simulation results show that three intervention approaches may lead to lower cardiovascular risk compared to current clinical practice strategy:

In conclusion, the thesis showed, using Monte Carlo simulation techniques, three potential interventions to be considered in the clinical practice or antihypertensive drug labeling for better BP management, hence lower CVD risk in US adult population. These interventions were: BP calibration based on clinic visit time while considering drug specific PK/PD characteristics; patients should generally have post treatment clinic visit times between 11:00 AM ~ 3:00 PM; a high dose strategy for antihypertensive drug therapy; and setting a lower goal BP. The reduction in CVD risk was predicted to be substantial and these interventions should be entertained when considering changes to standard clinical practice in this area.

APPENDIX A: CODE FOR FIFTH CHAPTER

Generate 1000 sets of population parameters for baseline BP model

```
Dir <- "C:/YuYanJin/code/" # working direction
library(MIfuns) # load library

# parameter values from: Clin Pharmacol Ther, 1998. 64(6):p. 622-35
ThetaMean <-c(151,-0.067,0.029,-8.820)
ThetaCovar <- diag(c(8.8488,0.0001,0.00008,0.02465))
OmegaModeList <- list(diag(c(0.0029,0.184,6.35)),diag(c(56.7,1.5876)))
OmegaDfList <-c(10,40)# parameters are from 10 subjects, 4 occasions per subject, totally around 40
occasions
SigmaModeList <- 0.005
SigmaDfList <- 3000 # number of oberscations available
set.seed(123)
parameters <- CreateParametersForSimulation(nsim=1000,
ThetaMean=ThetaMean,ThetaCovar=ThetaCovar,
OmegaModeList=OmegaModeList,OmegaDfList=OmegaDfList,
SigmaModeList=SigmaModeList,SigmaDfList=SigmaDfList)
write.table(parameters,file=paste(Dir,"p.csv",sep=""),quote=F,sep=",",row.names=F)
```

1.2 Generate PK simulation dataset

```
set.seed(234)
Dir <- "C:/JINYU/"

dose.daily <- 300 # ug
dose.interval <- 24 # QD dosing regimen
ni <- length(id <- 1:3840) # 3840 subjects for simulation
month <- 1  # PK simulation period is one month
time1 <- seq(0,24,by=0.2) # day 30,one data point every twelve mins
tempt1 <- (month*30-1)*24
tempt2 <- month*30*24
time2 <- seq(tempt1,tempt2,by=0.2)# day 30,one data point every twelve mins
time <- c(time1,time2)
TIME <- rep(time,times=ni)
length(time)
ID <- rep(id, each=length(time))
d <- data.frame(ID,TIME)
d$CONC <- rep(0,times=length(d$TIME))
d$AMT <- ifelse(d$TIME==8, dose.daily, 0) # dosing time: 8:00 AM
d$II <- ifelse(d$TIME==8, dose.interval, 0)

addl <- month*30*24/dose.interval-1 # number of additional dose
d$ADDL <- ifelse(d$TIME==8,addl, 0)
d$MDV <- ifelse(d$TIME==8,1,0)
mean.clcr <- 100 # population mean creatinine clearance is 100 ml/min
sd.clcr <- 20 # standard deviation of creatinine clearance 20ml/min
clcr <- rnorm(ni, mean= mean.clcr,sd=sd.clcr)
d$CLCR <- rep(clcr,each=length(time))
d$CLCR <- round(d$CLCR, digits=3)
names(d) <- c("CID","TIME","DV","AMT","II","ADDL","MDV","CLCR")

write.table(d,file=paste(Dir,"pkdata300.csv",sep=""),sep=",",quote=F,col.names=T,row.names=F,na='.')
```

1.3 NONMEM code for simulation of moxonidine concentration

```
$PROBLEM Moxoniding concentration simulation
$DATA pkdata300.csv IGNORE=C
$INPUT ID TIME DV=CONC AMT II ADDL MDV CLCR
```

```
$SUBROUTINE ADVAN6 TOL=5
$MODEL
COMP(DOSE,DEFDOSE)
COMP(CENTRAL,DEFOBS)
COMP(EFFECT)
$PK
  TVCL=THETA(1)*(1+THETA(4)*(CLCR-90))
  CL=TVCL*EXP(ETA(1))
  V2=THETA(2) ;
  KA=THETA(3)*EXP(ETA(2)) ;
  K20=CL/V2  ;
  KE0=THETA(5)*EXP(ETA(3));
  S2=V2;
 $DES
DADT(1) = -KA*A(1)
DADT(2) =  KA*A(1) - K20*A(2)
CP=A(2)/V2
DADT(3) = KE0*(CP-A(3))
$ERROR
CE=A(3);
IPRED=F ;
Y=F+ERR(1)*F ;
$SIM (225 NEW) (012345678 UNIFORM) SUB=1 ONLYSIM
$THETA
(0,35)
(0,132)
(0,2.30)
(0,0.00671)
(0,0.198)
$OMEGA
0.018225
0.9801
1.00
$SIGMA
0 FIX;
$TABLE NOPRINT ONEHEADER FILE=1.tab
ID AMT TIME DV CLCR MDV CE CL V2 KA K20 KE0
```

1.4 R code for figure 2

```
library(MIfuns)
Dir <- "C:/YuYanJin/code/" # working direction

# Calculate percentage of patients with |measured delta BP - true delta BP| >= 10 or 5 mm Hg
percentile <- function (x){
        d1 <- length(x[x>=10])/length(x)
        d2 <- length(x[x<=-10])/length(x)
        d3 <- length(x[x>=5])/length(x)
        d4 <- length(x[x<=-5])/length(x)
        d5 <- d1+d2
        d6 <- d3+d4
        d <-c(d5,d6)
        return (d)
}

## imput 1000 sets of population parameters in baseline BP model
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
## import simulated moxonidine concentration from NONMEM output
pk <- read.table(file=paste(Dir,"1.tab",sep=""),as.is=T,header=T,skip=1)
pk <- pk[,c('ID','TIME','CONC','CE')]

## import 24-h mean SBP values without any treatment from three NDAs accross four study
theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]
```

```
ni <- length(id<-1:length(theta1))     # total number of subjects
month <- 1     # time period of the simulation
sderror <- 5   # standard deviation of Cuff BP measurement error
set.seed(234) # set a seed
seeds <- round(runif(1000, min=1, max=20000)) # generate 1000 seeds for 1000 clinical trials

n <- 1          # sequence of runs
nsim <- 1000       # replicates per run
nsim1 <- (n-1)*nsim+1    # starting replicate (clinical trial) in the specific run
nsim2 <- n*nsim          # ending replicate (clinical trial) in the specific run
resp <- NULL

# k represent kth clinical trial
for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)

## baseline BP model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
## taking kth row from file p.csv as population parameter values in baseline BP model
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3) # variance covariance
matrix for interindividual variability
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)   # generate individual level inter-individual
variability values

mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2) # variance covariance matrix for interoccasion
variability
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) # generate individual level inter-occasion
variability, two occasions per individual (day one and day 30)

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term

eta2 <- iiv[,2]   ### vector: interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### vector: interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### vector: interindividual variability on clock time (hr)

eta.k1d <- iov[,1]  ### vector: interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### vector: interoccasion variability on clock time (hr)

theta2 <- THETA2*(1+eta2) ### generate individual values (n=3840)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3840)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term

temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

for (i in 1:ni){
     # simulate BP cirdadian rhythm for day one
     t <- seq(0,24,by=0.2)
        t.length <- length(t)
                cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                temp.id <- c(temp.id,rep(i,times=t.length))
                temp.time <- c(temp.time,t)
                temp.bsl <- c(temp.bsl,BSL)
```

```
      # simulation BP circadian rhythm for day 30th
      cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data

## merge baseline BP and PK concentration into one file
bpk <- merge ( x=pk, y=data, by.x=c("ID","TIME"), by.y=c("ID","TIME"), all=T)
bpk <- bpk[order(bpk$ID,bpk$TIME),]

### PD parameters from Table IV in "Clin Pharmacol Ther,1998.64(6):p.622-35"
## emax and its interindividual variability
EMAX <- 0.167 # population mean of Emax
ETA.EMAX <- 0.502 # population parameter of inter individual variability in Emax
emax <- signif(EMAX*exp(rnorm(ni,0,ETA.EMAX)),digits=4) # simulate individual values of Emax
## ec50 and its interindividual variability
EC50 <- 0.945
ETA.EC50 <- 1.3
ec50 <- signif(EC50*exp(rnorm(ni,0,ETA.EC50)),digits=4)

## merge individual PD parameter values with simulated PK data and baseline BP

bpk$EMAX <- rep(emax, each=length(bpk$TIME[bpk$ID==1]))
bpk$EC50 <- rep(ec50, each=length(bpk$TIME[bpk$ID==1]))

## simulate one month BP values with moxonidine treatment based on PD model
bpk$BP <- bpk$BSL*(1-bpk$EMAX*bpk$CE/(bpk$EC50+bpk$CE))
bpk$CE <- round(bpk$CE,digits=4)
bpk$BP <- signif(bpk$BP,digits=4)

## add placebo effect ~ N(4,2)
d1 <- bpk
pb <- rnorm(ni,mean=4, sd=2) # simulate placebo effects
d2 <- data.frame(id,pb)
names(d2) <- c("ID","PB")
d <- merge(d1,d2,by=("ID"),all=T)
d <- transform(d,SBP=BP-PB)
d <- d[,c("ID","TIME","BSL","SBP")]
dd1 <- aggregate(list(BSL.median=d$BSL),by=list(TIME=d$TIME),median) # calculate population mean of
Baseline SBP (day one) at kth clinical trial
dd2 <- aggregate(list(SBP.median=d$SBP),by=list(TIME=d$TIME),median) # calculate population mean of
post treatment SBP (day 30th) at kth clinical trial
dd <- merge(dd1,dd2,by=("TIME"),all=T)
dd$REP <- k
resp <- rbind(resp,dd)# add kth clinical trial results with all previous trials results
}

resp <- data.frame(resp)
write.table(resp,
file=paste(Dir,"sbp","_",nsim1,"_",nsim2,"_data2",".csv",sep=""),quote=F,sep=",",row.names=F)
```

1.5 R code for figure 4

```
library(MIfuns)
Dir <- "C:/YuYanJin/code/" # working direction
```

```
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}

## imput 1000 sets of population parameters
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")

## import simulated concentration NONMEM output
pk <- read.table(file=paste(Dir,"1.tab",sep=""),as.is=T,header=T,skip=1)
pk <- pk[,c('ID','TIME','CONC','CE')]

## import SBP baseline values from three NDAs accross four study
theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]

ni <- length(id<-1:length(theta1))     # total number of subjects
month <- 1     # time period of the simulation
sderror <- 5   # standard deviation of Cuff BP measurement error

set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
n <- 1         # sequence of runs
nsim <- 1000       # replicates per run
nsim1 <- (n-1)*nsim+1    # starting replicates in the specific run
nsim2 <- n*nsim          # ending replicates in the specific run
bp.bsl <- NULL
dev <- NULL

for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
# parameter from table III data set 2 in "Clin Pharmacol Ther,1998.64(6):p.622-35"

mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) #one occasion for each individual

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term

eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                    ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                    ### no variability in phase shift in second cosine term

temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

# generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
for (i in 1:ni){
```

```
      t <- seq(0,24,by=0.2)
                  t.length <- length(t)
                  cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                  cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                  BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                  temp.id <- c(temp.id,rep(i,times=t.length))
                  temp.time <- c(temp.time,t)
                  temp.bsl <- c(temp.bsl,BSL)
      cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data

## merge baseline BP and PK concentration into one file
bpk <- merge ( x=pk, y=data, by.x=c("ID","TIME"), by.y=c("ID","TIME"), all=T)
bpk <- bpk[order(bpk$ID,bpk$TIME),]

## PD parameters from Table IV in "Clin Pharmacol Ther,1998.64(6):p.622-35"
## emax and its interindividual variability
EMAX <- 0.167
ETA.EMAX <- 0.502
emax <- signif(EMAX*exp(rnorm(ni,0,ETA.EMAX)),digits=4)
## ec50 and its interindividual variability
EC50 <- 0.945
ETA.EC50 <- 1.3
ec50 <- signif(EC50*exp(rnorm(ni,0,ETA.EC50)),digits=4)
## merge individual PD parameter values with simulated PK data and baseline BP
bpk$EMAX <- rep(emax, each=length(bpk$TIME[bpk$ID==1]))
bpk$EC50 <- rep(ec50, each=length(bpk$TIME[bpk$ID==1]))
## simulate one month BP values with moxonidine treatment from equation 5
bpk$BP <- bpk$BSL*(1-bpk$EMAX*bpk$CE/(bpk$EC50+bpk$CE))
## export PKPD simulation results
bpk$CE <- round(bpk$CE,digits=4)
bpk$BP <- signif(bpk$BP,digits=4)
### add placebo effect ###
pb <- rnorm(ni,mean=4, sd=2)
pb <- data.frame(id,pb)
names(pb) <- c("ID","PB")
bpk.pb <- merge(bpk,pb,by="ID",all=T)
bpk.pb <- transform(bpk.pb, BP.pb=BP-PB)
bpk <- bpk.pb

d <- bpk[, c("ID","TIME","BSL","BP.pb")]
names(d) <- c("ID","TIME","BSL","BP")
temp.table <- NULL
### figure4a

bp1 <- NULL
bsl1 <- NULL
## extract baseline SBP from 8AM to 6PM for each subject
for (m in 1:ni){
  for (n in 8:18){
     temp.bsl1 <- d$BSL[d$ID==m & d$TIME==n]
     temp.bsl2 <- c(n,m,temp.bsl1)
     bsl1 <- rbind(bsl1,temp.bsl2)
}
```

```
}
bsl1 <- data.frame(bsl1)
names(bsl1)<- c("TIME","ID","BSL")
bsl1 <- transform(bsl1,BSL.mean=reapply(BSL,INDEX=TIME,FUN=mean)) # calculate baseline population
mean SBP at each clock time during 8AM~6PM
bsl2 <- bsl1[,c("TIME","BSL.mean")]
bsl2 <- unique(bsl2)

# extract post treatment SBP from 8AM to 6PM for each subject
for (m in 1:ni){
  for (n in 704:714){
      temp.bp1 <- d$BP[d$ID==m & d$TIME==n]
      temp.bp2 <- c(n,m,temp.bp1)
      bp1 <- rbind(bp1,temp.bp2)
}
}
bp1 <- data.frame(bp1)
names(bp1)<- c("TIME","ID","SBP")
bp1 <- transform(bp1,BP.mean=reapply(SBP,INDEX=TIME,FUN=mean),TIME=TIME-696) # calculate post
treatment population mean SBP at each clock time during 8AM~6PM
bp2 <- bp1[,c("TIME","BP.mean")]
bp2 <- unique(bp2)

temp.bp.bsl<- cbind(rep(k,length(bp2$TIME)),merge(bp2,bsl2,by="TIME",all=T))# merge baseline and post
treatment SBP
names(temp.bp.bsl) <- c("REP","TIME","BP.mean","BSL.mean")
bp.bsl <- rbind(bp.bsl,temp.bp.bsl) # row bind kth trial results with all previous trial results

### figure4b

dd <- temp.bp.bsl
temp.dev1 <- NULL
temp.dev2 <- NULL

# calculate SBP decreases from baseline at each clock time
# z represents baseline (before treatment) clinic visit time

for (z in 8:18){
   temp.dev1 <- rep(dd$BSL.mean[dd$TIME==z],length(dd$TIME))- dd$BP.mean
   temp.dev2 <- cbind(temp.dev2,temp.dev1)
}
temp.dev3 <- data.frame(rep(k,length(dd$TIME)),8:18,temp.dev2)

# column "TIME" represents post treatment clinic visit times from 8AM~6PM
# column "BSL8": measured SBP decreases from baseline at various follow up visit time if baseline visit was
at 8AM
# column "BSL18": measured SBP decreases from baseline at various follow up visit time if baseline visit
was at 6PM
names (temp.dev3) <- c("REP","TIME","BSL8","BSL9","BSL10","BSL11","BSL12",
              "BSL13","BSL14","BSL15","BSL16","BSL17","BSL18")
 dev <- rbind(dev,temp.dev3) # conbine kth clinical trial results with all previous trial results
}

bp.bsl <- data.frame(bp.bsl)
dev <- data.frame(dev)
write.table(bp.bsl, file=paste(Dir,"bp.bsl","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(dev,file=paste(Dir,"dev","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

1.6 R code for figure 5

```
## the code is almost same as figure4.R (see denote in figure4.R)
library(MIfuns)
Dir <- "W:/final_sa/aim1/figure5/"
ni <- length(id<-1:3840)
```

```
month <- 1

reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}

p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))

n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim
bp.bsl <- NULL
dev <- NULL
theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]

for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
## model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) #one occasion for each individual

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term

eta1 <- iiv[,1]
eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term

temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

for (i in 1:ni){
       t <- seq(0,24,by=0.2)
                        t.length <- length(t)
                        cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                        cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                        BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                        temp.id <- c(temp.id,rep(i,times=t.length))
                        temp.time <- c(temp.time,t)
                        temp.bsl <- c(temp.bsl,BSL)
     cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
             cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
```

```
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data

sft <- rnorm(ni,mean=11.8, sd=10.9)
sft1 <- data.frame(id,sft)
names(sft1) <- c("ID","RESP")
data.sft <- merge(data,sft1,by="ID",all=T)
data.sft <- transform(data.sft, BP=BSL-RESP)
bpk <- data.sft
bpk <- bpk[,c("ID","TIME","BSL","BP")]
names(bpk) <- c("ID","TIME","BSL","BP")
d <- bpk

bp1 <- NULL
bsl1 <- NULL
for (m in 1:ni){
  for (n in 704:714){
     temp.bp1 <- d$BP[d$ID==m & d$TIME==n]
     temp.bp2 <- c(n,m,temp.bp1)
     bp1 <- rbind(bp1,temp.bp2)
}
}

for (m in 1:ni){
  for (n in 8:18){
     temp.bsl1 <- d$BSL[d$ID==m & d$TIME==n]
     temp.bsl2 <- c(n,m,temp.bsl1)
     bsl1 <- rbind(bsl1,temp.bsl2)
}
}

bp1 <- data.frame(bp1)
bsl1 <- data.frame(bsl1)
names(bp1)<- c("TIME","ID","SBP")
names(bsl1)<- c("TIME","ID","BSL")

bp1 <- transform(bp1,BP.mean=reapply(SBP,INDEX=TIME,FUN=mean),TIME=TIME-696)
bp2 <- bp1[,c("TIME","BP.mean")]
bp2 <- unique(bp2)

bsl1 <- transform(bsl1,BSL.mean=reapply(BSL,INDEX=TIME,FUN=mean))
bsl2 <- bsl1[,c("TIME","BSL.mean")]
bsl2 <- unique(bsl2)

temp.bp.bsl<- cbind(rep(k,length(bp2$TIME)),merge(bp2,bsl2,by="TIME",all=T))
names(temp.bp.bsl) <- c("REP","TIME","BP.mean","BSL.mean")

bp.bsl <- rbind(bp.bsl,temp.bp.bsl)
dd <- temp.bp.bsl

temp.dev1 <- NULL
temp.dev2 <- NULL
for (z in 8:18){
   temp.dev1 <- rep(dd$BSL.mean[dd$TIME==z],length(dd$TIME))- dd$BP.mean
   temp.dev2 <- cbind(temp.dev2,temp.dev1)
}
```

```
temp.dev3 <- data.frame(rep(k,length(dd$TIME)),8:18,temp.dev2)
names (temp.dev3) <- c("REP","TIME","BSL8","BSL9","BSL10","BSL11","BSL12",
            "BSL13","BSL14","BSL15","BSL16","BSL17","BSL18")
dev <- rbind(dev,temp.dev3)
}

bp.bsl <- data.frame(bp.bsl)
dev <- data.frame(dev)
write.table(bp.bsl, file=paste(Dir,"bp.bsl","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(dev,file=paste(Dir,"dev","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

1.7 R code for table 2 and table 3

```
library(MIfuns)
Dir <- "C:/YuYanJin/code/" # working direction
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
# Calculate percentage of patients with |measured delta BP - true delta BP| >= 10 or 5 mm Hg
percentile <- function (x){
         d1 <- length(x[x>=10])/length(x)
         d2 <- length(x[x<=-10])/length(x)
         d3 <- length(x[x>=5])/length(x)
         d4 <- length(x[x<=-5])/length(x)
         d5 <- d1+d2
         d6 <- d3+d4
         d <-c(d5,d6)
         return (d)
}

# imput 1000 sets of population parameters
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
# import simulated concentration from NONMEM output
pk <- read.table(file=paste(Dir,"1.tab",sep=""),as.is=T,header=T,skip=1)
pk <- pk[,c('ID','TIME','CONC','CE')]
# import SBP baseline values from three NDAs accross four study
theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]
ni <- length(id<-1:length(theta1))     # total number of subjects
month <- 1     # time period of the simulation
sderror <- 5   # standard deviation of Cuff BP measurement error
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))

n <- 1          # sequence of runs
nsim <- 1000       # replicates per run
nsim1 <- (n-1)*nsim+1    # starting replicates in the specific run
nsim2 <- n*nsim          # ending replicates in the specific run

table2 <- NULL
table3 <- NULL

# kth replicate of clinical trial
for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
## model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
```

```
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)   # inter-individual variability
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) # inter-occasion variability two occasion (day
one and day 30) for each individual

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term

eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term

temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

for (i in 1:ni){
     t <- seq(0,24,by=0.2)
                    t.length <- length(t)
                    cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                    cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                    BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                    temp.id <- c(temp.id,rep(i,times=t.length))
                    temp.time <- c(temp.time,t)
                    temp.bsl <- c(temp.bsl,BSL)
     cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
          cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
          BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
          t.new <- (occ-1)*24 + t
          temp.id <- c(temp.id,rep(i,times=t.length))
          temp.time <- c(temp.time,t.new)
          temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data
# merge baseline BP and PK concentration into one file
bpk <- merge ( x=pk, y=data, by.x=c("ID","TIME"), by.y=c("ID","TIME"), all=T)
bpk <- bpk[order(bpk$ID,bpk$TIME),]

### PD parameters from Table IV in "Clin Pharmacol Ther,1998.64(6):p.622-35"
## emax and its interindividual variability
EMAX <- 0.167
ETA.EMAX <- 0.502
emax <- signif(EMAX*exp(rnorm(ni,0,ETA.EMAX)),digits=4)
## ec50 and its interindividual variability
EC50 <- 0.945
ETA.EC50 <- 1.3
ec50 <- signif(EC50*exp(rnorm(ni,0,ETA.EC50)),digits=4)
## merge individual PD parameter values with simulated PK data and baseline BP
bpk$EMAX <- rep(emax, each=length(bpk$TIME[bpk$ID==1]))
bpk$EC50 <- rep(ec50, each=length(bpk$TIME[bpk$ID==1]))
## simulate one month BP values with moxonidine treatment from equation 5
bpk$BP <- bpk$BSL*(1-bpk$EMAX*bpk$CE/(bpk$EC50+bpk$CE))
bpk$CE <- round(bpk$CE,digits=4)
```

```
bpk$BP <- signif(bpk$BP,digits=4)
### add placebo effect ~ N(4,2)
pb <- rnorm(ni,mean=4, sd=2)
pb <- data.frame(id,pb)
names(pb) <- c("ID","PB")
bpk.pb <- merge(bpk,pb,by="ID",all=T)
bpk.pb <- transform(bpk.pb, BP.pb=BP-PB)
bpk <- bpk.pb
bpk <- bpk[,c("ID","TIME","BSL","BP.pb")]
names(bpk) <- c("ID","TIME","BSL","BP")
### calculate office hour mean SBP at baseline
d <- bpk
d1 <- d[d$TIME>=8,]
dd <- d1[d1$TIME<=18,]
dd <- transform(dd,bsl=reapply(BSL,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","bsl")]
dd <- unique(dd)
dd1 <- dd
### calculate office hour mean SBP at day30 BP
d <- bpk
d1 <- d[d$TIME>=704,]
dd <- d1[d1$TIME<=714,]
dd <- transform(dd,mbp=reapply(BP,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","mbp")]
dd <- unique(dd)
dd2 <- dd
## true BP change with drug effect ###
d <- merge(dd1,dd2,by="ID",all=T)
d <- transform(d, delta=bsl-mbp)
d <- d[order(d$ID),]

### calculate cuff measured BP changes from baseline at random clinic visit time ##
# generate random clinic visit times
visittime1 <- round(runif(ni, min=8, max=18)) # random clinic visit time at baseline
visittime2 <- round(runif(ni, min=704, max=714)) # first random clinic visit time on day 30th
visittime3 <- round(runif(ni, min=704, max=714)) # second random clinic visit time on day 30th

# extract true BP at the clinic visit time based on PKPD simulation results
temp.true1 <- NULL
temp.true2 <- NULL
temp.true3 <- NULL

for (m in 1:ni){
        true1 <- bpk$BSL[bpk$ID==m & bpk$TIME==visittime1[m]]
        temp.true1 <- c(temp.true1,true1)
        true2 <- bpk$BP[bpk$ID==m & bpk$TIME==visittime2[m]]
        temp.true2 <- c(temp.true2,true2)
    true3 <- bpk$BP[bpk$ID==m & bpk$TIME==visittime3[m]]
        temp.true3 <- c(temp.true3,true3)
}

true.obs <- data.frame(1:ni,temp.true1,temp.true2,temp.true3)
names(true.obs) <- c("ID","truebsl","truebp1","truebp2")
# generate cuff measurement error ~ N(0,5)
true.obs$errorBP1 <- rnorm(ni,sd=sderror)
true.obs$errorBP2 <- rnorm(ni,sd=sderror)
true.obs$errorBP3 <- rnorm(ni,sd=sderror)
# generate observed cuff BP at clinic visit times
true.obs <- transform(true.obs,
cuffbsl=truebsl+errorBP1,
cuffbp1=truebp1+errorBP2,
cuffbp2=truebp2+errorBP3)
## calculate cuff measured BP decrease from baseline
true.obs <- transform(true.obs,cuff5delt=cuffbsl-cuffbp1,cuff0delt=truebsl-truebp1)
```

```
## merge measured BP decrease with true BP decrease from baseline ##
bp <- merge(true.obs,d,by="ID",all=T)
bp <- transform(bp,dev5=cuff5delt-delta,dev0=cuff0delt-delta,dev12=cuffbp1-cuffbp2)
## percent of patients with deviation greater than 5 mm Hg or 10 mm Hg
temp.table2 <- c(k,mean(bp$delta),sd(bp$delta),mean(bp$cuff0delt),sd(bp$cuff0delt),
          mean(bp$cuff5delt),sd(bp$cuff5delt),percentile(bp$dev0),percentile(bp$dev5),percentile(bp$dev12))
table2 <- rbind(table2,temp.table2)

## TABLE 3

# calculate cuff measured BP changes from baseline at matched clinic visit times for both baseline and after
30 days treatment ##

for (q in 8:18)
{ spetime <- 24*29+q
  temp.true<- NULL
  true.obs <- NULL
  temp.bsl <- NULL
  for (j in 1:ni){
            true <- bpk$BP[bpk$ID==j & bpk$TIME==spetime]
            temp.true <- c(temp.true,true)
     bsl <- bpk$BSL[bpk$ID==j & bpk$TIME==q]
     temp.bsl <- c(temp.bsl,bsl)
     }
  true.obs <- data.frame(1:ni,temp.true,temp.bsl)
  names(true.obs) <- c("ID","true","bsl")
  true.obs$errorBP1 <- rnorm(ni,sd=sderror)## generate cuff measurement error with SD
  true.obs$errorBP2 <- rnorm(ni,sd=sderror)## generate cuff measurement error with SD

  true.obs <- transform(true.obs,cuffbsl=bsl+errorBP1,cuffbp=true+errorBP2) ## generate observed cuff BP
at clinic visit times

  true.obs <- transform(true.obs,cuff0delt=bsl-true, cuff5delt=cuffbsl-cuffbp)
  true.obs$time <- q
  dd <- merge(true.obs,d,by="ID",all=T)
  dd <- dd[,c("ID","time","delta","cuff0delt","cuff5delt")]
  dd <- transform(dd,dev0=cuff0delt-delta,dev5=cuff5delt-delta,ddev=cuff5delt-cuff0delt)
r <-
c(k,q,mean(dd$delta),sd(dd$delta),mean(dd$cuff0delt),sd(dd$cuff0delt),mean(dd$cuff5delt),sd(dd$cuff5delt)
,percentile(dd$dev0),percentile(dd$dev5),percentile(dd$ddev))
table3 <- rbind(table3,r)
}
}
write.table(table2, file=paste(Dir,"table2","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(table3, file=paste(Dir,"table3","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

APPENDIX B: CODE FOR SIXTH CHAPTER

2.1 R code for figures (type I antihypertensive agents: moxonidine)

```
library(MIfuns)
Dir <- "C:/yuyan/code/"
ni <- length(id<-1:3840)
month <- 1

#functions
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
# write a function which will divide continous BP into four different BP groups based on JNC guidline
JNC_BP <- function(x) {
          y<- ifelse(x<120 & x>=0,1,
               ifelse(x<140&x>=120,2,
                 ifelse(x<160 & x>=140,3,4)))
          return(y)
        }
# round observed BP to nearest 5 or 10
nround5 <- function(x) {
          temp <- round(x,0)
          unit <- temp/10
          temp1 <- floor(unit)*10+5
          temp1[(unit-floor(unit)) <=0.2] <- floor(unit[(unit-floor(unit))<=0.2])*10
          temp1[(unit-floor(unit)) >=0.8] <- ceiling(unit[(unit-floor(unit))>=0.8])*10
          return(temp1)
}
# Calculate % of patients whose difference in measured BP and true BP were greater than 10 or 5 mm Hg
percentile <- function (x){
          d1 <- length(x[x>=10])/length(x)
          d2 <- length(x[x<=-10])/length(x)
          d3 <- length(x[x>=5])/length(x)
          d4 <- length(x[x<=-5])/length(x)
          d5 <- d1+d2
          d6 <- d3+d4
          d <-c(d5,d6)
          return (d)
}

p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
pk <- read.table(file=paste(Dir,"1.tab",sep=""),as.is=T,header=T,skip=1)
pk <- pk[,c('ID','TIME','CONC','CE')]
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim
fig1 <- NULL
fig2 <- NULL

theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]

for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <- 5
```

```
## parameter from table III data set 2 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = 2*ni, mu2, Omega2, empirical = FALSE) #one occasion for each individual

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term

eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3              ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5              ### no variability in phase shift in second cosine term

temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

# generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
for (i in 1:ni){
      t <- seq(0,24,by=0.2)
                        t.length <- length(t)
                        cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                        cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                        BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                        temp.id <- c(temp.id,rep(i,times=t.length))
                        temp.time <- c(temp.time,t)
                        temp.bsl <- c(temp.bsl,BSL)
      cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data

## merge baseline BP and PK concentration into one file
bpk <- merge ( x=pk, y=data, by.x=c("ID","TIME"), by.y=c("ID","TIME"), all=T)
bpk <- bpk[order(bpk$ID,bpk$TIME),]
## PD parameters from Table IV in "Clin Pharmacol Ther,1998.64(6):p.622-35"
## emax and its interindividual variability
EMAX <- 0.167
ETA.EMAX <- 0.502
emax <- signif(EMAX*exp(rnorm(ni,0,ETA.EMAX)),digits=4)

## ec50 and its interindividual variability
EC50 <- 0.945
ETA.EC50 <- 1.3
ec50 <- signif(EC50*exp(rnorm(ni,0,ETA.EC50)),digits=4)
```

```
## merge individual PD parameter values with simulated PK data and baseline BP
bpk$EMAX <- rep(emax, each=length(bpk$TIME[bpk$ID==1]))
bpk$EC50 <- rep(ec50, each=length(bpk$TIME[bpk$ID==1]))
## simulate one month BP values with moxonidine treatment from equation 5
bpk$BP <- bpk$BSL*(1-bpk$EMAX*bpk$CE/(bpk$EC50+bpk$CE))
## export PKPD simulation results
bpk$CE <- round(bpk$CE,digits=4)
bpk$BP <- signif(bpk$BP,digits=4)
## add placebo effect ###
pb <- rnorm(ni,mean=4, sd=2)
pb <- data.frame(id,pb)
names(pb) <- c("ID","PB")
bpk.pb <- merge(bpk,pb,by="ID",all=T)
bpk.pb <- transform(bpk.pb, BP.pb=BP-PB)
bpk <- bpk.pb
bpk <- bpk[,c("ID","TIME","BSL","BP.pb")]
names(bpk) <- c("ID","TIME","BSL","BP")

## calculate office hour mean at day0 baseline
d <- bpk
d1 <- d[d$TIME>=8,]
d2 <- d1[d1$TIME<=18,]
dd <- d2
dd <- transform(dd,bsl=reapply(BSL,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","bsl")]
dd <- unique(dd)
dd1 <- dd

### calculate office hour mean at day30 BP
d <- bpk
d1 <- d[d$TIME>=704,]
d2 <- d1[d1$TIME<=714,]
dd <- d2
dd <- transform(dd,mbp=reapply(BP,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","mbp")]
dd <- unique(dd)
dd2 <- dd

## true BP change with drug effect
d <- merge(dd1,dd2,by="ID",all=T)
d <- transform(d, delta=bsl-mbp)
d <- d[order(d$ID),]

## precise clinic visit time to each clock time/ one measurement per visit

## generate two random clinic visits for each patients on day 30th ###
for (q in 8:18){
visittime <- 24*29+q

## extract true BPs at the clinic visits from PKPD simulation results
temp.true <- NULL
for (i in 1:ni){
        true <- bpk$BP[bpk$ID==i & bpk$TIME==visittime]
        temp.true <- c(temp.true,true)
}

## generate a data frame with information of ID, visit time, true BP
true.obs <- data.frame(1:ni,visittime, temp.true)
names(true.obs) <- c("ID","VISITTIME","TRU")
## generate a data frame with information of ID, visit time, true BP ##
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,d,by="ID",all=T)
names(true.obs) <- c("ID","VISITTIME","TRU","BSL","TBP","DEL")
```

```
true.obs$errorBP<- rnorm(ni,sd=sderror)
true.obs <- transform(true.obs,OBS1=TRU+errorBP,OBS2=nround5(TRU+errorBP))
true.obs <- transform( true.obs, T=JNC_BP(TBP),B1=JNC_BP(OBS1),B2=JNC_BP(OBS2))

## compare consistancy of true BPs and "observed" BPs at two visits ##
true.obs$CONSIS1 <- ifelse(true.obs$T==true.obs$B1,0,1)
true.obs$CONSIS2 <- ifelse(true.obs$T==true.obs$B2,0,1)
## calculate the inconsistant decision making between two visits ###
P1 <- length(true.obs$CONSIS1[true.obs$CONSIS1==1])/length(true.obs$CONSIS1)
P2 <- length(true.obs$CONSIS2[true.obs$CONSIS2==1])/length(true.obs$CONSIS2)
## conbine results into one file ##
P<- c(k,q,P1,P2)
fig1 <- rbind(fig1,P)
}

# precise clinic visit time to each clock time/ two measurements per visit
for (q in 8:18){
visittime <- rep(24*29+q,ni)
# generate random clinic visit time on day 30th between 8AM-6PM ##
# visittime <- runif(ni, min=24*29+8, max=24*29+18)
ms1 <- visittime
ms2 <- ms1 + 0.2 # measure #2 occurs 12 mins after measure 1

# extract true BP at the clinic visit time based on PKPD simulation results
temp.true1 <- NULL
temp.true2 <- NULL
for (i in 1:ni){
        true1 <- bpk$BP[bpk$ID==i & bpk$TIME==ms1[i]]
        temp.true1 <- c(temp.true1,true1)
        true2 <- bpk$BP[bpk$ID==i & bpk$TIME==ms2[i]]
        temp.true2 <- c(temp.true2,true2)
}
true.obs1 <- data.frame(1:ni,ms1,temp.true1)
true.obs2 <- data.frame(1:ni,ms2,temp.true2)
names(true.obs1) <- c("ID","TIME","TRU")
names(true.obs2) <- c("ID","TIME","TRU")
true.obs <- rbind(true.obs1,true.obs2)
true.obs <- true.obs[order(true.obs$ID,true.obs$TIME),]

## generate cuff measurement error with various SD
true.obs$errorBP <- rnorm(2*ni,sd=sderror)
## generate observed cuff BP at clinic visit times
true.obs <- transform(true.obs,OBS1=TRU+errorBP,OBS2=TRU+errorBP)

true.obs <- transform(
    true.obs,
    avebp1=reapply(OBS1,INDEX=ID,FUN=mean),
    avebp2=reapply(OBS2,INDEX=ID,FUN=mean)
)

true.obs <- true.obs[,c("ID","avebp1","avebp2")]
true.obs <- unique(true.obs)
true.obs <- merge(true.obs,d,by="ID",all=T)
names(true.obs) <- c("ID","OBS1","OBS2","BSL","TBP","DEL")
true.obs <- transform(true.obs,B1=JNC_BP(OBS1),B2=JNC_BP(OBS2),T=JNC_BP(TBP))

## compare "observed" BP with "true" BP at various measurement error

true.obs$CONSIS1 <- ifelse(true.obs$B1==true.obs$T,0,1)
true.obs$CONSIS2 <- ifelse(true.obs$B2==true.obs$T,0,1)
### calculate the percent of patients with BP misclassification
P1 <- length(true.obs$CONSIS1[true.obs$CONSIS1==1])/length(true.obs$CONSIS1)
P2 <- length(true.obs$CONSIS2[true.obs$CONSIS2==1])/length(true.obs$CONSIS2)
```

```
P <- c(k,q,P1,P2)
fig2 <- rbind(fig2,P)
}
}

fig1 <- data.frame(fig1)
fig2 <- data.frame(fig2)
write.table(fig1,
file=paste(Dir,"aim22_one","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(fig2,
file=paste(Dir,"aim22_two","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

2.2 R code for tables (type I antihypertensive agents: moxonidine)

```
library(MIfuns)
Dir <- "C:/Yuyan/code/"

ni <- length(id<-1:3840)
month <- 1

# functions
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
## write a function which will divide continous BP into four different BP groups based on JNC guidline
JNC_BP <- function(x) {
          y<- ifelse(x<120 & x>=0,1,
               ifelse(x<140&x>=120,2,
                 ifelse(x<160 & x>=140,3,4)))
          return(y)
        }
# round observed BP to nearest 5 or 10
nround5 <- function(x) {
          temp <- round(x,0)
          unit <- temp/10
          temp1 <- floor(unit)*10+5
          temp1[(unit-floor(unit)) <=0.2] <- floor(unit[(unit-floor(unit))<=0.2])*10
          temp1[(unit-floor(unit)) >=0.8] <- ceiling(unit[(unit-floor(unit))>=0.8])*10
          return(temp1)
}
# Calculate percentage of patients whose difference in measured BP and true BP were greater than 10 or 5
mm Hg
percentile <- function (x){
          d1 <- length(x[x>=10])/length(x)
          d2 <- length(x[x<=-10])/length(x)
          d3 <- length(x[x>=5])/length(x)
          d4 <- length(x[x<=-5])/length(x)
          d5 <- d1+d2
          d6 <- d3+d4
          d <-c(d5,d6)
          return (d)
}

p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")

## import simulation results from NONMEM output
pk <- read.table(file=paste(Dir,"1.tab",sep=""),as.is=T,header=T,skip=1)
pk <- pk[,c('ID','TIME','CONC','CE')]

set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
n <- 1
```

```
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim

table1 <- NULL
table2 <- NULL

theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]

for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <- 5

# parameter from table III data set 2 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = 2*ni, mu2, Omega2, empirical = FALSE) #one occasion for each individual

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term
eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term
temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

# generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
for (i in 1:ni){
      t <- seq(0,24,by=0.2)
                  t.length <- length(t)
                  cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                  cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                  BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                  temp.id <- c(temp.id,rep(i,times=t.length))
                  temp.time <- c(temp.time,t)
                  temp.bsl <- c(temp.bsl,BSL)
     cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
          cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
          BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
          t.new <- (occ-1)*24 + t
          temp.id <- c(temp.id,rep(i,times=t.length))
          temp.time <- c(temp.time,t.new)
          temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data
```

```
## merge baseline BP and PK concentration into one file
bpk <- merge ( x=pk, y=data, by.x=c("ID","TIME"), by.y=c("ID","TIME"), all=T)
bpk <- bpk[order(bpk$ID,bpk$TIME),]
## PD parameters from Table IV in "Clin Pharmacol Ther,1998.64(6):p.622-35"
## emax and its interindividual variability
EMAX <- 0.167
ETA.EMAX <- 0.502
emax <- signif(EMAX*exp(rnorm(ni,0,ETA.EMAX)),digits=4)
## ec50 and its interindividual variability
EC50 <- 0.945
ETA.EC50 <- 1.3
ec50 <- signif(EC50*exp(rnorm(ni,0,ETA.EC50)),digits=4)

## merge individual PD parameter values with simulated PK data and baseline BP
bpk$EMAX <- rep(emax, each=length(bpk$TIME[bpk$ID==1]))
bpk$EC50 <- rep(ec50, each=length(bpk$TIME[bpk$ID==1]))
## simulate one month BP values with moxonidine treatment from equation 5
bpk$BP <- bpk$BSL*(1-bpk$EMAX*bpk$CE/(bpk$EC50+bpk$CE))
## export PKPD simulation results
bpk$CE <- round(bpk$CE,digits=4)
bpk$BP <- signif(bpk$BP,digits=4)

## add placebo effect ###
pb <- rnorm(ni,mean=4, sd=2)
pb <- data.frame(id,pb)
names(pb) <- c("ID","PB")
bpk.pb <- merge(bpk,pb,by="ID",all=T)
bpk.pb <- transform(bpk.pb, BP.pb=BP-PB)
bpk <- bpk.pb
bpk <- bpk[,c("ID","TIME","BSL","BP.pb")]
names(bpk) <- c("ID","TIME","BSL","BP")

## calculate office hour mean at day0 baseline
d <- bpk
d1 <- d[d$TIME>=8,]
dd <- d1[d1$TIME<=18,]
dd <- transform(dd,bsl=reapply(BSL,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","bsl")]
dd <- unique(dd)
dd1 <- dd

## calculate office hour mean at day30 BP
d <- bpk
d1 <- d[d$TIME>=704,]
dd <- d1[d1$TIME<=714,]
dd <- transform(dd,mbp=reapply(BP,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","mbp")]
dd <- unique(dd)
dd2 <- dd

## true BP change with drug effect
d <- merge(dd1,dd2,by="ID",all=T)
d <- transform(d, delta=bsl-mbp)
d <- d[order(d$ID),]

## generate two random clinic visits for each patients on day 30th
visittime1 <- runif(ni, min=24*29+8, max=24*29+18)
visittime1 <- round(visittime1)
visittime2 <- runif(ni, min=24*29+8, max=24*29+18)
visittime2 <- round(visittime2)

## extract true BPs at the clinic visits from PKPD simulation results
temp.true1 <- NULL
for (i in 1:ni){
```

```
        true <- bpk$BP[bpk$ID==i & bpk$TIME==visittime1[i]]
        temp.true1 <- c(temp.true1,true)
}
temp.true2 <- NULL
for (i in 1:ni){
        true <- bpk$BP[bpk$ID==i & bpk$TIME==visittime2[i]]
        temp.true2 <- c(temp.true2,true)
}

## generate a data frame with information of ID, visit time, true BP
true.obs <- data.frame(1:ni,visittime1, temp.true1, visittime2, temp.true2)
names(true.obs) <- c("ID","VISITTIME1","TRU1","VISITTIME2","TRU2")
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,d,by="ID",all=T)
names(true.obs) <- c("ID","VISITTIME1","TRU1","VISITTIME2","TRU2","BSL","TBP","DEL")
## simulate measurement error for each individual visit assuming sd of measure error =5
true.obs$errorBP1 <- rnorm(ni,sd=sderror)
true.obs$errorBP2 <- rnorm(ni,sd=sderror)
true.obs <-
transform(true.obs,OBS1=TRU1+errorBP1,OBS2=TRU2+errorBP2,OBS3=nround5(TRU1+errorBP1),OBS4
=nround5(TRU2+errorBP2))

## divide observed BP and "true" BP at clinic visits into BP groups
true.obs <- transform(
true.obs,T=JNC_BP(TBP),B1=JNC_BP(OBS1),B2=JNC_BP(OBS2),B3=JNC_BP(OBS3),B4=JNC_BP(OBS
4))

## compare consistancy of true BPs and "observed" BPs at two visits
true.obs$CONSIS1 <- ifelse(true.obs$T==true.obs$B1,0,1)
true.obs$CONSIS2 <- ifelse(true.obs$T==true.obs$B3,0,1)
true.obs$CONSIS3 <- ifelse(true.obs$B1==true.obs$B2,0,1)
true.obs$CONSIS4 <- ifelse(true.obs$B3==true.obs$B4,0,1)

## calculate the BP misclassification rate
P1 <- length(true.obs$CONSIS1[true.obs$CONSIS1==1])/length(true.obs$CONSIS1)
P2 <- length(true.obs$CONSIS2[true.obs$CONSIS2==1])/length(true.obs$CONSIS2)
P3 <- length(true.obs$CONSIS3[true.obs$CONSIS3==1])/length(true.obs$CONSIS3)
P4 <- length(true.obs$CONSIS4[true.obs$CONSIS4==1])/length(true.obs$CONSIS4)
P <- c(k,P1,P2,P3,P4)
table1 <- rbind(table1,P)

## generate random clinic visit time on day 30th between 8AM-6PM
visittime <- runif(ni, min=24*29+8, max=24*29+18)
ms1 <- round(visittime)
ms2 <- ms1 + 0.2 # measure #2 occurs 12 mins after measure 1

## extract true BP at the clinic visit time based on PKPD simulation results
temp.true1 <- NULL
temp.true2 <- NULL

for (i in 1:ni){
        true1 <- bpk$BP[bpk$ID==i & bpk$TIME==ms1[i]]
        temp.true1 <- c(temp.true1,true1)
        true2 <- bpk$BP[bpk$ID==i & bpk$TIME==ms2[i]]
        temp.true2 <- c(temp.true2,true2)
}

true.obs1 <- data.frame(1:ni,ms1,temp.true1)
true.obs2 <- data.frame(1:ni,ms2,temp.true2)
names(true.obs1) <- c("ID","TIME","TRU")
names(true.obs2) <- c("ID","TIME","TRU")
true.obs <- rbind(true.obs1,true.obs2)
true.obs <- true.obs[order(true.obs$ID,true.obs$TIME),]
```

```
## generate cuff measurement error with various SD ###
true.obs$errorBP <- rnorm(2*ni,mean=0,sd=sderror)

## generate observed cuff BP at clinic visit times
true.obs <- transform(true.obs,OBS1=TRU+errorBP,OBS2=nround5(TRU+errorBP))
true.obs <- transform(
    true.obs,
    avebp1=reapply(OBS1,INDEX=ID,FUN=mean),
    avebp2=reapply(OBS2,INDEX=ID,FUN=mean)
)

## divide observed BP and "true" BP at clinic visit into BP groups    ###
true.obs <- true.obs[,c("ID","avebp1","avebp2")]
true.obs <- unique(true.obs)
true.obs <- merge(true.obs,d,by="ID",all=T)
names(true.obs) <- c("ID","OBS1","OBS2","BSL","TBP","DEL")
true.obs <- transform(true.obs,B1=JNC_BP(OBS1),B2=JNC_BP(OBS2),T=JNC_BP(TBP))
## compare "observed" BP with "true" BP at various measurement error
true.obs$CONSIS1 <- ifelse(true.obs$B1==true.obs$T,0,1)
true.obs$CONSIS2 <- ifelse(true.obs$B2==true.obs$T,0,1)
## calculate the percent of patients with BP misclassification
P1 <- length(true.obs$CONSIS1[true.obs$CONSIS1==1])/length(true.obs$CONSIS1)
P2 <- length(true.obs$CONSIS2[true.obs$CONSIS2==1])/length(true.obs$CONSIS2)
P <- c(k,P1,P2)
table2 <- rbind(table2,P)
}
table1 <- data.frame(table1)
table2 <- data.frame(table2)
write.table(table1,
file=paste(Dir,"aim21_one","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(table2,
file=paste(Dir,"aim21_two","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

2.3 R code for both figures and tables (type II antihypertensive agents)

```
library(MIfuns)
Dir <- "Z:/yuyan/aim2_CI/"
ni <- length(id<-1:3840)
month <- 1

# functions
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}

# write a function which will divide continous BP into four different BP groups based on JNC guidline
JNC_BP <- function(x) {
          y<- ifelse(x<120 & x>=0,1,
               ifelse(x<140&x>=120,2,
                 ifelse(x<160 & x>=140,3,4)))
          return(y)
        }
# round observed BP to nearest 5 or 10
nround5 <- function(x) {
          temp <- round(x,0)
          unit <- temp/10
          temp1 <- floor(unit)*10+5
          temp1[(unit-floor(unit)) <=0.2] <- floor(unit[(unit-floor(unit))<=0.2])*10
          temp1[(unit-floor(unit)) >=0.8] <- ceiling(unit[(unit-floor(unit))>=0.8])*10
          return(temp1)
}
```

```
# Calculate percentage of patients whose difference in measured BP and true BP were greater than 10 or 5
mm Hg
percentile <- function (x){
        d1 <- length(x[x>=10])/length(x)
        d2 <- length(x[x<=-10])/length(x)
        d3 <- length(x[x>=5])/length(x)
        d4 <- length(x[x<=-5])/length(x)
        d5 <- d1+d2
        d6 <- d3+d4
        d <-c(d5,d6)
        return (d)
}
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))

n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim
table1 <- NULL
fig1 <- NULL
theta1 <- read.table(file=paste(Dir,"m24sbp.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
theta1 <- theta1[,c("SBP")]

for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <-5

## parameter from table III data set 2 in "Clin Pharmacol Ther,1998.64(6):p.622-35"

mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = 2*ni, mu2, Omega2, empirical = FALSE) #one occasion for each individual

THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term

eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term

temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

### generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
for (i in 1:ni){
     t <- seq(0,24,by=0.2)
                  t.length <- length(t)
                  cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
```

```
                cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                temp.id <- c(temp.id,rep(i,times=t.length))
                temp.time <- c(temp.time,t)
                temp.bsl <- c(temp.bsl,BSL)
        cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}

temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data

## assuming drug response is normally distributed ~ N(11.8,10.9)
sft <- rnorm(ni,mean=11.8, sd=10.9)
sft1 <- data.frame(id,sft)
names(sft1) <- c("ID","RESP")
data.sft <- merge(data,sft1,by="ID",all=T)
data.sft <- transform(data.sft, BP=BSL-RESP)
bpk <- data.sft
bpk <- bpk[,c("ID","TIME","BSL","BP")]
names(bpk) <- c("ID","TIME","BSL","BP")

## calculate office hour mean at day0 baseline
d <- bpk
d1 <- d[d$TIME>=8,]
d2 <- d1[d1$TIME<=18,]
dd <- d2
dd <- transform(dd,bsl=reapply(BSL,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","bsl")]
dd <- unique(dd)
dd1 <- dd

## calculate office hour mean at day30 BP
d <- bpk
d1 <- d[d$TIME>=704,]
d2 <- d1[d1$TIME<=714,]
dd <- d2
dd <- transform(dd,mbp=reapply(BP,INDEX=ID,FUN=mean))
dd <- dd[,c("ID","mbp")]
dd <- unique(dd)
dd2 <- dd

## true BP change with drug effect
d <- merge(dd1,dd2,by="ID",all=T)
d <- transform(d, delta=bsl-mbp)
d <- d[order(d$ID),]

## generate two random clinic visits for each patients on day 30th
visittime1 <- runif(ni, min=24*29+8, max=24*29+18)
visittime1 <- round(visittime1)
visittime2 <- runif(ni, min=24*29+8, max=24*29+18)
visittime2 <- round(visittime2)

## extract true BPs at the clinic visits from PKPD simulation results
temp.true1 <- NULL
for (i in 1:ni){
        true <- bpk$BP[bpk$ID==i & bpk$TIME==visittime1[i]]
```

```
        temp.true1 <- c(temp.true1,true)
}
temp.true2 <- NULL
for (i in 1:ni){
        true <- bpk$BP[bpk$ID==i & bpk$TIME==visittime2[i]]
        temp.true2 <- c(temp.true2,true)
}

## generate a data frame with information of ID, visit time, true BP
true.obs <- data.frame(1:ni,visittime1, temp.true1, visittime2, temp.true2)
names(true.obs) <- c("ID","VISITTIME1","TRU1","VISITTIME2","TRU2")
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,d,by="ID",all=T)
names(true.obs) <- c("ID","VISITTIME1","TRU1","VISITTIME2","TRU2","BSL","TBP","DEL")

## simulate measurement error for each individual visit assuming sd of measure error =5
true.obs$errorBP1 <- rnorm(ni,sd=sderror)
true.obs$errorBP2 <- rnorm(ni,sd=sderror)
true.obs <-
transform(true.obs,OBS1=TRU1+errorBP1,OBS2=TRU2+errorBP2,OBS3=nround5(TRU1+errorBP1),OBS4
=nround5(TRU2+errorBP2))
## divide observed BP and "true" BP at clinic visits into BP groups
true.obs <- transform(
true.obs,T=JNC_BP(TBP),B1=JNC_BP(OBS1),B2=JNC_BP(OBS2),B3=JNC_BP(OBS3),B4=JNC_BP(OBS
4))

## compare consistancy of true BPs and "observed" BPs at two visits
true.obs$CONSIS1 <- ifelse(true.obs$T==true.obs$B1,0,1)
true.obs$CONSIS2 <- ifelse(true.obs$T==true.obs$B3,0,1)
true.obs$CONSIS3 <- ifelse(true.obs$B1==true.obs$B2,0,1)
true.obs$CONSIS4 <- ifelse(true.obs$B3==true.obs$B4,0,1)

## calculate the BP misclassification rate
P1 <- length(true.obs$CONSIS1[true.obs$CONSIS1==1])/length(true.obs$CONSIS1)
P2 <- length(true.obs$CONSIS2[true.obs$CONSIS2==1])/length(true.obs$CONSIS2)
P3 <- length(true.obs$CONSIS3[true.obs$CONSIS3==1])/length(true.obs$CONSIS3)
P4 <- length(true.obs$CONSIS4[true.obs$CONSIS4==1])/length(true.obs$CONSIS4)
P <- c(k,P1,P2,P3,P4)
table1 <- rbind(table1,P)

for (q in 8:18){
visittime <- 24*29+q
# extract true BPs at the clinic visits from PKPD simulation results
temp.true <- NULL
for (i in 1:ni){
        true <- bpk$BP[bpk$ID==i & bpk$TIME==visittime]
        temp.true <- c(temp.true,true)
}

## generate a data frame with information of ID, visit time, true BP
true.obs <- data.frame(1:ni,visittime, temp.true)
names(true.obs) <- c("ID","VISITTIME","TRU")

## generate a data frame with information of ID, visit time, true BP
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,d,by="ID",all=T)
names(true.obs) <- c("ID","VISITTIME","TRU","BSL","TBP","DEL")

## simulate measurement error for each individual visit assuming sd of measure error =5
sderror <- 5
true.obs$errorBP<- rnorm(ni,sd=sderror)
true.obs <- transform(true.obs,OBS1=TRU+errorBP,OBS2=nround5(TRU+errorBP))

## divide observed BP and "true" BP at clinic visits into BP groups
```

```
true.obs <- transform( true.obs, T=JNC_BP(TBP),B1=JNC_BP(OBS1),B2=JNC_BP(OBS2))

## compare consistancy of true BPs and "observed" BPs
true.obs$CONSIS1 <- ifelse(true.obs$T==true.obs$B1,0,1)
true.obs$CONSIS2 <- ifelse(true.obs$T==true.obs$B2,0,1)

## calculate BP misclassfication rate
P1 <- length(true.obs$CONSIS1[true.obs$CONSIS1==1])/length(true.obs$CONSIS1)
P2 <- length(true.obs$CONSIS2[true.obs$CONSIS2==1])/length(true.obs$CONSIS2)

## conbine results into one file
P<- c(k,q,P1,P2)
fig1 <- rbind(fig1,P)
}
}
table1 <- data.frame(table1)
fig1 <- data.frame(fig1)

write.table(table1,
file=paste(Dir,"type_II_table","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(fig1, file=paste(Dir,"type_II_fig","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

APPENDIX C: CODE FOR SEVENTH CHAPTER

3.1 Strategy I

```
library(MIfuns)
Dir <- "/home/jiny/final_sa/aim3_CI/I/" # working direction
cvr <- read.table(file=paste(Dir,"cvr.csv",sep=""),sep=",",header=T,skip=0) # import virtual subject
characteristics
# function will be used later
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
theta1 <- cvr[,c("SBP")]
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim

table <- NULL
table90 <- NULL

for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <- 5
ni <- length(id <- 1:length(cvr$ID)) #  number of subjects for simulation
m6 <- NULL
m7 <- NULL
# Model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) #one occasion for each individual
THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term
eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term
temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
month <- 1  # PK simulation period is one month
occ <- month*30

# generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35" ##
for (i in 1:ni){
```

```
      t <- seq(0,24,by=0.2)
                        t.length <- length(t)
                        cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                        cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                        BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                        temp.id <- c(temp.id,rep(i,times=t.length))
                        temp.time <- c(temp.time,t)
                        temp.bsl <- c(temp.bsl,BSL)
      cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data

# generates dose-response
d <- data.frame(ID=id,al=rnorm(ni,6,12),am=rnorm(ni,3,2),ah=rnorm(ni,2,2),
                  al2=rnorm(ni,6,12),am2=rnorm(ni,3,2),ah2=rnorm(ni,2,2),
                  al3=rnorm(ni,6,12),am3=rnorm(ni,3,2),ah3=rnorm(ni,2,2),
                   bl=rnorm(ni,5,12),bm=rnorm(ni,2,2),bh=rnorm(ni,2,2),
                   cl=rnorm(ni,5,12),cm=rnorm(ni,2,2),ch=rnorm(ni,2,2),
                   dl=rnorm(ni,5,12),dm=rnorm(ni,2,2),dh=rnorm(ni,2,2))
res <- merge(cvr,merge(d,BSL,by="ID",all=T),by="ID",all=T)

# A low
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al,d$BSL-d$al-d$bl)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
temp.true <- NULL
for (i in 1:ni){
            true <- d$BP[d$ID==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.true <- c(temp.true,true)
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(1:ni,visittime, temp.true, temp.obs)
names(true.obs) <- c("ID","VISITTIME","TRU","OBS")
true.obs <- merge(true.obs,cvr,by="ID",all=T)
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=1,MONTH=1)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l11 <- true.obs[true.obs$class==1,]
l12 <- true.obs[true.obs$class==2,]
l13 <- true.obs[true.obs$class==3,]
m6 <- NULL
if (length(l11$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l11[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
```

```
## l13
if (length(l13$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al2,d$BSL-d$al)
d <- merge(d,l13,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l13_1 <- true.obs[true.obs$class==1,]
l13_2 <- true.obs[true.obs$class==2,]
l13_3 <- true.obs[true.obs$class==3,]

if(length(l13_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

m7 <- NULL
if (length(l13_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}

# l13_3
if (length(l13_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al3,d$BSL-d$al2)
d <- merge(d,l13_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
```

```
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                    ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                       ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l13_3_1 <- true.obs[true.obs$class==1,]
l13_3_2 <- true.obs[true.obs$class==2,]
l13_3_3 <- true.obs[true.obs$class==3,]

if (length(l13_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

if (length(l13_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}

## l13_3_3
if (length(l13_3_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL,d$BSL-d$al3)
d <- merge(d,l13_3_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")

true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                    ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                       ifelse(true.obs$OBS<90,3,1)))
```

```
#1:control;  2:not control;  3:dose is too
l13_3_3_1 <- true.obs[true.obs$class==1,]
l13_3_3_2 <- true.obs[true.obs$class==2,]
l13_3_3_3 <- true.obs[true.obs$class==3,]

if (length(l13_3_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

if (length(l13_3_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}

if (length(l13_3_3_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
}
}

## level 12 month 2
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am,d$BSL-d$al-d$bl-d$am)
d <- merge(d,l12,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l12$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                   ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l21 <- true.obs[true.obs$class==1,]
l22 <- true.obs[true.obs$class==2,]
```

```
l23 <- true.obs[true.obs$class==3,]

m1 <- merge(d[,c("ID","TIME","BP")],l21[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

if (length(l23$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l23[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=2)
m7 <- rbind(m7,m1)
}
## l22
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah,d$BSL-d$al-d$bl-d$am-d$ah)
d <- merge(d,l22,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l22$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l31 <- true.obs[true.obs$class==1,]
l32 <- true.obs[true.obs$class==2,]
l33 <- true.obs[true.obs$class==3,]

m1 <- merge(d[,c("ID","TIME","BP")],l31[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

if (length(l33$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l33[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=3)
m7 <- rbind(m7,m1)
}
```

```
##l32
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl,d$BSL-d$al-d$bl-d$am-d$ah-d$bm)
d <- merge(d,l32,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l32$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                   ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l41 <- true.obs[true.obs$class==1,]
l42 <- true.obs[true.obs$class==2,]
l43 <- true.obs[true.obs$class==3,]
m1 <- merge(d[,c("ID","TIME","BP")],l41[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

if (length(l43$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l43[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=4)
m7 <- rbind(m7,m1)
}

## l42
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm,d$BSL-d$al-d$bl-d$am-d$ah-d$bm-d$bh)
d <- merge(d,l42,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l42$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
```

```
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l51 <- true.obs[true.obs$class==1,]
l52 <- true.obs[true.obs$class==2,]
l53 <- true.obs[true.obs$class==3,]

m1 <- merge(d[,c("ID","TIME","BP")],l51[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

if (length(l53$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l53[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=5)
m7 <- rbind(m7,m1)
}
## l52
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh,d$BSL-d$al-d$bl-d$am-d$ah-d$bm-
d$bh-d$cl)
d <- merge(d,l52,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l52$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l61 <- true.obs[true.obs$class==1,]
l62 <- true.obs[true.obs$class==2,]
l63 <- true.obs[true.obs$class==3,]

m1 <- merge(d[,c("ID","TIME","BP")],l61[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

m1 <- merge(d[,c("ID","TIME","BP")],l62[,c("ID","MONTH")],by="ID",all.y=T)
```

```
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

if (length(l63$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l63[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=6)
m7 <- rbind(m7,m1)
}

if (length(m7$ID)>0){
m7 <- transform(m7, REP=k)
table90 <- rbind(table90,m7)
}

## CALCULATE CV RISK AT BASELINE

t1 <- res[res$TIME<=18,]
t1 <- t1[t1$TIME>=8,]
t1 <- transform(t1,MBP=reapply(BSL,INDEX=ID,FUN=mean),HYP=0)
risk1 <- t1[,c("ID","MBP","SEX","AGE","TCH","HDL","DIAB","HYP","SMK","SBP")]
risk1 <- unique(risk1)
d<- risk1
d$x <- ifelse(d$SEX==1& d$HYP==0, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.93303*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
       ifelse(d$SEX==1& d$HYP==1, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.99881*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
       ifelse(d$SEX==2& d$HYP==0, 2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.76157*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB,
       2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.82263*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB)))
d$p <- ifelse(d$SEX==1, 1-0.88936^exp(d$x-23.9802),
         1-0.95012^exp(d$x-26.1931))
risk1 <- d

## CALCULATE CV RISK MONTH 6 POST TREATMENT

cvr$MBP <- NULL
t2 <- merge(m6,cvr,by="ID",all.x=T)
t2 <- transform(t2,HYP=0)
d <-t2

d$x <- ifelse(d$SEX==1& d$HYP==0, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.93303*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
       ifelse(d$SEX==1& d$HYP==1, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.99881*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
       ifelse(d$SEX==2& d$HYP==0, 2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.76157*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB,
       2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.82263*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB)))

d$p <- ifelse(d$SEX==1, 1-0.88936^exp(d$x-23.9802),
         1-0.95012^exp(d$x-26.1931))
risk2 <- d
table <- rbind(table,c(k,mean(risk1$p),mean(risk2$p)))
}
table <- data.frame(table)
names(table) <- c("REP","risk0","risk6_l")
```

```
table90 <- data.frame(table90)
write.table(table, file=paste(Dir,"risk_I","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(table90, file=paste(Dir,"IDs
toxi","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

3.2 Strategy II

```
library(MIfuns)
Dir <- "W:/final_sa/aim3_CI/II/"
cvr <- read.table(file=paste(Dir,"cvr.csv",sep=""),sep=",",header=T,skip=0)
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
month <- 1  # PK simulation period is one month
theta1 <- cvr[,c("SBP")]
# ONE MONTH BASELINE BP PROFILE SIMULATION
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
# the part need to be changed
n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim
table <- NULL
table90 <- NULL
for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <- 5
ni <- length(id <- 1:length(cvr$ID)) #  subjects for simulation
m6 <- NULL
m7 <- NULL
#### Model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) #one occasion for each individual
THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term
eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                    ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                    ### no variability in phase shift in second cosine term
temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30

## generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
for (i in 1:ni){
      t <- seq(0,24,by=0.2)
                  t.length <- length(t)
```

```
                    cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                    cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                    BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                    temp.id <- c(temp.id,rep(i,times=t.length))
                    temp.time <- c(temp.time,t)
                    temp.bsl <- c(temp.bsl,BSL)
      cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data
## generate dose response relationship
d <- data.frame(ID=id,al=rnorm(ni,6,12),am=rnorm(ni,3,2),ah=rnorm(ni,2,2),
                  al2=rnorm(ni,6,12),am2=rnorm(ni,3,2),ah2=rnorm(ni,2,2),
                  al3=rnorm(ni,6,12),am3=rnorm(ni,3,2),ah3=rnorm(ni,2,2),
                   bl=rnorm(ni,5,12),bm=rnorm(ni,2,2),bh=rnorm(ni,2,2),
                   cl=rnorm(ni,5,12),cm=rnorm(ni,2,2),ch=rnorm(ni,2,2),
                   dl=rnorm(ni,5,12),dm=rnorm(ni,2,2),dh=rnorm(ni,2,2))
res <- merge(cvr,merge(d,BSL,by="ID",all=T),by="ID",all=T)
#### A low
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
temp.true <- NULL
for (i in 1:ni){
            true <- d$BP[d$ID==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.true <- c(temp.true,true)
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(1:ni,visittime, temp.true, temp.obs)
names(true.obs) <- c("ID","VISITTIME","TRU","OBS")
true.obs <- merge(true.obs,cvr,by="ID",all=T)

true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=1,MONTH=1)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                    ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                       ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l11 <- true.obs[true.obs$class==1,]
l12 <- true.obs[true.obs$class==2,]
l13 <- true.obs[true.obs$class==3,]
m6 <- NULL
if (length(l11$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l11[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
## l13
if (length(l13$ID)>0){
d <- res
```

```
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am,d$BSL-d$al-d$am-d$bl-d$bm)
d <- merge(d,l13,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l13_1 <- true.obs[true.obs$class==1,]
l13_2 <- true.obs[true.obs$class==2,]
l13_3 <- true.obs[true.obs$class==3,]

if(length(l13_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

m7 <- NULL
if (length(l13_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
## l13_3
if (length(l13_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al,d$BSL-d$al-d$bl)
d <- merge(d,l13_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
```

```
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l13_3_1 <- true.obs[true.obs$class==1,]
l13_3_2 <- true.obs[true.obs$class==2,]
l13_3_3 <- true.obs[true.obs$class==3,]

if (length(l13_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l13_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
## l13_3_3
if (length(l13_3_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al2,d$BSL-d$al)
d <- merge(d,l13_3_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l13_3_3_1 <- true.obs[true.obs$class==1,]
l13_3_3_2 <- true.obs[true.obs$class==2,]
l13_3_3_3 <- true.obs[true.obs$class==3,]
if (length(l13_3_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
```

```
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

if (length(l13_3_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}

if (length(l13_3_3_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
}
}
### level 12 month 2
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-
d$bh-d$cl-d$cm-d$ch)
d <- merge(d,l12,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l12$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                    ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l21 <- true.obs[true.obs$class==1,]
l22 <- true.obs[true.obs$class==2,]
l23 <- true.obs[true.obs$class==3,]
if (length(l21$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l21[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
```

```
m6 <- rbind(m6,m1)
}
if (length(l23$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l23[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=2)
m7 <- rbind(m7,m1)
}
### l22
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$ch,
            d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$ch-d$dl-d$dm-d$dh)
d <- merge(d,l22,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l22$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l31 <- true.obs[true.obs$class==1,]
l32 <- true.obs[true.obs$class==2,]
l33 <- true.obs[true.obs$class==3,]
if (length(l31$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l31[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l33$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l33[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=3)
m7 <- rbind(m7,m1)
}
##l32
d <- res
d$BP <- d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$ch-d$dl-d$dm-d$dh
d <- merge(d,l32,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l32$ID)
d$flag <- rep(1:ni,each=242)
```

```
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=140 & true.obs$DIAB==0,2,
                  ifelse(true.obs$OBS>=130 & true.obs$DIAB==1,2,
                     ifelse(true.obs$OBS<90,3,1)))
#1:control;  2:not control;  3:dose is too
l41 <- true.obs[true.obs$class==1,]
l42 <- true.obs[true.obs$class==2,]
l43 <- true.obs[true.obs$class==3,]
if (length(l41$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l41[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l42$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l42[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l43$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l43[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=4)
m7 <- rbind(m7,m1)
}
if (length(m7$ID)>0){
m7 <- transform(m7, REP=k)
table90 <- rbind(table90,m7)
}
#### CALCULATE CV RISK AT MONTH 6
cvr$MBP <- NULL
t2 <- merge(m6,cvr,by="ID",all.x=T)
t2 <- transform(t2,HYP=0)
d <-t2
d$x <- ifelse(d$SEX==1& d$HYP==0, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.93303*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
      ifelse(d$SEX==1& d$HYP==1, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.99881*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
      ifelse(d$SEX==2& d$HYP==0, 2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.76157*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB,
      2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.82263*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB)))
d$p <- ifelse(d$SEX==1, 1-0.88936^exp(d$x-23.9802),
```

```
        1-0.95012^exp(d$x-26.1931))
risk2 <- d
table <- rbind(table,c(k,mean(risk2$p)))
}
table <- data.frame(table)
names(table) <- c("REP","risk6_II")
table90 <- data.frame(table90)
write.table(table, file=paste(Dir,"risk_II","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(table90, file=paste(Dir,"IDs
toxi","_II_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

3.3 Strategy III

```
library(MIfuns)
Dir <- "W:/final_sa/aim3_CI/III/"
cvr <- read.table(file=paste(Dir,"cvr.csv",sep=""),sep=",",header=T,skip=0)
## functions
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
month <- 1  # PK simulation period is one month
theta1 <- cvr[,c("SBP")]
## ONE MONTH BASELINE BP PROFILE SIMULATION
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
# the part need to be changed ##
n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim
table <- NULL
table90 <- NULL
for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <- 5
ni <- length(id <- 1:length(cvr$ID)) #  subjects for simulation
m6 <- NULL
m7 <- NULL
## Model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) #one occasion for each individual
THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term
eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                  ### no variability in phase shift in first cosine term
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                  ### no variability in phase shift in second cosine term
temp.id <- NULL
temp.time <- NULL
```

```
temp.bsl <- NULL
occ <- month*30
## generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35"
for (i in 1:ni){
      t <- seq(0,24,by=0.2)
                  t.length <- length(t)
                  cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                  cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                  BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                  temp.id <- c(temp.id,rep(i,times=t.length))
                  temp.time <- c(temp.time,t)
                  temp.bsl <- c(temp.bsl,BSL)
      cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
            cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
            BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
            t.new <- (occ-1)*24 + t
            temp.id <- c(temp.id,rep(i,times=t.length))
            temp.time <- c(temp.time,t.new)
            temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data
d <- data.frame(ID=id,al=rnorm(ni,6,12),am=rnorm(ni,3,2),ah=rnorm(ni,2,2),
                  al2=rnorm(ni,6,12),am2=rnorm(ni,3,2),ah2=rnorm(ni,2,2),
                  al3=rnorm(ni,6,12),am3=rnorm(ni,3,2),ah3=rnorm(ni,2,2),
                   bl=rnorm(ni,5,12),bm=rnorm(ni,2,2),bh=rnorm(ni,2,2),
                   cl=rnorm(ni,5,12),cm=rnorm(ni,2,2),ch=rnorm(ni,2,2),
                   dl=rnorm(ni,5,12),dm=rnorm(ni,2,2),dh=rnorm(ni,2,2))
res <- merge(cvr,merge(d,BSL,by="ID",all=T),by="ID",all=T)
#### A low
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al,d$BSL-d$al-d$bl)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
temp.true <- NULL
for (i in 1:ni){
            true <- d$BP[d$ID==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.true <- c(temp.true,true)
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(1:ni,visittime, temp.true, temp.obs)
names(true.obs) <- c("ID","VISITTIME","TRU","OBS")
true.obs <- merge(true.obs,cvr,by="ID",all=T)
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=1,MONTH=1)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                    ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l11 <- true.obs[true.obs$class==1,]
l12 <- true.obs[true.obs$class==2,]
l13 <- true.obs[true.obs$class==3,]
if (length(l11$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l11[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
## l13
```

```
if (length(l13$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al2,d$BSL-d$al)
d <- merge(d,l13,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
            ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l13_1 <- true.obs[true.obs$class==1,]
l13_2 <- true.obs[true.obs$class==2,]
l13_3 <- true.obs[true.obs$class==3,]
if(length(l13_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

if (length(l13_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
## l13_3
if (length(l13_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al3,d$BSL-d$al2)
d <- merge(d,l13_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
```

```
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l13_3_1 <- true.obs[true.obs$class==1,]
l13_3_2 <- true.obs[true.obs$class==2,]
l13_3_3 <- true.obs[true.obs$class==3,]
dim(l13_3_1)
dim(l13_3_2)
dim(l13_3_3)
if (length(l13_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l13_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
## l13_3_3
if (length(l13_3_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL,d$BSL-d$al3)
d <- merge(d,l13_3_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
sderror <- 5
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l13_3_3_1 <- true.obs[true.obs$class==1,]
l13_3_3_2 <- true.obs[true.obs$class==2,]
l13_3_3_3 <- true.obs[true.obs$class==3,]
if (length(l13_3_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
```

```
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l13_3_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
if (length(l13_3_3_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
}
}
### level 12 month 2
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am,d$BSL-d$al-d$bl-d$am)
d <- merge(d,l12,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l12$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120 ,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l21 <- true.obs[true.obs$class==1,]
l22 <- true.obs[true.obs$class==2,]
l23 <- true.obs[true.obs$class==3,]
if (length(l21$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l21[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l23$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l23[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
```

```
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=2)
m7 <- rbind(m7,m1)
}
### l22
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah,d$BSL-d$al-d$bl-d$am-d$ah)
d <- merge(d,l22,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l22$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l31 <- true.obs[true.obs$class==1,]
l32 <- true.obs[true.obs$class==2,]
l33 <- true.obs[true.obs$class==3,]
m1 <- merge(d[,c("ID","TIME","BP")],l31[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
if (length(l33$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l33[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=3)
m7 <- rbind(m7,m1)
}
###l32
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl,d$BSL-d$al-d$bl-d$am-d$ah-d$bm)
d <- merge(d,l32,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l32$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
```

```
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l41 <- true.obs[true.obs$class==1,]
l42 <- true.obs[true.obs$class==2,]
l43 <- true.obs[true.obs$class==3,]
m1 <- merge(d[,c("ID","TIME","BP")],l41[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)

if (length(l43$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l43[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=4)
m7 <- rbind(m7,m1)
}
### l42
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm,d$BSL-d$al-d$bl-d$am-d$ah-d$bm-d$bh)
d <- merge(d,l42,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l42$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                    ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l51 <- true.obs[true.obs$class==1,]
l52 <- true.obs[true.obs$class==2,]
l53 <- true.obs[true.obs$class==3,]
m1 <- merge(d[,c("ID","TIME","BP")],l51[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
if (length(l53$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l53[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
```

```
m1 <- transform(m1,MONTH=5)
m7 <- rbind(m7,m1)
}
## l52
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh,d$BSL-d$al-d$bl-d$am-d$ah-d$bm-
d$bh-d$cl)
d <- merge(d,l52,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l52$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l61 <- true.obs[true.obs$class==1,]
l62 <- true.obs[true.obs$class==2,]
l63 <- true.obs[true.obs$class==3,]
m1 <- merge(d[,c("ID","TIME","BP")],l61[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
m1 <- merge(d[,c("ID","TIME","BP")],l62[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
if (length(l63$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l63[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=6)
m7 <- rbind(m7,m1)
}
if (length(m7$ID)>0){
m7 <- transform(m7, REP=k)
table90 <- rbind(table90,m7)
}
#### CALCULATE CV RISK AT MONTH 6
cvr$MBP <- NULL
t2 <- merge(m6,cvr,by="ID",all.x=T)
t2 <- transform(t2,HYP=0)
d <-t2
d$x <- ifelse(d$SEX==1& d$HYP==0, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.93303*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
```

```
      ifelse(d$SEX==1& d$HYP==1, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.99881*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
      ifelse(d$SEX==2& d$HYP==0, 2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.76157*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB,
      2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.82263*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB)))
d$p <- ifelse(d$SEX==1, 1-0.88936^exp(d$x-23.9802),
        1-0.95012^exp(d$x-26.1931))
risk3 <- d
table <- rbind(table,c(k,mean(risk3$p)))
}
table <- data.frame(table)
names(table) <- c("REP","risk3_III")
table90 <- data.frame(table90)
write.table(table, file=paste(Dir,"risk_III","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(table90, file=paste(Dir,"IDs
toxi_III","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

3.4 Strategy IV

```
library(MIfuns)
Dir <- "W:/final_sa/aim3_CI/IV/"
cvr <- read.table(file=paste(Dir,"cvr.csv",sep=""),sep=",",header=T,skip=0)
## function
reapply <- function(x,INDEX,FUN,...){
        y <- tapply(x,INDEX)
        z <- tapply(x,INDEX,FUN,...)
        z[y]
}
month <- 1  # PK simulation period is one month
theta1 <- cvr[,c("SBP")]
p <- read.table(file=paste(Dir,"p.csv",sep=""),as.is=T,header=T,skip=0,sep=",")
set.seed(234)
seeds <- round(runif(1000, min=1, max=20000))
n <- 1
nsim <- 1000
nsim1 <- (n-1)*nsim+1
nsim2 <- n*nsim
table <- NULL
table90 <- NULL
for (k in nsim1:nsim2){
seed <- seeds[k]
set.seed(seed)
sderror <- 5
ni <- length(id <- 1:length(cvr$ID)) #  subjects for simulation
m6 <- NULL
m7 <- NULL
## baseline model from "Clin Pharmacol Ther,1998.64(6):p.622-35"
mu1 <- c(0,0,0)
Omega1 <- matrix(c(p[k,5],p[k,6],p[k,8],p[k,6],p[k,7],p[k,9],p[k,8],p[k,9],p[k,10]),3,3)
mu2 <- c(0,0)
Omega2 <- matrix(c(p[k,11],p[k,12],p[k,12],p[k,13]),2,2)
iiv <- mvrnorm(n = ni, mu1, Omega1, empirical = FALSE)
iov <- mvrnorm(n = ni*2, mu2, Omega2, empirical = FALSE) #one occasion for each individual
THETA2 <- p[k,2]  ### population mean for amplitude of first cosine term
THETA3 <- 0       ### phase shift in first cosine term
THETA4 <- p[k,3]  ### population mean for amplitude of second cosine term
THETA5 <- p[k,4]  ### phase shift in second cosine term
eta2 <- iiv[,2]   ### interindividual variability in amplitude of first cosine term(THETA2)
eta4 <- iiv[,2]   ### interindividual variability in amplitude of second cosine term(THETA4)
eta.t <- iiv[,3] ### interindividual variability on clock time (hr)
eta.k1d <- iov[,1]  ### interoccasion variability in baseline (mm Hg)
eta.k2d <- iov[,2]  ### interoccasion variability on clock time (hr)
theta2 <- THETA2*(1+eta2) ### generate individual values (n=3642)in amplitude of first cosine term
theta3 <- THETA3                    ### no variability in phase shift in first cosine term
```

```
theta4 <- THETA4*(1+eta4) ### generate individual values (n=3642)in amplitude of second cosine term
theta5 <- THETA5                    ### no variability in phase shift in second cosine term
temp.id <- NULL
temp.time <- NULL
temp.bsl <- NULL
occ <- month*30
# generate one month baseline BP profile from equation 3 in "Clin Pharmacol Ther,1998.64(6):p.622-35" ##
for (i in 1:ni){
          t <- seq(0,24,by=0.2)
                    t.length <- length(t)
                    cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[i])/12-theta3)
                    cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[i])/12-theta5)
                    BSL <- eta.k1d[i]+theta1[i]*(1+cos1+cos2)
                    temp.id <- c(temp.id,rep(i,times=t.length))
                    temp.time <- c(temp.time,t)
                    temp.bsl <- c(temp.bsl,BSL)
     cos1 <- theta2[i]*cos(pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta3)
          cos2 <- theta4[i]*cos(2*pi*(t + eta.t[i] + eta.k2d[ni+i])/12-theta5)
          BSL <- eta.k1d[ni+i]+theta1[i]*(1+cos1+cos2)
          t.new <- (occ-1)*24 + t
          temp.id <- c(temp.id,rep(i,times=t.length))
          temp.time <- c(temp.time,t.new)
          temp.bsl <- c(temp.bsl,BSL)
}
temp.bsl <- signif(temp.bsl,digits=5)
data <- data.frame(temp.id,temp.time,temp.bsl)
names(data) <- c("ID","TIME","BSL")
BSL <- data
d <- data.frame(ID=id,al=rnorm(ni,6,12),am=rnorm(ni,3,2),ah=rnorm(ni,2,2),
                  al2=rnorm(ni,6,12),am2=rnorm(ni,3,2),ah2=rnorm(ni,2,2),
                  al3=rnorm(ni,6,12),am3=rnorm(ni,3,2),ah3=rnorm(ni,2,2),
                   bl=rnorm(ni,5,12),bm=rnorm(ni,2,2),bh=rnorm(ni,2,2),
                   cl=rnorm(ni,5,12),cm=rnorm(ni,2,2),ch=rnorm(ni,2,2),
                   dl=rnorm(ni,5,12),dm=rnorm(ni,2,2),dh=rnorm(ni,2,2))
res <- merge(cvr,merge(d,BSL,by="ID",all=T),by="ID",all=T)
#### A low
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
temp.true <- NULL
for (i in 1:ni){
          true <- d$BP[d$ID==i & d$TIME==visittime[i]]
          obs <- true + errorBP[i]
          temp.true <- c(temp.true,true)
          temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(1:ni,visittime, temp.true, temp.obs)
names(true.obs) <- c("ID","VISITTIME","TRU","OBS")
true.obs <- merge(true.obs,cvr,by="ID",all=T)
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=1,MONTH=1)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                    ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l11 <- true.obs[true.obs$class==1,]
l12 <- true.obs[true.obs$class==2,]
l13 <- true.obs[true.obs$class==3,]
m6 <- NULL
if (length(l11$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l11[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
```

```
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
### l13
if (length(l13$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am,d$BSL-d$al-d$am-d$bl-d$bm)
d <- merge(d,l13,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13$ID)
d$flag <- rep(1:ni,each=242)

visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l13_1 <- true.obs[true.obs$class==1,]
l13_2 <- true.obs[true.obs$class==2,]
l13_3 <- true.obs[true.obs$class==3,]
if(length(l13_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
m7 <- NULL
if (length(l13_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
# l13_3
if (length(l13_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al,d$BSL-d$al-d$bl)
d <- merge(d,l13_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
```

```
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l13_3_1 <- true.obs[true.obs$class==1,]
l13_3_2 <- true.obs[true.obs$class==2,]
l13_3_3 <- true.obs[true.obs$class==3,]
if (length(l13_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l13_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
## l13_3_3
if (length(l13_3_3$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al2,d$BSL-d$al)
d <- merge(d,l13_3_3,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l13_3_3$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l13_3_3_1 <- true.obs[true.obs$class==1,]
l13_3_3_2 <- true.obs[true.obs$class==2,]
l13_3_3_3 <- true.obs[true.obs$class==3,]

if (length(l13_3_3_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
```

```
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}

if (length(l13_3_3_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
if (length(l13_3_3_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l13_3_3_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
}
}
#### level 12 month 2
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-
d$bh-d$cl-d$cm-d$ch)
d <- merge(d,l12,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l12$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l21 <- true.obs[true.obs$class==1,]
l22 <- true.obs[true.obs$class==2,]
l23 <- true.obs[true.obs$class==3,]
if (length(l21$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l21[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l23$ID)>0){
```

```
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$bl-d$bm,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$cl-d$cm)
d <- merge(d,l23,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l23$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l23_1 <- true.obs[true.obs$class==1,]
l23_2 <- true.obs[true.obs$class==2,]
l23_3 <- true.obs[true.obs$class==3,]
if (length(l23_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l23_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l23_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l23_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
if (length(l23_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l23_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
### l22
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$ch,
         d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$ch-d$dl-d$dm-d$dh)
d <- merge(d,l22,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l22$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
```

```
temp.obs <- NULL
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l31 <- true.obs[true.obs$class==1,]
l32 <- true.obs[true.obs$class==2,]
l33 <- true.obs[true.obs$class==3,]
if (length(l31$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l31[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l33$ID)>0){
d <- res
d$BP <- ifelse(d$FLAG==1,d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$cl-d$cm,
          d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$dl-d$dm)
d <- merge(d,l33,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l33$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                   ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l33_1 <- true.obs[true.obs$class==1,]
l33_2 <- true.obs[true.obs$class==2,]
l33_3 <- true.obs[true.obs$class==3,]
if (length(l33_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l33_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l33_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l33_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
```

```
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
if (length(l33_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l33_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
###l32
d <- res
d$BP <- d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$ch-d$dl-d$dm-d$dh
d <- merge(d,l32,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
ni <- length(id <- l32$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
        true <- d$BP[d$flag==i & d$TIME==visittime[i]]
        obs <- true + errorBP[i]
        temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l41 <- true.obs[true.obs$class==1,]
l42 <- true.obs[true.obs$class==2,]
l43 <- true.obs[true.obs$class==3,]
if (length(l41$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l41[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l42$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l42[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l43$ID)>0){
d <- res
d$BP <- d$BSL-d$al-d$am-d$ah-d$bl-d$bm-d$bh-d$cl-d$cm-d$dl-d$dm
d <- merge(d,l43,by.x=c("ID"),by.y=c("ID"),all.y=T)
d <- d[order(d$ID,d$TIME),]
```

```
ni <- length(id <- l43$ID)
d$flag <- rep(1:ni,each=242)
visittime <- runif(ni, min=24*29+8, max=24*29+18)
visittime <- round(visittime)
errorBP <- rnorm(ni,sd=sderror)
temp.obs <- NULL
for (i in 1:ni){
            true <- d$BP[d$flag==i & d$TIME==visittime[i]]
            obs <- true + errorBP[i]
            temp.obs <- c(temp.obs, obs)
}
true.obs <- data.frame(id,visittime, temp.obs)
names(true.obs) <- c("ID","VISITTIME","OBS")
true.obs <- transform(true.obs,CTIME=VISITTIME-696,VISIT=2,MONTH=2)
true.obs <- true.obs[order(true.obs$ID),]
true.obs <- merge(true.obs,cvr,by=c("ID"),all.x=T)
true.obs$class <- ifelse(true.obs$OBS>=120,2,
                  ifelse(true.obs$OBS<90,3,1))
#1:control;  2:not control;  3:dose is too
l43_1 <- true.obs[true.obs$class==1,]
l43_2 <- true.obs[true.obs$class==2,]
l43_3 <- true.obs[true.obs$class==3,]

if (length(l43_1$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l43_1[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m6 <- rbind(m6,m1)
}
if (length(l43_2$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l43_2[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
if (length(l43_3$ID)>0){
m1 <- merge(d[,c("ID","TIME","BP")],l43_3[,c("ID","MONTH")],by="ID",all.y=T)
m1 <- m1[order(m1$ID,m1$TIME),]
m1 <- m1[m1$TIME>=704 & m1$TIME<=714,]
m1 <- transform(m1,MBP=reapply(BP,INDEX=ID,FUN=mean))
m1 <- unique(m1[,c("ID","MBP","MONTH")])
m1 <- transform(m1,MONTH=1)
m7 <- rbind(m7,m1)
}
}
if (length(m7$ID)>0){
m7 <- transform(m7, REP=k)
table90 <- rbind(table90,m7)
}
######### CALCULATE CV RISK AT MONTH 6
cvr$MBP <- NULL
t2 <- merge(m6,cvr,by="ID",all.x=T)
t2 <- transform(t2,HYP=0)
d <-t2
d$x <- ifelse(d$SEX==1& d$HYP==0, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.93303*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
      ifelse(d$SEX==1& d$HYP==1, 3.06117*log(d$AGE)+1.12370*log(d$TCH)-
0.93263*log(d$HDL)+1.99881*log(d$MBP)+0.65451*d$SMK+0.57367*d$DIAB,
```

```
      ifelse(d$SEX==2& d$HYP==0, 2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.76157*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB,
      2.32888*log(d$AGE)+1.20904*log(d$TCH)-
0.70833*log(d$HDL)+2.82263*log(d$MBP)+0.52873*d$SMK+0.69154*d$DIAB)))
d$p <- ifelse(d$SEX==1, 1-0.88936^exp(d$x-23.9802),
         1-0.95012^exp(d$x-26.1931))
risk2 <- d
table <- rbind(table,c(k,mean(risk2$p)))
}
table <- data.frame(table)
names(table) <- c("REP","risk6_II")
table90 <- data.frame(table90)
write.table(table, file=paste(Dir,"risk_II","_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
write.table(table90, file=paste(Dir,"IDs
toxi","_II_",nsim1,"_",nsim2,".csv",sep=""),quote=F,sep=",",row.names=F)
```

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