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Population Pharmacokinetics Studies with Nonlinear Mixed Effects Modeling

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ABSTRACT

Population pharmacokinetics is the application of our statistical and programming expertise to convert data into knowledge. Pharmacokinetic and PK/PD models applied to drug concentration-time data can provide crucial information in early and late stage clinical development or post-marketing development. Population PK modeling uses population-based approaches to identify and interpret the demographic, genetic, physiologic and pathologic factors and so on. This paper presents how population PK can estimate the typical PK and/or PD parameters in a population of patients, investigate and identify sources of variability (covariates) that explain differences in the parameters between patients, and quantify the impact of these covariates. SAS NLMIXED procedure is also introduced.

INTRODUCTION

Population pharmacokinetics is the study of the sources and correlates of variability in drug concentrations among individuals who are the target patient population receiving clinically relevant doses of the drug. Certain patient demographical, pathophysiological, and therapeutic features, such as body weight, excretory and metabolic functions, and the presence of other therapies, can regularly alter dose-concentration relationships. For example, steady-state concentrations of drugs eliminated mostly by the kidney are usually greater in patients suffering from renal failure than they are in patients with normal renal function who receive the same drug dosage. Population pharmacokinetics seeks to identify the measurable pathophysiologic factors that cause changes in the dose-concentration relationship and the extent of these changes so that, if such changes are associated with clinically significant shifts in the therapeutic index, dosage can be appropriately modified.

In traditional PK, subjects are usually healthy volunteers or highly selected patients, and the average behavior of a group has been the main focus of interest. Inter-individual variability in pharmacokinetics is minimized, often through complex study designs and control schemes, or through restrictive inclusion/exclusion criteria. In fact, information on the variability that occurs during a clinical trial is very critical, but it is obscured by these restrictions. In contrast to traditional PK, the population PK approach encompasses some or all of the following features.
Table 1. Comparison: Traditional versus Population PK Approaches

<table>
<thead>
<tr>
<th></th>
<th>Traditional</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
<td>Healthy volunteers</td>
<td>Target patient population (pediatric, elderly, AIDS)</td>
</tr>
<tr>
<td></td>
<td>Highly selected Patients</td>
<td></td>
</tr>
<tr>
<td><strong>Study Size</strong></td>
<td>Small</td>
<td>Large or integrated (observational, experimental)</td>
</tr>
<tr>
<td><strong>Sampling Data</strong></td>
<td>Dense (typically 1 to 6 time points) following drug administration.</td>
<td>Sparse, few samples for many patients</td>
</tr>
<tr>
<td><strong>Inter-individual Variability</strong></td>
<td>Minimized through restrictive criteria</td>
<td>Demographics Pathophysiological Concomitant medications</td>
</tr>
<tr>
<td><strong>Relationships of concentration, PK/PD</strong></td>
<td>Limited</td>
<td>Extensive, make predictions about future events - steady state concentrations and efficacy. guide dosage adjustments. determine therapeutic window. guide dosage for safety</td>
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**IMPORTANCE OF POPULATION PK ANALYSIS**

- Summarize
- assess effects of covariates (age, weight, lab values, concomitant medications, other diseases, etc.) on PK and/or PK/PD
- To define optimal dosing regimen that will maximize efficacy and/or safety, guidance for choosing dosing regimens for pivotal studies and labeling and for future studies (hepatic status, drug-drug interaction).
- To estimate the parameters listed above with either dense and/or sparse data
- To estimate the magnitude of inter-patient variability
- To estimate the random residual variability (including – intra-patient measurement error)
- To assist in developing a preclinical, clinical pharmacokinetic program for an NDA submission:
  - To provide Bayesian priors for forecasting in Randomized Concentration Controlled Trials and in the refinement of patient dosing regimens
- To help explain failed or less than successful trial based on PK and/or PK/PD relationships
TYPES OF POPULATION PK ANALYSIS

PK is the study of drug absorption, distribution, metabolism and elimination, while PD studies the relationship between drug concentration and response. PK and PD are linked directly or indirectly.

- CL – drug clearance (L/hr), CL is calculated as Dose*F/AUC, where F is bioavailability.
- V or Vd – volume of distribution, a larger number means compound is distributed to a greater extent into the rest of the body from the circulating blood.
- D – actual dose amount, D = Oral dose*F.
- Emax – maximum possible effect.
- ED50 – dose to get 50% of the maximum effect.

1. Naïve Pooled Data combines all the data as if they came from a single reference individual and fit into a model using classical fitting procedures. It is simple, but can not investigate fixed effect sources of variability, distinguish between variability within and between individuals.

2. Two-Stage Approach It first estimates the individual subjects PK and/or pharmacodynamic (PD) parameters from dense data using classical fitting procedures (e.g. WLS), then estimate the population parameters across subjects (mean, variance, covariance). The standard error of the estimates for the coefficients can be calculated. However, it is only suitable for dense data. The variance-covariance across subjects (inter-individual, intra-individual variability) may be biased. Since the imbalance is ignored, the estimates of interindividual errors are upwardly biased as residual error increases. Mixed-effects modeling results in less biased estimates when residual error is present.

3. Bayesian Estimation

The prior distribution of the parameters across a population of subjects and the actual data from an individual are used when estimating the parameters for an individual. The estimation of parameters in the individual uses the posterior probability of the parameters. The prior distribution determines individual parameters. It needs the estimates of the priors for the parameters (mean, covariance). The fit may be dependent on priors (and uncertainty in the priors).

4. Nonlinear Mixed Effects Modeling This approach can be used in situations where extensive measurements will not be made on all or any of the subjects. In sparse data situations, where the traditional two-stage approach is not applicable because estimates of individual parameters are, a priori, out of reach, a single-stage approach, such as nonlinear mixed-effects modeling, should be used. This approach considers the population study sample, rather than the individual, as a unit of analysis for the
estimation of the distribution of parameters and their relationships with covariates within the population. The approach uses individual PK data of the observational type, which may be sparse, unbalanced, and fragmentary. Analysis according to the nonlinear mixed-effects model provides estimates of population characteristics that define the population distribution of the PK and/or PD parameters. The collection of population characteristics is composed of population mean values (derived from fixed-effects parameters) and their variability within the population (generally the variance-covariance values derived from random-effects parameters). The mixed-effects modeling approach is referred to below as the population PK approach.

Nonlinear mixed effects modeling uses one stage analysis that simultaneously estimates all parameters (e.g. mean parameters, fixed effect parameters, inter-individual variability, random residual error). It also provides estimates of the precision of these parameters. It linearizes the individual’s profile by taking a first order Taylor series expansion around the population parameter value and then proceeds with maximum likelihood estimation. A simple model as example:

\[
\begin{align*}
\ln \bar{C}_i &= \mu_{C_1} + \eta_{C_1} \\
\ln \bar{V}_i &= \mu_{V} + \eta_{V}
\end{align*}
\]

Population Mean parameters: \(\mu_{C_1}, \mu_{V}\)

Variance parameters : measure inter-individual variability

\[
\Omega = \begin{pmatrix} \sigma_{C_1}^2 & \sigma_{C_1}\sigma_{V}\rho \\ \sigma_{C_1}\sigma_{V}\rho & \sigma_{V}^2 \end{pmatrix}
\]

An example model including covariates such as body weight, age:

\[
\begin{align*}
\ln \bar{C}_i &= \mu_{C_1} + \theta_{BW_i} + \theta_{age_i} + \eta_{C_1} \\
\ln \bar{V}_i &= \mu_{V} + \eta_{V}
\end{align*}
\]

There are multiple estimation methods to be chosen depending on the assumptions.

(1). First-order method – FO estimates the typical value for each parameter (or fixed effect coefficient), the variance-covariance (\(\omega_2\)’s) of the interindividual random effect (\(\eta\)) for each parameter and the variance (\(\sigma^2\)) for the residual error (\(\epsilon\)). The individual \(\eta\)s are NOT estimated.

(2). POSTHOC Estimation – Uses empirical Bayes methods to estimate \(\eta\)s for each individual.

(3). First-order conditional estimation – Individual estimates of the \(\eta\)’s are evaluated for every iteration of the regression, thereby influencing the final estimates of the mean parameters and the variance-covariance matrix.

(4). Laplacian Conditional Estimation – Instead of performing a first-order expansion, a Laplacian estimation (second order) is used. This may be a better procedure for very nonlinear models.

(5). HYBRID Estimation - Performs FOCE to estimate \(\eta\)s, except for those \(\eta\)s which are designated by the user to be estimated using the FO method. This method may allow a considerable time savings over the full FOCE when not all \(\eta\)s require full conditional estimation.
DATA AND INFORMATION REQUIRED FOR POPULATION PK ANALYSIS

1. Data Input
(1) Accurate dosing information and history such as dose formulation, dosage.
(2) Plasma/blood concentrations from a validated assay (sparse or dense)
(3) Pharmacodynamic measurements and safety profiles (e.g., ECG, side effects)
(4) Covariate data – demographics, lab values, concomitant meds, metabolizer status, disease, fasting.
(5) Accurate capture of time/date associated with above items

2. Prior Knowledge and Information
Certain preliminary pharmacokinetic information and the drug's major elimination pathways in humans already should be known. Preliminary studies should establish the basic pharmacokinetic model of the drug because the sparse data collected during population PK studies may not provide adequate information for discriminating among pharmacokinetic models. When properly performed, population PK studies combined with suitable mathematical/statistical analysis can be a valid alternative to extensive studies.

(1) Previous PK information: pharmacokinetic modeling (compartmental model, parameter estimates, relative proportion of inter-patient to intrapatient and/or residual.), summary statistics.
(2) Impact of patient covariates (e.g. age, body weight, medical conditions), if any. For example, creatinine clearance and drug clearance, much of the drug is eliminated by the kidney without being metabolized (unchanged) or much of the drug undergoes metabolism.

What's the SAS programmer's role
(1) Collects the raw data and produces derived data sets for the listings
SAS can import data from Excel spreadsheet, dBASE file (DBMS identifier of DBF), Microsoft Access database, Lotus spreadsheet, delimited file (with comma, tab or blank as delimiter).
SAS programmer's role.
2). merging concentration and time values to calculate actual clock time, and check actual time not within acceptable range of nominal time point, since time affects accuracy of calculated PK parameters. Input dataset usually in "vertical" format, one observation per sample
(3). Produces the critical data set or customized spreadsheet or output files for PK analysis program (such as WinNonLin). PROC EXPORT or DATA _NULL_ (especially good for customization)
MODELING CONSIDERATIONS

1. Data editing. Data editing means using a set of procedures for detecting and correcting errors in the data. Data may be usable or unusable (e.g., time of blood sampling missing, dosing information with no associated concentrations, concentrations with missing dosing information). The issue of any missing covariate data should be addressed. Missing data represent a potential source of bias. Thus, every effort should be made to in the collection and management of data, to reduce the amount of missing data. Many subjects may be rich in covariate data, and some may be missing only a small sample of covariates. Excluding all subjects with any covariate data missing will vastly decrease the sample size. In certain situations, it may be better to impute missing covariate values for some subjects rather than to delete those subjects from the data set. Some simple methods of imputation, including the use of median, mean, or mode for missing values, may be biased and inefficient when predictors are correlated. Multiple imputations using maximum likelihood are better for imputation. The sensitivity of the results of the analysis to the method of imputation of missing data should be tested, especially if the number of missing values is substantial. If there is a pattern to the missing data, appropriate statistical procedures should be used to address the problem. If the concentration data are missing randomly, the process that caused the missing data can be ignored and the observed data can be analyzed without regard to the missing data.

Outlier. The reasons for declaring a data point to be an outlier should be statistically convincing. A distinction should be made between outlying individuals (inter-subject variability) and outlier data points (intra-subject variability). It would be possible to perform model building on the reduced data set (i.e., the data set without outliers) to reanalyze the entire data set (including the outliers) using the final population model, and to discuss the difference in the results. Including extreme outliers is not a good practice when using least-squares or normal-theory type estimation methods, as such outliers inevitably have a disproportionate effect on estimates. Also, it is well known that for most biological phenomena, outlying observations are far more frequent than suggested by the normal distribution (i.e., biological distributions are heavy-tailed). Some robust methods of population analysis have recently been suggested, and these may allow outliers to be retained without giving them undue weight.

2. Error Specification
Observed concentrations are subject to error. Information about the medication (the drug, dose, timing of doses, and compliance) would be considered as the Fixed Effects Factors. In the nonlinear regression model one has to account for a structure of the error variance and additional modeling of the error e.g. through heteroscedasticity. The precision of the kinetic parameter estimates is improved when random effects and covariates are participated.

Random Effects Factors, which cannot be determined or measured, consist of recording errors, unknown pathophysiology, analytical variations. It can be divided into:
- Inter-individual random effects - differences between subjects
– Intra-individual/inter-occasion random effects - account for changes within an individual
– Residual error - measurement error, model misspecification

Covariates can explain the variability in model parameters, exclude covariate relationships that lack importance, increase the mechanistic interpretability of the model, increase the predictive performance of the model, generate hypotheses and discriminate between alternate hypotheses.

- Weight effected both volume and clearance
- Clearance in males ~ 40% greater than females.
- Average weight male had an AUC less than average weight female
- Baseline creatinine clearance in patients with renal impairment

Without CrCL as a covariate 27% With CrCL as a covariate 19%.

<table>
<thead>
<tr>
<th>Type</th>
<th>Covariate</th>
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<tbody>
<tr>
<td>Demographics –categorical</td>
<td>Gender, Race, Smoking, Alcohol-consuming</td>
</tr>
<tr>
<td>Demographics -numeric</td>
<td>Age, Weight, Height, BMI, BSA</td>
</tr>
<tr>
<td>Lab</td>
<td>Hepatic or renal function</td>
</tr>
<tr>
<td></td>
<td>Food / fasting, phenotype/genotype</td>
</tr>
<tr>
<td>characteristics</td>
<td>Concomitant medications</td>
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3. Tools of Computational Statistics for PK Analysis

The effects of factors that are related to the classification of subjects into subpopulations by a mixture model on clearance were evaluated by software. The models can be developed through the following available softwares:

- NONMEM
- S-Plus – \textit{nlme} function
- SAS - NLINMIX macro (pre- v8), PROC NLMIXED.
- WinNonmix
- WinBUGS, PKBUGS / Pharmaco

SAS NLMIXED procedure fits nonlinear mixed models, in which both fixed and random effects enter nonlinearly. One of the most common is pharmacokinetics. PROC NLMIXED fits nonlinear mixed models by maximizing an approximation to the likelihood integrated over the random effects. Different integral approximations are available to maximize the likelihood function.

- First-order Taylor series approximation (like NONMEM).
- Gaussian quadrature (numerical integration).
- Importance sampling (Monte-Carlo integration).

Successful convergence of the optimization problem results in parameter estimates along with their approximate standard errors based on the second derivative matrix of the likelihood function. PROC NLMIXED enables you to use the estimated model to construct predictions of arbitrary functions using empirical Bayes estimates of the random effects. You can also estimate arbitrary functions of the nonrandom parameters,
and PROC NLMIXED computes their approximate standard errors using the delta method. PROC NLMIXED uses SAS datasets directly, and pulls out appropriate statistics by ODS. An example of NLMIXED application is below.

```
p, nlmixed data=theoph;
  parms beta1=-3.22 beta2=0.47 beta3=-2.45 s2b1=0.03 cb12=0 s2b2=0.4 s2=0.5;
  cl = exp(beta1 + b1);
  ka = exp(beta2 + b2);
  ke = exp(beta3);
  pred = dose*ka*ke*(exp(-ke*time)-exp(-ka*time))/cl/(ka-ke);
  model conc ~ normal(pred,s2);
  random b1 b2 ~ normal([0,0],[s2b1,cb12,s2b2]) subject=subject;
run;
```

**POPULATION MODEL DEVELOPMENT**

1. **Exploratory Data Analysis**

Exploratory data analysis isolates and reveals patterns and features in the population data set using graphical and statistical techniques. It also serves to uncover unexpected departures from familiar models. An important element of the exploratory approach is its flexibility, both in tailoring the analysis to the structure of the data and in responding to patterns that are uncovered by successive analysis steps. Most population PK analysis procedures are based on explicit assumptions about the data, and the validity of the analyses depends upon the validity of assumptions. Exploratory data analysis techniques provide powerful diagnostic tools for confirming assumptions or, when the assumptions are not met, for suggesting corrective actions. Exploratory data analysis should be coupled with more sophisticated population modeling techniques. SAS programmer's role Produces summary statistics for the parameters calculated by PK program Produces summary statistics for concentration-time profiles and urinary excretion data Produces tables, listings and figures/graphs (TLFs or TLGs) for individual patient and summary data.

2. **Population PK Modeling**

Non-linear model describes change in response across repeated measures (time, concentration, dose etc.). The sources of variability in non-linear model includes within subjects (model misspecification, measurement error) and between subjects (differences between subjects covariates, possible influence of unmeasured covariates). Non-linear mixed effects modeling fits the best model to the population as a whole, and also can incorporate covariate values to account for systematic differences between groups of patients (e.g. bodyweight, gender, genotype, other covariates).

The steps taken to develop a population model should be outlined clearly in the population analysis report to permit the reproducibility of the analysis. The criteria and
rationale for model building procedures dealing with confounding, covariate, and parameter redundancy should be stated clearly. The criteria and rationale for model reduction to arrive at the final population model should be explained clearly.

• Define the PK/PD structural model
• Define the preliminary random error models (intersubject, random residual error)
• Determine initial estimates of all parameters (for structural and error models)
• Estimate parameters - no covariates
• Estimate the parameters for each individual
• Determine initial parameter-covariate relationships (SAS, S-Plus), covariate selection.
• Determine the final model by adding covariates (stepwise addition and deletion)
• Evaluate the population model: For obtaining parsimonious nonlinear kinetic models one has used the Akaike criterion which penalizes the likelihood by the number of parameters and the Schwartz criterion which penalizes the likelihood by the number of parameters multiplied by the square root of the number of observations. Influence of single measurements and single individuals can be investigated by importance sampling and a sensitivity analysis using MCMC

3. Model Validation
The objective of model validation is to examine whether the model is a good description of the validation data set in terms of its behavior and of the application proposed. Validation can be defined as the evaluation of the predictability of the model developed

A. Diagnostic plots
(1). Predicted vs. Observed Concentrations plot gives an overall sense of the goodness of fit, the concentration-time points should fall randomly (without bias) and closely (precisely) to the line of unity throughout the concentration range.

(2). Weighted Residuals vs. Predicted Concentrations plots have a unit variance and are uncorrelated, the statistical and structural models are “removed” – any pattern observed in these plots is definitely not accounted for by the current model (should be no bias or trend), if residual error model is adequate, should have equal and random distribution of points at throughout the prediction range.

(3). Observed and Predicted Concentrations vs. Time plot allows the user to see if the predicted concentrations are similar to the observed concentrations over time

(4). Weighted Residuals vs. Time plot allows the user to see if the discrepancies change over time, the weighted residuals should be random over time

(5). Plotting residuals against covariates is a method related to the prediction errors on concentration approach; the method differs in the sense that no statistic is computed and no statistical tests are performed. A simple plot of residuals obtained by freezing the final model and predicting into a validation data set against covariates can yield information on the clinical significance of the model in terms of a covariate or subpopulation.

B. Model Evaluation
(1). Data-splitting is a useful internal validation technique for creating a validation data set to test the predictive performance of a model when it is not practical to collect new data to be used as a validation data set. A random subset of the data (two-thirds, i.e., the index data set) should be used for model building, and the remaining data should be used for model validation. The disadvantage of data-splitting is that, in general, the predictive accuracy of the model is a function of the sample size resulting from the data-splitting. To maximize the predictive accuracy of data-splitting, it is recommended that the entire sample be used for model development and assessment. Data-splitting may not validate the final. Another technique of internal validation is re-sampling. There are two ways to perform re-sampling: cross-validation and bootstrapping. The bootstrap approach may be appropriate. Under that approach, the mean parameter values obtained by repeatedly fitting the final population model to a reasonable number of bootstrap replicates (e.g., at least 200 bootstrap replicates) are compared to the final population model parameter estimates obtained without bootstrap replication. Alternatively, cross-validation can be used though it is sometimes inefficient.

(2). External evaluation is another type of evaluation, which is driven by the expected use of model, such as, the application of the developed model to a new data set from another study.

Because the maximum likelihood procedure is sensitive to bizarre observations, the stability of the model should be checked. It is important to evaluate the quality of the results of a population PK study or analysis for robustness. Evaluation for robustness can be approached using sensitivity analysis and leverage analysis; the use of case deletion diagnostics is also encouraged. Evidence of robustness demonstrates that the results are reasonable and independent of the analyst.

**DISCUSSION**

The nonlinear mixed-effects modeling approach is especially helpful in certain adaptive study designs, such as dose-ranging studies (e.g., so called titration, or effect controlled designs). Population modeling is most likely to add value when a reasonable a priori expectation exists that inter-subject kinetic variation may warrant altered dosing for some subgroups in the target population. The population PK approach can be used to estimate population parameters of a response surface model in phase 1 and late phase 2b of clinical drug development, where information is gathered on how the drug will be used in subsequent stages of drug development. The population PK approach can increase the efficiency and specificity of drug development by suggesting more informative designs and analyses of experiments. In phase 1 and, perhaps, much of phase 2b, where patients are sampled extensively, complex methods of data analysis may not be needed. Two-stage methods can be used to analyze the data, and standard regression methods can be used to model dependence of parameters on covariates. Alternatively, data from individual studies in phases 1 and 2b can also be pooled and analyzed using the nonlinear mixed-effects modeling approach.
The population PK approach can also be used in early phase 2a and phase 3 of drug development to gain information on drug safety (efficacy) and to gather additional information on drug pharmacokinetics in special populations, such as the elderly. This approach can also be useful in post-marketing surveillance (phase 4) studies. Studies performed during phases 3 and 4 of clinical drug development lend themselves to the use of a full population pharmacokinetic sampling study design (few blood samples drawn from several subjects at various time points. This sampling design can provide important information during new drug evaluation, regulatory decision making, and drug labeling.

Physiologically-Based PK Model (PBPK) is a drug kinetics in terms of the physiology, anatomy and biochemistry of the organism and are composed of compartments which represent body organs and tissues. Further assumptions concern drug uptake, clearance and allometric scaling. The body compartments are linked together by a flow network. A PBPK model is defined by a system of deterministic kinetic equations (mass balance equations) of the amount or the concentration of the drug in the compartments as a function of time and initial dose. PBPK models are more complex than compartment models and they involve usually a large number of parameters.

APPLICATIONS

Population PK modeling for metoprolol confirmed CYP2D6 poor metabolizers (PM) have significantly lower CL/F as compared to extensive metabolizers (EM) and smokers have significantly larger V/F as compared to nonsmokers. Population PK/PD modeling, confirmed that CYP2D6 phenotype and smoking do not alter the EIHR effect of metoprolol. The predictive ability of population PK/PD models may be assessed by the posterior predictive check (PPC) approach. Classification of subjects by population mixture model helps in detecting subpopulations not explained by known covariates.

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