Development of a population PK model using

NONMEM® - Case Study I

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[Creating a data file](#)

The comma delimited data file consists of data obtained from 100 individuals who were randomized to either 100 or 250mg dose. A sample NONMEM data file for this study looks the following:

C	One compartment intravenous bolus study									
C	No. of subjects= 100, Dose = 100 or 250mg,DV=Plasma concentration, ug/ml									
C	Time = hrs, CL = L/hr, V=L									
CID	TIME	CONC	AMT	DOSE	MDV	AGE	WT	SCR	ISM	CLCR
1	0	0	100	100	1	34.823	38.212	1.1129	0	42.635
1	0.25	13.026	0	100	0	34.823	38.212	1.1129	0	42.635
1	0.5	14.984	0	100	0	34.823	38.212	1.1129	0	42.635
1	0.75	14.16	0	100	0	34.823	38.212	1.1129	0	42.635
1	1	19.316	0	100	0	34.823	38.212	1.1129	0	42.635
1	1.5	13.146	0	100	0	34.823	38.212	1.1129	0	42.635
1	2	12.921	0	100	0	34.823	38.212	1.1129	0	42.635
1	2.5	8.485	0	100	0	34.823	38.212	1.1129	0	42.635
1	3	16.437	0	100	0	34.823	38.212	1.1129	0	42.635
1	4	10.724	0	100	0	34.823	38.212	1.1129	0	42.635
1	6	8.7352	0	100	0	34.823	38.212	1.1129	0	42.635
1	8	7.697	0	100	0	34.823	38.212	1.1129	0	42.635
1	12	4.479	0	100	0	34.823	38.212	1.1129	0	42.635
1	16	2.4183	0	100	0	34.823	38.212	1.1129	0	42.635
1	20	4.7586	0	100	0	34.823	38.212	1.1129	0	42.635
1	24	2.3655	0	100	0	34.823	38.212	1.1129	0	42.635
2	0	0	100	100	1	32.765	74.838	0.8846	1	126
2	0.25	7.4203	0	100	0	32.765	74.838	0.8846	1	126

Explanation of the data file columns

Column name	Description	Required or optional
ID	subject identification number. It is written as CID, where C = default NONMEM recognized symbol for ignoring any data from data file.	Required item
TIME	Blood sampling time. Should be chronologically arranged.	Required
CONC = DV	Plasma concentration in this case, could be any dependant variable i.e., biomarker concentration or tissue concentration	Required
AMT	Dose administered at dosing time or zero for observation records	Required
DOSE	Dose administered for each patient, included for graphical requirements	User defined - Optional
MDV	Missing dependant variable, takes value as 1, when observation is missing , otherwise zero.	Optional
AGE, WT, ISM, CLCR	Covariate information for each indidvidual. AGE = age in yrs, WT= Weight in kg, ISM = is male (1), female =0, CLCR=Creatinine clearance = ml/min	User defined - Optional

The data file created is stored as .csv file in any working directory for the project or in the same folder as the control stream.

Click here for link to the data file

[Control stream for base model](#)

The control stream (with explanations for each line) for the structural model, which is a one-compartment i.v bolus model based on preliminary data analysis, is given below. Analysis in NONMEM® was performed by using a first order (FO) estimation method. Results from other estimation methods namely, first order conditional estimation (FOCE) and FOCE with interaction are also provided in the end for comparison.

[Click here for the control stream](#) (Download the control stream, copy the contents in Notepad and save it in the working directory with a .ctl extension)

[Performing the NONMEM run](#)
- NONMEM execution was done using Wings for NONMEM (WFN) developed by Dr.Nick Holford, available at http://wfn.sourceforge.net/ WFN is a set of DOS batch command files and awk scripts. The various features of WFN are described at the above mentioned website.
- Click the command prompt icon configured for WFN (This could be done when installing WFN) and open the working directory (The path could also be stored).
  - When using WFN, Type nmgo XXXX (The name of the control stream, No need to type the .ctl extension) to perform the NONMEM run.

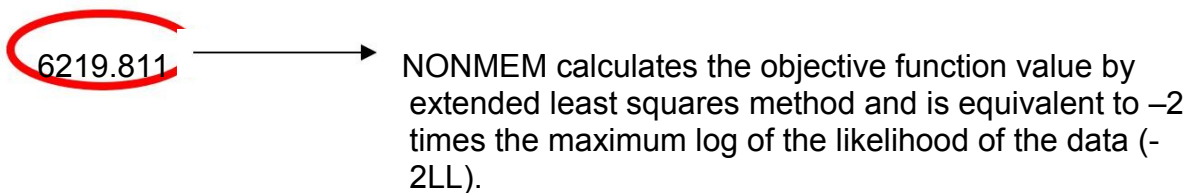[Viewing and interpreting the output file](#)
- When each control stream is run, an output folder is created in the same working directory from where the NONMEM run was performed.

- In the output folder, there is a summary file with a **.smr** extension, a detailed output file with **.lst** extension and the table output in a **.fit** extension.

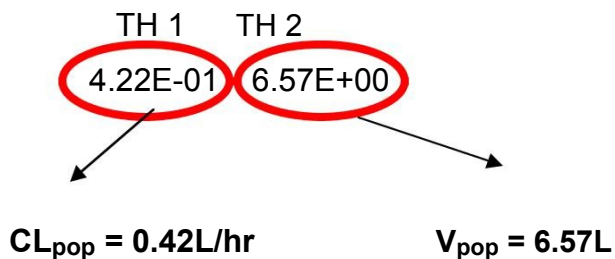For this case study, the output from the **.lst** file is explained and interpreted. Diagnostic plots are created using the table output with a **.fit** extension using S-Plus®.

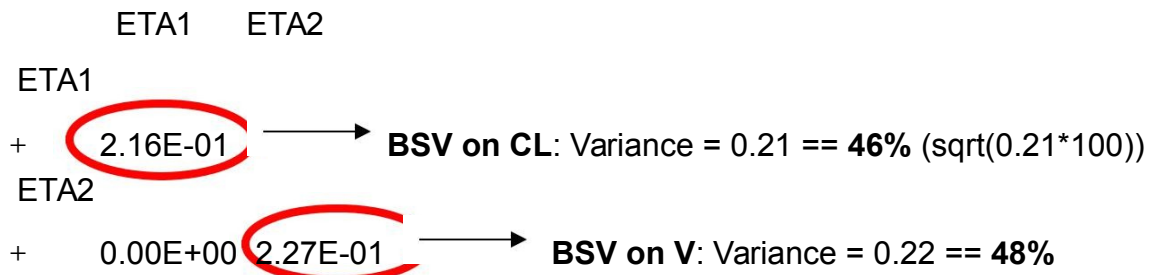## MINIMUM VALUE OF OBJECTIVE FUNCTION

6219.811 $\longrightarrow$ NONMEM calculates the objective function value by extended least squares method and is equivalent to –2 times the maximum log of the likelihood of the data (-2LL).

FINAL PARAMETER ESTIMATE OF BASE MODEL

THETA - VECTOR OF FIXED EFFECTS PARAMETERS

TH 1   TH 2

4.22E-01 6.57E+00

CL_{pop} = 0.42L/hr   **V_{pop} = 6.57L**

OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS

    ETA1   ETA2

ETA1

+   2.16E-01 $\longrightarrow$ **BSV on CL**: Variance = 0.21 == **46%** (sqrt(0.21*100))

ETA2

+   0.00E+00 2.27E-01 $\longrightarrow$ **BSV on V**: Variance = 0.22 == **48%**

NONMEM takes the random effects as variance and not as standard deviation. The BSV parameter given by NONMEM is omega and should not be confused

with ETA. Similarly, the residual error is sigma and not to be confused with epsilon.

SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS

EPS1 EPS2

EPS1

+ 2.08E-02 ⟶ **WSV (Proportional error)**: variance = 0.02 == 14%

EPS2

+ 0.00E+00 7.99E+00 ⟶ **WSV (Additive error)**: variance = 7.99

= 2.82ug/ml

Diagnostic plots for structural model and interpretation

The first plot to look at would be a plot of concentration (observed (DV), individual predicted (IPRED) and population predicted (PRED)) versus time for all the individuals. NONMEM obtains IPRED values by the Bayesian POSTHOC option. Using the population mean estimate of parameters (prior) and each individual data (likelihood), NONMEM obtains the individual parameter estimates (posterior). From the individual parameter estimates, individual predicted concentrations (IPRED) are obtained. A representative plot of few individuals is provided below. This plot would give an overall trend of fitted concentrations. It could be seen that for certain individuals the population predictions are underpredicted or overpredicted.
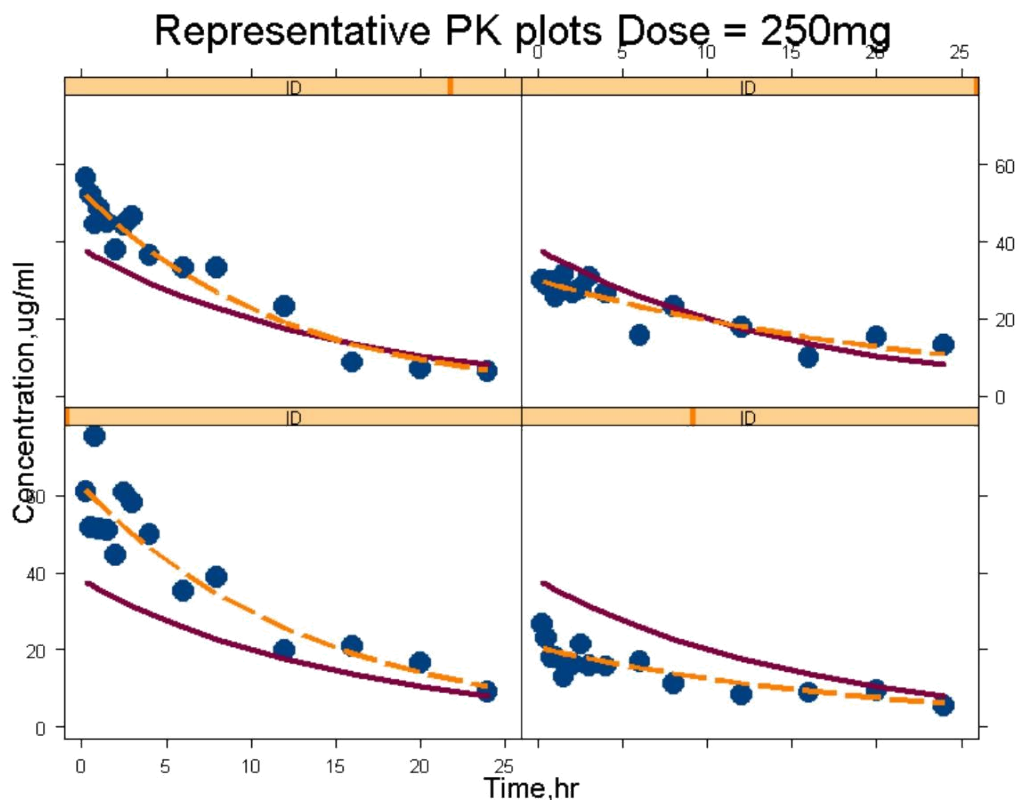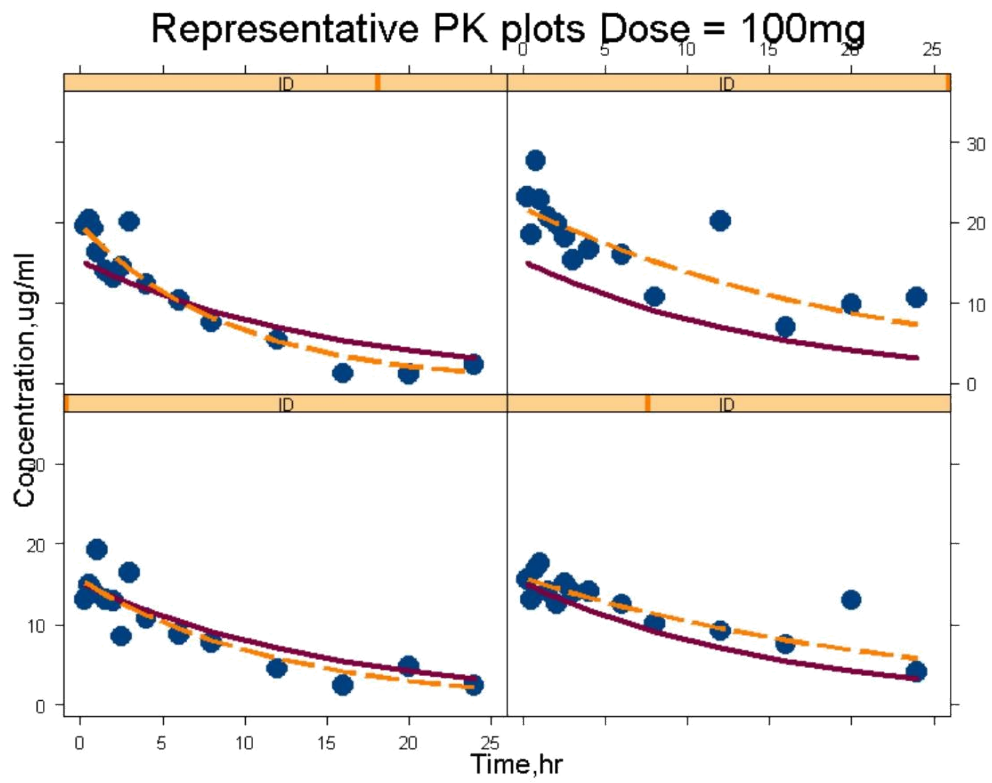
Representative PK plots Dose = 100mg

Representative PK plots Dose = 250mg

Figure 1: Plot of observed (•), individual predicted (-----)and population predicted (-----) concentration versus time.

The second set of diagnostic plots would be the following.

- · DV vs IPRED and DV vs PRED: These graphs could be looked for any bias in the predictions. Ideally the points should be uniformly distributed along the line of identity. Normally DV vs IPRED is much better than DV vs PRED as PRED contains unexplained variability. Here we could see that PRED is biased at higher observed concentrations. The reason for it needs to be explored.

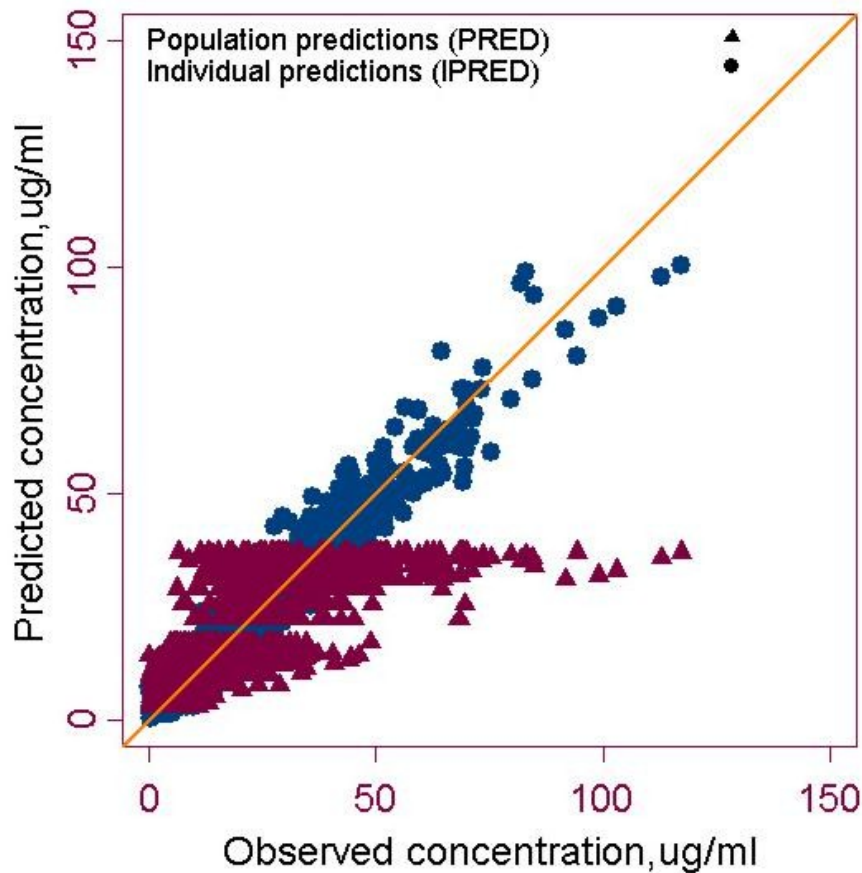## Observed vs predicted concentrations - PK

Figure 2a: Goodness of fit plots (• - Observed vs individual predicted concentration, ▲ - Observed vs population predicted)

- RES vs PRED and WRES vs PRED: Residual plots are looked for any unaccounted heterogenity in the data.

- Note: Though residual plots are some of the goodness of fits plot explored, the utility of these residual plots is not well documented.
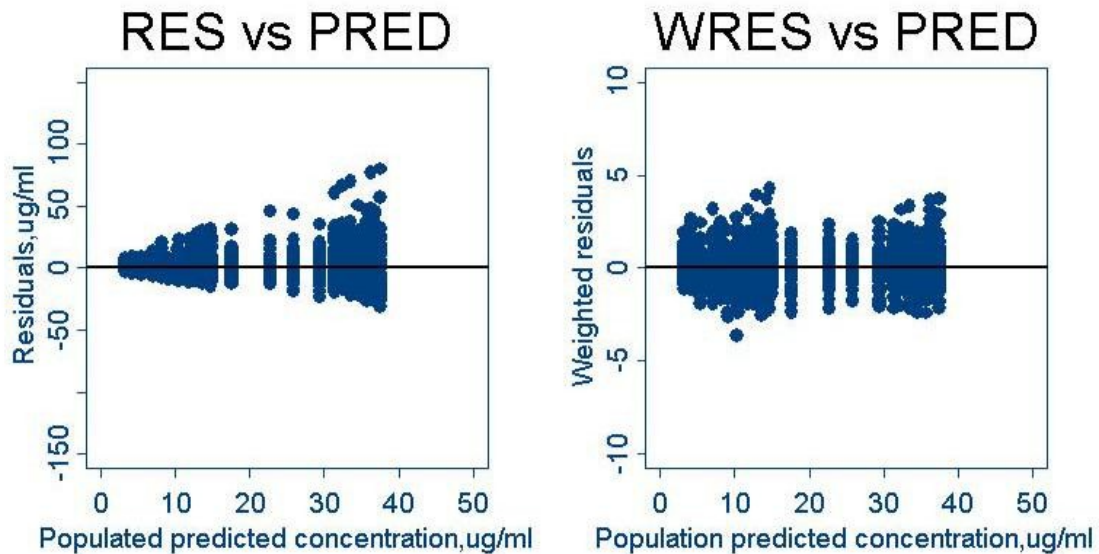

Figure 2b: Goodness of fit plots (Residuals & Weighted residuals vs Population predicted concentration)

As the DV vs PRED plots look biased, it would be necessary to explore covariate parameter relationship. According to background information of the drug, the drug is said to be renally eliminated and a general range of CLCR values from the data is from 20 to 130 ml/min.
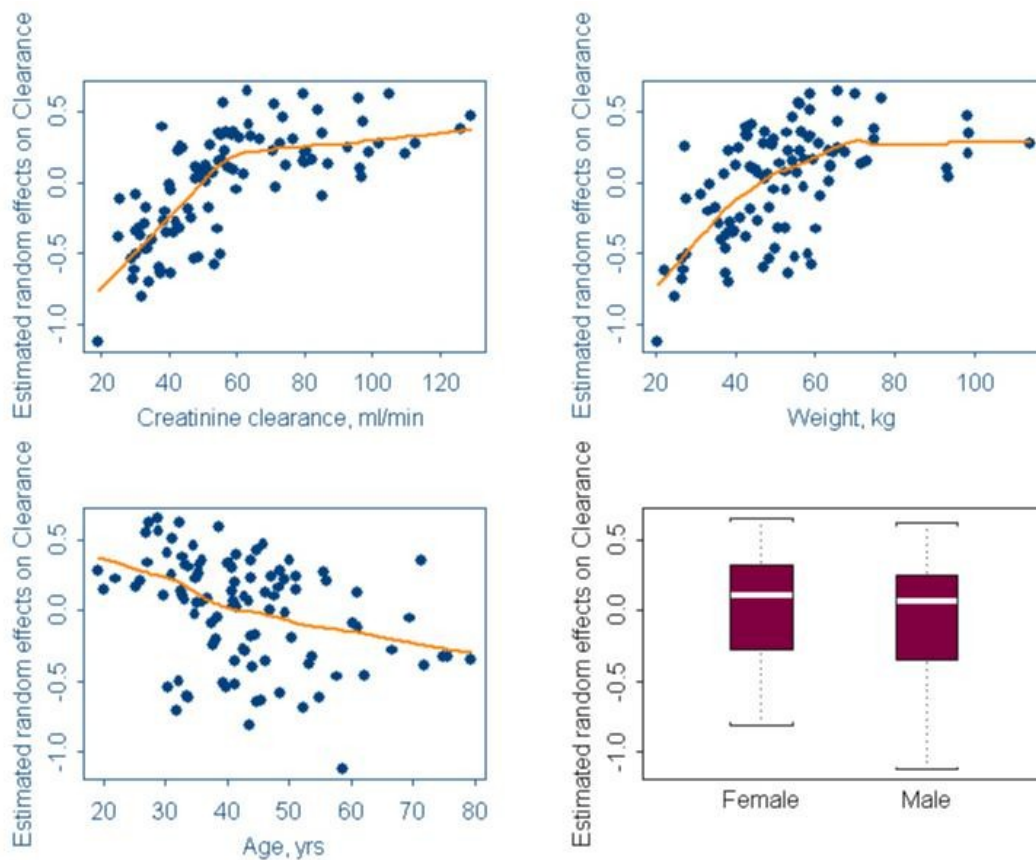

Figure 3: Empirical Bayes estimates of η_{CL} ((BSV$_{CL}$:ETA1) versus covariates (Age, weight and CLCR, gender)

It could be seen from the graph that η_{CL} and CLCR have a correlation, which is expected in our case and needs to be explored in the covariate model.

Correlation between age and CLCR and Weight and CLCR is expected, but since one of the correlational covariates (CLCR) based on pre-existing physiological reasons will be explored, there is no need to explore weight and age as covariates for clearance.

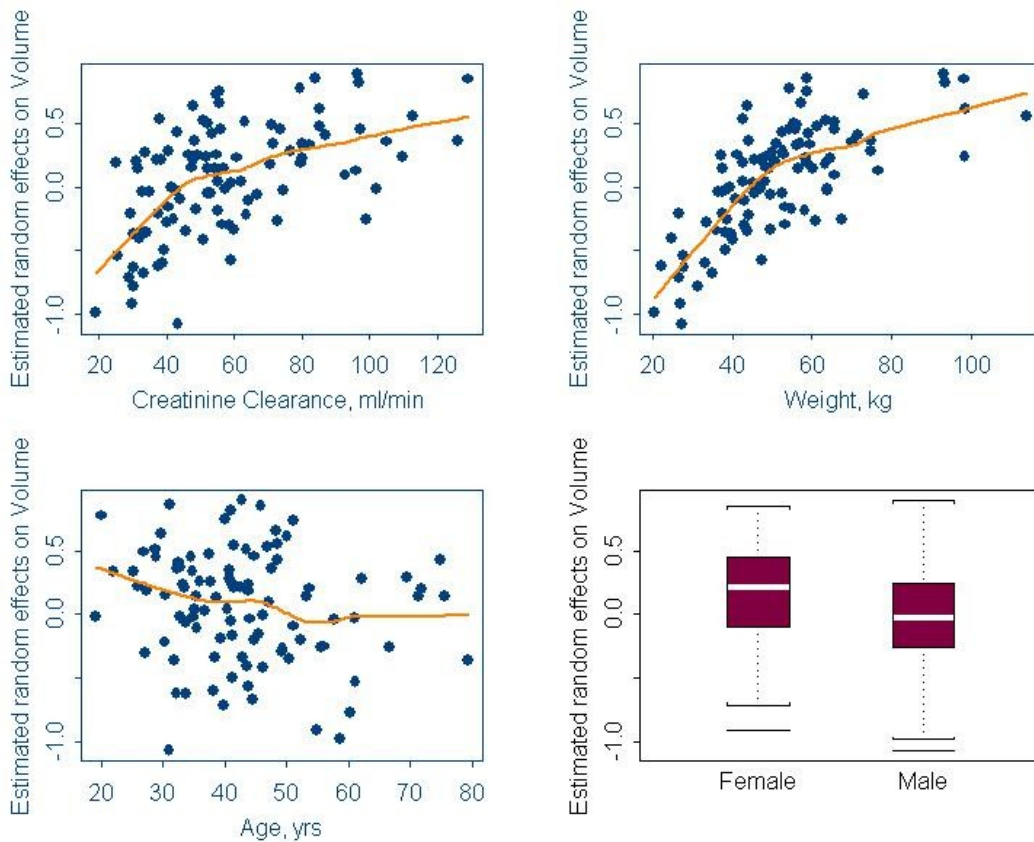Similarly a scatter plot of η_V and the covariates needs to be plotted.

Figure 4: Empirical Bayes estimates of EtaV ((BSV$_V$:ETA2) versus covariates (Age, wt and CLCR and gender)

In the case of volume, based on existing clinical reasoning, weight could be an influential covariate and needs to be explored in the covariate model. Although in the scatter plot, ηV and CLCR looks to have some relation, it does not carry any physiological meaning.

Covariate model

Based on graphical analysis, in our covariate model we will include CLCR as a covariate on CL and weight as a covariate on V. The covariate model with explanation is shown below.

Click here for control stream for covariate model

Covariate model results and interpretation

Again, the results are obtained from *.lst* file of the output folder.

MINIMUM VALUE OF OBJECTIVE FUNCTION

5927.895 ⟶ Objective function value decreased from the base model by 291.0 points. A log-likelihood ratio (LLR) (See the theory section for more details) test is used to compare between base and covariate model (nested models). Here the inclusion of CLCR as a covariate on CL and weight as a covariate on V are significant (p<0.001).

PARAMETER ESTIMATES FROM FINAL MODEL (COVARIATE MODEL)

THETA - VECTOR OF FIXED EFFECTS PARAMETERS

TH 1   TH 2   TH 3
9.05E-01 8.69E-01 9.76E+00

CL_{pop} = 0.9L/hr V_{pop} = 9.8L

THETA (2) = A shape parameter which explains the relationship between CL and CLCR according to the equation used in the model.

$$CL = CL_{pop}*(CLCR/120)**THETA(2)$$

OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS

            ETA1   ETA2

ETA1

+   6.32E-02   ⟶   **BSV on CL**: 25% (decreased from 46%)

ETA2

+   0.00E+00   8.15E-02   ⟶   **BSV on V**: 28% (decreased from 48%)

Inclusion of significant covariates namely CLCR and Weight has decreased the unexplained variability in the data.

SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS

          EPS1   EPS2

EPS1

+   1.60E-02

EPS2   Same as before (Page no: 4)

+   0.00E+00   8.22E+00

STANDARD ERROR ESTIMATE FOR PARAMETERS

Inclusion of $COVARIANCE in the control stream calculates the precision of each parameter estimate. Larger the standard error is, less precise is the estimate. Standard errors are used to compute the 95% confidence intervals for the parameters. The output for standard errors is also found in the *.lst* file. **Standard error should not be confused with BSV. Even BSV has a standard error.**

THETA - VECTOR OF FIXED EFFECTS PARAMETERS

TH 1	TH 2	TH 3
6.92E-02	7.61E-02	3.33E-01

Relative standard error (RSE) as reported by NONMEM is calculated as:

%RSE for CL = (SE/Final parameter estimate)*100 = 0.0692/0.905*100 = 7.6%

Similarly for shape parameter and Volume,
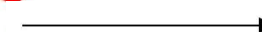
%RSE for Shape parameter = 0.0761/0.869*100 =

8.7% %RSE for V = 0.33/9.76*100 = 3.3%

OMEGA - COV MATRIX FOR RANDOM EFFECTS - ETAS

	ETA1	ETA2
ETA1		
+	1.21E-02	
ETA2		
+	.........1.06E-02	

%RSE for ηCL = 0.012/0.0632*100 = 19%

SIGMA - COV MATRIX FOR RANDOM EFFECTS - EPSILONS ****

	EPS1	EPS2
EPS1		
+	1.71E-03	
EPS2		
+	.........5.82E-01	

1. An important plot to look at would be the plot of DV vs PRED and IPRED.

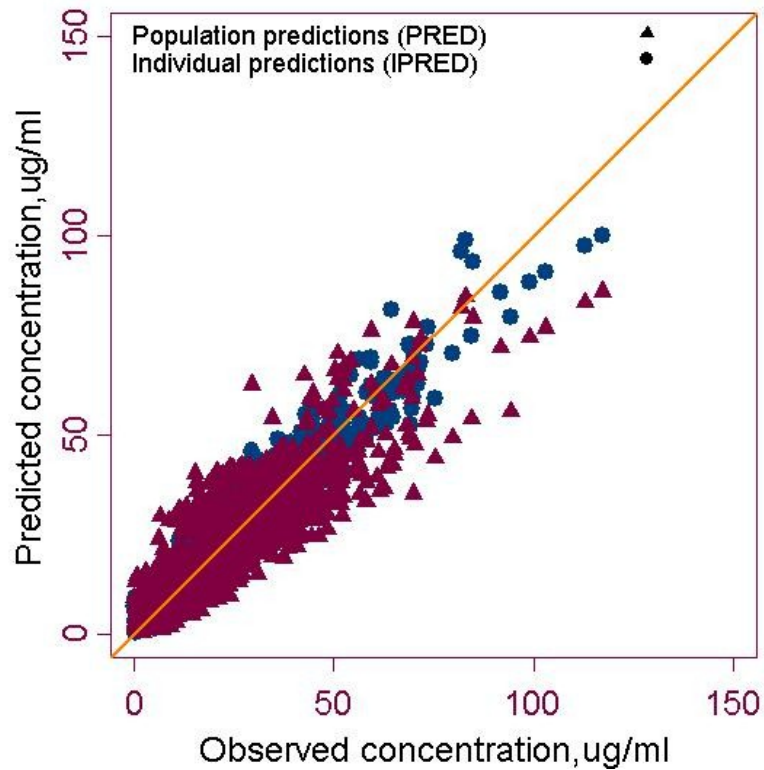## Observed vs predicted concentrations - PK

Figure 5: Goodness of fit plots after covariate modeling (DV – Observed concentration, PRED-Population predicted, IPRED – Individual predicted)

It could be seen that DV vs PRED shows uniformly distributed points on either sides of lines of identity. The bias seen before (see Fig 2) is not seen here.

2. Scatter plot of eta's of CL and V versus the covariates

Please note that ETACL versus CLCR and ETAV versus weight does not have any trend.
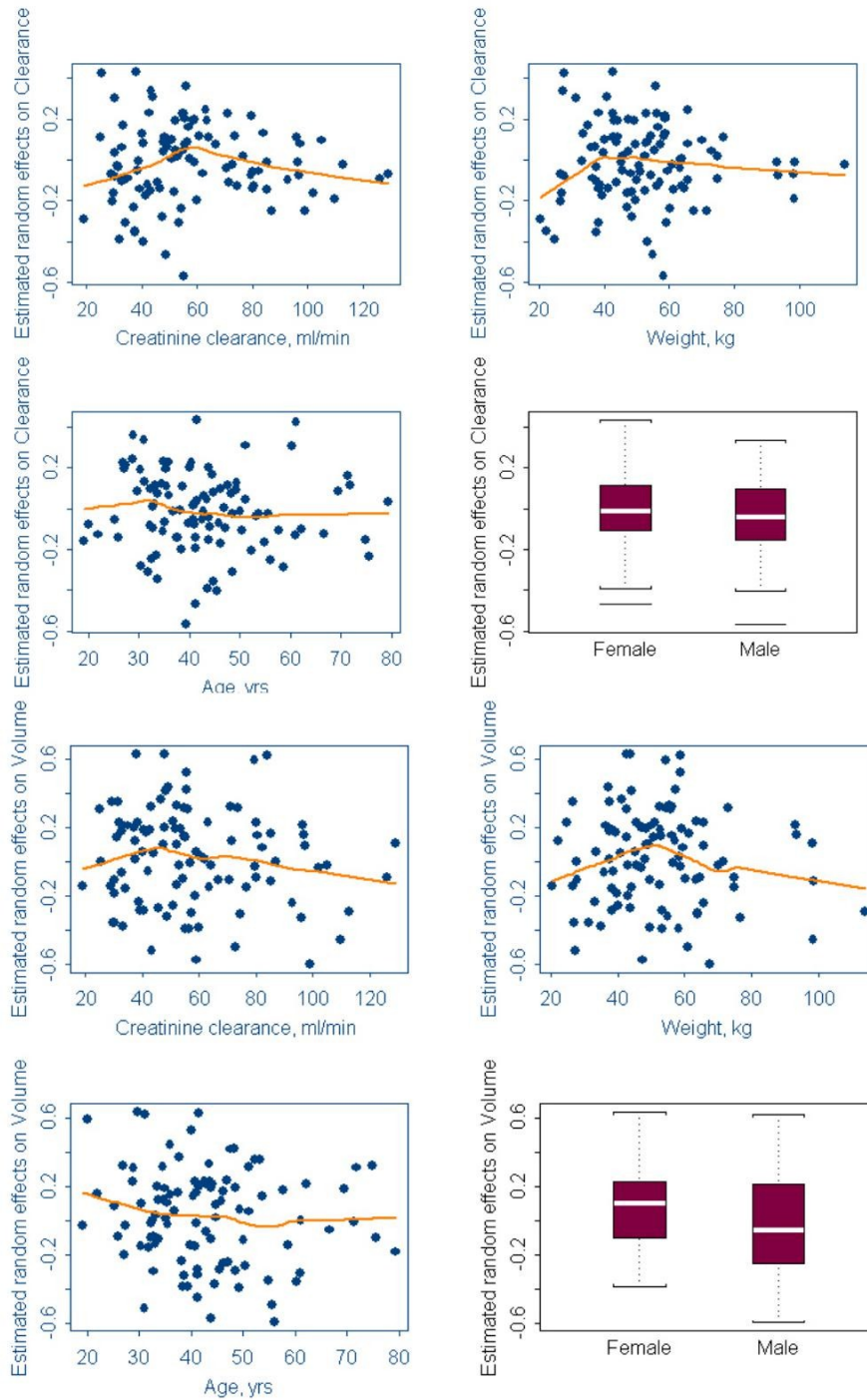
No trend seen, compare with Fig3

Compare with fig 4

Figure 6 : Empirical Bayes estimates of EtaCL & EtaV versus covariates (Age, wt and CLCR and gender) after covariate modeling.

The results obtained could be compiled and documented according to the user's documentation procedure. Below, the results obtained from by first order (FO) approximation are provided.

Model	OFV	Population estimate (%SE)	Between subject variability (%SE)
Base model iv 1 cmt $CL = CL_{pop} \cdot \eta CL$ $V = V_{pop} \cdot \eta V$	6219.81		
Final model iv 1 cmt with covariates $CL = CL_{pop} \cdot (CLCR/120)^{\theta 2} \cdot \eta CL$ $V = V_{pop} \cdot (WT/70) \cdot \eta V$	5927.89		
CL (L/hr)		0.90 (7.6)	25 % (19)
V (L)		9.8 (3.3)	28 % (13)
Residual variability			
Proportional error	12% (10)		
Additive error	2.86 ug/ml (7)		

Results from FOCE approximation in NONMEM®

Model	OFV	Population estimate (%SE)	Between subject variability (%SE)
Base model iv 1 cmt $CL = CL_{pop} \cdot \eta CL$ $V = V_{pop} \cdot \eta V$	6109.70		
Final model iv 1 cmt with covariates $CL = CL_{pop} \cdot (CLCR/120)^{\theta 2} \cdot \eta CL$ $V = V_{pop} \cdot (WT/70) \cdot \eta V$	5890.18		
CL (L/hr)		0.87 (6.6)	24 % (17.9)
V (L)		10.1 (3.1)	29 % (13.5)
Residual variability			
Proportional error	12.8% (11)		
Additive error	2.83 ug/ml (7.2)		

Note: Objective function value should not be compared between different estimation methods. (FO vs FOCE vs FOCE-inter)

Results from FOCE with interaction approximation in NONMEM®

Model	OFV	Population estimate (%SE)	Between subject variability (%SE)
Base model iv 1 cmt $CL = CL_{pop} \cdot \eta CL$ $V = V_{pop} \cdot \eta V$	6046.46		
Final model iv 1 cmt with covariates $CL = CL_{pop} \cdot (CLCR/120)^{\theta 2} \cdot \eta CL$ $V = V_{pop} \cdot (WT/70) \cdot \eta V$	5887.59		
CL (L/hr)		0.88 (6.6)	23.1 % (17.4)
V (L)		10.2 (3.0)	29.3 % (13.6)
Residual variability			
Proportional error	12.1% (10.8)		
Additive error	2.92 ug/ml (6.9)		

Appendix –I

S-Plus codes for generating some of the graphs shown in the tutorial are provided below:

```
#Case Study -1, ACCP website tutorial
cs1_iv1estpar<-importData(file="C:\\.. location\\cs1_iv1est_par.fit", type="ASCII",
startRow=2, colNameRow=2)
cs1iv1estpar<-cs1_iv1estpar[cs1_iv1estpar$TIME>0,]         #To remove time=0

##Select a few ID and plot the individual plot
cs1iv1estind<-
cs1iv1estpar[cs1iv1estpar$ID==1|cs1iv1estpar$ID==15|cs1iv1estpar$ID==90|cs1iv1estpar$ID==
95,] # select few ID's
graphsheet()
par(mar=c(5,5,4,4))
xyplot(CONC~TIME|ID, data=cs1iv1estind,xlim=c(0,25),ylim=c(0,35), xlab="", ylab="",
                panel=function(x,y){
              panel.xyplot(x,y,cex=2,pch=16,col=2)})
title(main="Representative PK plots", xlab="Time,hr", ylab="Concentration,ug/ml",
cex=1.3)
par(new=T,xaxs="d") #To overlay PRED in the same plot
xyplot(PRED~TIME|ID, data=cs1iv1estind, xlab="",ylab="",xlim=c(0,25),ylim=c(0,35),
                panel=function(x,y){
              panel.xyplot(x,y,cex=2,type="l",lty=1,lwd=5,col=3)})
par(new=T,xaxs="d")    #To overlay IPRED in the same plot
xyplot(IPRE~TIME|ID, data=cs1iv1estind, xlab="",ylab="",xlim=c(0,25),ylim=c(0,35),
                panel=function(x,y){
              panel.xyplot(x,y,cex=2,type="l",lty=4,lwd=5,col=5)})
export.graph(FileName = "C:\\..location.. \\indgood.jpg", Name = "GSD2",ExportType
="JPG")  #Export graph function

# To create DV,PRED,IPRED vs Time plot
graphsheet()                   #create a new graphsheet
par(mar = c(5, 5, 4, 4), cex = 1.5, pty="s") ##plot area(pty="s")
plot(cs1iv1estpar$CONC,cs1iv1estpar$IPRE,xlab="", ylab="",xlim=c(0,150), ylim=c(0,150),
col=2,pch=16,cex=1.5)
par(new=T,xaxs="d")
plot(cs1iv1estpar$CONC,cs1iv1estpar$PRED, xlab="", ylab="", xlim = c(0,150), ylim
=c(0,150),col=3,pch=17,cex=1.5)
abline(0,1, lwd = 3, col = 5)
title(main="Observed vs predicted concentrations - PK",xlab="Observed
concentration, ug/ml", ylab="Predicted concentration,ug/ml", cex=1.5)
key(text = c("Population predictions (PRED)", "Individual predictions (IPRED)"),
points = list(pch = list(17, 16)), corner = c(0, 1), cex = 1.15)
```

```
export.graph(FileName = C:\\.. location…\\predvsobsgood.jpg", Name = "GSD2",ExportType
="JPG")

#Plot of random effects versus covariates
par(mfrow=c(2,2), mar = c(5,5,4,4))
plot(cs1iv1estpar$CLCR,cs1iv1estpar$ETA1, xlab="Creatinine clearance, ml/min",
ylab = "Estimated random effects on Clearance",col=2, pch = 16, cex =1.0)
lines(lowess(cs1iv1estpar$CLCR, cs1iv1estpar$ETA1), col = 5, lwd = 3)
plot(cs1iv1estpar$WT,cs1iv1estpar$ETA1, xlab="Weight, kg", ylab = "Estimated
random effects on Clearance", col=2, pch = 16, cex = 1.0)
lines(lowess(cs1iv1estpar$WT, cs1iv1estpar$ETA1), col = 5, lwd = 3)
plot(cs1iv1estpar$AGE,cs1iv1estpar$ETA1, xlab="Age, yrs", ylab = "Estimated random
effects on Clearance", col=2, pch = 16, cex = 1.0) lines(lowess(cs1iv1estpar$AGE,
cs1iv1estpar$ETA1), col = 5, lwd = 3)
boxplot(split(cs1iv1estpar$ETA1,cs1iv1estpar$ISM),names=c("Female","Male"),
ylab = "Estimated random effects on Clearance", cex = 1.0)
export.graph(FileName = "C:\\.. location ….\\covvsrandeffcl.jpg", Name =
"GSD2",ExportType ="JPG")
```