Ralph, Lorraine D. (2004) *The influence of hepatic function on the pharmacokinetics and dose requirements of epirubicin.*

PhD thesis

http://theses.gla.ac.uk/3956/

Copyright and moral rights for this thesis are retained by the author

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

This thesis cannot be reproduced or quoted extensively from without first obtaining permission in writing from the Author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the Author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Glasgow Theses Service
http://theses.gla.ac.uk/
theses@gla.ac.uk

The influence of hepatic function on the pharmacokinetics and dose requirements of epirubicin

by

Lorraine D Ralph BSc(Hons) MSc

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow

Department of Medicine and Therapeutics
Department of Medical Oncology

May 2004

DECLARATION

I declare that this thesis has been written by myself and is a record of work performed by myself. It has not been submitted previously for a higher degree. The research was carried out in the Departments of Medicine and Therapeutics and Medical Oncology under the supervision of Doctor Alison Thomson and Doctor Chris Twelves.

May 2004 L.D. Ralph

SUMMARY

Epirubicin is a cytotoxic anti-cancer agent that is mainly used in the treatment of breast cancer. Epirubicin is predominantly cleared by the liver and as many patients with breast cancer also have liver metastases, it is necessary to reduce the dose in these patients to avoid toxicity. The current UK dosage guidelines recommend that epirubicin is administered according to the body surface area (BSA) of the patient and their bilirubin concentration. However, one study found no significant correlation between epirubicin clearance and bilirubin and several studies have found no relationship between any pharmacokinetic parameter and BSA. A survey that included 173 UK oncologists found that many oncologists do not follow the current UK dosage guidelines for epirubicin. The aims of this thesis were to develop new dosage guidelines for epirubicin.

In this thesis, a population analysis performed using a UK data set, including 109 patients with breast cancer treated with single-agent epirubicin, identified aspartate aminotransferase (AST) as the only covariate that had a clinically significant influence on epirubicin CL. The population model for epirubicin CL was as follows: CL (L/h) = 72.9 x (1- (0.135 x (ln AST)). Inclusion of AST reduced the inter-individual variability in CL from 49 to 39 %.

Internal techniques recommended in the FDA guidelines to validate population models, including sensitivity analysis, jacknife analysis and cross validation, were used to assess the stability and robustness of the population model. The results of these analyses found the model to be stable, not based on outliers or spurious data and suggested favorable predictive performance of CL in patients with AST above 150 U/L.

Using the population model, new dosage guidelines were proposed to achieve a target AUC of 4000 ng.h/ml, as identified from the literature. The following doses were predicted to achieve this exposure with the greatest precision: AST<150 U/L = 125 mg; AST 150-250 U/L = 90 mg; AST 250-500 U/L = 60 mg; AST> 500 U/L = 30 mg. In the UK data set, the new guidelines achieved the target with greater precision (root mean squared error (rmse) = 39.0 %) than the current UK guidelines, current USA guidelines or an earlier equation based on AST (rmse = 63 %, 62 % and 59 %, respectively). Furthermore, as the

proposed dosing guidelines do not require adjustment according to BSA, they could reduce dosage preparation time and minimise the potential for prescribing and dispensing errors.

The most stringent test for the validity of a population model is to measure the predictive performance of that model using a different data set. This is known as external validation. The predictive performance of the model was evaluated using two external data sets. In 18 patients with either breast cancer or hepatocellular carcinoma, the population model estimated CL values with poor precision (rmse = 82 %). Similarly, in a Swedish data set including 79 patients with breast cancer, the population model also estimated CL values with poor precision (rmse = 43 %). A comparison of CL values in patients with normal liver function showed that the median CL in patients from the Swedish data set was nearly twice that in the UK data set. The reason for the differences in CL between the two data sets was unclear; possible explanations include a drug interaction between the concomitant medication (cyclophosphamide and fluorouracil) administered to patients in the Swedish data set or differences in general health status of the two patient groups.

The Swedish and UK data sets were combined and a new population model using all the data was developed. Despite the increase in patient numbers, AST was still the only clinical factor that was identified as influencing epirubicin PK.

An alternative approach to dose individualisation, *a posteriori* dose-adjustment, was investigated. This method involves taking blood samples and measuring drug concentrations to enable estimation of individual PK parameters that can be used for dose adjustment. Using D-optimality to identify optimum blood sampling times, limited sampling designs were developed to facilitate estimation of epirubicin CL by MAP Bayesian estimation techniques from only 2 or 3 blood samples. These limited sampling designs combined with MAP Bayesian estimation were found to estimate epirubicin CL with greater precision than both the covariate model and a previous limited sampling model derived by multiple linear regression. A simulation analysis indicated that the proposed limited sampling designs were robust with respect to errors in the recording of

sampling times, suggesting that they will work well in clinical practice. In contrast to the dosage guidelines developed using AST, these limited sampling designs were shown to predict CL precisely in an independent data set including 18 patients with breast cancer or hepatocellular carcinoma. An obvious disadvantage of the *a posteriori* method is that it cannot be used to determine the first dose.

On the basis of the analysis presented, a suggested future dosing strategy for epirubicin is to calculate the first dose from clinical characteristics and then adjust subsequent doses according to *a posteriori* methods using the proposed limited sampling designs. However, due to the high level of unexplained variability in epirubicin CL, further work to identify additional clinical characteristics that might influence the pharmacokinetics of epirubicin is warranted.

ACKNOWLEDGEMENTS

I wish to thank the following persons for their help with the research and preparation of this thesis:

Firstly I wish to thank my supervisors Dr Alison Thomson and Prof Chris Twelves for their encouragement, helpful discussions and time spent reviewing this thesis.

I would like to thank my advisor Dr Andrew Kelman for his support and advice.

I would also like to thank Ms Nicola Dobbs and Dr Marie Sandstrom for providing me with the clinical data sets, without which this research would not have been possible.

I would like to express my gratitude to Quintiles Ltd for funding this research, particularly Dr Charlie Brindley and Graham Murray for supporting the substantial time I have spent away from the office to study.

Finally, I would like to thank my husband John for all his loving support and understanding during the time spent working on this thesis.

TABLE OF CONTENTS

Title Page i
Declaration ii
Summary iii
Acknowledgements vi
Table of Contents vii
List of Tables xiii
List of Figures xv
List of Publications and Presentations xviii
List of Abbreviations xix

Chapter 1: Introduction 1
1.1 Pharmacokinetics 2
1.1.1 Factors influencing pharmacokinetics of drugs 2
1.2 Methods of quantifying liver dysfunction 3
1.2.1 Biochemical tests 3
1.2.2 Child-Pugh classification 4
1.2.3 Quantitative liver function tests 5
1.3 Population pharmacokinetics 6
1.3.1 Uses and application of the population approach 6
1.3.2 Two-stage approach 6
1.3.3 Mixed effect modelling approach 7
1.4 Pharmacokinetics and pharmacodynamics 8
1.4.1 The importance of defining a PK-PD relationship 8
1.4.2 Difficulties of measuring the PK-PD relationship with anti-cancer drugs 9
1.4.3 Using haematological toxicity as a marker of therapeutic response 9
1.4.4 Relationships between tumour response and plasma concentrations for anti- cancer drugs 11
1.5 Dose individualisation of anti-cancer drugs 12
1.5.1 Why dose individualise? 12
1.5.2 Individual dose adjustment a priori 12
1.5.3 Individual dose adjustment a posteriori 15
1.6 Epirubicin 19
1.6.1 Clinical use 19
1.6.2 Maximum tolerated dose 19
1.6.3 Mechanism of action 20
1.6.4 Metabolism and excretion 21
1.6.5 Pharmacokinetics 21
1.6.6 Factors influencing the pharmacokinetics of epirubicin 24
1.6.7 Population pharmacokinetics of epirubicin 25
1.6.8 PK-PD relationships for epirubicin 26
1.6.9 Dosage guidelines for epirubicin 27
1.7 Aims 29

Chapter 2: General Methods **30**
2.1 Pharmacokinetic models 31
2.2 Population pharmacokinetic analysis 35
2.2.1 Modelling inter-individual variability in PK parameters 35
2.2.2 Modelling residual variability 36
2.3 NONMEM 39
2.3.1 Program 39
2.3.2 NONMEM data file structure 39
2.3.3 Control stream format 40
2.3.4 Estimation methods in NONMEM 43
2.4 Model selection with NONMEM 45
2.4.1 Objective function value 45
2.4.2 Selecting the best structural model 45
2.4.3 Selecting the best residual error model 46
2.4.4 Screening for covariates of interest 47
2.4.5 Addition of covariates to the model 48
2.5 Simulation 49
2.6 Estimation of bias and imprecision 50

Chapter 3: Population analysis of an epirubicin data set **52**
3.1 Introduction to chapter 53
3.2 Methods 54
3.2.1 Patient data 54
3.2.2 Exploratory analysis of data set 55
3.2.3 Assessment of structural and statistical models 55
3.2.4 Identification of covariates for model building with NONMEM 56
3.2.5 Population model building using NONMEM 56
3.2.6 Assessment of variance-covariance matrix 57
3.3 Results 57
3.3.1 Patient data 57
3.3.2 Exploratory analysis of data set 57
3.3.3 Assessment of structural and statistical models 60
3.3.4 Identification of covariates for model building with NONMEM 66
3.3.5 Population model building using NONMEM 75
3.3.6 Assessment of variance-covariance matrix 82
3.4 Discussion 84

Chapter 4: Validation of population model for epirubicin using internal techniques **88**
4.1 Introduction to chapter 89
4.2 Methods 92
4.2.1 The effect of removing concentration data collected before 0.1 h post-dose 92
4.2.2 Jacknife analysis 92
4.2.3 Likelihood based method for the detection of individuals influencing covariate selection 93

4.2.4 Cross validation analysis 94
4.2.5 Sensitivity of the model to 10 % errors in AST and plasma concentrations 95
4.3 Results 96
4.3.1 The effect of removing concentration data collected before 0.1 h post-dose 96
4.3.2 Jacknife analysis 96
4.3.3 Likelihood based method for the detection of individuals influencing covariate selection 99
4.3.4 Cross validation analysis 106
4.3.5 Sensitivity of the model to 10 % errors in AST and plasma concentrations 110
4.4 Discussion 112

Chapter 5: Development of new dosage guidelines 115
5.1 Introduction to chapter 116
5.2 Methods 116
5.2.1 Investigation of PK/PD relationships 116
5.2.2 Development of new dosage guidelines 117
5.2.3 Comparison of dosage guidelines 117
5.2.4 Validation of population model using an external data set 118
5.3 Results 120
5.3.1 Investigation of PK/PD relationships 120
5.3.2 Development of new dosage guidelines 125
5.3.3 Comparison of dosage guidelines 125
5.3.4 Validation of population model using an external data set 134
5.4 Discussion 142

Chapter 6: Validation of UK population model using a Swedish data set... 144
6.1 Introduction to chapter 145
6.2 Methods 145
6.2.1 Patient data 145
6.2.2 Predictions of concentrations in Swedish patients using UK population model 146
6.2.3 Prediction of CL values in Swedish patients using UK population model 146
6.3 Results 147
6.3.1 Patient data 147
6.3.2 Predictions of concentrations in Swedish patients using UK population model 147
6.3.3 Prediction of CL values in Swedish patients using UK population model 151
6.4 Discussion 153

Chapter 7: Investigation into the differences in pharmacokinetics between the UK and Swedish data sets 155
7 Introduction to chapter 156
7A Comparison of epirubicin pharmacokinetics in UK and Swedish data sets 156
7A.1 Methods 156
7A.1.1 Estimation of population parameters for Swedish data set 156
7A.1.2 Estimation of population parameters for the combined data set (UK and Swedish) 157
7A.1.3 Comparison of individual Bayesian CL estimates in UK and Swedish patients 157
7A.2 Results 158
7A.2.1 Estimation of population parameters for Swedish data set 158
7A.2.2 Estimation of population parameters for the combined data set (UK and Swedish) 162
7A.2.3 Comparison of individual Bayesian CL estimates in UK and Swedish patients 163
7A.3 Discussion 165
7B Investigation into the differences in liver function and dosage regimens between the UK and Swedish data sets 167
7B.1 Introduction 167
7B.2 Methods 168
7B.2.1 Comparison of CL distributions and concentrations in Swedish and UK data sets in patients with normal liver function 168
7B.2.2 Assessment of the relationship between AST and CL in the Swedish data set 168
7B.2.3 The effect of varying duration of infusion on concentration-time profiles 168
7B.3 Results 169
7B.3.1 Comparison of CL distributions and concentrations in Swedish and UK data sets in patients with normal liver function 169
7B.3.2 Assessment of the relationship between AST and CL in the Swedish data set 169
7B.3.3 The effect of varying duration of infusion on concentration-time profiles 169
7B.4 Discussion 174
7C Assessment of CL estimates in the Swedish data set 176
7C.1 Introduction 176
7C.2 Methods 176
7C.2.1 Comparison of Bayesian CL estimates in Swedish data set obtained by 3 different methods 176
7C.2.2 Simulation analysis to assess 3 methods of attaining Bayesian estimates of CL 177
7C.2.3 Simulation analysis to assess whether inclusion of a 48 h sample effects the accuracy and precision of CL values estimated by 3 methods 178
7C.3 Results 179

7C.3.1 Comparison of Bayesian CL estimates in Swedish data set obtained by 3 different methods....179
7C.3.2 The accuracy and imprecision of Bayesian CL estimates by 3 different methods....179
7C.3.3 Simulation analysis to assess whether inclusion of a 48 h sample effects the accuracy and precision of CL values estimated by 3 methods....183
7C.4 Discussion....185

Chapter 8: Population analysis of combined UK and Swedish data sets....186
8.1 Introduction to chapter....187
8.2 Methods....187
8.2.1 Generation of the data set....187
8.2.2 Model building....187
8.3 Results....188
8.3.1 Selection of the basic model....188
8.3.2 Model building with covariates....188
8.4 Discussion....198

Chapter 9: Development of a limited sampling strategy....200
9 Introduction to chapter....201
9A Development and validation of limited sampling designs to estimate epirubicin CL....202
9A.1 Methods....202
9A.1.1 Identification of optimum sampling times....202
9A.1.2 Assessment of limited sampling designs....202
9A.1.3 Selection of optimum times using the minimum and maximum CL estimates in the data sets....203
9A.1.4 Validation of limited sampling designs using a new patients group.203
9A.2 Results....204
9A.2.1 Identification of optimum sampling times....204
9A.2.2 Assessment of limited sampling designs....204
9A.2.3 Selection of optimum times using the minimum and maximum CL estimates in the data sets....211
9A.2.4 Validation of limited sampling designs using a new patients group.211
9A.3 Discussion....217
9B Sensitivity of limited sampling designs to recording errors of blood sampling times....220
9B.1 Methods....220
9B.1.1 Simulation of reference data sets and estimation of PK parameters..220
9B.1.2 Simulation of data sets with recording errors and estimation of CL..220
9B.2 Results....222
9B.2.1 Estimation of PK parameters from reference data sets....222
9B.2.2 Estimation of CL from data sets with recording errors....231
9B.3 Discussion....232
9C Investigation into reason for bias in CL estimates....234
9C.1 Methods....234
9C.1.1 Assessment of the influence of residual error on bias....234

9C.1.2 Investigation into whether bias occurred by chance.....234
9C.1.3 Investigation into the influence of blood sampling times on bias.....235
9C.2 Results.....236
9C.2.1 Assessment of the influence of residual error on bias.....236
9C.2.2 Investigation into whether bias occurred by chance.....236
9C.2.3 Investigation into the influence of blood sampling times on bias.....236
9C.3 Discussion.....241
9D Assessment of Jakobsen's multiple linear regression limited sampling approach.....243
9D.1 Introduction.....243
9D.2 Methods.....243
9D.3 Results.....245
9D.4 Discussion.....247
9 Conclusions to Chapter.....248

Chapter 10: General Conclusions.....249

References.....256

Appendix.....274

LIST OF TABLES

Chapter 1

Table 1.1 Mean epirubicin pharmacokinetic parameter estimates reported in the literature 23

Chapter 3

Table 3.1 Summary of demographics and clinical characteristics of patients ..58
Table 3.2 Objective function values obtained by two- and three-compartment models with proportional and combined error 62
Table 3.3 GAM analysis results: best three models identified for CL, V_1, Q_2, Q_3 and V_3 73
Table 3.4 Summary of models evaluated using FOCE-INTER 76
Table 3.5 Parameter estimates from basic, full and final model 81
Table 3.6 Summary of models using FOCE-INTER with full variance-covariance matrix 83

Chapter 4

Table 4.1 Parameter estimates including and excluding concentration data before 0.1 h 97
Table 4.2 Estimates of population estimates and standard errors using NONMEM and Jacknife analysis 98
Table 4.3 Population estimates from complete data set and from training data sets 107
Table 4.4 Imprecision (rmse %) of population CL estimates calculated with basic and final models 108
Table 4.5 Population parameter estimates following sensitivity analysis 111

Chapter 5

Table 5.1A Linear regression of survival fraction for haemoglobin, white blood cells, neutrophils and platelets against AUC, C_{max}, dose (mg) and dose (mg/m^2) 121
Table 5.1B Linear regression of log survival fraction for haemoglobin, white blood cells, neutrophils and platelets against AUC, C_{max}, dose (mg) and dose (mg/m^2) 121
Table 5.2 Linear regression of survival fraction for haemoglobin, white blood cells, neutrophils and platelets against time above a threshold concentration 124
Table 5.3A New dosage guidelines based on AST concentration 126
Table 5.3B New dosage guidelines based on AST concentration and BSA 126
Table 5.3C New dosage guidelines based on AST concentration with additional 25 % dose reduction 126
Table 5.4 Imprecision and bias of AUC estimates using different dosage guidelines 128

Table 5.5 Summary of clinical characteristics of patients in validation data set....138
Table 5.6 Population parameter estimates for the validation data set....140

Chapter 6

Table 6.1 Summary of clinical characteristics in Swedish and UK patients...148

Chapter 7

Table 7.1 Population parameter estimates for the UK data set, the Swedish data set and the combined data set....161
Table 7.2 Bias and imprecision of Bayesian CL values in simulated data sets estimated using 3 different methods....181
Table 7.3 Bias and imprecision of Bayesian CL estimates obtained using 3 different methods when an additional 48 h sample was included...184

Chapter 8

Table 8.1 Population parameter estimates for the combined UK and Swedish data sets calculated using the first order method....190
Table 8.2 Summary of clearance models tested using the combined data set 194
Table 8.3 Summary of volume models tested using the combined data set....195
Table 8.4 Population parameter estimates from final model....196

Chapter 9

Table 9.1 Optimum sampling times (h) selected following administration of epirubicin as a bolus injection, as a 3 minute infusion and as a 19 minute infusion....205
Table 9.2 Limited sampling designs....206
Table 9.3 Me and rmse estimates with 95 % confidence intervals for each limited sampling design....210
Table 9.4 Comparison of optimal sampling times selected using population PK parameters to those selected using the PK parameters of individuals with minimum and maximum CL....212
Table 9.5 Me and rmse estimates with 95 % confidence intervals for each limited sampling design using the validation data set....216
Table 9.6 The precision and bias of CL estimates from limited sampling designs with and without recording errors....224
Table 9.7 The precision and bias of V_3 and Q_3 estimates from limited sampling designs....230
Table 9.8 The precision and bias of CL estimates from 5 simulated data sets in which blood samples were taken up to 48 h post-dose....237
Table 9.9 The precision and bias of CL estimates from 5 simulated data sets in which blood samples were taken up to 96 h post-dose....237
Table 9.10 The precision and bias of CL estimates from 5 simulated data sets in which blood samples were taken up to 168 h post-dose....237

LIST OF FIGURES

Chapter 1

Figure 1.1 Metabolic pathways of epirubicin profile 22

Chapter 2

Figure 2.1A A 2-compartment model with a corresponding concentration-time profile 32
Figure 2.1B A 3-compartment model with a corresponding concentration-time profile 32
Figure 2.2A Diagram illustrating the relationship between TVCL, CL_i and η_{CLi} .37
Figure 2.2B Distribution of ηs for all subjects within the population 37
Figure 2.3A Diagram illustrating the relationship between observed concentrations, predicted concentration and residual error 38
Figure 2.3B Distribution of ϵ_{ij} for all concentrations in all subjects in the data set 38
Figure 2.4 Excerpt from a NONMEM data file 41
Figure 2.5 Example of a control stream 42

Chapter 3

Figure 3.1 Matrix plot to assess relationships between covariates 59
Figure 3.2 Measured and individual predicted concentration versus time plots for patients from Trial 1, 2 and 3 using a 3 compartment model with combined error 61
Figure 3.3 Weighted residuals versus time for 2 and 3 compartment models with combined error 63
Figure 3.4 Population predicted and individual predicted concentrations versus measured concentrations calculated using a 3 compartment model with combined error 64
Figure 3.5 The modulus of individual weighted residuals versus individual predicted concentrations for a 3 compartment model with proportional or combined error 65
Figure 3.6 Individual weighted residuals versus time for a 3 compartment models with proportional error using FO and FOCE-INTER 67
Figure 3.7 Scatter plots of CL versus clinical characteristics 68
Figure 3.8 Box plots of CL versus clinical characteristics 70
Figure 3.9 Akaike values of the best 30 models for CL identified by GAM analysis 72
Figure 3.10 Cook's distance versus leverage following GAM analysis on bayesian estimations of CL 74
Figure 3.11 Influence of AST, dose and BSA on the CL model 77
Figure 3.12 Influence of liver metastases on the CL model 78
Figure 3.13 Influence of liver metastases and dose on Q_3 model 80

Chapter 4

Figure 4.1 Population parameter estimates from jacknife samples.....100
Figure 4.2 Likelihood-based method to identify individuals influencing covariate selection: change in OFV jacknife versus patient ID number.....105
Figure 4.3A Prediction errors from basic CL model versus AST.....109
Figure 4.3B Prediction errors from final CL model versus AST.....109

Chapter 5

Figure 5.1 Relationship between toxicity and AUC 122
Figure 5.2 Relationship between toxicity and dose.....123
Figure 5.3 Predicted AUC values associated with AST values following no dose-adjustment or dose-adjustment according to new proposed dosage guidelines.....127
Figure 5.4A Predicted AUC values for patients in UK data set administered a dose according to new AST guidelines.....130
Figure 5.4B Predicted AUC values for patients in UK data set administered a dose according to new AST guidelines with additional 25 % dose reduction group 130
Figure 5.5 Comparison of AST and bilirubin concentrations 131
Figure 5.6 Comparison of AST and bilirubin concentrations and resulting doses according to current UK or new AST guidelines 132
Figure 5.7 Proposed doses using UK guidelines, USA guidelines, new AST guidelines and the Dobbs and Twelves equation.....133
Figure 5.8 Estimated AUC values for patients administered a dose according to the UK guidelines, USA guidelines or Twelves' equation.....135

Figure 5.9 Concentration-time profiles from validation data set 136
Figure 5.10 Goodness of fit plots for validation data set 139
Figure 5.11 Scatter plot of the CL values estimated from AST values versus 'true' CL values in the validation data set 141

Chapter 6

Figure 6.1 Concentrations predicted in Swedish patients using UK population model plotted versus measured concentrations 149
Figure 6.2 Predicted concentrations obtained using the UK population model and measured concentrations in Swedish patients plotted versus time 150
Figure 6.3 CL estimates for Swedish patients obtained using the UK population model plotted against Bayesian CL estimates 152

Chapter 7

Figure 7.1 Swedish data set population analysis: WRES versus time for 2- and 3-compartment models 159
Figure 7.2 Population predicted and individual predicted concentrations plotted against measured concentrations for Swedish data set 160
Figure 7.3 Box plots of individual CL estimates in Swedish and UK data sets estimated from population analysis of the combined data sets 164
Figure 7.4 Comparison of CL distributions in Swedish and UK data sets in patients with normal liver function 170
Figure 7.5 Dose-adjusted concentration time profiles in Swedish patients and UK patients with normal liver function 171
Figure 7.6 Predicted epirubicin concentration-time profiles in a typical individual assuming a 100 mg dose administered as a 3 min or a 1 hour infusion 172
Figure 7.7 Bayesian CL estimates in Swedish patients obtained by 3 different methods 180
Figure 7.8 Bayesian CL estimates for simulated data sets estimated using 3 different methods plotted against true CL values (with and without additional 48 h sample) 182

Chapter 8

Figure 8.1 Goodness of fit plots for combined UK and Swedish data sets using the first order method 189
Figure 8.2 Scatter plots of individual estimates of CL versus clinical characteristics 191
Figure 8.3 Box-plots of CL, V_1, Q_2, Q_3, and V_3 in UK and Swedish patients .192
Figure 8.4 Individual CL estimates fitted with the final population model 197

Chapter 9

Figure 9.1 Scatter plots of the CL estimates from the truncated data sets versus CL estimates from full data set 207
Figure 9.2 Scatter plots of the CL estimates from the validation truncated data sets versus CL estimates from full validation data set 213
Figure 9.3 CL estimates using full data set against 'true' CL values 223
Figure 9.4 CL estimates from limited sampling designs with and without recording errors against 'true' CL values 225
Figure 9.5 V_3 (above) and Q_3 (below) estimates from full data set against 'true' values 227
Figure 9.6 V_3 estimates from limited sampling designs against 'true' V_3 values 228
Figure 9.7 Q_3 estimates from limited sampling designs against 'true' Q_3 values 229
Figure 9.8 Bias calculated for CL values above geometric mean and below geometric mean in data sets with concentrations simulated up to 48, 96 and 168 h post-dose 238
Figure 9.9 CL values estimated by two-sample and one-sample equations plotted versus true CL values 246

LIST OF PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Ralph LD, Thomson AH, Dobbs NA, Twelves C (2003). A population model of epirubicin pharmacokinetics and application to dosage guidelines. Cancer Chemother Pharmacol. 52 (1): 34-40

Ralph LD, Thomson AH, Dobbs NA, Twelves C (2004). Maximum a posteriori bayesian estimation of epirubicin clearance by limited sampling. Br J Clin Pharmacol. *In Press.*

PRESENTATIONS

Oral Presentation - PKUK, Wales 2001. Population pharmacokinetic analysis of epirubicin in breast cancer patients with liver metastases

Oral Presentation - EUFEPS, Djuronaset, Sweden 2002. New dosage guidelines for epirubicin – a population approach

Poster Presentation - PAGE, Verona, Italy 2003. Bayesian estimation of epirubicin clearance by limited sampling

Oral Presentation - PKUK, Liverpool 2003. Validation of a population model for epirubicin in breast cancer patients.

LIST OF ABBREVIATIONS

Symbol	Definition
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALL	Acute lymphocytic leukaemia
AIC	Akaike information criteria
AUC	Area under the curve
BSA	Body surface area
C_{max}	Maximum concentration
CL	Clearance
CrCL	Creatinine clearance
CI	Confidence interval
CV	Coefficient of variation
DV	Dependent variable
FEC	Fluorouracil, epirubicin and cyclophosphamide (combination therapy)
5-FU	Fluorouracil
FDA	Food and drug administration
FO	First order estimation
FOCE	First order conditional estimation
GAM	Generalised additive modelling
GFR	Glomerular filtration rate
GGT	γ-glutamyl transpeptidase
IPRED	Individual predicted concentration
IRES	Individual residual
IWRES	Individual weighted residual
LOQ	Limit of quantification
LVRT	Liver volume replaced by tumour
MAP	Maximum a posteriori
me	mean error
MLR	Multiple linear regression
MPE	Mean prediction error
MTD	Maximum tolerated dose
OFV	Objective function value
PD	Pharmacodynamics
PK	Pharmacokinetics
Pe	Prediction error
PRED	Population predicted concentration
RES	Residual
rmse	Root mean squared error
RSE	Relative standard error
SPC	Summary of product characteristics
TDM	Therapeutic drug monitoring
TV	Typical value
WBC	White blood cell
WHO	World Health Organisation
WRES	Weighted residual

Symbol	Definition
ϵ	residual error, difference between model predicted and measured concentration
η	interindividual error, difference between individual pharmacokinetic parameter and population value
σ^2	variance of ϵ
ω^2	variance of η

CHAPTER 1

INTRODUCTION

INTRODUCTION TO CHAPTER

This Chapter gives a general background to the research performed in this thesis. Firstly, a brief introduction to pharmacokinetics is given in Section 1.1, then factors that can influence pharmacokinetics are described, focussing mainly on the influence of liver disease. Section 1.2 describes the various methods used to quantify liver dysfunction. An introduction to population pharmacokinetics is given in Section 1.3, followed by Section 1.4, which describes the importance of defining PK-PD relationships and the practical difficulties encountered in oncology. Information on why dose-individualisation in cancer treatment may be required and various approaches undertaken is provided in Section 1.5. In Section 1.6, a background to the clinical use, toxicity, pharmacokinetics and current dosage guidelines of epirubicin is provided. Finally, the aims of the analysis performed in this thesis is given.

1.1 PHARMACOKINETICS

Pharmacokinetics is the study of the time-course of drug concentrations within the body and more specifically drug absorption, distribution and elimination. Pharmacokinetic models are fitted to drug concentration-time data to summarise the results and to predict outcomes under different circumstances. Knowledge of the pharmacokinetics of a drug can enable optimum dosage regimens to be designed. Further details of the methodology used for pharmacokinetic modelling are provided in Chapter 2.

1.1.1 Factors influencing pharmacokinetics of drugs

The pharmacokinetics of a drug are largely determined by the structure of the drug molecule. However, there are many factors that influence the pharmacokinetics of drugs, resulting in variations in drug handling between different individuals (inter-individual variability). For example, the rate and extent of absorption can be influenced by diet, disease and co-administration of other drugs. Distribution can be influenced by obesity and physiological differences in tissue binding. Elimination may vary between individuals due to differences in blood flow and enzyme activity as a result of differences in genetics, age, disease or co-administration of other drugs. Similarly, the pharmacokinetics may vary within the same individual on different

dosing occasions (inter-occasional variability) due to a variety of factors including differences in diet, temperature and exercise.

1.1.1.1 The effect of liver disease on pharmacokinetics

Liver disease can lead to a reduction in the number of liver cells, a reduction in enzyme level or activity, a reduced exchange across the endothelial lining, impaired oxygen uptake and a reduction in portal vein perfusion as a result of intra- and extrahepatic portal-systemic shunting (Morgan & McLean 1995; Verbeeck & Horsmans 1998). Furthermore, biliary obstruction can occur, resulting in a reduced secretion of bile into the duodenum. These disease-induced changes in the physiology of the liver can lower the hepatic clearance of drugs. Hepatocellular damage can cause a reduction in the synthesis of proteins, including albumin and α_1-acid-glycoprotein. This can lead to a reduction in the plasma protein binding of drugs and hence an increase in the fraction unbound (Rowland & Tozer 1995). In liver disease there may also be an accumulation of endogenous compounds, such as bilirubin, that can inhibit the binding of drugs to plasma proteins (Verbeeck & Horsman 1998). An increase in the fraction unbound can result in an increased volume of distribution and an increase in the total clearance of some drugs. An increase in the fraction unbound could also lead to an increased uptake by target tissues and an altered pharmacodynamic response.

1.2 METHODS OF QUANTIFYING LIVER DYSFUNCTION

1.2.1 Biochemical tests

Biochemical tests are routinely performed in patients and can be used to assess the severity of liver disease (Gholson & Bacon 1993; Kumar & Clarke 1998). The various tests reflect different pathophysiological processes including necrosis and inflammation, cholestasis and a reduced synthetic ability. These tests are markers of hepatocellular damage and are therefore not markers of metabolising activity or capacity; however, they may still be useful as indirect markers of liver function.

Necrosis and inflammation of the liver can cause aspartate aminotransferase (AST) and alanine aminotransferase (ALT) to leak into the blood from damaged liver cells and thus levels of these enzymes become elevated. Elevated concentrations of

ALT are specific to hepatocellular damage, whereas elevated levels of AST may also reflect damage to the heart, skeletal muscle, kidney and brain.

In cholestasis, where cholesterol and bile acid secretion are impaired, serum levels of alkaline phosphatase and γ-glutamyl transpeptidase (GGT) are elevated. Concentrations of alkaline phosphatase are increased in liver disease due to increased synthesis by liver cells but elevated levels are also observed in bone disease. The highest serum levels of alkaline phosphatase caused by liver disease are associated with hepatic metastases and primary biliary cirrhosis. GGT is present in many tissues as well as the liver and its activity is also increased following chronic administration of enzyme inducers such as phenytoin and alcohol.

Severe cholestasis can lead to a decrease in the concentration of bile salts in the intestine, resulting in poor absorption of vitamin K. The resulting deficiency in vitamin K may cause an increase in prothrombin time, which can be corrected by administration of vitamin K. Prothrombin synthesis may be reduced in severe hepatocellular injury and in this case the increase in prothrombin time cannot be corrected by administration of vitamin K.

In chronic liver disease, the synthesis of albumin may be impaired and its concentration will decrease slowly with time. As the half-life of serum albumin is 2-3 weeks, concentrations do not immediately reflect liver damage and changes may not be observed in acute disease.

Bilirubin is mainly generated from the extra-hepatic degradation of the haem pigment from erythrocytes. Bilirubin binds non-covalently to albumin and is transported to the liver where it is conjugated with glucuronic acid and excreted in the bile. Consequently, serum bilirubin concentrations can become elevated due to hepatocellular damage, biliary obstruction or extrahepatic diseases.

1.2.2 Child-Pugh classification

The Child-Pugh grading system uses clinical and biochemical measurements to classify the severity of liver disease (Pugh et al. 1973). The classification was first used in 38 patients requiring an emergency operation for bleeding oesophageal varices and gave a good prediction of post-operative risk. The factors used were encephalopathy, ascites, bilirubin concentration, albumin concentration and

prothrombin time. Points were scored (1 to 3) with increasing abnormality for each of the five factors.

As with biochemical tests, the Child-Pugh grading system does not measure the physiological factors that are important for the hepatic clearance of drugs. The method of scoring means that patients with different types of liver damage may have the same score yet may not necessarily have the same capacity to clear a particular drug. The Child-Pugh grading system is a crude measure of hepatic disease; encephalopathy, ascites, reduced levels of albumin and increased prothrombin time are insensitive markers of hepatic damage and are only observed in severe and chronic hepatic disease.

1.2.3 Quantitative liver function tests

A variety of substrates can be administered to an individual so that hepatic blood flow or the metabolic capacity of the liver can be estimated. The substrate is administered to the patient, blood samples are taken at various time points post-dose and plasma concentrations of the substrate are measured in order to calculate a clearance value. The total clearance estimated is assumed to be a measure of hepatic clearance and thus extrahepatic clearance of the chosen substrate should be minimal.

For drugs with high extraction ratios, hepatic clearance is limited by blood flow. For such drugs, highly extracted substrates, including galactose, sorbitol and indocyanine green, can be used as probes to measure hepatic blood flow as they are highly extracted by the liver, and hepatic blood flow is assumed to correspond to their estimated clearances (Verbeeck & Horsmans 1998). Drugs with low extraction ratios are more dependent on changes in metabolic capacity. Clearance estimates of substrates that are enzyme capacity limited, such as caffeine and antipyrine, can be used as probes to measure the metabolic capacity of the liver. Antipyrine is a substrate for CYP1A2, CYP2C and CYP3A whereas caffeine is a substrate for CYP1A2, arylamine N-acetyltransferase and xanthine oxidase (Brockmoller & Roots, 1994). Erythromycin is a substrate for CYP3A4 and a breath test has been developed that measures the rate of erythromycin metabolism, and hence CYP3A4 activity (Watkins et al. 1989). The breath test involves intravenous administration of erythromycin radiolabelled with ^{14}C and subsequent measurement of $^{14}CO_2$ exhaled in the breath.

Docetaxel is extensively metabolised by CYP3A4, and the erthyromycin breath test was shown to predict docetaxel CL more precisely than biochemical tests (Hirth et al. 2000).

These quantitative liver function tests enable estimation of hepatic blood flow or the metabolic capacity of the liver and thus have potential advantages over the biochemical tests that reflect hepatocellular damage. However, clearance of a drug is dependent on a unique combination of factors including the activity of specific phase I isozymes, phase II enzymes, blood flow, biliary excretion and concentrations of various plasma proteins, and therefore, a single probe compound may not be able to predict the pharmacokinetics of a particular drug accurately. One solution could be to administer to each patient, a cocktail of probe compounds, each of which has clearance affected by different metabolic enzymes and other physiological factors (Brockmoller & Roots 1994). However, the complications of drug-drug interactions and the safety implications of administering a cocktail of drugs to patients with hepatic disease do not favor this approach as a solution.

1.3 POPULATION PHARMACOKINETICS

1.3.1 Uses and application of the population approach

"Population pharmacokinetics" is the term given to a data analysis approach that can be used to determine the typical pharmacokinetics of a drug in a population of individuals and to provide estimates of inter-individual and residual variability. Knowledge of the typical PK parameter values and their variability in the population can be used to develop dosage guidelines that will achieve target drug concentrations in the patient population. The population approach can also identify clinical factors influencing the pharmacokinetics of a drug, such as age or liver function, and thus can indicate if there is a requirement for dose adjustment in certain subpopulations.

1.3.2 Two-stage approach

In traditional pharmacokinetic studies, such as those in Phase I clinical trials, a large number of blood samples are taken from each individual (eg. 10 to 20 blood samples) and thus the data is described as dense. A full concentration-time profile can be

characterised for each individual and enables pharmacokinetic parameters to be calculated using a traditional methods such as compartmental analysis. Dense data permits the use of the two-stage population approach so called because in the first stage pharmacokinetic parameters are calculated and in the second stage the individual pharmacokinetics parameters are used to estimate average PK parameters and inter-individual variability. The sources of variability in the pharmacokinetics can be investigated using statistical tools such as linear regression. This method is easy to perform and requires minimal skills.

A disadvantage of the two-stage approach is that it requires a large number of samples to be taken per individual. Although such data is readily available from Phase I trials, these studies generally include a small number of homogenous subjects. As the aims of population analyses is to estimate inter-individual variability in the population and identify clinical factors influencing the pharmacokinetics, data collected from Phase II and Phase III clinical trials are more appropriate for population analyses as these studies have fewer entrance restrictions and include a larger number of heterogeneous patients. However, in Phase II and III trials only a small number of blood samples are usually taken per subject, commonly termed sparse data, and thus they do not permit the two-stage approach. Another disadvantage of the two-stage approach is that errors associated with the estimated PK parameters are ignored and this results in over estimation of inter-individual variability (Sheiner & Beal 1980).

1.3.3 Mixed effect modelling approach

To overcome the difficulties experienced with the two-stage approach Beal and Sheiner (1992) developed a software package called NONMEM that uses mixed effect modelling techniques to perform population pharmacokinetic analysis. NONMEM simultaneously fits all the data, which may be sparse and unbalanced, to attain values for the typical pharmacokinetics parameters and their distribution in the population. Using these estimates and the concentration data for each individual, NONMEM uses Bayesian methods to obtain individual estimates of pharmacokinetics parameters (details are provided in Chapter 2).

Mixed effect models are so called because they include the influence of both fixed effects and random effects. Fixed effects are factors that can be measured or

determined, e.g. pharmacokinetic parameters and covariates. Random effects are factors that cannot be observed, such as unknown differences in physiology or bioanalytical errors. In population pharmacokinetic models, random effects can be subdivided into 3 types: Inter-individual variability (differences between individuals); inter-occasion variability (differences within an individual between different dosing occasions, which cannot be explained by covariates); and residual error, which is the difference between the observed and predicted concentrations as a result of measurement error, model misspecification or other unknown errors.

Population analyses using mixed effect models estimate individual pharmacokinetic parameters with more precision and less bias than the two-stage approach (Sheiner & Beal 1980; Sheiner & Beal 1981a). Another advantage of mixed effect modelling is that it provides errors of the parameter estimates. However, a disadvantage of mixed effect modelling is that it is less user friendly and requires substantial training.

1.4 PHARMACOKINETICS AND PHARMACODYNAMICS

1.4.1 The importance of defining a PK-PD relationship

It is generally assumed that there is a relationship between the concentration-time profile of a drug in the plasma (as determined by the dosage regimen and the pharmacokinetics) and the observed clinical effect of the drug (pharmacodynamics), albeit often complex. For example, it may be possible to identify a threshold concentration or area under the concentration-time curve, above which the chances of a response increase. If we are to adjust the dosage regimen according to a patient's pharmacokinetics (PK) to achieve the desired pharmacodynamic (PD) effect, we need to understand the relationship between the PK and the PD of the drug. Unfortunately, due to the complex nature of neoplastic disease, this PK-PD relationship is often very difficult to define.

1.4.2. Difficulties of measuring the PK-PD relationships for anti-cancer drugs

Therapeutic responses to chemotherapy are measured by changes in the size of lesions, time to disease progression and survival (Hayward et al. 1977). Therapeutic responses to antineoplastic drugs are often difficult to relate to measured drug concentrations as an outcome is often not observed for many months or even years. It is also difficult to attribute the pharmacodynamic effect to a single dose cycle as many dose cycles are usually given and generally many different drugs are administered. Furthermore, patients who show a tumour response to an anticancer agent are more likely to receive further treatments, resulting in a biased relationship between total systemic exposure and tumour response.

1.4.3. Using haematological toxicity as a marker of therapeutic response

Due to the complications associated with using tumour response as a pharmacodynamic endpoint, many pharmacodynamic studies of anti-cancer drugs have used haematological toxicity as a marker for the pharmacodynamic effect. This would seem a valid assumption as most cytotoxics are antiproliferative and their effect on proliferating bone marrow may mirror that of tumour tissue. In a study of 211 patients with advanced breast cancer, it was shown that patients with lower leukocyte nadirs had a significantly better response to chemotherapy (distance disease free and overall survival) then those with higher nadirs (Saarto et al. 1997). For most anti-cancer agents a maximum tolerated dose (MTD) is sought, which is defined as the maximum dose that can be given with acceptable toxicity in most patients. It is assumed that administration of drug at the maximum tolerated dose will maximise the chances of a tumour response. Relationships between systemic exposure, as measured by area under the curve (AUC), and white blood cell (WBC) count have been identified for many anti-cancer agents including 5-fluorouracil (5-FU) (Santini et al. 1989), 9-aminocamptothecin lactone (de Jonge et al. 1999) and etoposide (Stewart et al. 1991), doxorubicin (Piscitelli et al. 1993) and epirubicin (Jakobsen et al. 1991b; Dobbs et al. 1998b). These studies used a single nadir blood cell count as the pharmacodynamic

end point. The relationship identified between epirubicin AUC and WBC count is discussed in more detail in Section 1.68.

Patients who have prolonged leukopenia have been shown to be at a greater risk of infection than those who have a more a rapid recovery (Bodey et al. 1966) and thus the measurement of several blood cell counts over a period of time is considered a better marker of toxicity that a single nadir WBC measurement. Several researchers have developed models that incorporate the change in blood counts with time. The pharmacodynamic response of etoposide, measured by leukopenia, was described by a cubic-spline function, which fitted the average response of the population versus time (Karlsson et al. 1995). The model included a lag-time (length of time before WBC count decline is observed) and the duration of time below the WBC count baseline. However, the parameters estimated from this mathematical model are difficult to relate to physiological processes. A more physiological model was developed for paclitaxel, which relates the drug concentration-time profile with the time course of leukopenia (Minami et al. 1998). This physiological indirect-response model has 2 compartments corresponding to the leukocyte pool in the bone marrow, the site of drug action, and the peripheral blood, where the leukocytes are measured. An E_{max} model was used which relates the extent of inhibition of leukocyte production in bone marrow cells to the extent of systemic exposure to paclitaxel, measured by AUC. More recently, a physiological model was successfully used to describe the time course of neutropenia following administration of 2'-deoxy-2'-methylidenecytidine (DMDC) to patients with colorectal or non-small-cell lung cancer (Friberg et al. 2000). The model imitated time delays of the bone marrow maturation process and consisted of nine proliferating transient compartments that were sensitive to DMDC, 5 non-proliferative, non-sensitive compartments and one compartment for circulating neutrophils. The advantages of time-dependent pharmacodynamic models are that they use information from many cell counts, rather than just the nadir, as a measure of response and they allow flexibility on when cell counts are made.

It should be emphasized that measures of haematological toxicity are not direct measures of drug efficacy. For most cancer drugs, doses are administered close to the maximum tolerated dose, however, an increase in toxicity may not necessarily result in an increase in tumour response.

1.4.4 Relationships between tumour response and plasma concentrations for anti-cancer drugs

Despite the difficulties described above, there have been a small number of studies that have used tumour response as a pharmacodynamic end point. In a large study of 1028 patients administered carboplatin for the treatment of ovarian cancer, the likelihood of a tumour response at a given AUC was described by the modified Hill equation (Jodrell et al. 1992). The likelihood of a response did not increase above an AUC of 7 mg.min/ml; however, the likelihood of leukopenia and thrombocytopenia did continue to increase above this threshold. This emphasizes the limitations of using haematological toxicity as a pharmacodynamic marker of tumour response.

In patients with Kaposi's sarcoma associated with acquired immunodeficiency syndrome (AIDS), who were treated with pegylated-liposomal doxorubicin, classification and regression tree (CART) analysis showed that lesion response, evaluated 3 weeks after treatment, was significantly related to the average daily maximum doxorubicin concentration (Amantea et al. 1997). Doses of up to 80 mg/m^2 pegylated-liposomal doxorubicin have been administered in previous solid tumour trials, however, in this study it was suggested that a dose of 20 to 30 mg/m^2 every three weeks should be efficacious in this patient group. Although higher doses may result in an increased risk of toxicity, they did not appear to result in an increased tumour response.

Systemic exposure to docetaxel, as measured by AUC, was investigated as a potential indicator of tumour response using multivariate regression, in a study with 189 patients with non-small cell lung cancer (Bruno et al. 1998). They showed that the risk of tumour progression decreased with an increase in the AUC measured during the first cycle of treatment.

Patients with acute lymphocytic leukaemia being treated with methotrexate, who had had median methotrexate serum concentrations less than 16 μM, were 3 times more likely to have a relapse during therapy (Evans et al. 1986). A further study in 182 children showed that patients who received individualized doses to achieve methotrexate concentrations greater than 16 μM had a significantly better response than patients who received conventional treatment, without an increase in the number of severe toxic events (Evans et al. 1998).

1.5 DOSE INDIVIDUALISATION OF ANTI-CANCER DRUGS

1.5.1 Why individualise doses?

As a result of pharmacokinetic variability, drug concentrations, and consequently the systemic exposure, of one patient may be much higher or lower than in another patient who is administered the same dose. Different doses may therefore be required in different individuals to achieve the same target systemic exposure, indicating the need for individualised dosing. Doses of anti-cancer agents are often decreased in response to toxic events but they are rarely increased if toxic effects don't occur. It is therefore likely that some patients will have a systemic exposure to anti-cancer compounds that is too low. Individualised dosing is particularly important for drugs with a narrow therapeutic window (where there is only a small difference in drug concentrations at which a therapeutic effect is expected and that at which toxicity is expected) and where inter-individual variability is high. These criteria are generally true for antineoplastic agents. For dose-individualisation to be successful, a clearly defined concentration-effect relationship is required to identify a target pharmacokinetic end-point such as the maximum plasma concentration (C_{max}) or AUC.

1.5.2 Individualised dose adjustment - a priori

With *a priori* dosing, the dose is determined from a knowledge of the relationship between pharmacokinetic parameters and the patient's clinical characteristics, such as age, weight, liver function or renal function, and on a desired pharmacokinetic end-point. This method of individualised dosing is simple, easy to implement and can reduce inter-individual variability in systemic exposure. However, it is not possible to ascertain if the dose for a given individual is too low. The success of this method depends upon how accurately the pharmacokinetics can be predicted from the clinical characteristics.

1.5.2.1 Dose adjustment according to body surface area

Doses of most anti-cancer agents are routinely adjusted accordingly to the patient's body surface area (BSA). The rationale behind this approach originates from studies

that showed that the MTD was similar in different species when the dose was adjusted for BSA. This suggested that inter-species scaling based on BSA could aid identification of a safe starting dose for Phase I clinical trials.

BSA is commonly calculated from height and weight using a formula proposed by Dubois (Dubois & Dubois 1916):

Predicted surface area (m^2) = weight $(kg)^{0.425}$ x height $(cm)^{0.725}$ x 0.007184

The actual BSA was measured by covering subjects in paper and then calculating the surface area of the paper. Although this formula was developed from only 9 individuals, they included a diverse range of body shapes and sizes.

There is some evidence to support scaling according to BSA between species but little evidence that dose adjustment based on BSA is of benefit within a species. Grochow and coworkers (1990) investigated the influence of weight, height and BSA on the pharmacokinetics of 6 anti-cancer compounds including an alkylating agent (busulfan), a microtubular toxin (taxol), three anti-metabolites (trimetrexate, dichloromethotrexate and brequinar), 2 intercalating agents (piroxantrone and menogaril) and 2 differentiating agents (N-methylformamide and hexamethylene-bis-acetamide). Clearance was only correlated with a measure of body size (height) for taxol ($r = 0.697$) and was not correlated with BSA for any of the other drugs investigated, indicating that dose adjustment according to BSA would not reduce inter-individual variability in systemic exposure.

1.5.2.2 Dose adjustment according to renal function

The clearance of drugs that are eliminated by the kidneys, such as carboplatin, usually depends on the glomerular filtration rate (GFR), which is correlated with creatinine clearance (CrCL). Several formulae have been proposed and validated prospectively, to estimate the optimal dose of carboplatin from GFR or CrCL. Egorin and coworkers (1985) used desired platelet nadir (dose-limiting toxicity), pre-treatment platelet count, BSA, status of prior chemotherapy and measured CrCL to calculate a dose whereas Calvert and coworkers (1989) used a measured GFR to calculate the dose required to obtain a target AUC. These equations require a direct measurement of CrCL or GFR. CrCL, and hence GFR, is commonly estimated from serum creatinine concentrations using formula such as the Cockcroft-Gault equation (1976), which is inaccurate at low creatinine concentrations (Caregaro et al. 1994). More recently, a formula has been

developed, using the population approach, to estimate GFR in patients with cancer from age, BSA, sex, serum creatinine and creatine kinase. This approach was less biased and more precise than the Cockcroft-Gault equation (Wright et al. 2001).

1.5.2.3 Dose adjustment according to hepatic function

Due to its complex nature, hepatic clearance is difficult to predict from clinical characteristics. Most studies have assessed the effects of liver dysfunction on the pharmacokinetics of anticancer drugs in the presence or absence of hepatic damage and have defined dosage guidelines by empirical methods. Few studies have established how the severity of liver damage affects the pharmacokinetics of anticancer drugs. This is likely to be due to the lack of an established liver function grading system and the small number of patients with liver dysfunction that are usually included in such studies. Biochemical markers such as ALT, AST, bilirubin and alkaline phosphatase have been used to identify the presence of liver damage and to indicate that dosage reduction may be necessary to avoid severe toxicity. Dose reductions have been recommended for patients with impaired liver function for a number of anti-cancer drugs including docetaxel (Bruno et al. 1998), vinorelbine (Robieux et al. 1996) and anthracyclines (Piscitelli et al. 1993, Dobbs et al. 2003).

Docetaxel is active in a wide variety of tumours including metastatic breast cancer and non-small cell lung cancer. Bruno et al. 1998, found that docetaxel clearance was 27 % lower in 26 patients with elevated transaminases and alkaline phosphatase compared to 614 patients with normal hepatic enzyme levels. As toxicity (neutropenia) was related to the clearance of docetaxel, the authors recommended a 25 % reduction in the dose for patients with elevated levels of transaminases and alkaline phosphatase.

In a study of 29 patients with breast cancer treated with vinorelbine, a significant correlation was found between drug clearance and prothrombin time, bilirubin concentration and albumin concentration (Robieux et al. 1996). Clearance of vinorelbine in patients who had 25 to 75 % liver volume replaced by tumour (LVRT) was similar to that in patients with no liver metastases, however, it was markedly reduced in patients estimated to have greater than 75 % LVRT. The authors recommended a dose reduction of at least 50 % for patients with severe liver dysfunction but no dosage alteration for patients with moderate liver dysfunction.

Following administration of doxorubicin (60 mg/m^2) to 82 patients with various cancers, plasma concentrations in 8 patients with liver disease were 4 to 5 times higher than those measured in patients with normal liver function (Benjamin et al. 1974). A significant increase in toxicity, as measured by WBC nadir, platelet nadir, mucositis and drug related deaths, was observed in patients with impaired liver function compared to those with normal liver function. Nine subsequent patients with moderate or severe liver dysfunction were administered doxorubicin at 50 % and 25 % of the normal doses, respectively. Following these dose reductions, the patients with liver dysfunction had similar plasma concentrations and toxicity to patients with normal liver function. The influence of liver dysfunction on epirubicin pharmacokinetics is discussed in Section 1.6.6.

1.5.3 Individualised dose adjustment - a posteriori

A posteriori dose adjustment requires blood samples to be taken from a patient so that individual pharmacokinetic parameters can be determined and used to adjust the next dose to achieve the target systemic exposure. *A posteriori* dose adjustment generally achieves the pharmacokinetic target with more precision than *a priori* methods (Sandstrom et al. 2001; Nguyen et al. 2002). Furthermore, it is possible to identify those individuals that have received subtherapeutic doses, and thus the dose can be increased accordingly. A limitation of the *a posteriori* method is that dose adjustment is not possible for the first dose, except for drugs administered as prolonged infusions, where blood samples taken early during the infusion can be used to adjust the infusion rate at later times. Traditional methods used to estimate pharmacokinetic parameters require a large number of blood samples, which is costly and inconvenient to patients and staff. Limited sampling methods have therefore been developed that enable pharmacokinetic parameters to be estimated from only 1 to 3 blood samples. These approaches include multiple linear regression (MLR) and maximum *a posteriori* (MAP) Bayesian analysis.

1.5.3.1 MLR as a limited sampling approach

Several studies have used multiple linear regression to identify the best 2 or 3 blood sampling times at which measured concentrations correlate most highly with the desired PK parameter. A PK parameter, such as AUC, that has previously been related

to a pharmacodynamic response is usually chosen. Following MLR analysis, an equation is generated which enables subsequent estimation of the PK parameter from 2 or 3 blood samples. The MLR approach has been used successfully to estimate the AUC of many anti-cancer compounds including vinblastine (Ratain & Vogelzang 1987), cyclophosphamide (Egorin et al. 1989), carboplatin (Sorensen et al. 1993), 9-amino-20(S)-camptothecin (Spareboom et al. 1999) and etoposide (Panetta et al. 2002). This simple technique enables medical staff to calculate doses quickly and easily using a pocket calculator. A disadvantage of the MLR technique is that the dosage schedule and times at which blood samples are collected must strictly adhere to those used to generate the equation, which may not be practical in routine clinical use. Furthermore, estimates of pharmacokinetic parameters at the extreme ends of the parameter distributions have been very poorly estimated using MLR (Panetta et al. 2002) and it is in these patients that dose adjustment is the most crucial.

1.5.3.2 Bayesian estimation for limited sampling

Individual pharmacokinetic parameters can be estimated from a small number of measured concentrations using Maximum *A Posteriori* (MAP) Bayesian estimation if there is prior knowledge of population values and their variability in the population (Sheiner et al. 1975). Unlike MLR limited sampling strategies, MAP Bayesian analysis offers more flexibility regarding blood sampling and the dosage administration (Rousseau et al. 2000). In addition, estimates of clearance (CL), and hence AUC, obtained using the Bayesian approach have been shown to be more accurate and precise than those determined using MLR (Panetta et al. 2002). One of the limitations of Bayesian estimation is that it requires the development of complex computer software and extensive training of medical staff.

Population analysis has been used to design and validate limited sampling strategies using MAP Bayesian analysis for a number of anti-cancer compounds including vinorelbine (Nguyen et al. 2002), docetaxel (Baille et al. 1997) and etoposide (Panetta et al. 2002). The limited sampling designs were found to give good predictions of CL in terms of accuracy and precision. Although blood samples do not need to be taken strictly at specific times for MAP Bayesian estimation, they do need

to be accurately recorded and some sampling time windows will result in more accurate estimations of PK parameters than others.

1.5.3.3 D-optimality

The accuracy and precision of individual pharmacokinetic parameter estimates depends on the number of blood samples and the times of sampling (D'Argenio 1981). The most popular method used to identify the optimal blood sampling times is D-optimality. D-optimality selects the same number of sampling times as the number of parameters in the pharmacokinetic model, e.g. for a two compartment model, 4 sampling times are selected. The optimum times are selected as those that minimize the determinant of the inverse Fisher Information Matrix (Box & Lucas 1959). Accurate identification of optimum sampling times using this method requires that both the structure of the pharmacokinetic model and the true model parameters are known, which of course does not occur in reality. D-optimality does not normally incorporate information on the population distribution of the pharmacokinetic parameters, i.e. inter-individual variability. However, the D-optimality criterion has recently been extended to include such information and therefore aid in the design of population pharmacokinetic studies (Mentre et al. 1995; Mentre et al. 1997).

1.5.3.4 Limited sampling of anthracyclines

Doxorubicin AUC and CL have been successfully estimated from 2 blood samples using both MLR and Bayesian estimation methods. Bayesian analysis using concentration measurements at 20 min and 24 h gave estimates of doxorubicin CL that were in close agreement with those obtained using 9 samples (Launay et al. 1989). MLR methods identified 2 and 48 h as the optimal times to estimate doxorubicin AUC (Ratain et al. 1991), which, although were in good agreement with the actual AUC values, were not as reliable as those obtained with the Bayesian method.

In 78 patients receiving epirubicin for treatment of advanced breast cancer, Jakobsen et al. (1991a) used MLR to identify the 2 and 24 h concentrations as the best combination and the 6 h concentration as the best single point to estimate epirubicin AUC. In addition, using data collected from 55 patients, they identified a positive correlation between AUC and log fractional WBC nadir and proposed a model that

could predict log fractional WBC nadir from only 1 or 2 blood samples (Jakobsen et al. 1991b).

1.5.3.5 Clinical outcome following a posteriori dose adjustment

Most studies have assessed the practicalities of *a posteriori* dose adjustment (or "therapeutic drug monitoring", TDM) retrospectively and there are few that have demonstrated the benefits of TDM in terms of patient outcome. This is probably due, at least in part, to the difficulties in measuring a therapeutic response and the large patient numbers required. Ideally, such studies require two groups of patients; one in which standard, non-adjusted doses are used and the other in which individualised doses are used. An encouraging example of the potential benefits of TDM in chemotherapy was a study in which 188 children with acute lymphocytic leukaemia (ALL) were randomised to receive either a standard or an individualised 24 h infusion of methotrexate (Evans et al. 1998). In patients who received individualised doses, 2 concentrations measured early during the infusion were used to obtain Bayesian CL estimates and the infusion rate was then adjusted so that the target systemic exposure was achieved. There was a significantly better response rate in patients who received individualised doses (76 %) compared to those who had standard therapy (66 %), without an increase in toxicity. This study highlighted that some patients do not respond to methotrexate therapy because they receive an inadequate dose rather than because their disease is resistant to treatment.

When MAP Bayesian estimation was used to adjust doses of teniposide in 21 paediatric patients with ALL, there was a 50 % increase in average systemic exposure compared to that given by conventional therapy, without any increase in dose-limiting toxicity (Rodman et al. 1993). Blood samples taken after an initial dose of teniposide were used to determine the pharmacokinetics and optimum dose of teniposide to achieve the desired AUC in subsequent courses. A three-fold range in dose was required to achieve an AUC that had previously been correlated with response (Rodman et al.1987).

In 81 patients with head and neck cancer, reduction of 5-FU infusion rate on Day 3 of a 5-day infusion in patients with high $AUC_{0\text{-}3days}$ values reduced the incidence of toxicity from 20 % to 12 % (Santini et al. 1989). Furthermore, the tumour response rate was higher in the patients who had dose adjustments than in those who received

conventional therapy. One explanation for this is that patients who received conventional therapy and had toxicity, subsequently had longer intervals between cycles and fewer cycles.

1.6 EPIRUBICIN

1.6.1 Clinical use

Epirubicin is a cytotoxic anti-cancer agent that is mainly used in the treatment of breast cancer. It has been shown to be highly active in advanced breast cancer when administered as a single agent or in combination with other drugs (Carmo-Pereira et al. 1991; Miller et al. 2000). Treatment of early breast cancer by administration of epirubicin in combination with fluorouracil and cyclophosphamide (CEF) resulted in prolonged relapse-free survival compared to combinations of cyclophosphamide, methotrexate and fluorouracil (CMF), thereby suggesting a role for epirubicin in adjuvant therapy (Coukell & Faulds 1997). Epirubicin has also been shown to be effective in the treatment of many other types of cancer including gastric, pancreatic and bladder (Wils 1986; Pavone-Macaluso et al. 1993; Calais da Silva et al. 1988).

1.6.2 Maximum tolerated dose

Epirubicin belongs to the anthracycline group of compounds. It has a structure identical to that of its prototype, doxorubicin, with the exception of the spatial orientation of the hydroxyl group at the 4' position of the daunosamine sugar (Arcamone et al. 1975). At equal doses, epirubicin has been shown to have similar efficacy to doxorubicin but with reduced toxic side effects (Robert. 1993). The dose limiting toxicity for epirubicin is neutropenia (Tjuljandin et al 1990). The maximum tolerated dose of epirubicin is 150 to 180 mg/m^2 (Tjuljandin et al. 1990; Miller et al. 2000) compared to approximately 90 mg/m^2 for doxorubicin (Reich 1978). Repeated administration of either doxorubicin or epirubicin can result in cardiotoxicity; however, the maximum tolerated cumulative dose of epirubicin (1000 mg/m^2) is much higher than that of doxorubicin (550 mg/m^2) (Plosker & Faulds 1993). Thus epirubicin can be administered at higher doses and/or for more treatment cycles than doxorubicin.

1.6.3 Mechanism of action

Although the exact mechanism of action of epirubicin is not fully understood, several biochemical effects have been observed following treatment with anthracyclines and it is thought that the cytotoxic activity of epirubicin may result from one or more of these mechanisms.

Anthracyclines are thought to intercalate between DNA base pairs inducing DNA fragmentation (Plosker & Faulds 1993). Furthermore, the complex formed with DNA inhibits the activity of topoisomerase II. Topoisomerases are intranuclear enzymes that enable the DNA double helix to unwind by transiently breaking and rejoining the double DNA strands. The intercalative binding of epirubicin to DNA also inhibits DNA helicase activity, which is required to separate double-stranded DNA (Bachur et al. 1992). Thus by interfering with the activity of topoisomerase II and DNA helicase, epirubicin inhibits DNA replication and transcription.

Anthracyclines react with a number of reducing agents including nicotinamide adenine dinucleotide (NADH) P-450 reductase, cytochrome b_5-reductase and xanthine oxidase to undergo electron transfer resulting in the generation of semiquinone free radicals (Doroshow 1983; Sinha & Politi, 1990). The semiquinone free radicals can further react with oxygen to generate superoxide anion radicals, hydrogen peroxide and hydroxyl radicals (Goodman & Gilman, 1992). The free radicals and other reactive species are believed to result in cytotoxicity by alkylating DNA, causing DNA strand breaks, peroxidation of membrane phospholipids and reactions with other cellular macromolecules (Sinha & Politi, 1990).

Several mechanisms of resistance to anthracyclines have been identified. The most consistent finding in anthracycline resistant cells is the over expression of p-glycoprotein, an efflux pump that reduces intracellular drug accumulation (Mouridsen et al. 1990). Addition of inhibitors of p-glycoprotein, such as verapamil, to cell lines previously resistant was shown to restore the sensitivity of these cells to epirubicin (Plosker & Faulds, 1993). A significant increase in glutathione peroxidase and glutathione transferases was observed in a breast cancer cell line (MCF-7) that was resistant to doxorubicin (Sinha & Politi 1990). These enzymes are believed to detoxify the cytotoxic reactive species generated by anthracyclines. It has also been postulated

that interference with apoptotic pathways, such as over expression of the c-erbB-2 oncogene, could offer resistance to anthracyclines (Coukell & Faulds 1997).

1.6.4 Metabolism and excretion

The molecular structure of epirubicin and its major metabolic pathways are illustrated in Figure 1.1. Epirubicin is metabolised to epirubicinol and 4 aglycone metabolites by aldoketoreductase, predominantly in the liver, although metabolism also occurs in red blood cells (Weenan et al. 1983; Camaggi et al. 1988; Danesi et al. 2002). This route of metabolism is comparable to that of doxorubicin, which is metabolised to doxorubicinol and aglycones, however, epirubicin and epirubicinol can also undergo glucuronidation (Camaggi et al. 1988). Epirubicin glucuronide is the major metabolite found in both plasma and urine (Mross et al. 1988, Camaggi et al.1988). This additional route of metabolism for epirubicin could account for its more rapid elimination and hence its lower toxicity compared to doxorubicin. Epirubicinol is thought to be cytotoxic, whereas the aglycone metabolites are inactive and the activity of glucuronides is unknown (Plosker & Faulds 1993). Approximately 6 to 12 % of an epirubicin dose is excreted in the urine unchanged and a further 4 to 10 % is excreted in the urine as metabolites (Camaggi et al.1986; Camaggi et al.1988; Mross et al. 1988). The major route of epirubicin elimination is in the bile with approximately 22 % excreted as unchanged epirubicin and a further 13 % excreted as metabolites (Camaggi et al. 1986).

1.6.5 Pharmacokinetics

The plasma concentration-time profile of epirubicin is best described by a tri-exponential equation. A summary of pharmacokinetic parameter estimates reported in the literature is given in Table 1.1. Although the plasma clearance of epirubicin is rapid (50-85 L/h), the terminal half-life is relatively long (20 to 40 h) due to its large volume of distribution (500-1500 L/m^2).

Figure 1.1 Metabolic pathways of epirubicin

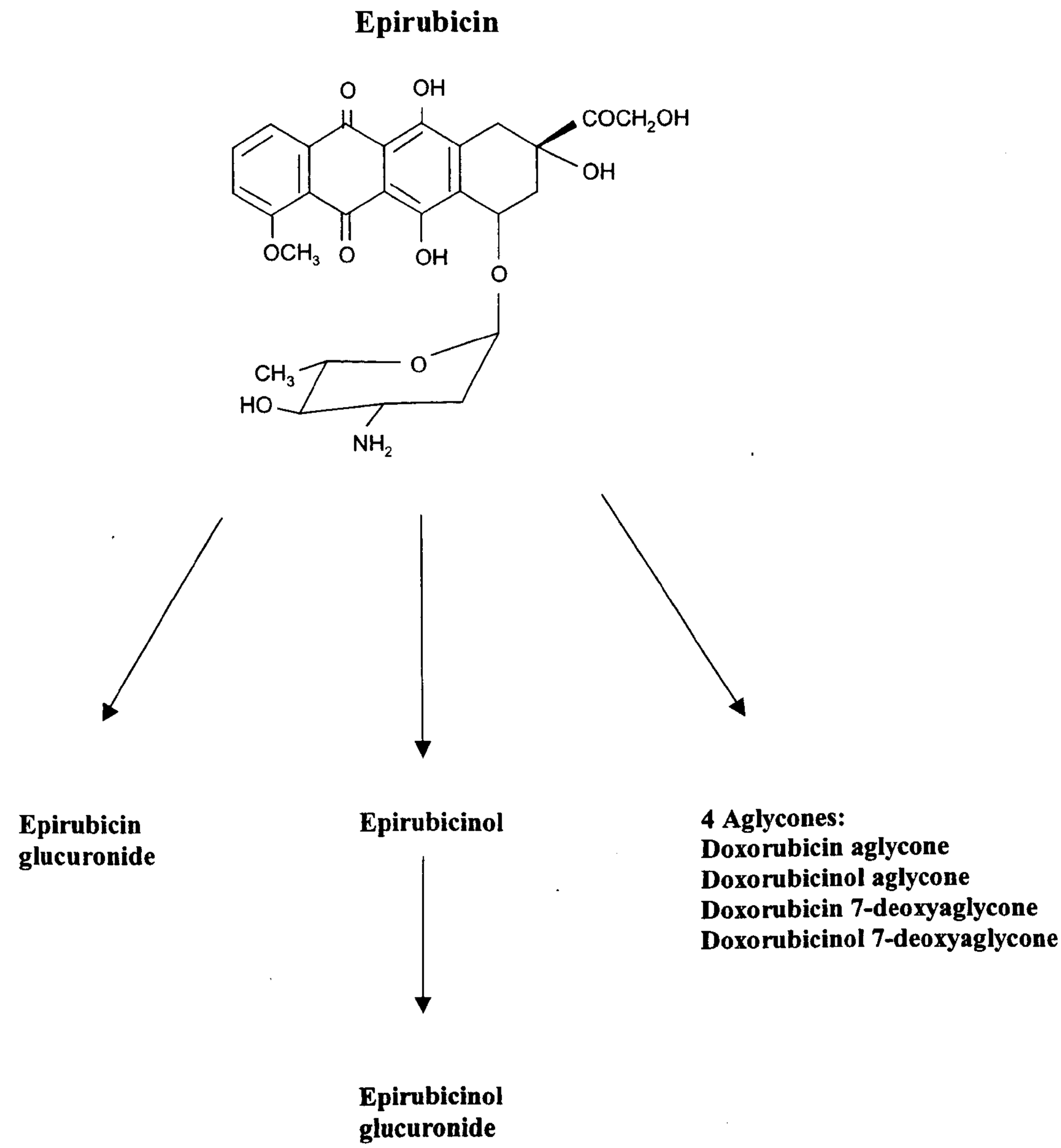


Table 1.1 Mean epirubicin pharmacokinetic parameter estimates reported in the literature

Reference	Dose range	Administration	Mean Pharmacokinetic Parameter Estimates					
			t1/2 α (min)	t1/2 β (h)	t1/2 γ (h)	V_{ss} (L/m^2)	CL (L/h)	n
Weenen (1983)	75-90 mg/m^2	NR	4.8	2.6	38	1430	NR	8
Mross (1988)	40-56 mg/m^2	bolus	1.8	0.49	15.3	609*	87.1	8
Robert (1992)	100 mg/m^2	bolus	2.43	1.95	21.7	851	52.6	5
Robert (1992)	100 mg/m^2	48 h infusion	NR	NR	27.8	699	46.7	5
Camaggi (1988)	60 mg/m^2	bolus	3	1.09	31.2	1309*	75	8
Danesi (2002)	90 mg/m^2	5 min infusion	3.6	1.37	16	480	74.4	6
Camaggi (1982)	60-90 mg/m^2	bolus	NR	NR	40	NR	52.9	11
Jakobsen (1991a)	40-135 mg/m^2	10 min infusion	8.4	1.79	19.5	NR	79.7*	55
Vrignaud (1985)	25-35 mg/m^2	3-6 min infusion	2.53	1.04	29.3	NR	71.9	10
Twelves (1992)	20-135 mg/m^2	bolus	2.94	1.28	22.7	NR	NR	22
Gurney (1998)	150 mg	120 h infusion	NR	NR	NR	NR	84.6	18

NR = Not reported

* = Units converted for comparative purposes, assuming a body weight of 70 Kg and a body surface area of 1.7 m^2

The pharmacokinetics of epirubicin in 78 patients with metastatic breast cancer were shown to be dose-proportional over the dose range of 40-135 mg/m^2 (Jackobsen et al. 1991a). In contrast, a greater than dose proportional increase in AUC and C_{max} values was reported in a study of 19 patients over the dose range of 90-150 mg/m^2 (Tjuljandin et al. 1990). However, this study included a smaller number of patients than that of Jakobsen and coworkers. In addition, the pharmacokinetic parameters were calculated using a 2-compartment model and the results appeared to be variable and inconsistent. Using a population approach, Wade and coworkers found that dose did not explain any of the inter-patient variability in the pharmacokinetics of epirubicin and therefore concluded that epirubicin had linear pharmacokinetics over the range of 25-100 mg/m^2 (Wade et al. 1992).

1.6.6 Factors influencing the pharmacokinetics of epirubicin

The AUCs of epirubicin and its metabolite epirubicinol increased by approximately 30 % and 100 %, respectively, when epirubicin was co-administered with paclitaxel/Cremophor EL (Danesi et al. 2002a). Both paclitaxel and Cremophor EL are substrates for P-glycoprotein and it has been suggested that these increases are due to a reduction in biliary excretion of epirubicin and epirubicinol resulting from competition for this carrier protein. Although co-administration of verapamil produced no changes in the pharmacokinetics of epirubicin and epirubicinol, significant increases (100 %) in the AUCs of the aglycones were observed (Mross et al. 1993). Verapamil also inhibits p-glycoprotein (Yusa & Tsuruo 1989), which may explain this effect. However, it is unclear why the pharmacokinetics of the aglycone metabolites were affected but not epirubicin or the other metabolites. The most likely explanation is that the small number of patients (n=10) included in this study was inadequate to obtain conclusive results. A 50 % increase in epirubicin and epirubicinol AUCs was observed following co-administration with cimetidine (Murray et al. 1998). The mechanism for this drug interaction remains unclear as although cimetidine is known to reduce blood flow, a decrease in liver blood flow was not observed.

Epirubicin clearance was found to be influenced by sex and age in a population analysis of epirubicin pharmacokinetics (Wade et al. 1992, see Section 1.6.7 for more details), however, a study of 27 patients with nasopharyngeal carcinoma found no

relationship between any pharmacokinetic parameter and sex or age (Hu et al. 1989). Patients with renal disease (n = 5) were found to have similar pharmacokinetics to patients with normal renal function (n = 11) following administration of epirubicin, however, a 40 % decrease in clearance was observed in patients with liver metastases (n = 6) (Camaggi et al. 1982). Abnormal liver function was also identified as influencing epirubicin clearance by Twelves et al. (1992), who observed a strong correlation between AST concentrations and epirubicin CL in 30 women with advanced breast cancer.

1.6.7 Population pharmacokinetics of epirubicin

A population analysis of epirubicin pharmacokinetics performed with NONMEM (Wade et al. 1992) found that CL was influenced by the sex of the patient and that age influenced CL in females but not in males. Limited data in males older than 50 years may have contributed to this observation. However, these results are inconsistent with the results from Hu et al (1989) who found no relationship between any pharmacokinetic parameter for epirubicin with sex or age. It should be noted that the population analysis performed by Wade and coworkers included only 36 patients, which is a very small number for a population analysis. Furthermore, their data were fitted with a 2-compartment rather than a 3-compartment model and the high residual error of 42 % suggests some model misspecification.

The population approach was also used to analyse data from 97 patients (177 cycles of treatment) with advanced breast cancer, who were administered epirubicin in combination with fluorouracil and cyclophosphamide (FEC) (Sandstrom 2002). A 3-compartment model was fitted to the data using NONMEM and covariates were added using an automated model building procedure. Albumin and bilirubin were found to have a statistically significant influence on epirubicin CL. However, as only 3% of the variability in CL was explained by these factors, this may have limited clinical significance. Since inter-occasional variability was estimated to be 7 %, which is low relative to the inter-individual variability (15 %) it suggested that therapeutic drug monitoring could be useful in treatment with epirubicin. Interindividual variability estimated in this study was much lower than that estimated in previous studies (29 to 75 %) (Camaggi et al 1982; Mross et al. 1988; Gurney et al. 1998; Danesi et al.

2002a). In most subjects only 2 to 4 blood samples were available per patient, which is perhaps insufficient to describe the concentration-time profile of a 3-compartment model. This could result in shrinkage of individual estimates of CL to the population values, resulting in an underestimation of interindividual variability.

1.6.8 PK-PD relationships for epirubicin

Jakobsen et al (1991b) identified a relationship between the epirubicin AUC and the fall in white blood cells (WBC), expressed as the logarithmic ratio of WBC nadir to pre-treatment WBC count, in 55 patients with advanced breast cancer. Dobbs and Twelves (1998b) also found statistically significant relationship between both WBC nadir and neutrophil nadir with epirubicin AUC, in 11 patients with advanced breast cancer. However, other studies have been unsuccessful in finding a relationship between AUC and response. No correlation was found between epirubicin AUC and WBC nadir in 22 patients with solid tumours (Camaggi et al. 1982) while in a study of 19 patients with various malignancies, linear regression analysis found no significant correlation between haematological toxicities (percentage change in granulocytes and neutrophils) and C_{max} or AUC (Tjuljandin et al. 1990). In addition, no significant relationship was observed between nonhaematological toxicities (stomatitis, nausea, vomiting and diarrhea) and C_{max} or AUC. However, both of these studies investigated relatively small numbers of subjects, and given the large variability in toxicity observed following treatment with anticancer agents, it is perhaps not surprising that no statistically significant relationships were observed between toxicity and pharmacokinetic parameters.

Semi-physiological models have been developed that successfully described the PK-PD relationships between WBC count-time profiles and plasma concentrations of fluorouracil-epirubicin-cyclophosphamide and of epirubicin-docetaxel (Sandstrom 2002). The models consisted of compartments that represent proliferating WBC, maturing WBC and circulating WBC. First order rate constants were used to describe the rate of proliferation, the maturation rate constant and the elimination rate of circulating cells. In the model, the drug acts on the proliferating compartment and effect is a function of drug plasma concentration and a drug-specific slope parameter. As the models were developed following treatment with combination therapy, it was

assumed that the individual influences of each drug on the WBC-time profile were additive. Despite this limitation, simulations based on the epirubicin model predicted WBC counts at 12 days post-dose that compared favourably with those reported by Jakobsen et al. (1991b) following administration of epirubicin as a single agent.

In a study of patients with nasopharyngeal carcinoma treated with epirubicin (Hu et al. 1989), the mean AUC in 6 complete responders was 4002 ng.h/ml which was approximately double that observed in 13 non-responders (1881 ng.h/ml). In contrast, no statistically significant difference in C_{max} was observed between responders and non-responders. Furthermore, no relationship between leukopenia, the dose-limiting toxicity, and any pharmacokinetic parameter was found. These results suggest that dose-adjustments according to an individual's estimated CL are likely to increase the chances of a tumour response.

1.6.9 Dosage guidelines for epirubicin

The current UK dosage guidelines recommend that the dose of epirubicin is based on the patient's BSA and bilirubin concentration (Epirubicin, Summary of Product Characteristics (SPC), UK). However, dosing according to BSA is "standard practice" in oncology and does not necessarily reflect scientific research. When 20 patients with BSAs ranging from 1.58 to 2.05 m^2 received a fixed epirubicin dose of 150 mg, no relationship was found between any pharmacokinetic parameter and BSA (Gurney et al. 1998). In addition, a population analysis of epirubicin pharmacokinetics in 79 patients with advanced breast cancer found that variability in epirubicin CL was not affected by BSA (Sandstrom 2002).

Following administration of epirubicin to 6 patients with liver metastases, a 40 % decrease in CL was observed compared to 11 patients with normal liver function, indicating that dose reductions may be required in patients with liver dysfunction (Camaggi et al. 1982). The UK dosage guidelines for epirubicin in patients with liver dysfunction are based on a study of doxorubicin (described in Section 1.5.2.3), performed 30 years ago (Benjamin et al. 1974). This study found that doxorubicin plasma concentrations in 8 patients with liver disease, as measured by elevated bilirubin concentrations, were 4 to 5 times higher than those measured in patients with normal liver function (n=82) and that this increase in systemic exposure to doxorubicin

was associated with a significant increase in toxicity. Nine subsequent patients with moderate (bilirubin 1.2 – 3 mg/100 ml) or severe (bilirubin > 3 mg/100ml) liver dysfunction were administered doxorubicin at 50 % and 25 % of the normal doses, respectively and their observed doxorubicin concentrations were similar to those observed in the patients with normal liver function. The proposed dosage guidelines were devised empirically rather than based on statistical evaluation of the relationships between PK parameters and markers of liver function, and furthermore, only included a relatively small number of patients with liver dysfunction. The dosage adjustments recommended in this study have been extended to form the dosage guidelines for all anthracyclines. Therefore, the current SPC guidelines for epirubicin recommend a 50 % reduction in dose for patients with moderately elevated bilirubin concentrations (bilirubin: 1.4 - 3 mg/100 ml) and a 75 % reduction for patients with severely elevated bilirubin levels (bilirubin: > 3 mg/100 ml). It should be noted that these dosage guidelines for epirubicin have not been validated.

More recently, a strong correlation was shown between serum AST levels and epirubicin CL in 30 women with abnormal liver function who received epirubicin for the treatment of advanced breast cancer (Twelves et al. 1992), however, there was no significant correlation with serum albumin, alkaline phosphatase or bilirubin. Thus, AST may be a better indicator for dose adjustment in hepatically-impaired patients than serum bilirubin. Another study in 16 patients with advanced breast cancer and abnormal liver tests also found a strong linear relationship between epirubicin CL and log AST (Dobbs & Twelves 2003) and used this relationship to produce a dosage nomogram to obtain a target AUC: dose = AUC (97.5-34.2 x log AST). The nomogram was used to determine epirubicin doses in a further 41 patients. Inter-individual variability in AUC values using the nomogram (CV = 26 - 30 %) was reduced compared to standard doses (CV = 47 %), however, the improvement was not statistically significant.

A survey that included 173 UK oncologists found that many oncologists do not follow the current UK dosage guidelines for epirubicin and showed considerable variability in prescribing habits between clinicians (Dobbs & Twelves 1998a). Therefore, further work is required to develop and validate new dosage guidelines for epirubicin.

1.7 AIMS

The aims of this thesis were to use a population approach to determine the typical pharmacokinetic parameters of epirubicin, evaluate their variability in the population, identify clinical characteristics that influence the pharmacokinetics of epirubicin and then use this information to develop new dosage guidelines. *A priori* and *a posteriori* methods for dose individualisation were investigated in this thesis. FDA guidelines on population pharmacokinetic analysis, recommend that population models, and particularly those intended to influence dosage guidelines, are validated. Therefore, a variety of techniques were utilised to validate the proposed dosage guidelines.

CHAPTER 2

GENERAL METHODS

INTRODUCTION TO CHAPTER 2

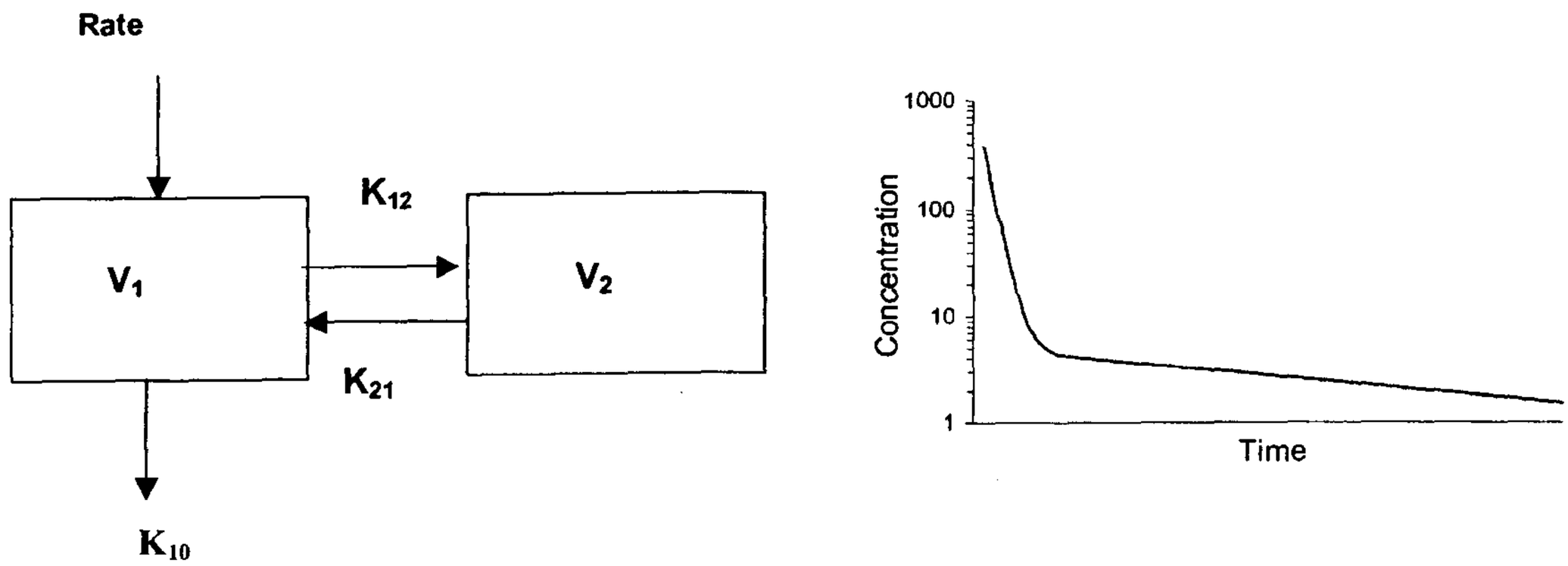
This Chapter provides details of pharmacokinetic and statistical methods that are common to subsequent Chapters. Firstly, a background to pharmacokinetic models is presented, then details are given of how the software package NONMEM was used for population pharmacokinetic analysis, MAP Bayesian estimation and simulation. Finally, some other statistical methods used in this thesis are summarised. Details of specific methods are provided as required within the later Chapters.

2.1 PHARMACOKINETIC MODELS

The most common approach to modelling pharmacokinetic data is to represent the body by one or more compartments. These compartments are often not representative of bodily tissues or organs but can be used to generate mathematical models to describe the data. The simplest model, a "one-compartment model", considers the body as a single unit in which the drug is rapidly and homogeneously distributed. Drug concentrations are observed to decrease with time in a monoexponential manner. However, most drugs do not distribute instantaneously throughout the entire body space but rather take some time to distribute into tissues. Drug concentrations during this distribution phase decline more rapidly than during the post-distribution phase and more complicated models with multiple compartments are needed to describe such profiles. In this thesis two and three compartment models were used to describe the pharmacokinetics of epirubicin.

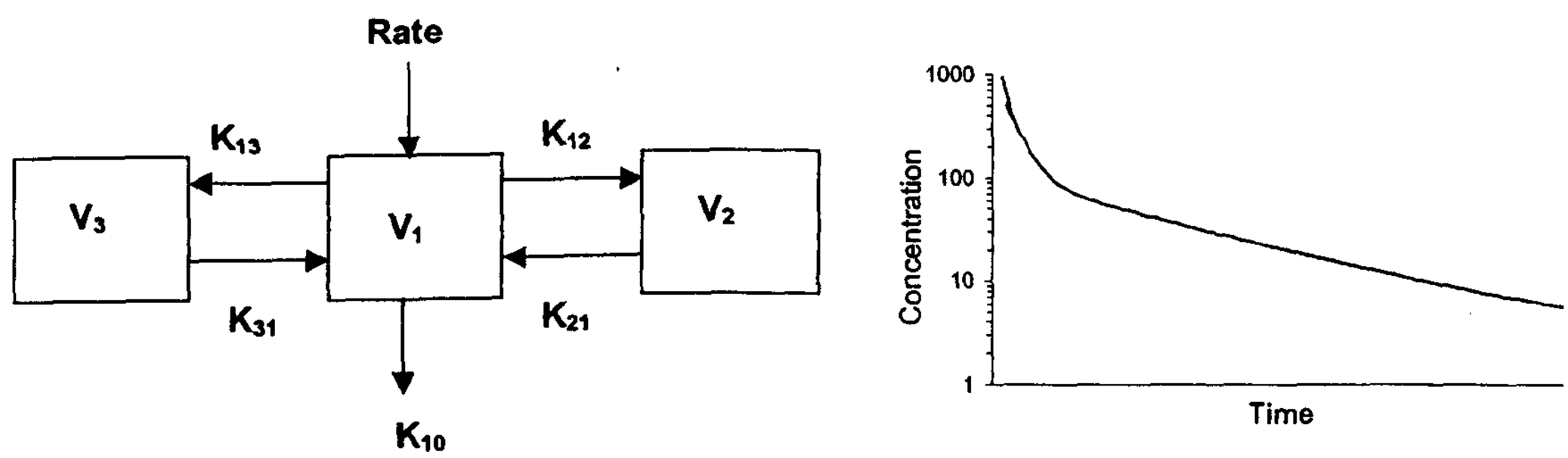
In a two compartment model, the body can be considered as having a "central" compartment (compartment 1) and a "peripheral" compartment (compartment 2). The central compartment represents the blood or organs that are in rapid equilibrium with the blood and the peripheral compartment represents tissues into which the drug distributes more slowly. Transfer of drug between compartments is assumed to be first order and, in this thesis, elimination is assumed to occur exclusively from the central compartment. A two-compartment model is illustrated in Figure 2.1.A, where V_1 and V_2 are the volumes of compartment 1 and compartment 2, respectively, k_{12} is the constant that describes the rate of transfer of drug from compartment 1 to compartment 2 and k_{21} describes the rate of transfer from compartment 2 to compartment 1.

Figure 2.1.A. A 2-compartment model with a corresponding concentration-time profile.



Key: V_1 is the volume of compartment 1, V_2 is the volume of compartment 2, k_{12} is the rate constant describing the movement of drug from compartment 1 to compartment 2 and k_{21} is the rate constant describing the movement of drug from compartment 2 to compartment 1

Figure 2.1.B. A 3-compartment model with a corresponding concentration-time profile



Key: V_1, V_2 and V_3 are the volumes of compartment 1, 2 and 3, respectively k_{12} is the rate constant describing the movement of drug from compartment 1 to compartment 2, k_{21} is the rate constant describing the movement of drug from compartment 2 to compartment 1, k_{13} is the rate constant describing the movement of drug from compartment 1 to compartment 3 and k_{31} is the rate constant describing the movement of drug from compartment 3 to compartment 1.

If A_1 is the amount of drug in compartment 1 and A_2 is the amount of drug in compartment 2, then the rate of change of drug in compartment 1 is described by the following equation:

$$dA_1/dt = k_{21}A_2 - k_{12}A_1 - k_{10}A_1$$

where t is time. The rate of change of drug in compartment 2 is described as follows:

$$dA_2/dt = k_{12}A_1 - k_{21}A_2$$

Solving the above equations for the amount of drug in the central compartment (A) at time t gives:

$$A_t = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t}$$

Concentrations can be calculated by dividing amount by volume, hence, the concentration at time t in the central compartment can be described as follows:

$$C_t = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t}$$

where λ_1 is the slope of the distribution phase of the concentration-time curve and λ_2 is the slope of the elimination phase of the concentration time curve. Figure 2.1A illustrates a concentration-time profile for a two-compartment model and shows how drug concentrations decrease with time in a bi-exponential manner. The distribution and elimination half-lives, i.e. the time taken for concentrations to half, can be calculated as follows:

Distribution half-life = $(\ln 2) / \lambda_1$

Elimination half-life = $(\ln 2) / \lambda_2$

Likewise, in a 3-compartment model, the body is represented by a central compartment and two peripheral compartments. A 3-compartment model is illustrated in Figure 2.1.B, where V_1, V_2, k_{12} and k_{21} are as above, V_3 is the volume of compartment 3 and k_{13} and k_{31} are rate constants describing the movement of drug from compartment 1 to compartment 3 and vice versa. If A_1, A_2 and A_3 are drug

amounts in compartments 1, 2 and 3 then the rate of change of drug in compartment 2 is as described above and the rate of change of drug in compartments 1 and 3 is described as follows:

Compartment 1

$dA_1/dt = k_{21}A_2 + k_{31}A_3 - k_{12}A_1 - k_{13}A_1 - k_{10}A_1$

Compartment 3

$dA_3/dt = k_{13}A_1 - k_{31}A_3$

Solving the above equations for the amount of drug in the central compartment (A) at time t gives:

$A_t = A_1e^{-\lambda_1 t} + A_2e^{-\lambda_2 t} + A_3e^{-\lambda_3 t}$

and as before, dividing through by volume gives:

$C_t = C_1e^{-\lambda_1 t} + C_2e^{-\lambda_2 t} + C_3e^{-\lambda_3 t}$

The distribution and elimination half-lives can be calculated as follows:

Distribution half-life 1 = $(\ln 2) / \lambda_1$

Distribution half-life 2 = $(\ln 2) / \lambda_2$

Terminal half-life = $(\ln 2) / \lambda_3$

Clearance (CL), defined as the proportionality factor relating drug elimination to plasma concentrations, can be calculated from $k_{10}V_1$. The analysis in this thesis focused on systemic exposure to epirubicin as measured by the area under the concentration time curve (AUC), and as

AUC = Dose/CL

it was more useful to parameterise the pharmacokinetic model in terms of CL rather than micro-rate constants. The two compartment model was parameterised to give estimates of CL, V_1, V_2 and intercompartmental clearance (Q_2). The three compartment model was parameterised to give estimates of CL, V_1, V_2, V_3 and intercompartmental clearance (Q_2 and Q_3) where Q_2 is intercompartmental CL between

compartments 1 and 2, and Q_3 is intercompartmental CL between compartments 1 and 3.

For a 2-compartment model, parameters CL, V_1, V_2 and Q_2 are related to the micro-rate constants as follows:

$CL = k_{10} * V_1$

$Q_2 = k_{12} * V_1$ *or* $Q_2 = k_{21} * V_2$

For a 3-compartment model, parameters V_1, V_2, V_3, Q_2 and Q_3 are related to the micro-rate constants as follows:

$CL = k_{10} * V_1$

$Q_2 = k_{12} * V_1$ *or* $Q_2 = k_{21} * V_2$

$Q_3 = k_{13} * V_1$ *or* $Q_3 = k_{31} * V_3$

2.2 POPULATION PHARMACOKINETIC ANALYSIS

Population pharmacokinetic analysis is used to determine the typical pharmacokinetics in the population and their variability within the population. The population approach can also identify clinical factors influencing the pharmacokinetics of a drug. In this thesis, population analysis was performed using mixed effect modelling. Mixed effect models are so called because they include the influence of both fixed effects and random effects. Fixed effects are factors that can be measured or determined, e.g. pharmacokinetic parameters and covariates. Random effects are factors that cannot be observed, such as unknown differences in physiology or bioanalytical errors. In population pharmacokinetic models, random effects can be subdivided into inter-individual variability, inter-occasion variability and residual error.

2.2.1 Modelling inter-individual variability in PK parameters

In this thesis, PK parameters were assumed to be log-normally distributed (Lacey et al. 1997). Inter-individual variability in PK parameters can be modelled as follows, using CL as an example:

$CL_i = TVCL*EXP(\eta_{CLi})$ which is equivalent to

$\ln(CL_i) = \ln(TVCL) + \eta_{CLi}$

where (CL_i) is the individual CL value, TVCL is the typical population value of CL and ETA (η_{CLi}) is the difference between ln(CL_i) and ln(TVCL). The relationship between ln(CL_i), ln(TVCL) and η_{CLi} is illustrated in Figure 2.2A The distribution of η_{Cli}s for all subjects within the population is assumed to be normal with a mean of zero and a variance of ω^2, as illustrated in Figure 2.2B.

2.2.2 Modelling residual variability

The residual errors (ϵ) represent the differences between the measured and model predicted concentrations and contain contributions from inter-occasional variability, assay error, model misspecification and other unknown errors. Figure 2.3A illustrates the jth measured concentration in the ith individual (c_{ij}), the corresponding model predicted concentration ($PRED_{ij}$) and the difference between them (ϵ_{ij}). Residual errors are unpredictable random quantities, however, the estimates of ϵ are assumed to follow a normal distribution with a mean of zero and a variance of σ^2. A normal distribution of ϵ is illustrated in Figure 2.3B.

Three residual error models were used in this thesis:

1) Additive $c_{ij} = pred_{ij} + \epsilon_{ij;}$

where the error on each concentration measurement is constant, regardless of the concentration value,

2) Proportional $c_{ij} = pred_{ij}\ (1+\epsilon_{ij})$

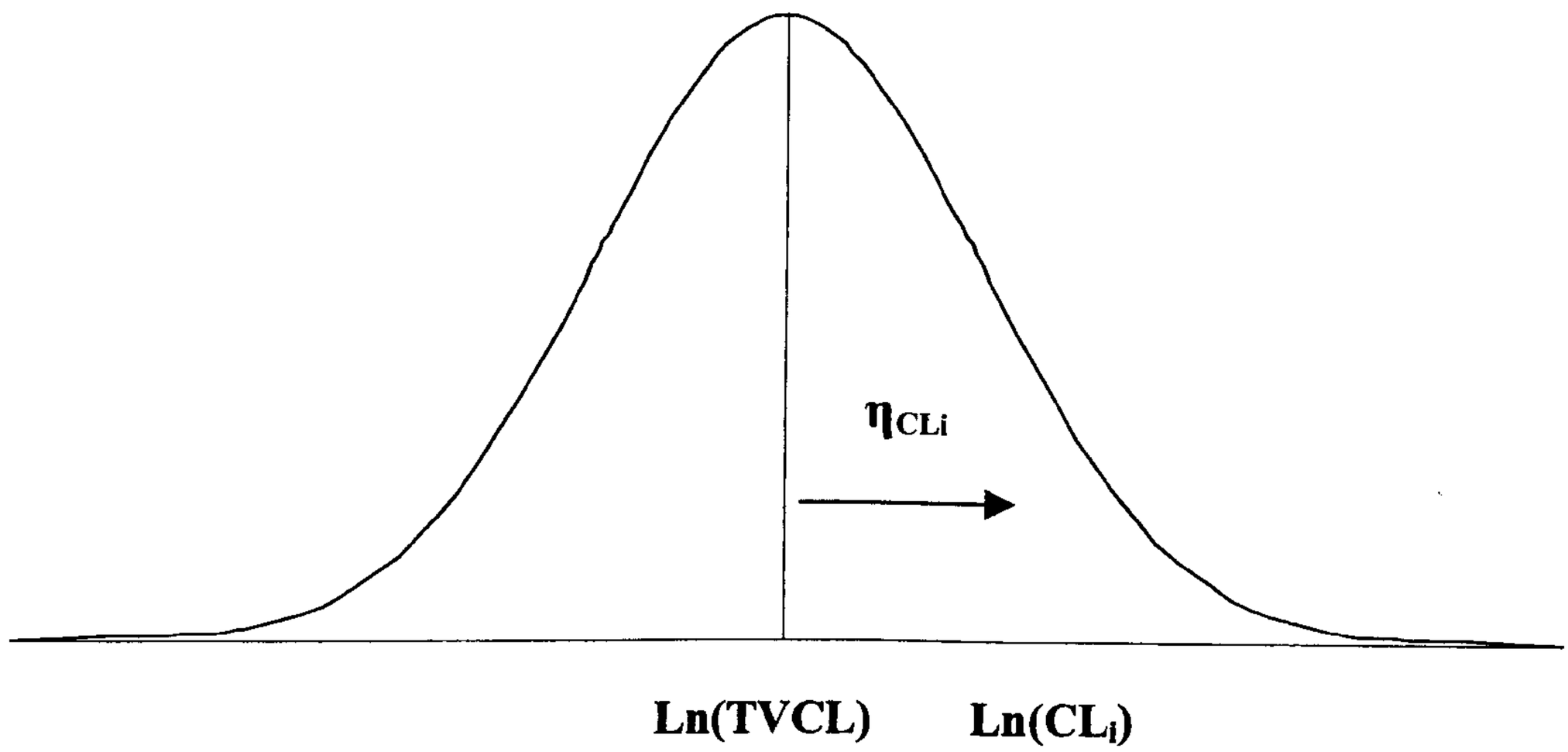
where the error is proportional to the concentration,

(N.B. the code for a proportional error model used in the control stream is given in Figure 2.4).

and 3) Combined $c_{ij} = pred_{ij} + pred_{ij}.\epsilon_{ij} + \epsilon_{2ij}$

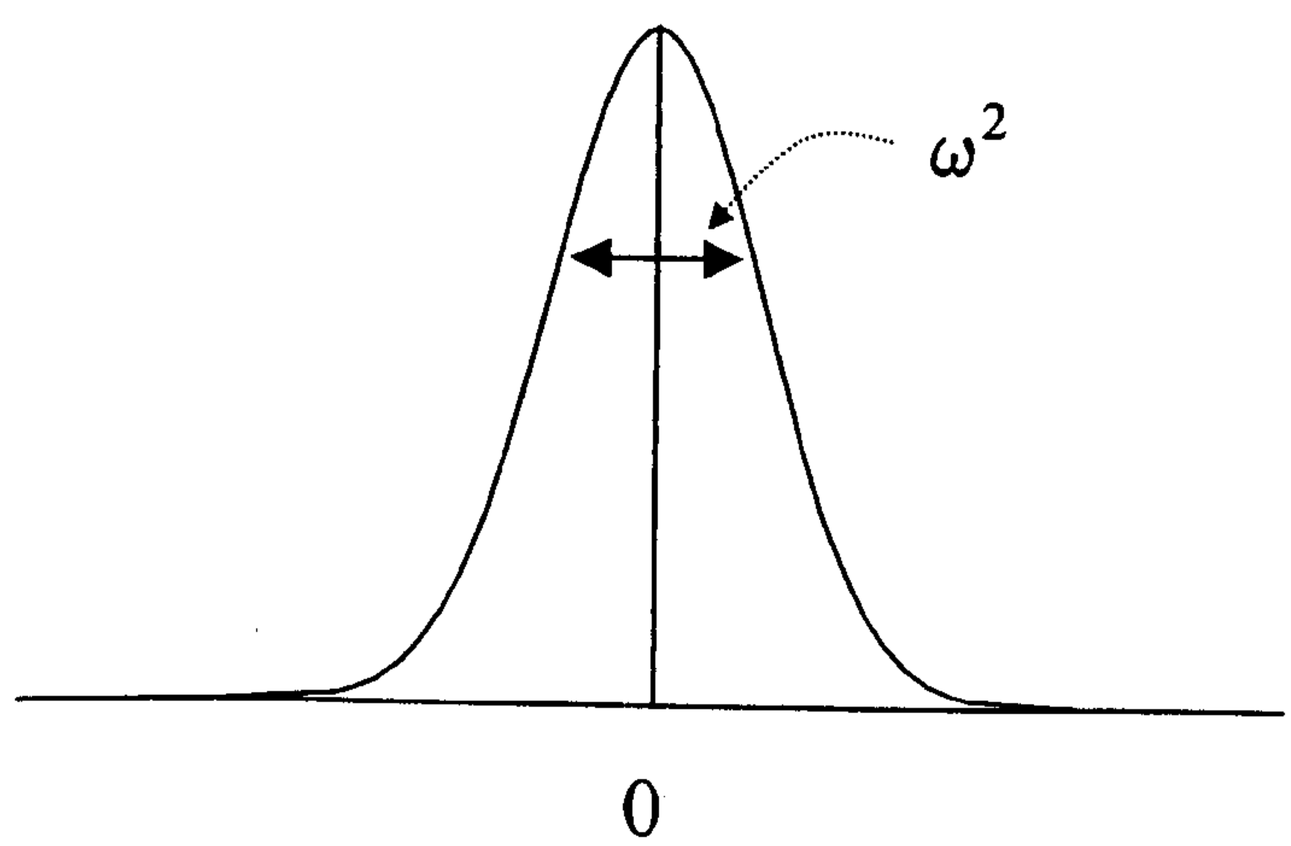
which has both additive and proportional components. At low concentrations the additive component dominates and at high concentrations the proportional component dominates.

Figure 2.2A. Diagram illustrating the relationship between TVCL, CL_i and η_{CLi}



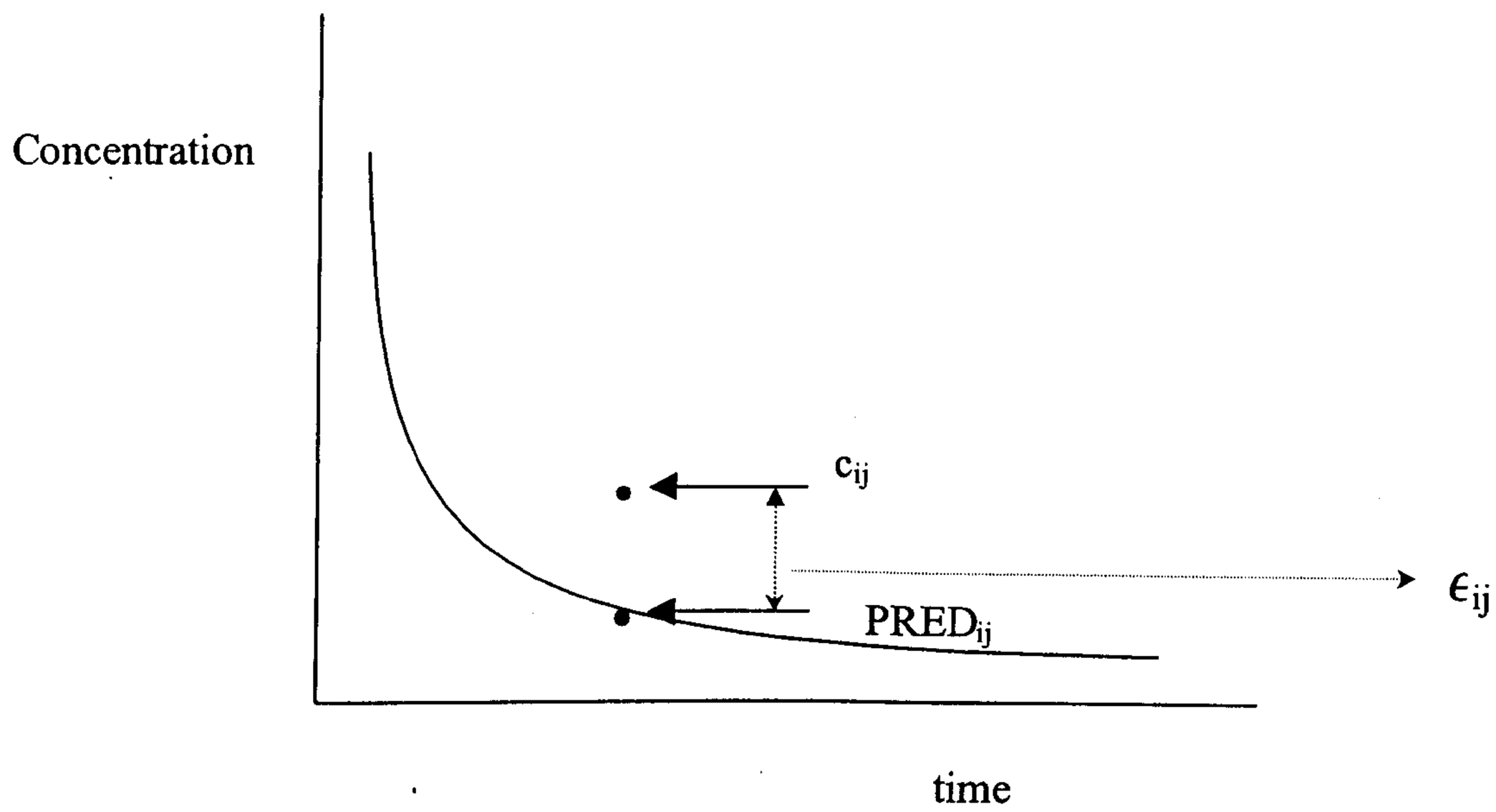
Key: CL_i is the estimate of CL for an individual, TVCL is the typical value of clearance in the population, η_{CLi} is the difference between the ln(TVCL) and ln(CL_i)

Figure 2.2B. Distribution of ηs for all subjects within the population



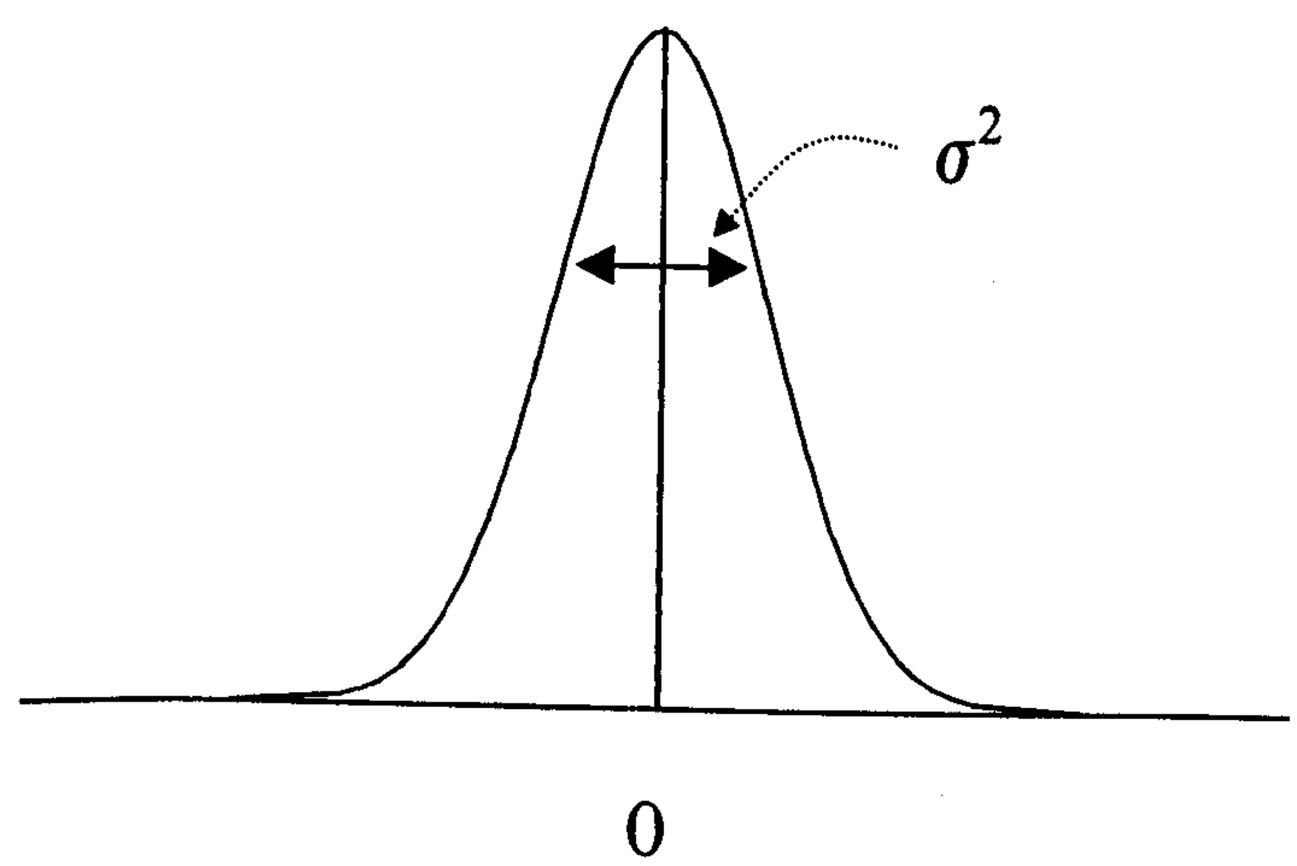
Legend: ηs values for all subjects are assumed to be normally distributed with a mean of zero and a variance of ω^2.

Figure 2.3A. Diagram illustrating the relationship between observed concentrations, predicted concentrations and residual error



Key: c_{ij} is the measured concentration, $PRED_{ij}$ is the predicted concentration, ϵ_{ij} is the residual error i.e. the difference between c_{ij} and $PRED_{ij}$

Figure 2.3B. Distribution of ϵ_{ij} for all concentrations in all subjects in the data set



Legend: ϵ_{ij} values for all concentrations in all subjects in the data set are assumed to be normally distributed with a mean of zero and a variance of σ^2.

2.3 NONMEM

2.3.1 Program

Population pharmacokinetic analysis was performed using NONMEM Version V (Beal & Sheiner 1992) with Compac Visual Fortran Compiler Version 6.0 on a computer with a Microsoft Windows 98 operating system and an AMD Duron processor. Visual NM (2.2) (Research and Development for Population Pharmacokinetics (R.D.P.P)) was used as a Windows interface for NONMEM to enable data management and aid graphical exploration. NONMEM is a FORTRAN computer program that was developed to fit NONlinear Mixed Effect Models to pharmacokinetic and pharmacodynamic data, especially data arising from population pharmacokinetic studies.

A collection of FORTRAN subroutines have been written for use with NONMEM, including ADVAN, TRANS, PK and ERROR. In PK, the user enters a model to estimate values for population pharmacokinetic parameters, individual pharmacokinetic parameters and inter-individual variability, whereas in ERROR, the user enters a model to describe the differences between measured and observed dependent variables e.g. concentrations. The ADVAN subroutines describe the structural model e.g. bolus input with monoexponential decline, first order input with biexponential decline, etc. In this thesis, two and three compartment models were fitted to the data. ADVAN3 is the subroutine for a two compartment model with basic parameters k_{10}, k_{12} and k_{21}. Using the translator subroutine, TRANS4, this was parameterised to provide estimates of CL, V_1, V_2 and Q_2. ADVAN11 is the subroutine that describes a three compartment model with basic parameters k_{10}, k_{12}, k_{21}, k_{13} and k_{31}. This was parameterised using TRANS4 to give estimates of CL, Q_2, Q_3, V_1, V_2 and V_3.

2.3.2 NONMEM data file structure

The clinical data to be analysed was arranged in columns. Information required in the data file included subject identification number (ID), dose administered (AMT), rate of dose input (RATE), blood sampling time (TIME) and observed concentration (DV, dependent variable). Additional columns were included for the covariate data e.g.

weight, age, bilirubin concentration, etc. An example of the NONMEM data file structure is given in Figure 2.4.

2.3.3 Control stream format

An example of a control stream is presented in Figure 2.5. The control stream is a non-formatted text file and is comprised of a series of records each initiated with "$". The first line of the control stream gives the title of the problem ($PROB), then the names of the column headers are listed under ($INPUT). Some of these column headers, such as ID, AMT, DV are specifically recognised by NONMEM, whereas others, such as BILI, AGE, etc. can be defined by the user. The name and path (location) of the data file is given in the next line under ($DATA). The next part of the file defines the model that will be used to fit the data. The subroutine used for the structural model, i.e. the ADVAN and TRANS subroutines are chosen under ($SUBROUTINE). In $PK the THETAs were assigned to describe the typical population values of PK parameters and their relationship with any covariates. ETAs were assigned to describe the random effects for the structural model i.e. inter-individual variability. A scale factor was also included in the $PK record to convert the amount of drug measured in a compartment to a concentration. A model to describe residual error was specified in the $ERROR record; further details of the approaches used to model residual variability are given in Section 2.2.2. Initial estimates for structural parameters ($THETA), variance of inter-individual error ($OMEGA) and variance of residual error ($SIGMA) are also provided. If covariance between ETAs was included in the model, the BLOCK command was used in the $OMEGA record. The number of ETA variables in the block was given e.g. 'BLOCK(3)' indicates that there are 3 ETA variables and that covariance is to be estimated between them. An example of a variance-covariance matrix is shown below.

Figure 2.4

Excerpt from a NONMEM data file

ID	AMT	TIME	DV	RATE	BILI	AST	ALB	HGT	WGT	MG	WHO	IWGT	AGE	CRCL
101	78	0	0	2364	7	36	31	155	56	78	1	98	71	62.8
101	0	0.2	1084	0	7	36	31	155	56	78	1	98	71	62.8
101	0	0.33	575	0	7	36	31	155	56	78	1	98	71	62.8
101	0	0.5	157	0	7	36	31	155	56	78	1	98	71	62.8
101	0	1.03	111	0	7	36	31	155	56	78	1	98	71	62.8
101	0	2.03	39	0	7	36	31	155	56	78	1	98	71	62.8
101	0	4.06	42.3	0	7	36	31	155	56	78	1	98	71	62.8
101	0	6.07	43.9	0	7	36	31	155	56	78	1	98	71	62.8
101	0	7.53	28.9	0	7	36	31	155	56	78	1	98	71	62.8
101	0	29.63	25.4	0	7	36	31	155	56	78	1	98	71	62.8
101	0	46.64	20.1	0	7	36	31	155	56	78	1	98	71	62.8
102	22	0	0	1100	2	20	48	160	66	22	0	111	56	81.2
102	0	0.2	317	0	2	20	48	160	66	22	0	111	56	81.2
102	0	0.333	83.1	0	2	20	48	160	66	22	0	111	56	81.2
102	0	0.5	56	0	2	20	48	160	66	22	0	111	56	81.2
102	0	1	47.2	0	2	20	48	160	66	22	0	111	56	81.2
102	0	2.5	39.2	0	2	20	48	160	66	22	0	111	56	81.2
102	0	3.96	26.3	0	2	20	48	160	66	22	0	111	56	81.2
102	0	22.73	10	0	2	20	48	160	66	22	0	111	56	81.2
102	0	31.86	7.5	0	2	20	48	160	66	22	0	111	56	81.2
102	0	46.93	3.8	0	2	20	48	160	66	22	0	111	56	81.2
103	105	0	0	1591	7	27	37	152	53	105	1	97	71	58.5
103	0	0.2	501.9	0	7	27	37	152	53	105	1	97	71	58.5
103	0	0.333	157.4	0	7	27	37	152	53	105	1	97	71	58.5
103	0	0.5	72.3	0	7	27	37	152	53	105	1	97	71	58.5
103	0	1.01	37.7	0	7	27	37	152	53	105	1	97	71	58.5
103	0	4.92	26	0	7	27	37	152	53	105	1	97	71	58.5
103	0	8.01	24.9	0	7	27	37	152	53	105	1	97	71	58.5
103	0	27.9	24.6	0	7	27	37	152	53	105	1	97	71	58.5
103	0	31.27	13.7	0	7	27	37	152	53	105	1	97	71	58.5
103	0	48	9.4	0	7	27	37	152	53	105	1	97	71	58.5
104	40	0	0	2000	6	33	32	148	59.5	40	2	114	67	48.6
104	0	0.15	577	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	0.2	241	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	0.25	142	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	0.333	87	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	0.5	66	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	0.75	51	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	1	36	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	2	26	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	6.5	12.3	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	31	5.9	0	6	33	32	148	59.5	40	2	114	67	48.6
104	0	37.1	3.7	0	6	33	32	148	59.5	40	2	114	67	48.6

Key: ID = patient identification number, AMT = dose, TIME = time post-start of infusion, DV = dependent variable (epirubicin plasma concentration), RATE = rate of drug infusion, BILI = bilirubin concentration, AST = AST concentration, ALB = albumin concentrations, HGT = height, WGT = weight, MG = dose (mg), WHO = performance status, IWGT = ideal body weight, CrCL = creatinine clearance.

Figure 2.5

Example of a control stream

```
$PROB EPIRUBICIN
$INPUT ID AMT TIME DV RATE BILI AST ALB HGT WGT MG WHO IWGT
AGE CRCL SY BSA LMET
$DATA C:\LORRAINE\MODEL4.PRN
$SUBROUTINE ADVAN11 TRANS4
$PK  TVCL=THETA(1)
     TVV1=THETA(2)
     TVQ2=THETA(3)
     TVV2=THETA(4)
     TVQ3=THETA(5)
     TVV3=THETA(6)
     CL=TVCL*EXP(ETA(1))
     V1=TVV1
     Q2=TVQ2
     V2=TVV2
     Q3=TVQ3*EXP(ETA(2))
     V3=TVV3*EXP(ETA(3))
     S1=V1/1000
$ERROR  IPRED=F
     DEL=0
     IF(F.EQ.0) DEL=1
     W=IPRED+DEL
     IRES=DV-IPRED
     IWRES=IRES/W
     Y=F+ERR(1)*W
$THETA (0,40) (0,10) (0,30) (0,40) (0,55) (0,800)
$OMEGA 0.2  0.2 0.2
$SIGMA 0.5
$EST MAX=9000 SIG=3 PRINT=5 NOABORT
$COV
$TABLE ID AMT TIME DV RATE BILI AST ALB HGT WGT MG WHO IWGT
AGE CRCL SY BSA LMET TVCL TVV3 TVQ3 CL V1 Q2 V2 Q3 V3 ETA(1) ETA(2)
ETA(3) IPRED IRES IWRES FILE=RUN1.PAR NOPRINT
```

	ETA1	ETA2	ETA 3
ETA1	0.2		
ETA 2	0.01	0.2	
ETA 3	0.01	0.01	0.2

In this example, the variance of the ETAs (diagonal values) are each estimated to be 0.2 and the covariance terms (off-diagonal values) are each estimated to be 0.01.

In the $ESTIMATION record, conditions for the estimation process are provided, such as the minimum number of significant figures to which accuracy of estimates is required, the maximum number of objective function evaluations (Section 2.4.1) allowed and the method of estimation eg. First Order or First Order Conditional Estimation (Section 2.3.4). A $COVARIANCE record was included to request that standard errors of parameters were generated. A $TABLE record was included to request generation of a table that included observation data, predicted concentrations, residuals, weighted residuals and other requested items.

2.3.4 Estimation methods in NONMEM

The best model to describe the data is the one that minimizes the differences between the measured concentrations and the model predicted concentrations. For non-linear models the best solution may not be unique ie. there may be more than one relative minimum. NONMEM uses linearization methods to approximate the non-linear model to a linear model during the fitting process. Two estimation algorithms were used in this thesis: the first-order method (FO) and the first-order conditional estimation method (FOCE).

The first-order method (FO) linearizes the non-linear model with respect to the random errors i.e. ETAs and epsilons, by using a first order Taylor-series expansion and is evaluated with the random effects set to zero ie. the individual ETAs are not estimated. This method attains estimates of the population model parameters including PK parameters, interindividual variability and residual error, but does not produce individual model parameters. ETAs for each individual are attained

subsequently using the 'POST-HOC' option (referred to as the posthoc estimates) by Bayes' methods using the estimated population parameters.

The first-order conditional estimation method (FOCE) is an extension of the FO method, but determines individual estimates of the ETAs at every iteration of the regression, thereby influencing the final estimates of the population parameters. Again, the first-order Taylor series expansion is used but the derivatives with respect to the ETAs are conditional on the individual estimates of ETA rather than fixing the ETAs to zero. Two estimation methods are available when using FOCE: 'no interaction' and 'interaction'. The default method 'no interaction' estimates the residual error from the mean parameter values. When 'interaction' is specified, residual error is estimated using the conditional estimates of ETAs. If the residual error model contains a proportional component, it is dependent on the predicted concentrations, which are in turn dependent on the individual ETAs, and therefore the 'interaction' option should be used. An additive residual error model is independent of the predicted concentrations, and therefore, the interaction option would not influence the parameter estimates.

In some cases, the FO method has resulted in a biased fit of the data (Beal & Sheiner 1992). Furthermore, a simulation analysis that compared the apparent significance level to the actual significance level, indicated that the FO method tends to over estimate the significance level of covariates and is likely to result in selection of false covariates (Wählby et al 2001). The FOCE method can produce parameter estimates that are less biased than the FO method and apparent significant levels are more consistent with actual significance levels (Beal & Sheiner 1992; Wählby et al 2001). The more complex algorithm used in the FOCE method results in an increased computation time and may result in increased convergence difficulties compared to the FO method. In this thesis, FOCE was the estimation method of choice, however, the FO method was used if convergence difficulties were encountered with FOCE.

In certain circumstances, such as in therapeutic drug monitoring, individual PK parameter estimates are required, but estimation of population parameters are not required. Individual PK parameters can be estimated from a small number of measured concentrations using Maximum *A Posteriori* (MAP) Bayesian estimation if there is prior knowledge of population values and their variability in the population (Sheiner et al. 1975). In this thesis, MAP Bayesian estimates of PK parameters were

obtained using NONMEM by including previously obtained estimates of population parameters in the control stream and using the 'MAXEVAL=0' option in the $ESTIMATION step to indicate that estimation of prior distributions of PK parameters was not required (an example of a control stream is provided in the Appendix).

2.4 MODEL SELECTION WITH NONMEM

2.4.1 Objective function value

NONMEM searches for the parameter values which best describe the data by a process of iteration. At each iteration, NONMEM predicts the concentrations at all sampling times given the current PK parameter estimates and then calculates the difference between the predicted and measured concentrations. The overall error between observations and predictions is based on the Extended Least Squares Objective Function Value (OFV):

$$OFV_{ELS} = \sum_{j} [\log \sigma^2 + (y_{ij} - M_{ij}(\theta, \eta_i))^2 / \sigma^2]$$

where σ^2 is the variance of the residual errors, y_{ij} is the jth observation from the ith individual and $M_{ij}(\theta, \eta_i)$ describes the jth population parameter in the ith individual. The process of iteration continues until the OFV is minimised. This value is based on the final model parameters and corresponds to –2 times the maximum log of the likelihood of the data (-2LL).

2.4.2 Selecting the best structural model

A reduction in the OFV was taken as an indication of improvement in the structural model. In addition, the following graphical tools were used to aid selection of the best structural model:

2.4.2.1 DV versus PRED/IPRED

Plotting measured concentrations versus predicted concentrations gives a general sense of the goodness of fit of the model. Predicted concentrations, estimated from the population model, are denoted as PREDs, whereas concentrations predicted from an

individual subject's pharmacokinetic parameter estimates are denoted IPREDs. Ideally, the points should be randomly (without bias) and closely (precisely) distributed around the line of unity throughout the concentration range.

2.4.2.2 Observed concentrations, PRED and IPRED versus time

One plot is produced per subject, which enables the analyst to compare the concentration-time profiles for the population predicted and individual predicted concentrations with the measured concentrations. These plots enable identification of any anomalous measurements and aid assessment of how well the structural model is fitting the data.

2.4.2.2 Residuals versus time

Residuals (RES) are the differences between the observed concentrations (DV) and the concentrations predicted by the population parameter estimates (DV-PRED). Individual residuals (IRES) are the differences between the observed concentrations and the concentrations predicted from the individual parameter estimates (DV-IPRED). Weighted residuals (WRES and IWRES) are produced by transforming the residuals so that all weighted residuals have unit variance and are uncorrelated. Weighted residuals should be randomly distributed around zero, over time. Biased predictions or trends in the WRES v. time plot indicates that the structural component of the model is inappropriate.

2.4.3 Selecting the best residual error model

The residual error model was selected with the help of the following graphical tools:

2.4.3.1 WRES or IWRES versus PRED

If the residual error model is adequate, weighted residuals should be equally and randomly scattered around zero when plotted against predicted concentration. WRES are approximately standard deviation units and WRES >3 or <-3 could indicate outliers.

2.4.4 Screening for covariates of interest

Once the optimum structural and residual error models were selected, the inclusion of covariates in the model was investigated. Testing the inclusion of covariates in NONMEM can be a time-consuming process, particularly if a large number of covariates are available. It is therefore useful to screen the covariates for those of potential interest to limit the number required for testing with NONMEM.

2.4.4.1 Graphical approaches

Covariates of potential interest were identified by plotting the individual estimates of each PK parameter against the covariates and looking for relationships. Plotting the PK parameters against the covariates can also give the analyst a feel for the 'shape' of the relationship e.g. linear, log linear.

2.4.4.2. Generalised additive modelling

Generalised additive model (GAM) analysis was also used to identify potentially important covariates. In the GAM analysis, the relationship between parameters and covariates can be described by the following equation:

$$P = \alpha + \sum_{j=1}^{p} f_j (X_j)$$

where P is the predicted pharmacokinetic parameter, α is a constant and $f_j(X_j)$ represents either a linear function or a smoothing spline.

In this thesis, GAM analysis was performed using the software packages Xpose Version 3.0 (Jonsson & Karlsson, 1999), which contains control files for S-PLUS 2000 (MathSoft International, 1999). The GAM analysis was performed separately on each PK parameter. Models were tested using a stepwise addition/deletion method. The GAM was set to model build with each covariate excluded and included as a linear regression model. If the linear regression model was found to be statistically significant, the covariate was then included as a natural cubic spline with one internal breakpoint. At each step, the addition or deletion of a covariate was determined as the one that resulted in the largest decrease in the Akaike information criterion (AIC) (Akaike, 1978). The addition/deletion stopped when the AIC reached a minimum value. The AIC value was calculated as follows:

$AIC = n_{obs}.\ Ln(WRSS) + 2n_{par}$

where n_{obs} is the number of observations, n_{par} is the number of parameters and WRSS is the weighted residual sum of squares.

Following GAM analysis with each pharmacokinetic parameter, Cook's distance was plotted against leverage. Cook's distance is a measure of the influence an individual has on the relationship and the leverage is a measure of how much an individual influences the certainty of the relationship (Cook 1977). An individual with a high value of Cook's distance and leverage indicates that they have a strong influence on the fit and may affect covariate selection. A model that is highly influenced by one or two unusual individuals may not be representative of the data set as a whole. Individuals with high leverage and/or Cook's values were removed from the data set and the GAM analysis was repeated.

2.4.5 Addition of covariates to the model

The covariates identified from the scatter plots and GAM analysis as influencing the PK parameters were tested for their inclusion in the model using NONMEM. Covariates were added into the NONMEM model, one at a time, as linear, power and $\log_e$ functions, as appropriate (an example of a control stream is provided in the Appendix).

2.4.5.1 Model comparison

The minimum value of the objective function provided in the NONMEM output was used as a tool to aid selection of the best model. The difference in the OFV between two hierarchical models indicates whether the full model is better than the reduced model. Two models are hierarchical if fixing the additional parameter(s) to zero produces the reduced model. The difference in the OFV between hierarchical models is approximately chi-squared distributed with n degrees of freedom, where n is the difference in the number of parameters between the models. For an α-level test, the change in the OFV can be compared to the $100(1-\alpha)$ percentile of the chi-squared distribution, given in statistical tables. For example, when n=1 (i.e. there is 1 additional parameter in the full model) for the improvement in the full model to be statistically significant at $p<0.05$ or $p<0.001$, the decrease in the OFV is required to be

at least 3.84 or 10.83, respectively. In this thesis, following the addition of a covariate to the model, a reduction in the OFV of greater than 3.84 was considered statistically significant ($p<0.05$, one degree of freedom) if FOCE was used. Wählby and coworkers (2001) previously showed that the FO method tends to over estimate the significance level of covariates and is more likely to result in false covariates being included in the model than if FOCE is used, therefore in this thesis if model building was performed using FO, the fall in OFV required to assume statistical significance was increased to 10.83.

The covariate found to have the greatest influence, as judged by the largest fall in OFV, was retained in the model and the other covariates were then added into the model singularly, again retaining the covariate with the lowest OFV. This was repeated until all the identified covariates were added to the model or until there was no further statistically significant reduction in OFV.

The effect of adding a covariate to the model was also assessed using graphics as follows, taking CL as an example. Delta CL for each patient was calculated by the subtraction of the typical population estimate of CL from the individual estimate of CL, estimated with and without the covariate of interest included in the model. Following the addition of the covariate to the model, if it has been adequately incorporated, no relationship between delta CL and covariate should be observed.

If inclusion of a covariate in the model resulted in a negligible reduction in interpatient variability, as measured by the coefficient of variation (CV%), it was considered to be of no clinical relevance and was removed from the model.

2.5 SIMULATION

Patient data sets were simulated by Monte Carlo methods using NONMEM. This approach takes into account inter-individual and residual error in a model and uses random inputs from the model parameter distributions to create a simulated data set comprising data points at the requested times for the desired number of patients. Information on the population model parameters was entered and a data file was prepared in the same format as a normal data file, containing patient identification numbers for the desired number of patients in the data set, with desired dosage history

and blood sampling times. Zero values were entered into the DV column. Individual PK parameters for the patients with corresponding concentration data were generated with NONMEM using the 'ONLY SIM' option in the $SIMULATION record with a random number entered as a seed (an example of a control stream is provided in the Appendix).

2.6 ESTIMATION OF BIAS AND IMPRECISION

Imprecision and bias of parameter estimates were calculated using the method described by Sheiner and Beal (1981). Prediction errors (P_{ej}) of estimates were expressed as follows:

P_{ej} (%) = (Estimate – Reference) / Reference x 100

The smaller the prediction error, the greater the precision of the estimate. The root mean squared prediction error (rmse), which describes the imprecision of the estimates, was calculated from the square-root of the mean squared P_{ej} (%) (mse) values ie.

$$mse = \frac{1}{N}\sum_{i=1}^{N} p_{ej}^{2} \qquad rmse = \sqrt{mse}$$

Bias is a measure of the amount by which the estimates are systematically too high or too low. The mean prediction error (me), which describes the bias of the estimates, was calculated from the mean of the P_{ej} (%) values.

The standard error ($se_{\overline{X}}$) of the me or mse was calculated from the following equation:

$$se_{\overline{X}} = \left[\frac{1}{N(N-1)}\sum_{i=1}^{N}(X_i - \overline{X})^2\right]^{1/2}$$

where for me, X_i are taken as P_{ej} and $\overline{X}$ is the mean of P_{ej} values (me). For mse, X_i are the individual squared P_{ej} values and $\overline{X}$ is the mean of squared P_{ej} values (mse). Confidence intervals (95 % CI) were calculated for me and mse using the following formula:

$$95\ \%\ \mathrm{CI} = \overline{X} \pm t_{0.975}(N-1)se_{\overline{X}}$$

where $t_{0.975}$(N-1) is the 97.5th percentile of the t-distribution with N-1 degrees of freedom, determined from statistical tables. A 95 % CI for rmse was calculated from the square root of the end points of the 95 % CI for mse.

CHAPTER 3

POPULATION ANALYSIS OF AN EPIRUBICIN DATA SET

3.1 INTRODUCTION TO CHAPTER

Epirubicin is predominantly cleared by the liver (Weenan et al. 1983; Camaggi et al. 1988) and a decrease in CL has been observed in patients with liver metastases (Camaggi et al. 1982). The current UK dosage guidelines recommend that the dose of epirubicin is based on the patient's BSA and bilirubin concentration (Epirubicin, Summary of Product Characteristics (SPC), UK). However, no significant relationship between epirubicin CL and bilirubin was observed in patients with abnormal liver function who received epirubicin for the treatment of advanced breast cancer (Twelves et al. 1992).

The aim of the work in this Chapter was to identify clinical characteristics that influence epirubicin CL. Previous studies that have investigated the influence of liver dysfunction on the pharmacokinetics of epirubicin have included only a small numbers of patients with liver dysfunction i.e. n=6 (Camaggi et al. 1982) and n=30 (Twelves et al. 1992). The analysis performed in this Chapter includes 109 patients, 72 of whom had liver metastases. Furthermore, the analysis in this Chapter was performed using mixed effect modelling, which is advantageous to the two-stage methods used in previous analyses as it provides errors of the parameter estimates, separate estimates of residual and inter-individual variability and estimates individual PK parameters with more precision and less bias than the two-stage approach (Sheiner & Beal 1980; Sheiner & Beal 1981a).

3.2 METHODS

3.2.1 Patient data

The data from 109 patients were collated from three trials in patients with cancer who were treated with epirubicin at Guys Hospital, London. Trial 1 included 50 patients (numbers 101 to 150) with advanced breast cancer who were considered to have normal liver function (less than 2.5-fold the reference AST level in the absence of liver metastases or less than 5-fold the reference AST level if liver metastases were present). Patients were administered epirubicin as a rapid intravenous infusion at doses in the range 12.5 to 120 mg/m^2, generally once every 3 weeks. However, for 8 patients, the first course of treatment was split into two doses of epirubicin, with the second dose administered 3 days after the first. In Trial 2, 23 patients (numbers 401-423) with advanced breast cancer and liver metastases were administered 25 mg/m^2 as a rapid intravenous infusion, once a week. In Trial 3, 37 patients (numbers 201 to 237) with advanced breast cancer and liver metastases were administered epirubicin, as a rapid intravenous infusion, at doses of 20 to 75 mg/m^2 every 3 weeks. The dose for patients in Trial 3 was determined according to the individual's AST concentration and performance status (classified according to the WHO criteria).

Blood samples were collected for the measurement of epirubicin concentrations on the first day of the first course of treatment with epirubicin. Plasma concentrations of epirubicin were measured using high performance liquid chromatography (HPLC) with fluorescence detection (Dobbs & Twelves 1991). The detection limit of the assay was 1 ng/ml. The precision of the assay (within-day and between-day), as measured by coefficients of variation, was less than 6 % at concentrations ranging from 5 to 2000 ng/ml.

The time of administration, length of infusion and blood sampling times were recorded. The following clinical measurements were performed on each patient: sex; age; height; weight; creatinine; albumin; bilirubin; AST; alkaline phosphatase; haemoglobin; white blood cell count; neutrophil count; and platelets. The presence or absence of liver metastases and bone metastases was recorded and whether the patient had received prior chemotherapy or endocrine treatment. Additional clinical factors were calculated, such as creatinine clearance (according to the Cockcroft-Gault equation (1976)), ideal body weight, body surface area, and performance status (classified according to the WHO criteria). The following pharmacodynamic data were

collected: response to treatment (progressive disease, stable disease, partial response, complete response); progression free interval; length of survival; pre-treatment haemoglobin count; pre-treatment white blood cell count; pre-treatment neutrophils count; pre-treatment platelet count; haemoglobin nadir; white blood cell nadir; neutrophil nadir; platelet nadir; haemoglobin on Day 21; white blood cell count on Day 21; neutrophil count on Day 21; and platelets on Day 21. These data were supplied by the clinical investigators in the form of an Excel spreadsheet.

3.2.2. Exploratory analyses of data set

Clinical variables were plotted against patient identification numbers in order to identify any gross errors such as a missing decimal place. Concentration-time profile plots and the data set spreadsheet were examined in order to identify any anomalous values. The frequency distribution of height, weight, ideal body weight, age, bilirubin, alkaline phosphatase, AST, albumin and creatinine clearance were plotted as histograms to assess normality and to identify any multi-modality. Any covariates that were identified as not normally distributed were log-transformed. Relationships between covariates were investigated by producing matrix plots using Minitab Version 13. For any covariates that were only normally distributed when log-transformed, the log-transformed values were used in the bivariate plots.

3.2.3. Assessment of structural and statistical models

Data were analysed with the population pharmacokinetic software package NONMEM Version V (Beal & Sheiner 1992). Two and three compartment models with zero order input were fitted to the epirubicin concentration-time data. The variance-covariance matrix was constrained to be diagonal. Using the first-order method (FO), individual Bayesian estimates of the pharmacokinetic parameters were obtained for each individual following population analysis using the 'post-hoc' option in NONMEM. The objective function values for each structural/error model and goodness of fit plots were compared to aid selection of the model that best fitted the data, as described in Chapter 2. Using the model that was determined to be the most appropriate, NONMEM analysis was repeated using the first-order conditional estimation method with interaction (FOCE-INTER) and compared to that using the first-order method

(FO) to see if there was an improvement in the fit of the model to the data, as judged by goodness of fit plots.

3.2.4. Identification of covariates for model building with NONMEM

Relationships between PK parameter estimates (estimated using the FO method) and continuous covariates (dose, height, weight, ideal body weight, BSA, age, creatinine clearance, bilirubin, AST, alkaline phosphatase and albumin) were investigated by visual inspection of scatter plots of individual PK parameters against covariates. A trend line (polynomial) was added to aid visual identification of any relationships. Box plots were produced to assess the influence of categorical variables, including presence of bone metastases, presence of liver metastases, WHO grade, prior endocrine treatment and prior chemotherapy on PK parameters. Statistical analysis of covariate-parameter relationships by Generalised Additive Modelling (GAM) was performed using the software packages Xpose Version 3.0 and S-plus 2000, as described in Chapter 2. The GAM analysis was performed using individual Bayesian estimates of PK parameters obtained using FO and FOCE.

3.2.5. Population model building using NONMEM

The population model building with NONMEM was performed using FOCE-INTER. The covariates identified from the scatter plots and GAM analysis (top 3 models) as influencing CL were tested using NONMEM for their inclusion in the CL model. Subjects with missing data for any of the clinical characteristics identified in the top GAM model were excluded from the analysis. Covariates were added into the model as linear functions. In addition, covariates that appeared to have a non-linear relationship with CL as judged from the scatter plots and the GAM analysis were added as power, exponential, reciprocal and $\log_e$ functions and the most appropriate function selected. The inclusion of covariates in the model for CL was determined by the objective function value (OFV), graphics and change in inter-individual variability as described in Chapter 2. A reduction in the OFV of greater than 3.84 was considered statistically significant ($p<0.05$, one degree of freedom). Covariates were then included into the models for V_3 and Q_3 using the same method of addition.

3.2.6 Assessment of variance-covariance matrix

The variance parameters were allowed to covary ie. a full variance-covariance matrix was included in the model and the model building process was repeated as described above.

3.3 RESULTS

3.3.1 Patient Data

Concentration-time data were available from 109 patients (Trials 1 to 3). Summary statistics for the demographics and clinical characteristics from these patients are displayed in Table 3.1. The ranges of bilirubin, AST and alkaline phosphatase levels were extended considerably beyond the normal reference range. Approximately 21 % of patients had bilirubin levels above the normal range, 69 % of patients had AST levels above the normal range and 69 % of patients had alkaline phosphatase levels above the normal range. Creatinine and albumin levels were generally within the normal range with only 6 % of patients with albumin concentrations below the normal range and 7 % of creatinine concentrations above the normal range. Following a single dose of epirubicin, a median of 11 blood samples was collected post-dose from each patient (ranging from 7 to 17). Typical blood sampling times were pre-dose and at 6, 10, 15 and 30 minutes post-dose and 1, 2, 4, 6, 12, 24, 48 hours post-dose. The median infusion time was 3 minutes.

3.3.2. Exploratory analyses of data set

The distribution of height, weight, age, creatinine and albumin were similar in all 3 trials. However, levels of bilirubin and AST were markedly elevated in more patients from Trials 2 and 3 compared to patients from Trial 1. There was no evidence of multi-modality (multiple populations) for any of the covariates. Histograms of the frequency of covariates showed that albumin, height, weight, ideal body weight and age were from a normal distribution whereas bilirubin, alkaline phosphatase, AST and creatinine appeared to be log normally distribution (data not shown). Positive correlations were observed between AST and bilirubin, AST and alkaline phosphatase, and bilirubin and alkaline phosphatase. Negative correlations were observed between albumin and AST, albumin and bilirubin, and albumin and alkaline phosphatase (Figure 3.1).

Table 3.1

Summary of demographics and clinical characteristics of patients

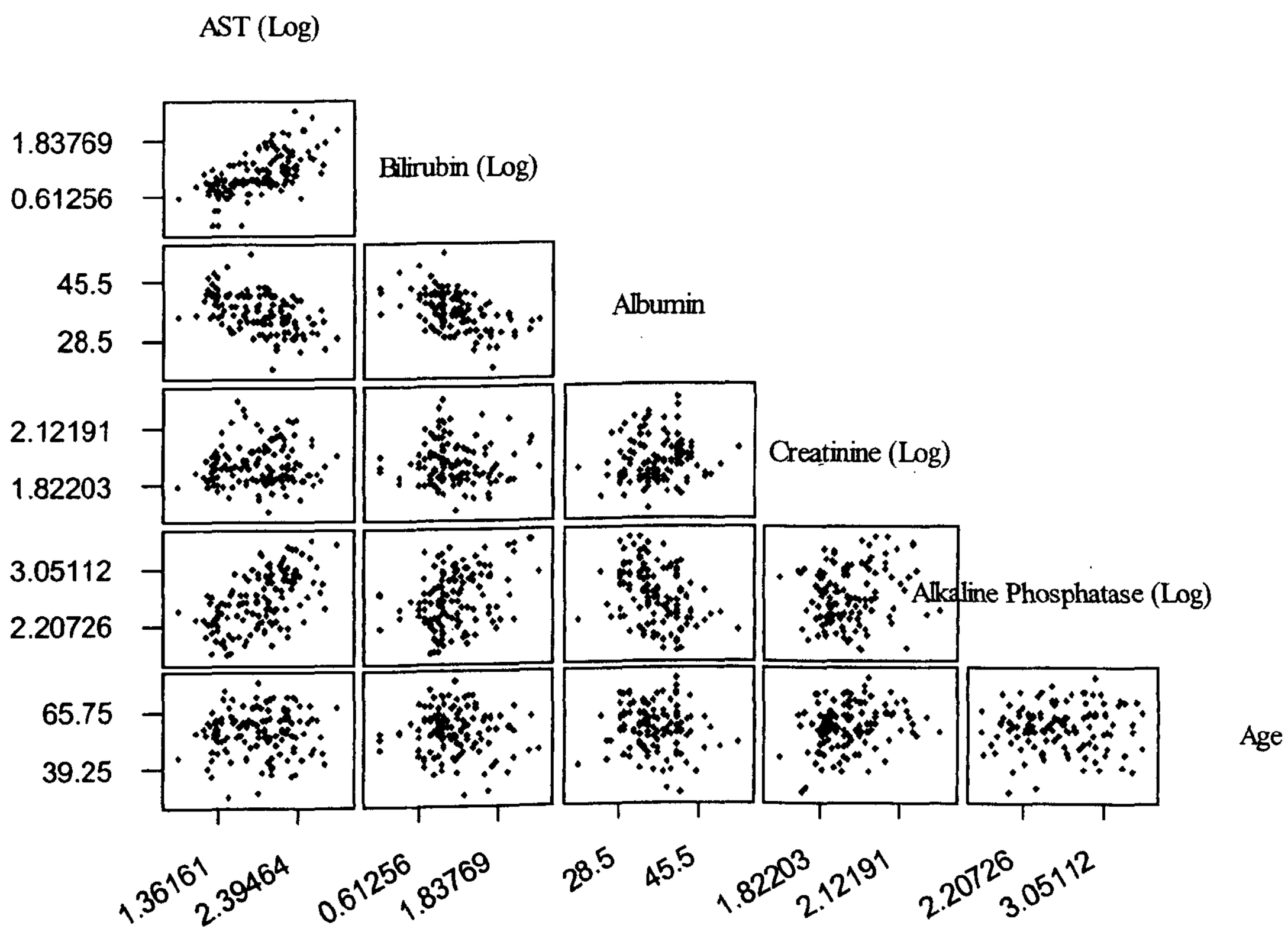
Clinical characteristics	Reference range	Median	Range	n
Dose (mg)		78	(20-228)	109
Age (years)		57	(35-79)	109
Height (cm)		157	(132-175)	108
Weight (Kg)		62.8	(37-89)	108
% Ideal body weight		110	(71-157)	108
Bilirubin ($\mu mol.l^{-1}$)	< 23	10	(1-282)	109
AST ($units.l^{-1}$)	< 43	90	(7-815)	109
Creatinine ($\mu mol.l^{-1}$)	50-130	82	(47-167)	104
Albumin ($g.l^{-1}$)	30-46	36.5	(25-54)	106
Alkaline phoshatase ($units.l^{-1}$)	< 255	403	(61-2972)	108
Haemoglobin ($g.dl^{-1}$)*	12-15	12.4	(8.4-17.1)	108
White blood cell ($x10^9.l^{-1}$)*	4-11	7.7	(3-22.1)	108
Neutrophil ($x10^9.l^{-1}$)*	2-8	5.91	(1.6-21.3)	99
Platelet ($x10^9.l^{-1}$)*	150-400	242	(38-587)	108

Clinical characteristics	Count	n
WHO performance status		109
0	11	
1	47	
2	41	
3	10	
Liver metastases (yes)	72	109
Bone metastases (yes)	66	109
Prior chemotherapy (yes)	24	86
Prior endocrine treatment (yes)	77	86

* = pre-treatment cell count

Figure 3.1

Matrix plot to assess relationships between covariates



3.3.3 Assessment of structural and statistical models

3.3.3.1 Assessment of structural and statistical models using FO

Successful convergence was achieved for both the 2 and 3 compartment models using FO. However, the estimate of the inter-individual variability of V_2 using the 3 compartment model was indeterminate, indicating that this model was over parameterised with respect to η's. The goodness of fit for the 2 and 3 compartment models, assessed by plotting individual predicted concentrations against measured concentrations, showed that concentrations below 1000 ng/ml, and hence, during the distribution and terminal phases of the concentration profile were relatively well estimated. In contrast, concentrations above 1000 ng/ml, which correspond to the "peak" plasma concentrations, were not estimated well. Examples of measured and individual predicted concentration-time profiles, estimated using the 3 compartment model with combined error, for a typical patient from each of the 3 trials are illustrated in Figure 3.2. Data up to the 6 minute time point were subsequently excluding from further analysis.

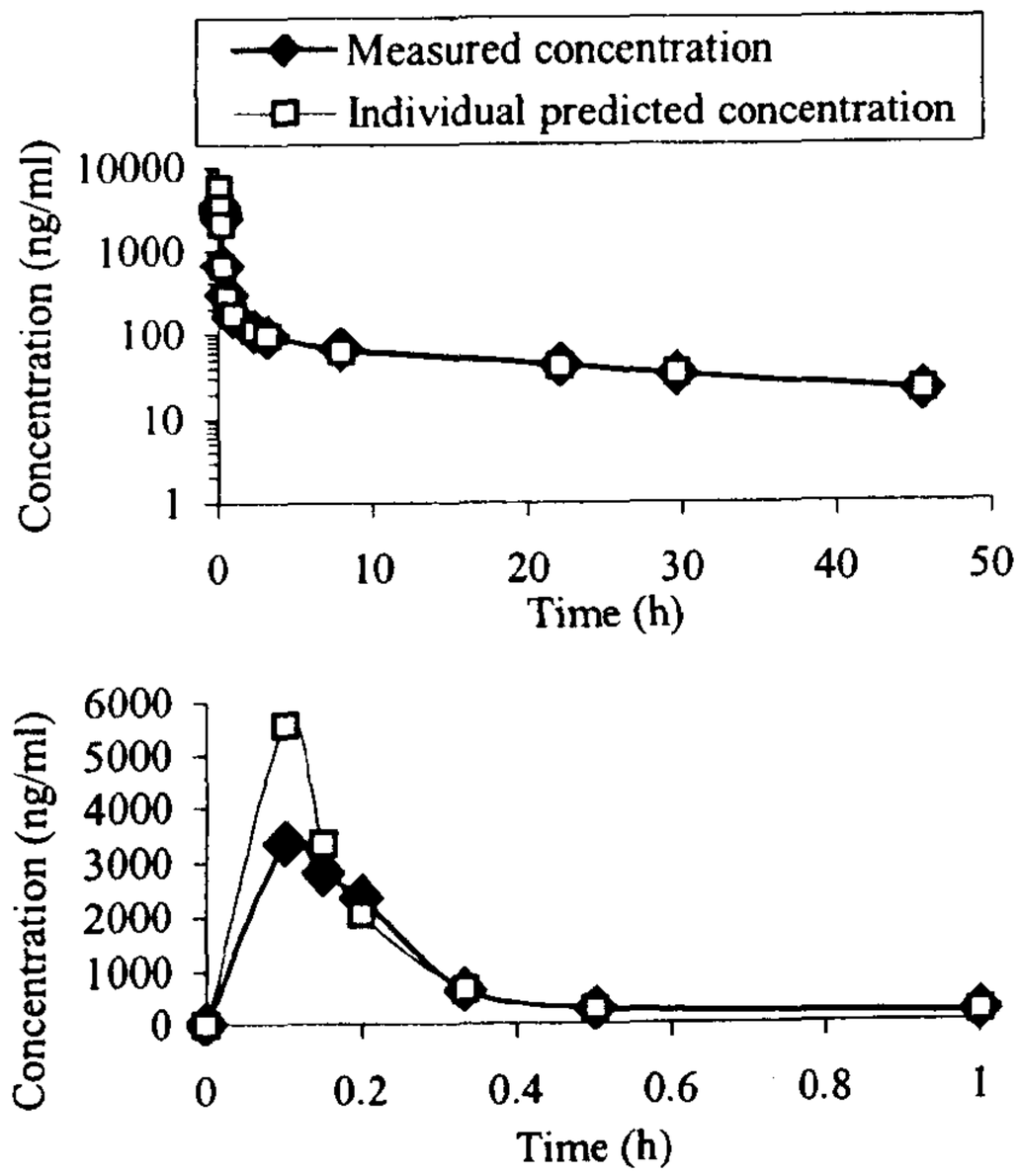
The 2 compartment model with proportional or combined residual error models had objective function values that were 818 and 715 units higher, respectively, than those obtained with the 3 compartment models (Table 3.2). With the 2 compartment model, there was a positive bias in weighted residuals at early time points, a negative bias from 2 to 24 h post-dose and a positive bias from 24 to 60 h post-dose (Figure 3.3). This pattern of the weighted residuals indicates model misspecification. A more random pattern of weighted residuals was obtained with the 3 compartment model (Figure 3.3). Therefore, it was concluded that the 3 compartment model best described the data. The predicted concentrations using typical population pharmacokinetic parameter estimates and individual pharmacokinetic parameter estimates using the 3 compartment model with combined error are illustrated in Figure 3.4.

The additive residual error model did not converge successfully. The objective function value obtained with the combined error model was slightly lower than the value obtained with the proportional error model (Table 3.2). However, the shape of the polynomial function fitted to the individual weighted residuals (modulus) versus the individual predicted concentrations was similar for the combined and proportional

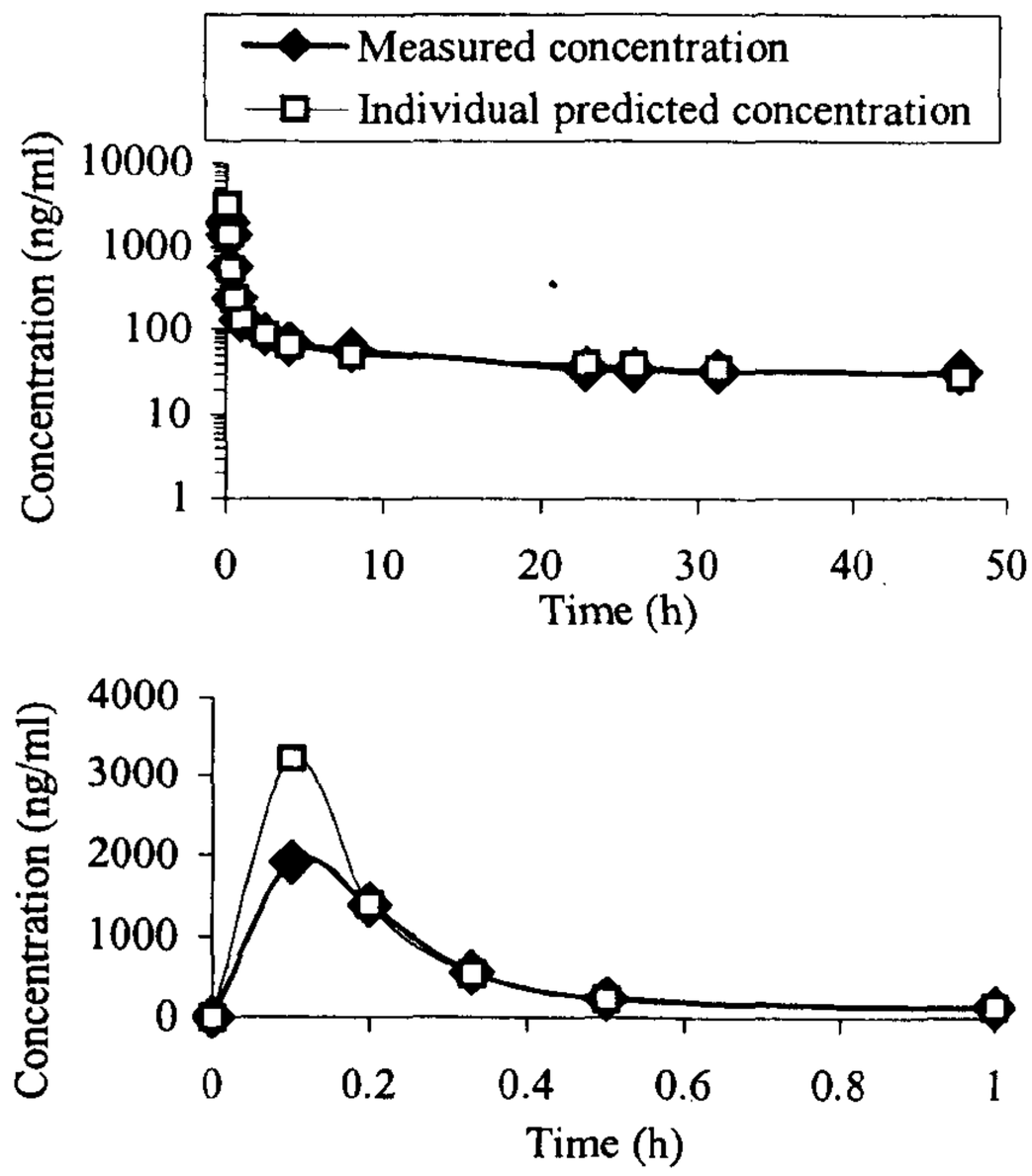
Figure 3.2

Measured and individual predicted concentration versus time plots for patients from Trial 1, 2 and 3 using a 3 compartment model with combined error

Patient 107



Patient 208



Patient 419

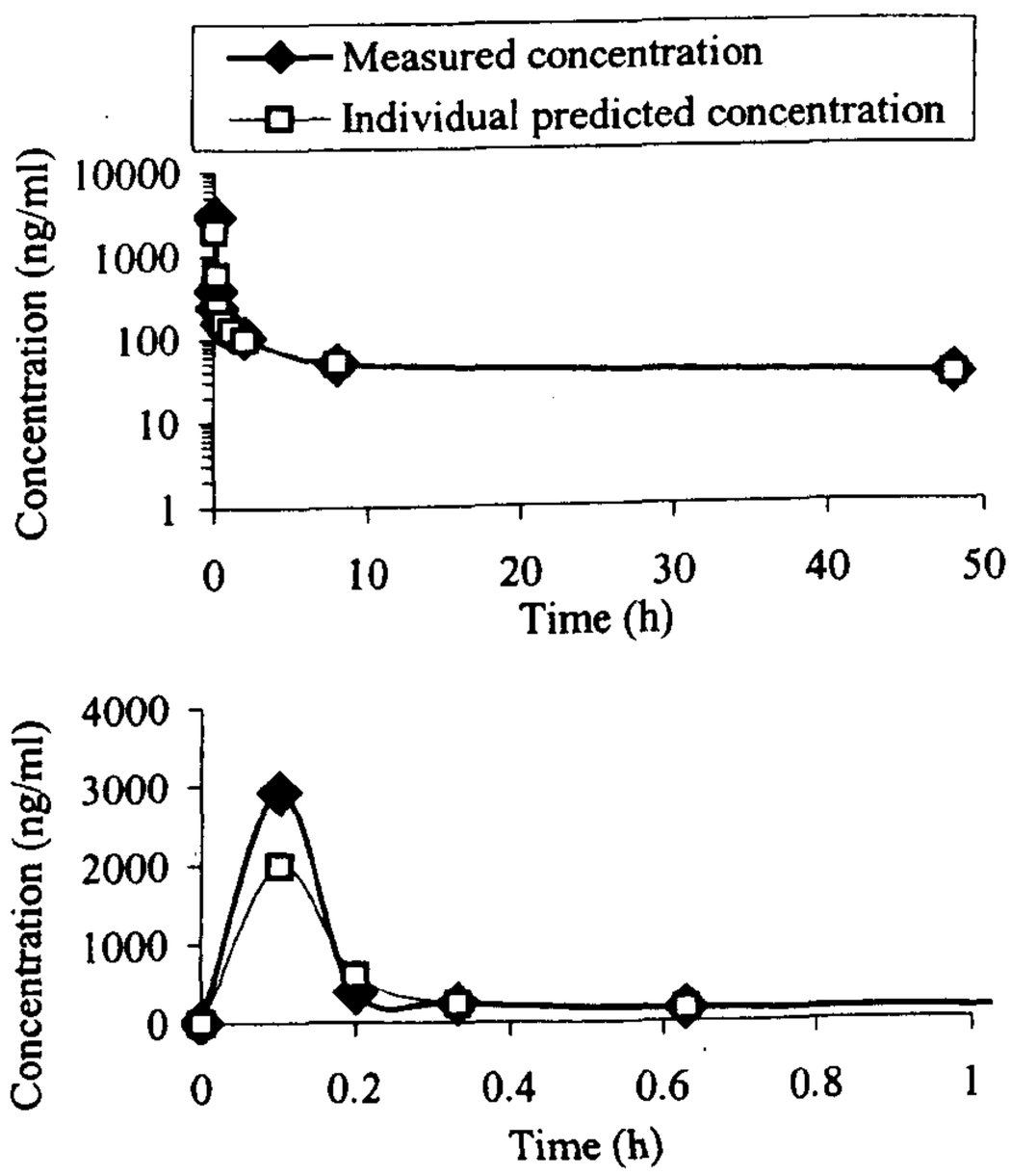


Table 3.2

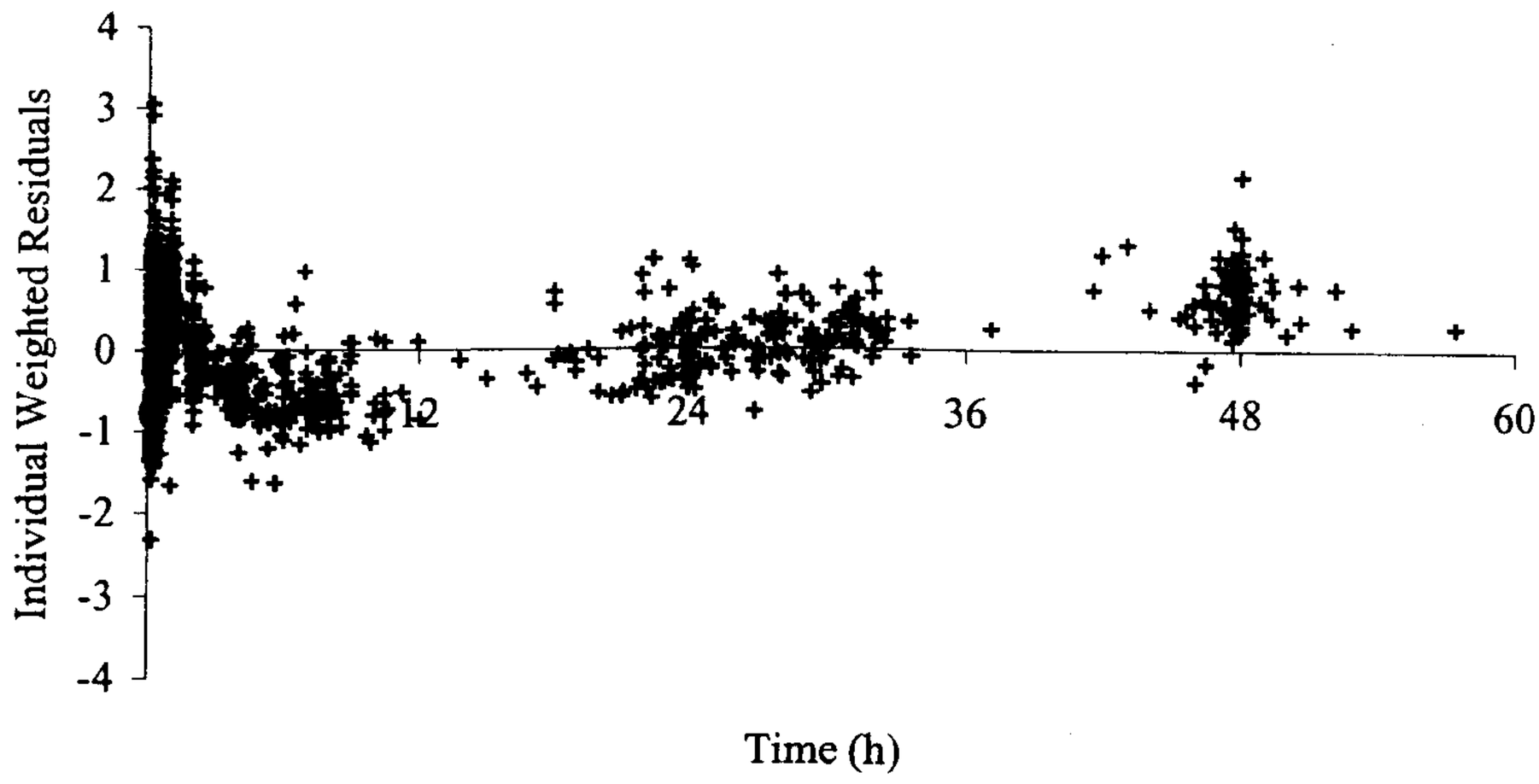
Objective function values obtained by two- and three-compartment models with proportional and combined error

Model	OFV
Two compartment with proportional error	9841
Two compartment with combined error	9728
Three compartment with proportional error	9023
Three compartment with combined error	9013

Figure 3.3

Weighted residuals versus time for 2 and 3 compartment models with combined error

2 compartment model



3 compartment model

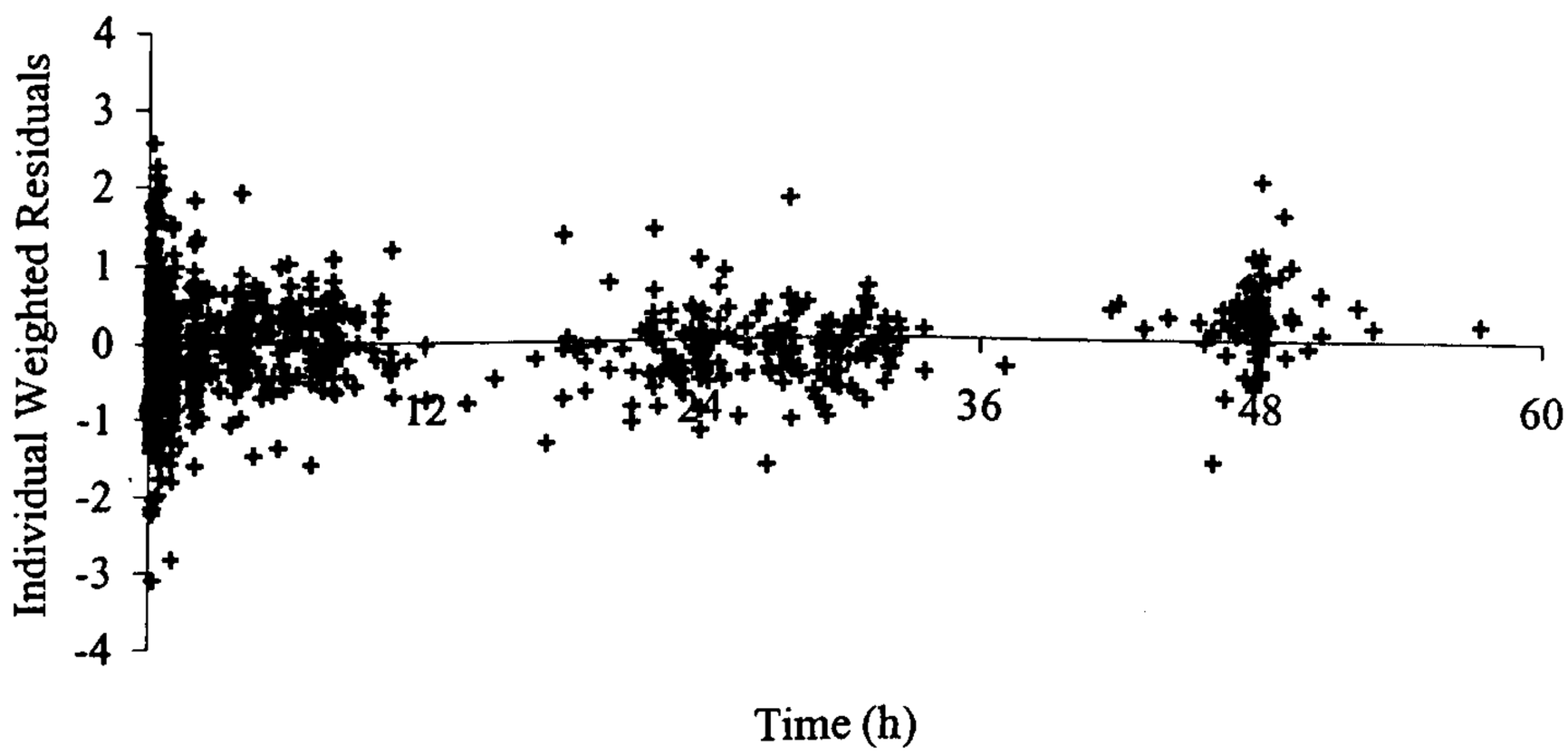
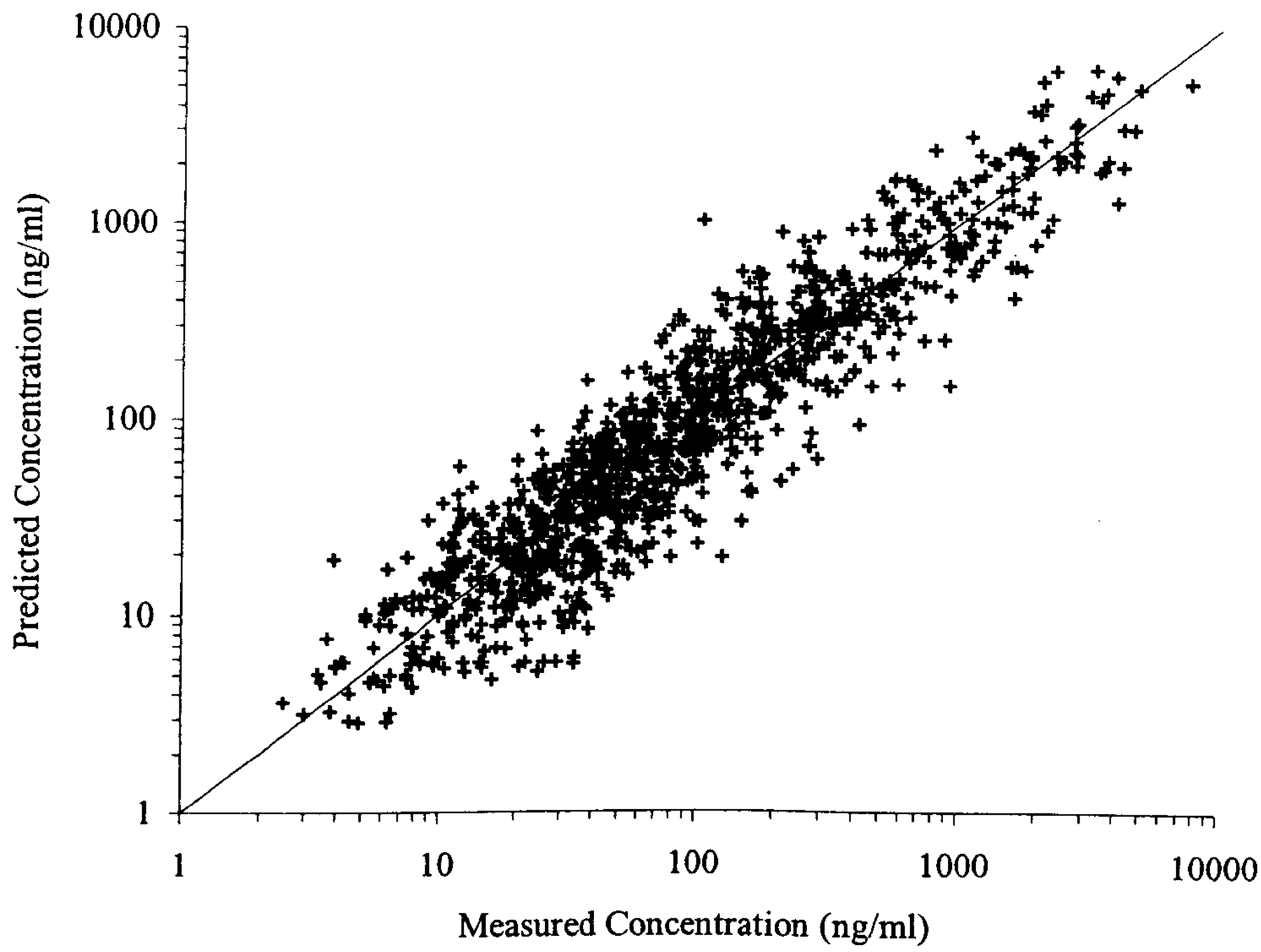


Figure 3.4

Population predicted and individual predicted concentrations versus measured concentrations calculated using a 3 compartment model with combined error



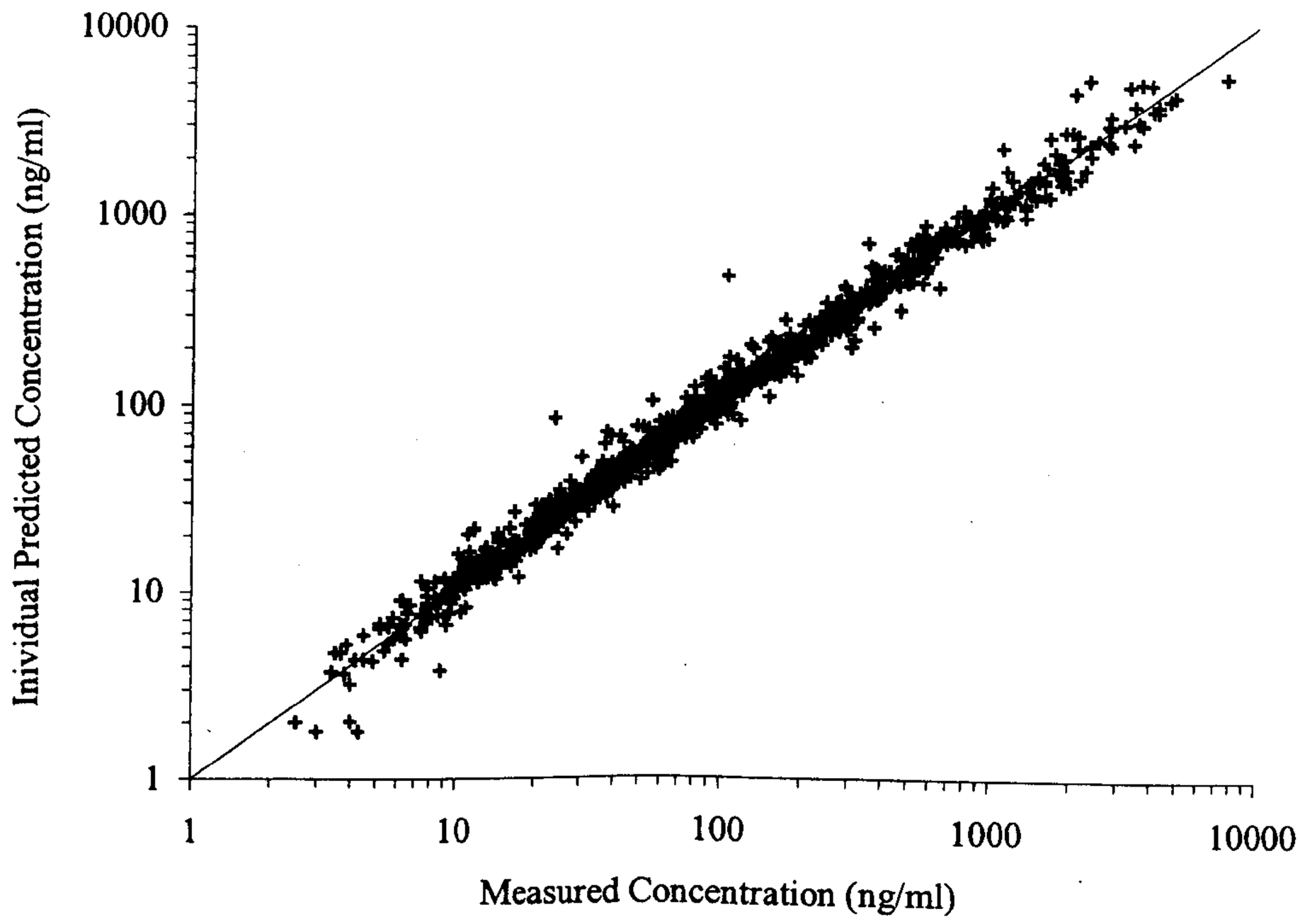
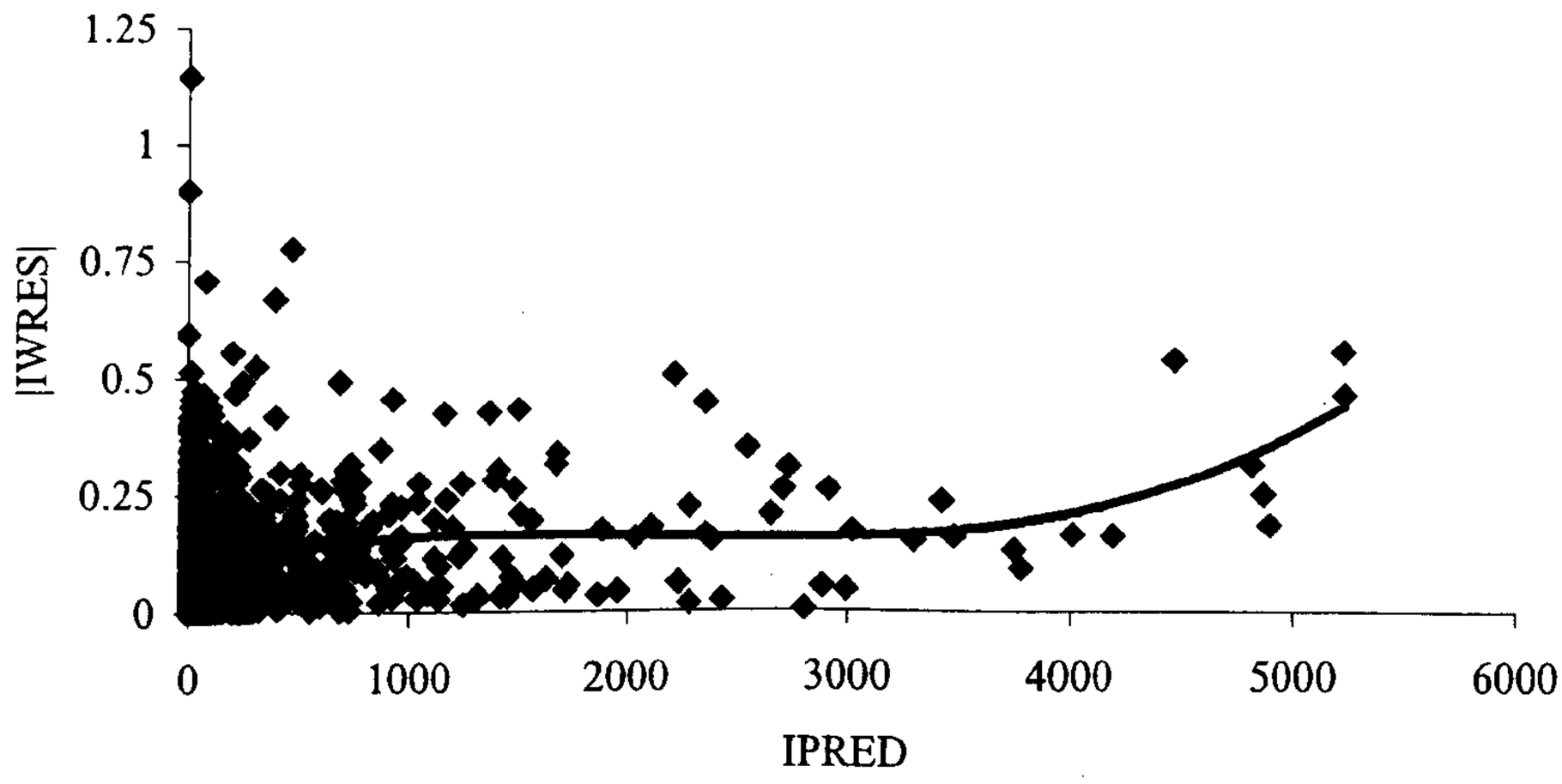


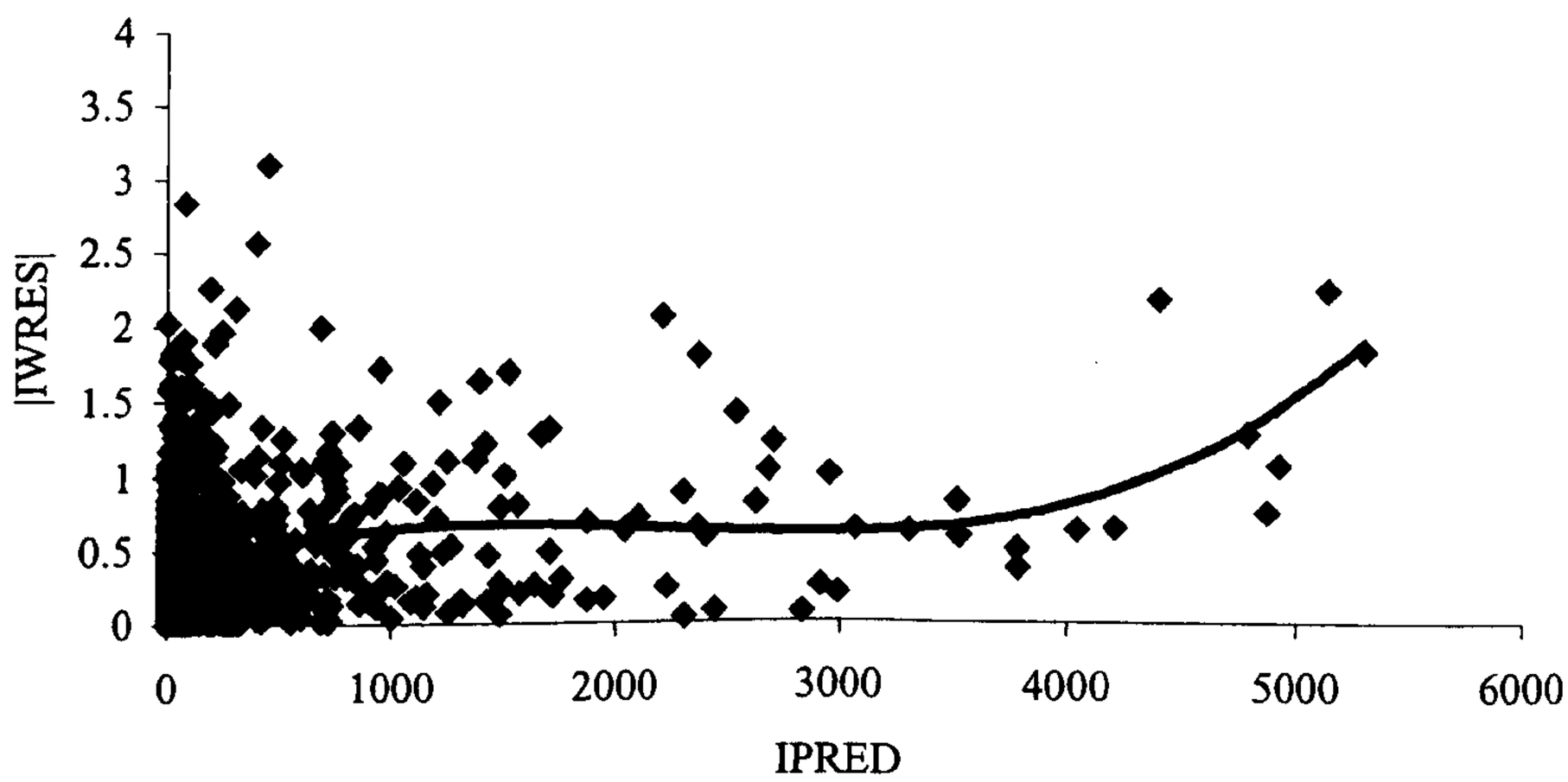
Figure 3.5

The modulus of individual weighted residuals versus individual predicted concentrations for a 3 compartment model with proportional or combined error

Proportional Error



Combined Error



models (Figure 3.5). For both error models, the individual weighted residuals were relatively constant up to 4000 ng/ml and increased at concentrations above 4000 ng/ml.

3.3.3.2 Assessment of the fit with the first-order conditional estimation method

The 3 compartment model with combined error was run with FOCE-INTER, to assess whether there was an improvement in the fit compared to the FO method. However, the 3 compartment model with combined error did not run successfully with the FOCE method. The 3 compartment model with proportional error did run successfully if inter-individual variability of V_1, Q_2 and V_2 were fixed to zero. The individual predicted concentration versus time plots were similar following runs with FO and FOCE, although there was less bias around 48 h post-dose when the model was run with FOCE compared to FO (Figure 3.6). Thus further model building with NONMEM was performed using FOCE-INTER.

3.3.4 Identification of covariates for model building with NONMEM

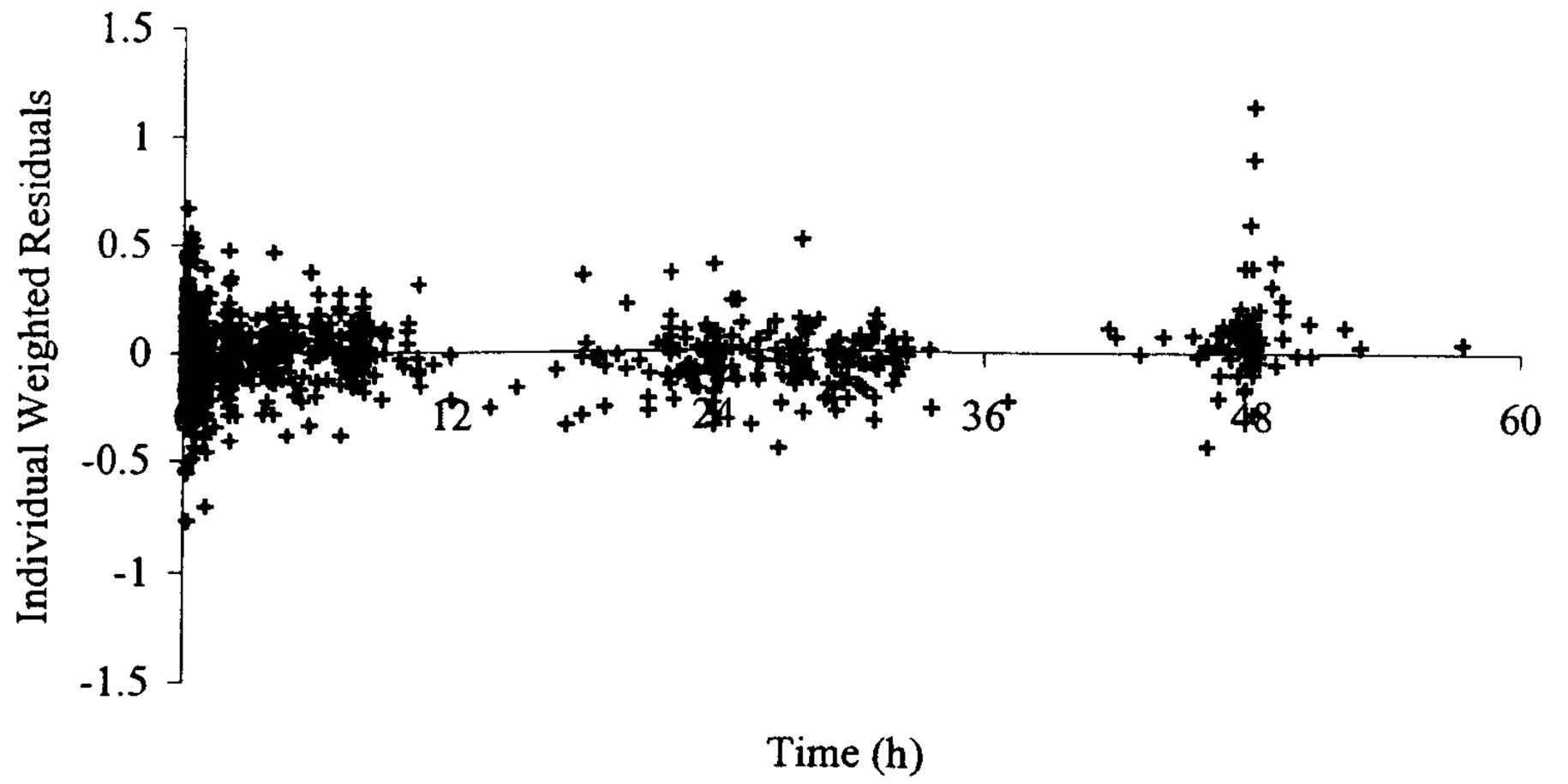
3.3.4.1 Graphical identification

Inter-individual variability could not be estimated for V_2 with FO and so covariate-parameter relationships could not be investigated for this parameter. Scatter plots of CL versus covariates are illustrated in Figure 3.7. The following covariates appeared to have a positive correlation with CL: albumin; dose; and creatinine clearance. The following covariates appeared to have a negative correlation with CL: bilirubin; AST; alkaline phosphatase; weight; and body surface area. From the box plots (Figure 3.8), it was noted that the median clearance value was lower in patients with liver metastases compared to those without liver metastases, however, due to the high variability, the CL values in the two groups overlapped considerably. There also appeared to be a trend towards a decrease in CL with increasing WHO grade. For the other pharmacokinetic parameters (Q_2, Q_3, V_1 and V_3), there were no parameter-covariate relationships identified from the plots (data not shown).

Figure 3.6

Individual weighted residuals versus time for a 3 compartment models with proportional error using FO and FOCE-INTER

FO



FOCE-INTER

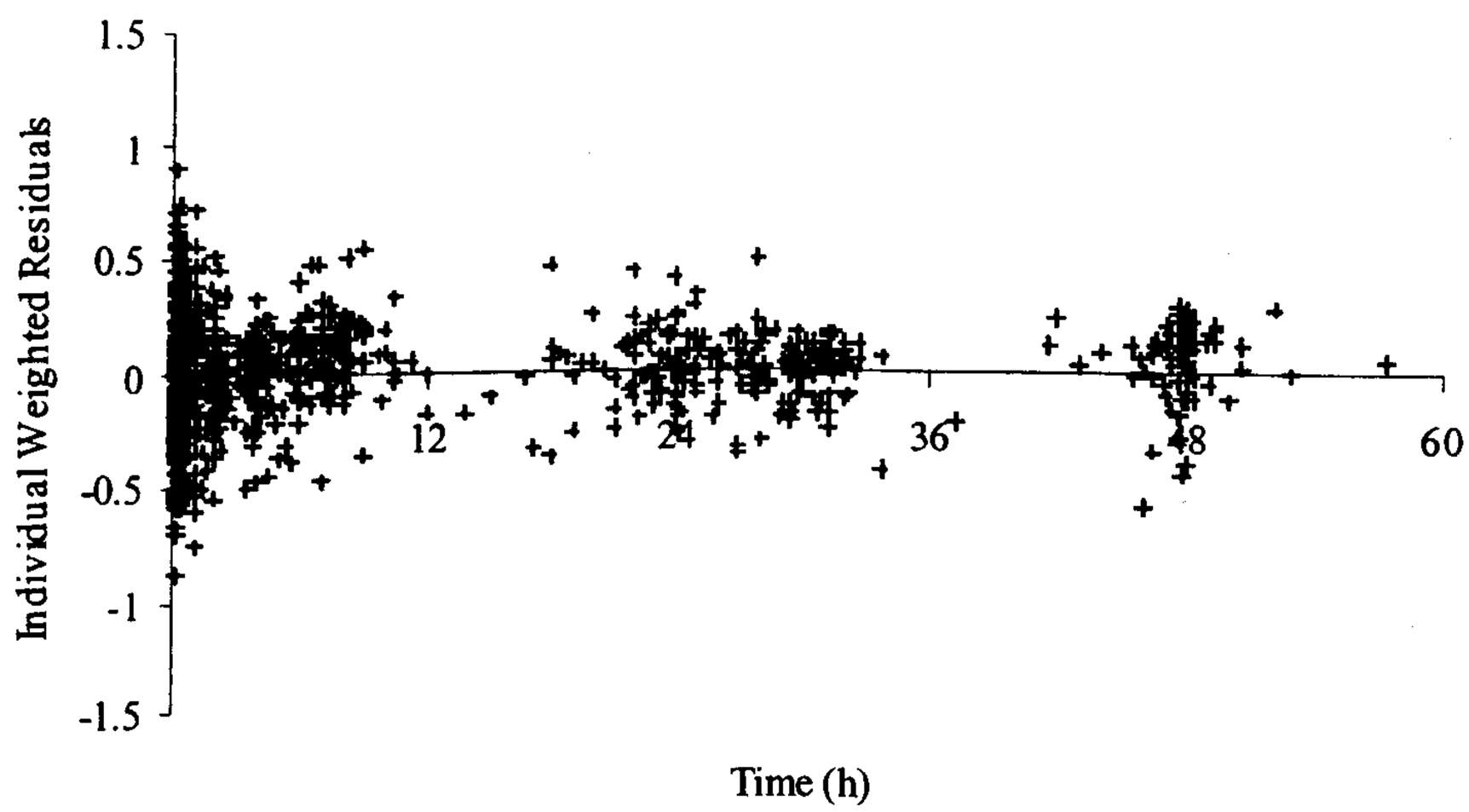


Figure 3.7 (1 of 2)

Scatter plots of CL versus clinical characteristics

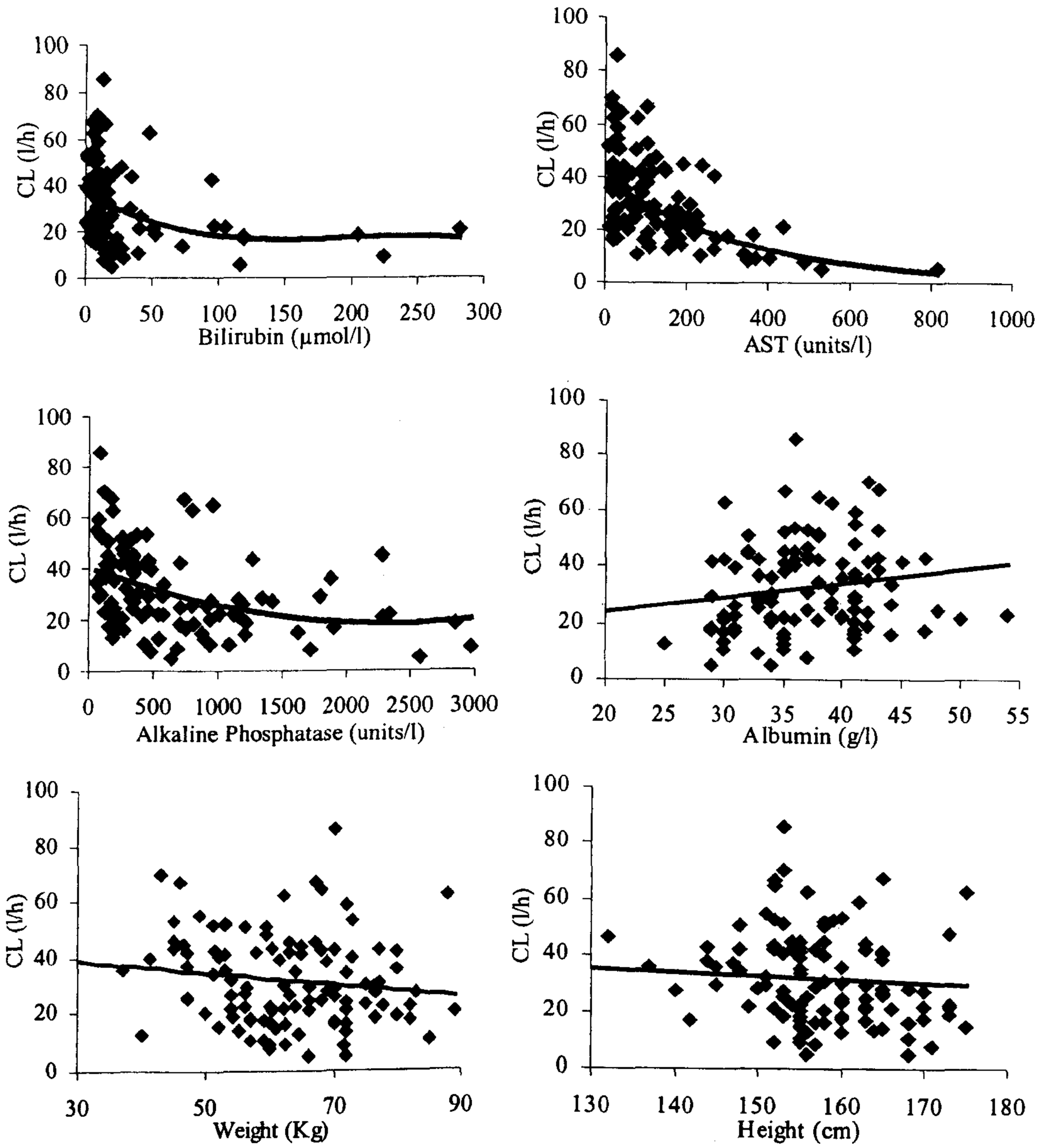


Figure 3.7 (2 of 2)

Scatter plots of CL versus clinical characteristics

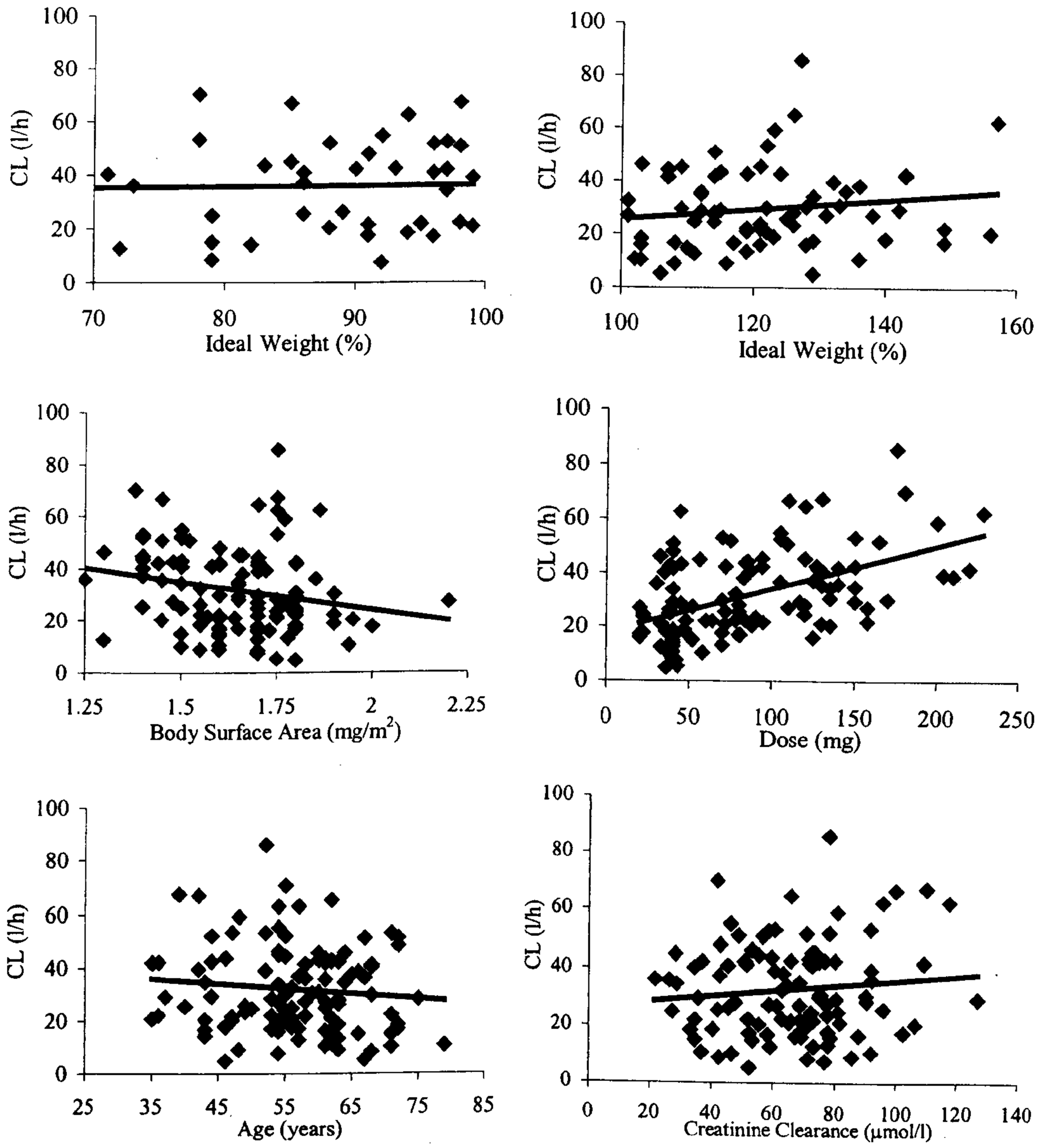
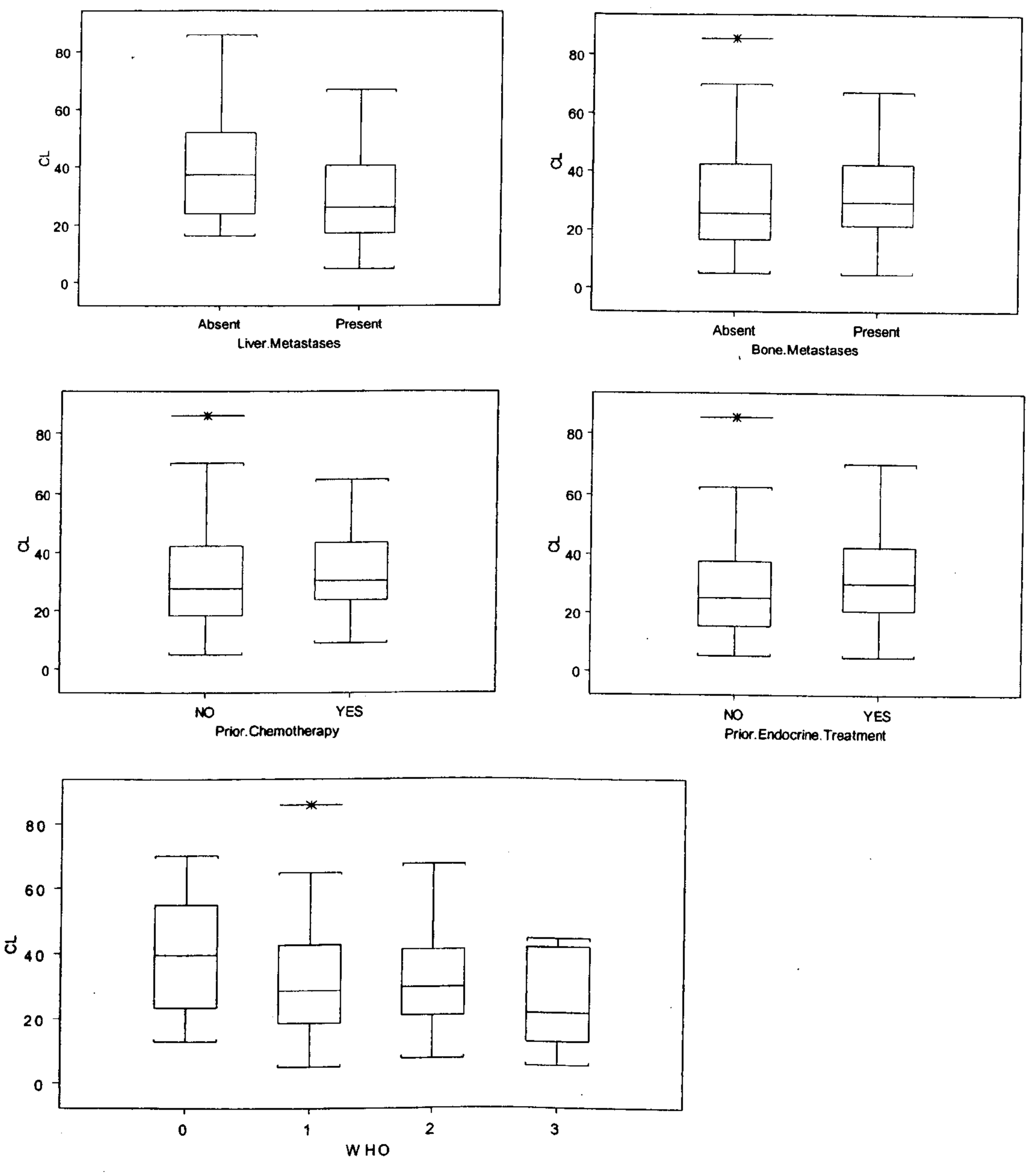


Figure 3.8

Box plots of CL versus clinical characteristics



CL = L/h

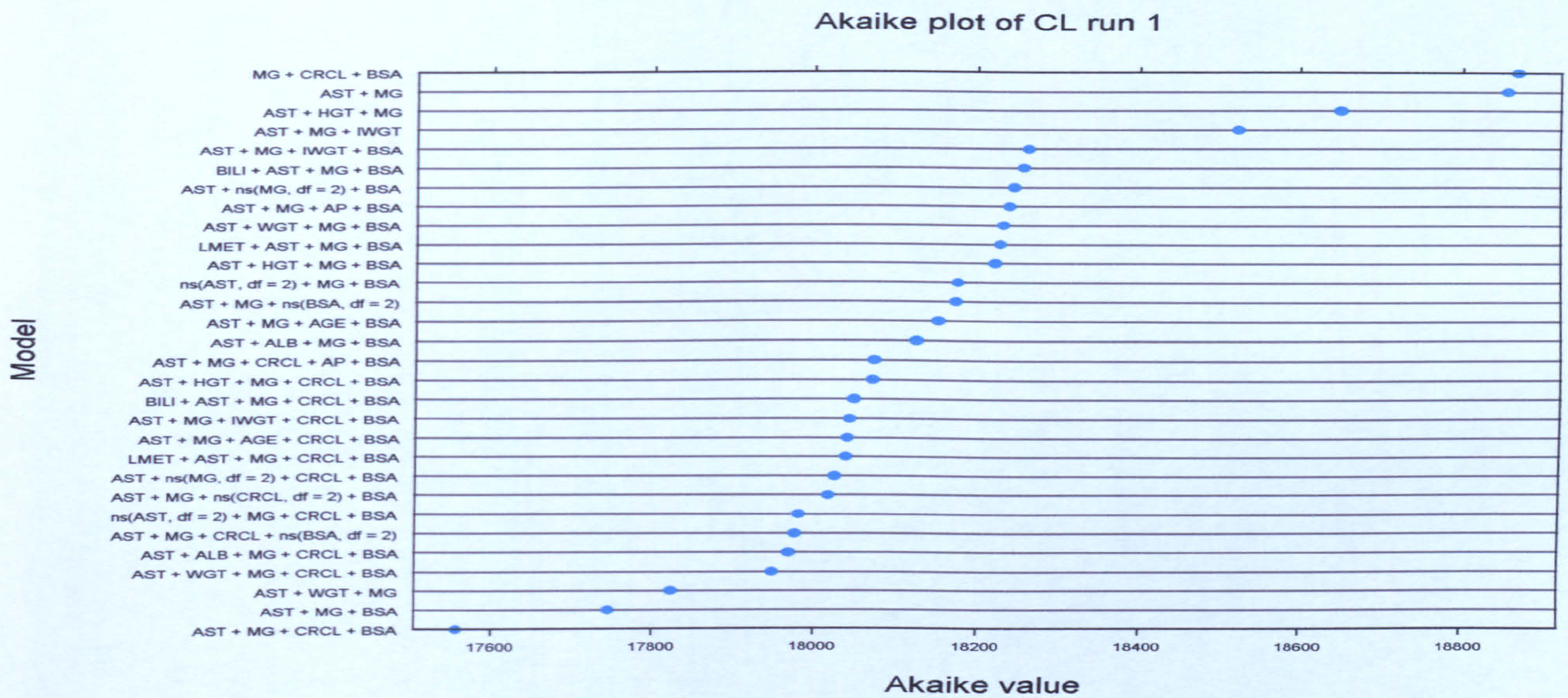
3.3.4.2 Generalised additive modelling

Inter-individual variability could not be estimated for V_2 with FO and could not be estimated for V_1, V_2, and Q_2 with FOCE-INTER and so covariate-parameter relationships could not be investigated for these parameters. Figure 3.9 illustrates the best 30 CL models tried in the stepwise search in the GAM analysis with FO estimates plotted against Akaike value. The top 3 models identified by GAM analysis for each PK parameter, estimated using FO, are reported in Table 3.3. The best model for CL included AST, dose, creatinine clearance and BSA as linear functions. Patient 413 was identified as having a high Cooks value and high leverage value, and therefore, could have a large influence on the covariates that were selected for CL (Figure 3.10); however, removal of this patient from the analysis did not affect the covariate selection of the top 3 models. For V_1, a non-linear function of albumin and linear functions of dose and weight were determined as being the most influential covariates; however, following removal of 3 patients with high leverage and Cooks values, the best model included only dose as a linear function (Table 3.3). GAM analysis on Q_2 identified dose and albumin as linear functions and age as a non-linear function as the best covariates to be included in the model, however on removal of one individual with a high Cook's value, only dose and age as linear functions were identified in the best model for Q_2. Dose as a non-linear function and liver metastases as a linear function were identified as the best model for Q_3 and this was not affected by the removal of 3 subjects with high leverage and/or Cooks values. AST and albumin included as linear functions were identified as being the best model for V_3 and this was not affected by the removal of data from patient 413, who had high leverage and Cooks values.

The results of the GAM analysis using the parameter estimates from FOCE were almost identical to those estimated using FO. The best model identified was the same for each PK parameter, however, for Q_3 the 3rd best model using FO estimates identified WHO grade as a covariate, whereas analysis using FOCE estimates did not identify WHO grade but instead identified albumin.

Figure 3.9

Akaike values of the best 30 models for CL identified by GAM analysis



KEY. ALB = Albumin; AP = Alkaline phosphatase; AST = Aspartate aminotransferase; BILI = Bilirubin; BSA = Body surface area; CRCL = Creatinine clearance; df = Degrees of freedom; HGT = Height; IWGT = Ideal body weight; LMET = Liver metastases; MG = Dose in mg; ns = Natural cubic spline; WGT = Weight.

Table 3.3

GAM analysis results: best three models identified for CL, V_1, Q_2, Q_3 and V_3

Equation	AIC
CL	26870
CL~AST+DOSE+CRCL+BSA	17556
CL~AST+DOSE+BSA	17745
CL~AST+DOSE+WEIGHT	17823

Equation	AIC
V_1	277
V_1~DOSE	247
V_1~DOSE+AGE	247
V_1~DOSE(ns)	249

*Subjects 147, 204 and 413 excluded

Equation	AIC
Q_2	29692
Q_2~DOSE+AGE	28470
Q_2~DOSE+AGE(ns)	28510
Q_2~ALBUMIN+DOSE+AGE	28560

*Subject 143 excluded

Equation	AIC
Q_3	36229
Q_3~LMETS+DOSE(ns)	33154
Q_3~LMETS+DOSE	33311
Q_3~WHO+LMETS+DOSE(ns)	33584

*Subjects 128, 132, 117 excluded

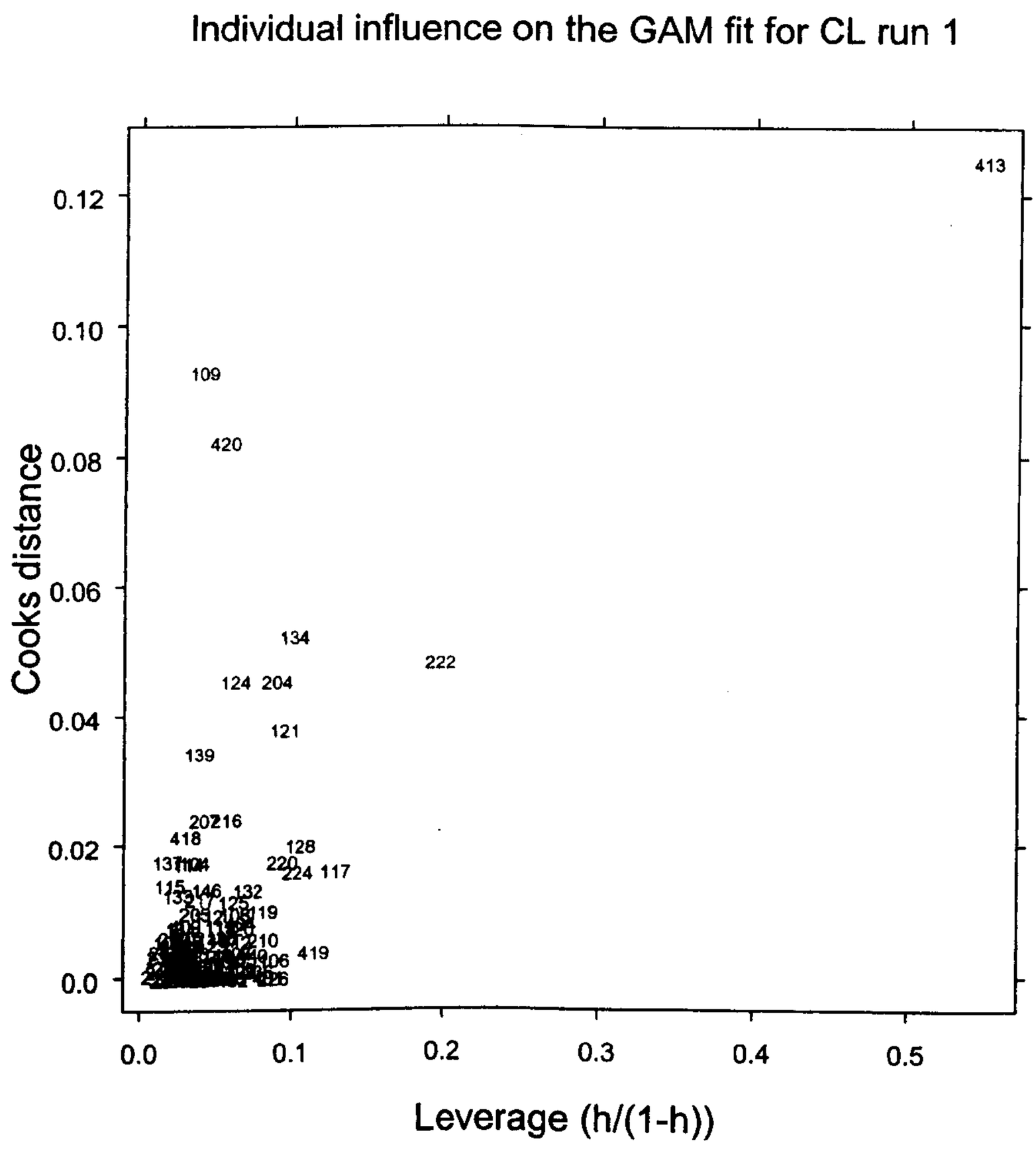
Equation	AIC
V_3	9184491
V_3~AST+ALBUMIN	8900254
V_3~BILIRUBIN+AST+ALBUMIN	8996226
V_3~AST+ALBUMIN+HEIGHT	9002008

*Subject 413 excluded

~ = 'Is a function of'
ns = natural cubic spline
* = Subjects with high Cooks/leverage values were excluded from the analysis
LMETS = liver metastases

Figure 3.10

Cook's distance versus leverage following GAM analysis on bayesian estimations of CL



3.3.5. Population model building using NONMEM

Patients 130, 416 and 421 were excluded from the analysis due to missing albumin data and Patient 221 was excluded due to missing weight data. Covariates tested for CL included AST, dose, BSA, weight, liver metastases, WHO grade and creatinine clearance (CrCL). A summary of the OFV obtained for the principal models tested is given in Table 3.4. AST, dose and liver metastases (absent/present) were found to result in a statistically significant reduction in the OFV (-49, -32 and -11, respectively), when added separately into the CL model, with ln AST having the greatest effect. CrCL data was not available for patients 223 and 228, thus the influence of CrCL was tested in only 103 patients. The inclusion of CrCL into the CL model was not statistically significant as judged by the change in the OFV (-1). Of the pairs of covariates tested, ln AST and dose resulted in the lowest OFV, with a fall in the OFV of 6, compared to ln AST alone. The addition of BSA onto this CL model resulted in a fall in the OFV of 7 and the further addition of liver metastases resulted in a further fall in the OFV of 15. Thus, the 'full model' for CL included ln AST, dose, BSA and liver metastases. The effect of adding ln AST, dose and BSA separately into the model for CL is illustrated in plots of delta CL versus each covariate (Figure 3.11). Delta CL for each patient was calculated by the subtraction of the typical population estimate of CL from the individual estimate of CL, estimated with and without the covariate of interest included in the model. Following the addition of the covariate to the model, if it has been adequately incorporated, no relationship between delta CL and covariate should be observed. A negative relationship between delta CL and AST, a positive relationship between delta CL and dose and a negative relationship between delta CL and BSA is observed before the addition of any covariates into the model. Following the inclusion of each covariate into the model, the influence of the covariate is accounted for in the estimation of the population estimate and thus the relationship between delta CL and each covariate is seen to disappear. The effect of adding liver metastases into the model is illustrated in the box-plots (Figure 3.12). Before the addition of liver metastases in the model the median delta CL in patients with liver metastases was less than that in patients without liver metastases. Following the inclusion of liver metastases into the model, its influence is accounted for in the estimation of the population estimate and thus the median values for delta CL in patients with and without liver metastases were similar.

Table 3.4

Summary of models evaluated using FOCE-INTER

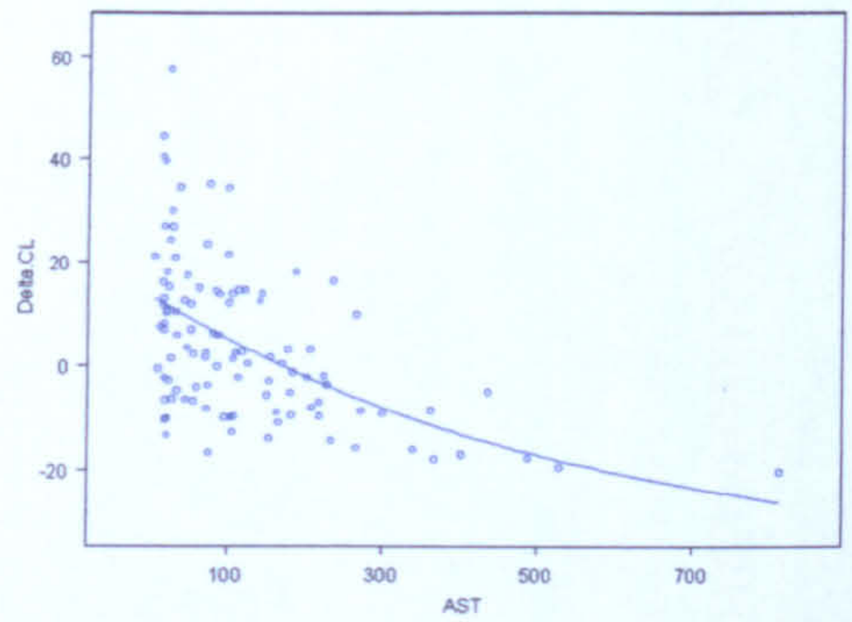
Model No.	CL Model	OFV	ΔOFV	cf. model No.
0	Base model	8088.65	-	-
1	**Ln AST**	**8039.26**	**-49.39**	**0**
2	**Dose**	**8056.90**	**-31.75**	**0**
3	BSA	8085.16	-3.49	0
4	WGT	8097.06	+8.41	0
5	**LMETS**	**8077.41**	**-11.24**	**0**
6	WHO*	8092.95	+4.30	0
7	WHO$	8087.50	-1.15	0
8	Basic (103 patients)#	7936.82	-	-
9	CrCL (103 patients)#	7935.94	-0.88	8
10	**Ln AST + Dose**	**8033.66**	**-5.60**	**1**
11	Ln AST + BSA	8036.73	-2.53	1
12	Ln AST + WGT	8037.16	-2.10	1
13	**Dose + BSA**	**8045.14**	**-11.76**	**2**
14	**Dose + WGT**	**8047.69**	**-9.21**	**2**
15	Dose + LMETS	8055.36	-1.54	2
16	**Ln AST + Dose + BSA**	**8026.84**	**-6.82**	**10**
17	Ln AST + Dose + WGT	8047.68	-14.02	10
18	**Ln AST + Dose + BSA + LMETS**	**8011.62**	**-15.22**	**16**
19	Ln AST + Dose + BSA + WHO$	8026.70	-0.14	16
	V_3 Model			
20	AST	8008.37	-3.25	18
21	Albumin	8007.81	-3.81	18
22	Height	8008.30	-3.32	18
23	Bilirubin	8009.90	-1.72	18
	Q_3 Model			
24	**LMETS**	**8004.69**	**-6.93**	**18**
25	WHO*	8006.95	-4.67	18
26	WHO$	8009.59	-2.03	18
27	**LnDose**	**8007.13**	**-4.49**	**18**
28	Albumin	8009.00	-2.62	18
29	**LMETS + LnDose**	**7993.35**	**-11.34**	**24**
30	LMETS + WHO*	8000.36	-4.33	24
31	LMETS +WHO$	8001.53	-3.16	24
32	LMETS + albumin	8004.43	-0.26	24
33	LMETS + LnDose+albumin	7993.34	-0.01	29
34	LMETS + LnDose+WHO$	7992.87	-0.48	29

KEY. AST = Aspartate aminotransferase; BSA = Body surface area; CrCL = Creatinine clearance; LMETS = Liver metastases; WGT = Weight; WHO = World Health Organisation (performance status)
* = Fall in OFV of 7.81 required for statistical significance ($p<0.05$)
$ = Grades 0 and 1 combined, grades 2 and 3 combined
# = patients 223 and 228 were excluded due to missing creatinine data
Bold text indicates models that gave a statistically significant improvement

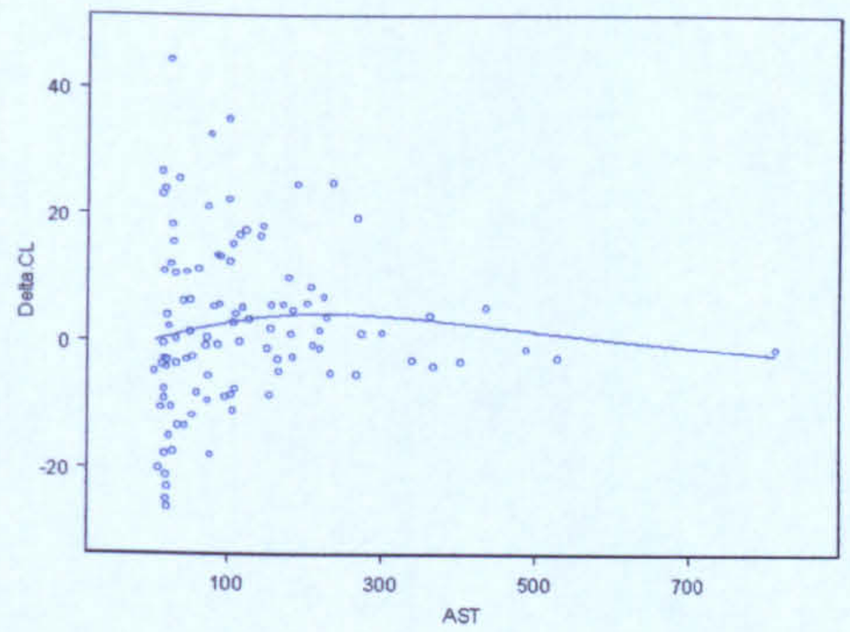
Figure 3.11

Influence of AST, dose and BSA on the CL model

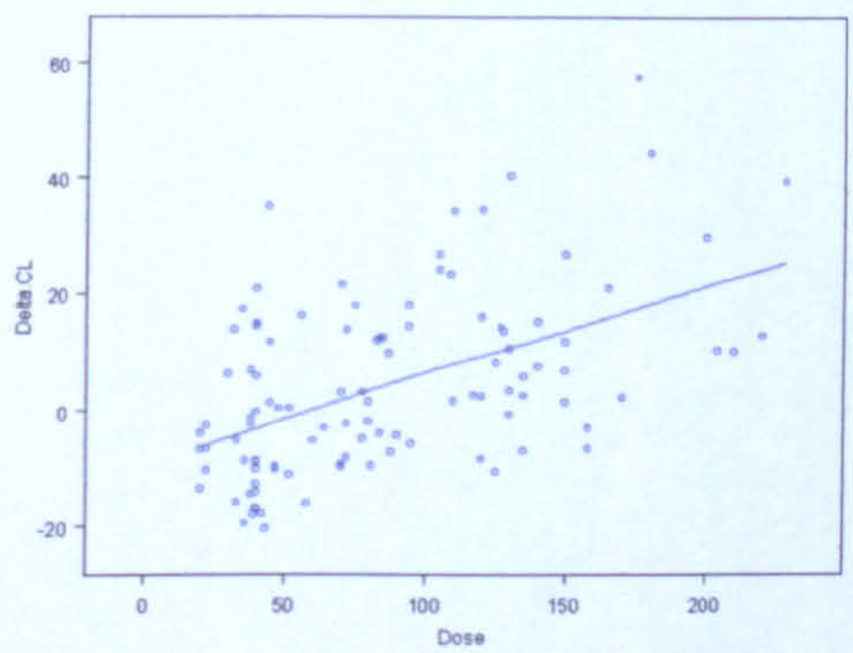
A. Basic Model. Influence of AST



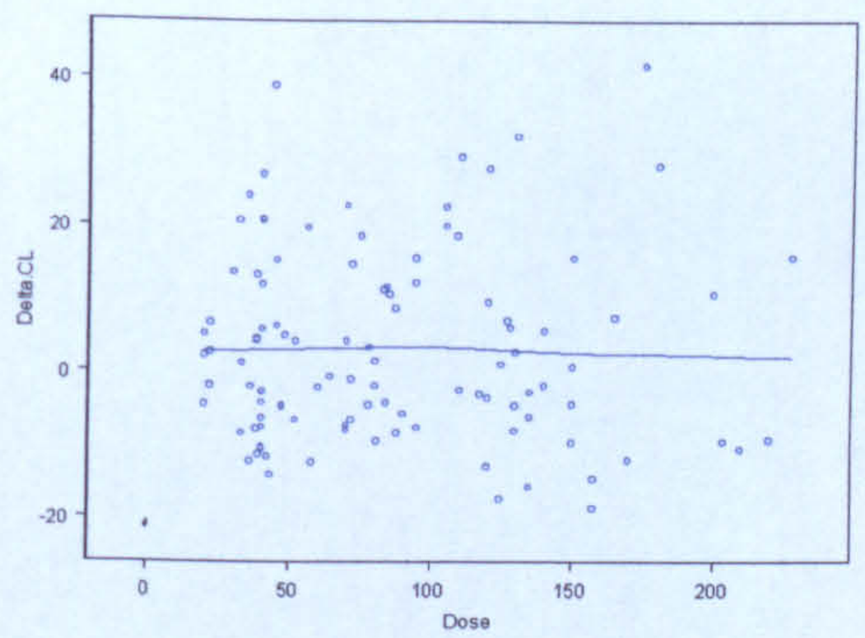
B. Ln AST Included in CL Model



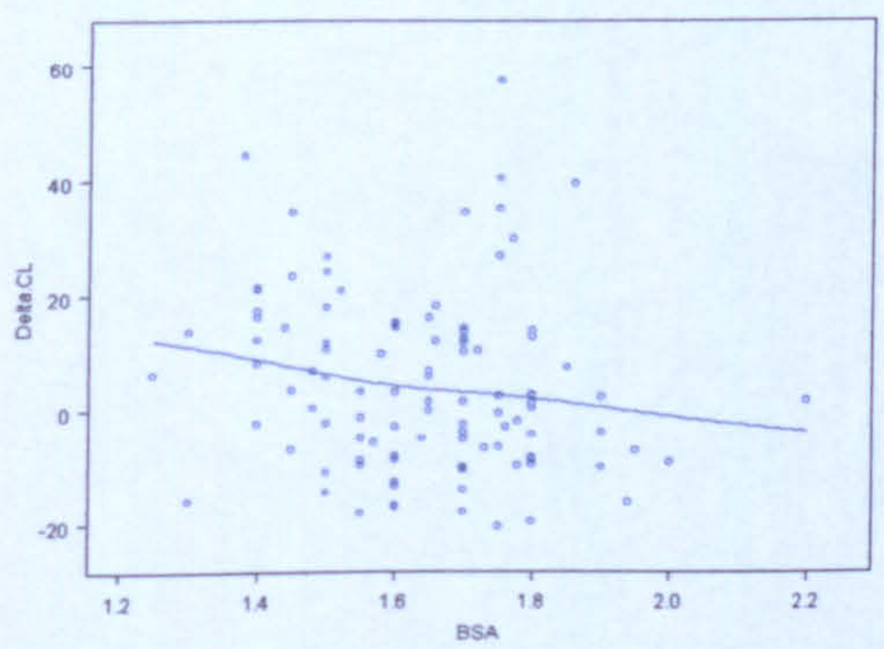
C. Basic Model. Influence of Dose



D. Dose Included in CL Model



E. Basic Model. Influence of BSA



F. BSA Included in CL Model

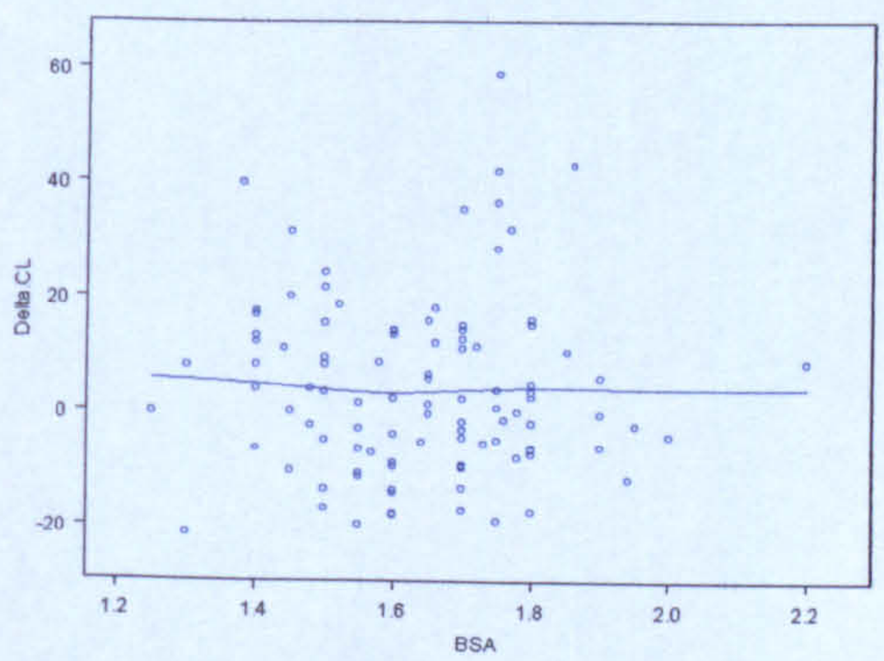
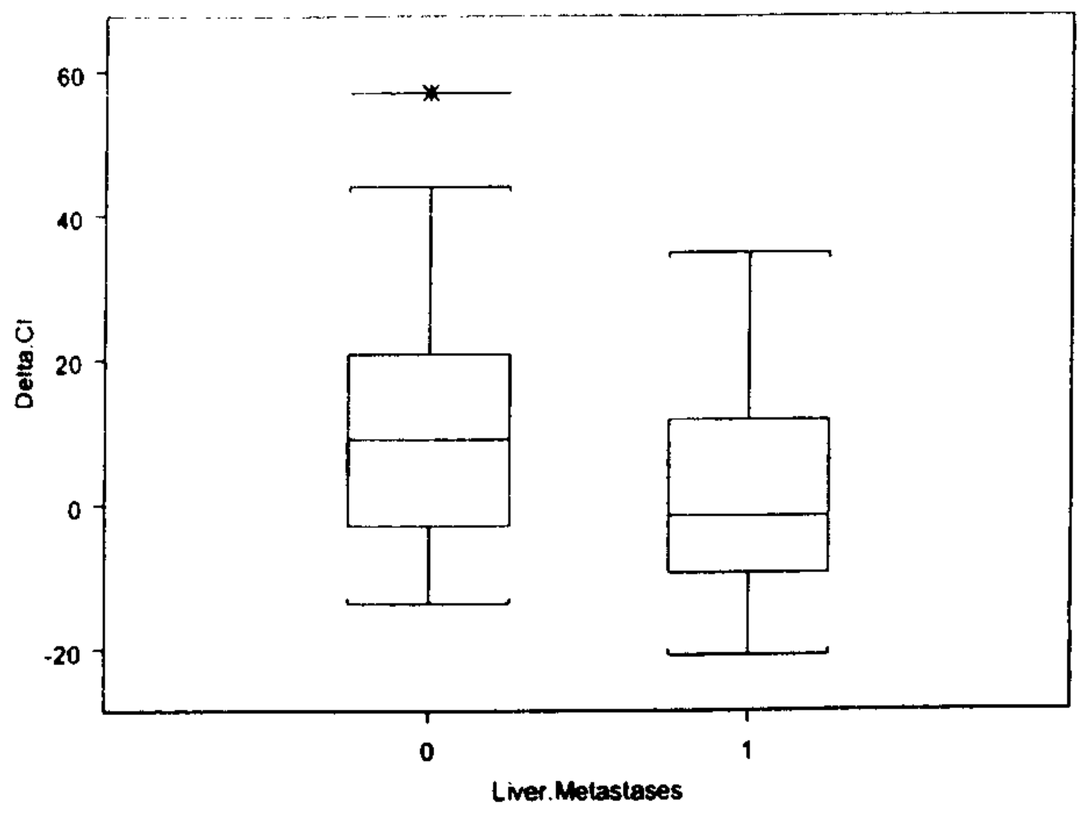


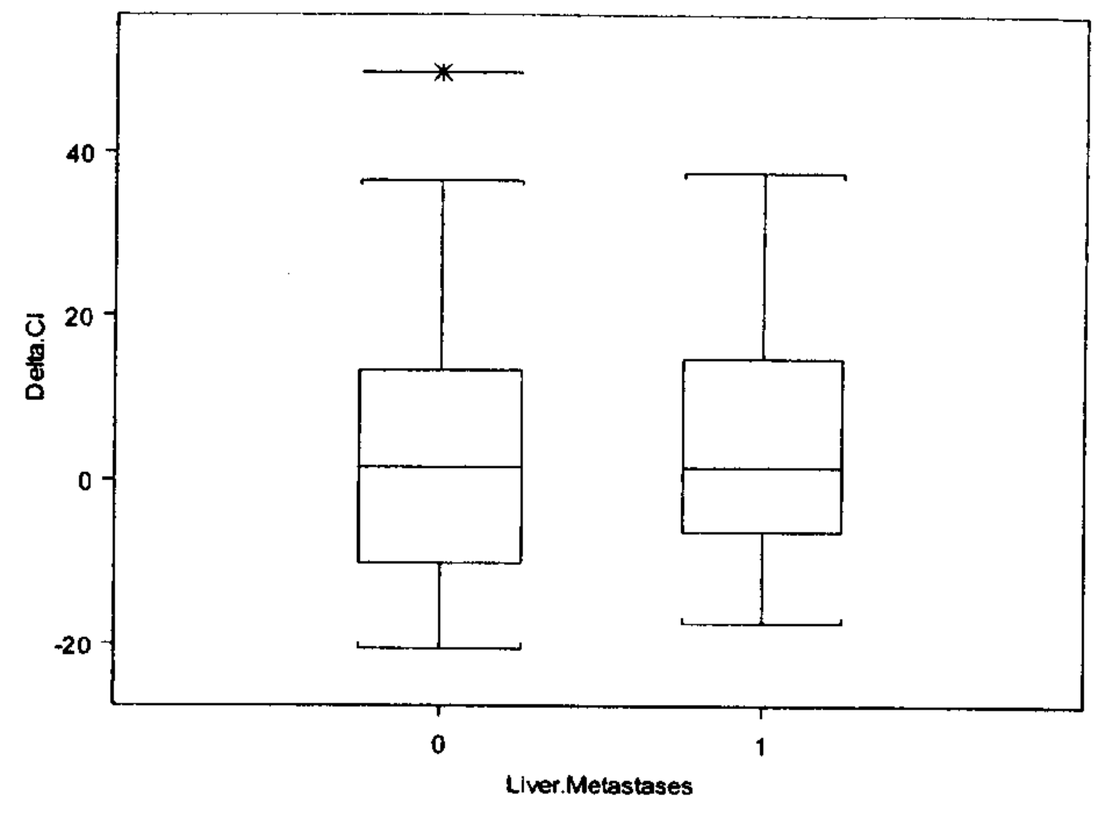
Figure 3.12

Influence of liver metastases on the CL model

A. Basic model



B. Liver metastases added into model



0 = Liver metastases absent
1 = Liver metastases present
* = outlier

Using the 'full model' for CL, model building was continued for V_3. AST, albumin, height and bilirubin were all added separately into the model for V_3, however, none of these covariates were found to result in a statistically significant improvement in OFV (Table 3.4). As a result of the GAM analysis for Q_3, WHO grade, liver metastases, dose and albumin were separately added into the model for Q_3. A significant fall in the OFV was observed following the addition of liver metastases (-7). Linear and various non-linear functions (as indicated by the GAM analysis) were tested for dose and the natural log function was the only function in which a statistically significant improvement in OFV was observed (-4). There was no further fall in OFV following addition of WHO grade or albumin into the model.

The influence of dose and liver metastases on Q_3 are presented graphically in Figure 3.13. Before the addition of liver metastases to the model, the median delta Q_3 in patients with liver metastases was greater than in patients without liver metastases. Following the inclusion of liver metastases into the model, its influence was accounted for in the estimation of the population estimate and thus the median values for delta Q_3 in patients with and without liver metastases were similar. In contrast, there did not appear to be an improvement in the delta Q_3 values following inclusion of dose as a natural log function.

The full model included CL as a function of ln AST, dose, BSA and liver metastases and Q_3 a function of ln dose and liver metastases with an OFV of 7993 compared to the basic model, without inclusion of covariates, which had an OFV of 8089. The parameter estimates of the basic and full models are given in Table 3.5.

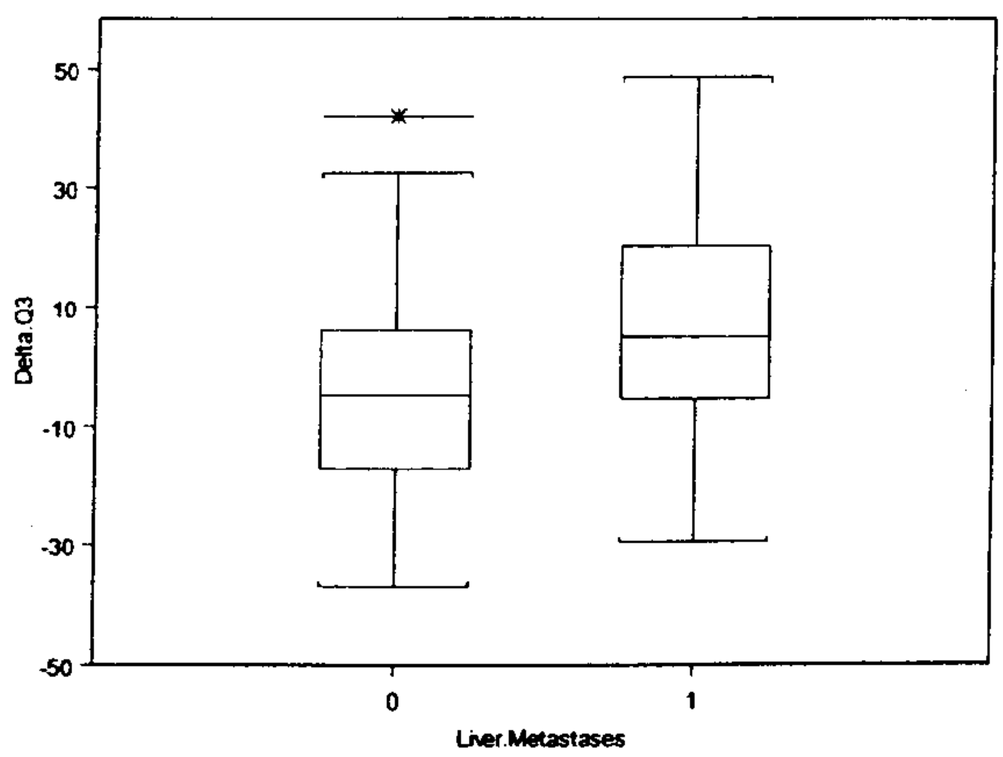
The inclusion of liver metastases and dose on Q_3 accounted for approximately 1.4 and 1.7 % of the variability of Q_3, respectively. The inclusion of dose, BSA and liver metastases each accounted for approximately 2 % of the variability of CL. The influence of these covariates in the population model for epirubicin was therefore deemed to be of little clinical significance. In contrast, the inclusion of AST on CL accounted for approximately 11 % of the variability in CL. Thus, the final model included CL as a natural log function of AST only:

$$CL=74.9 \times (1-(0.139 \times \ln AST))$$

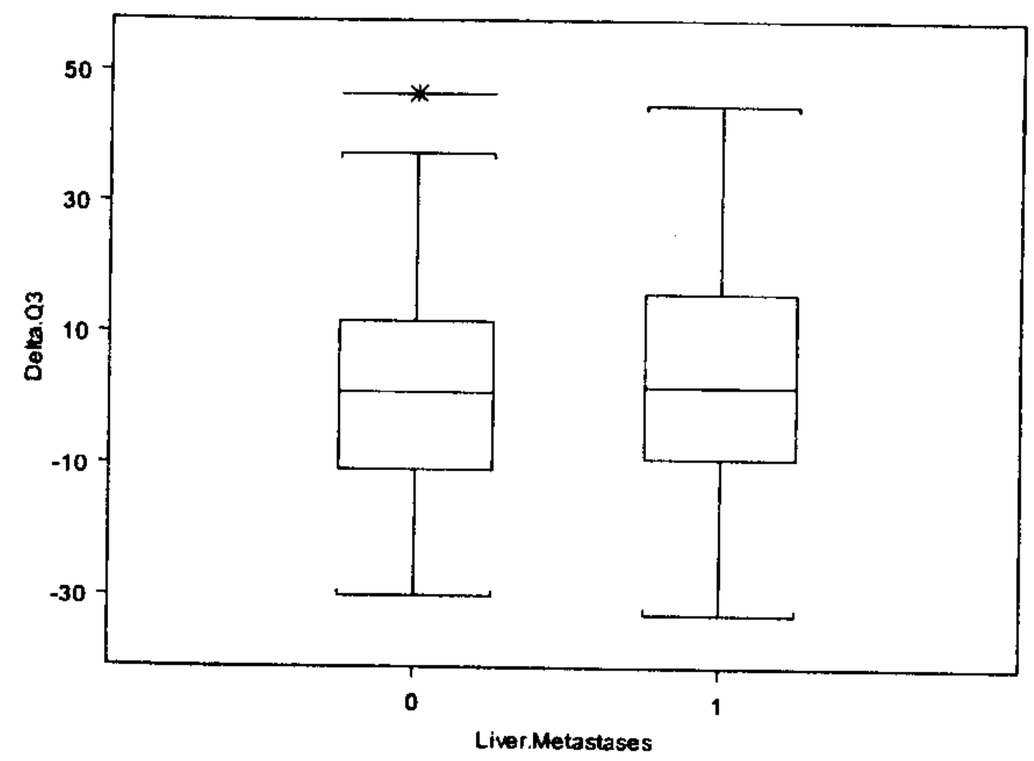
Figure 3.13

Influence of liver metastases and dose on Q_3 model

A. No covariates on Q_3

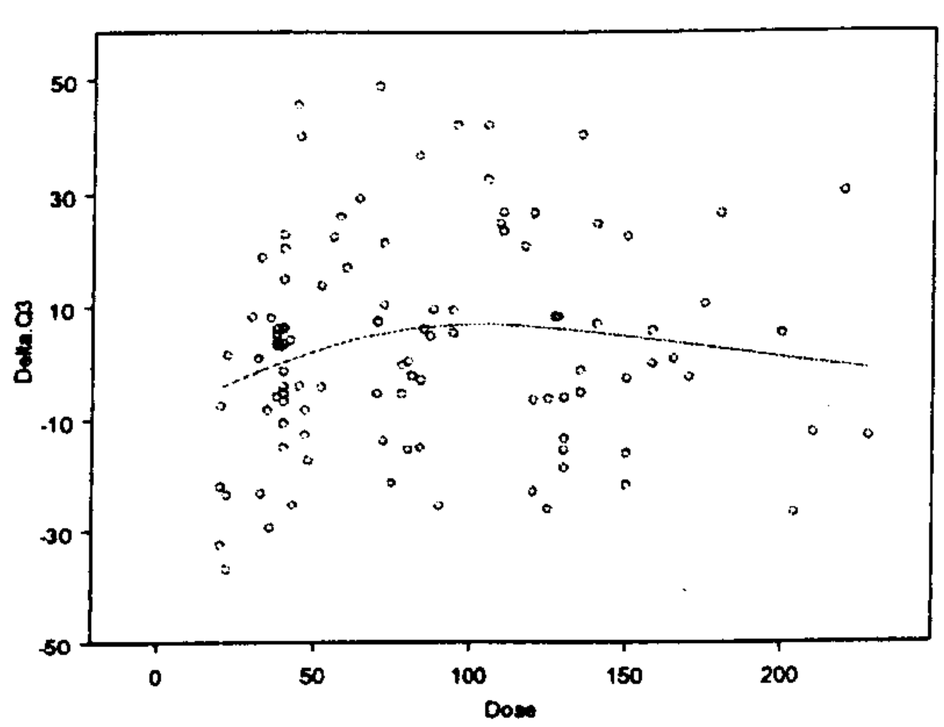


B. Liver metastases added into Q_3 model



0 = Liver metastases absent
1 = Liver metastases present
* = outlier

C. No covariates on Q_3



D. Ln dose added into Q_3 model

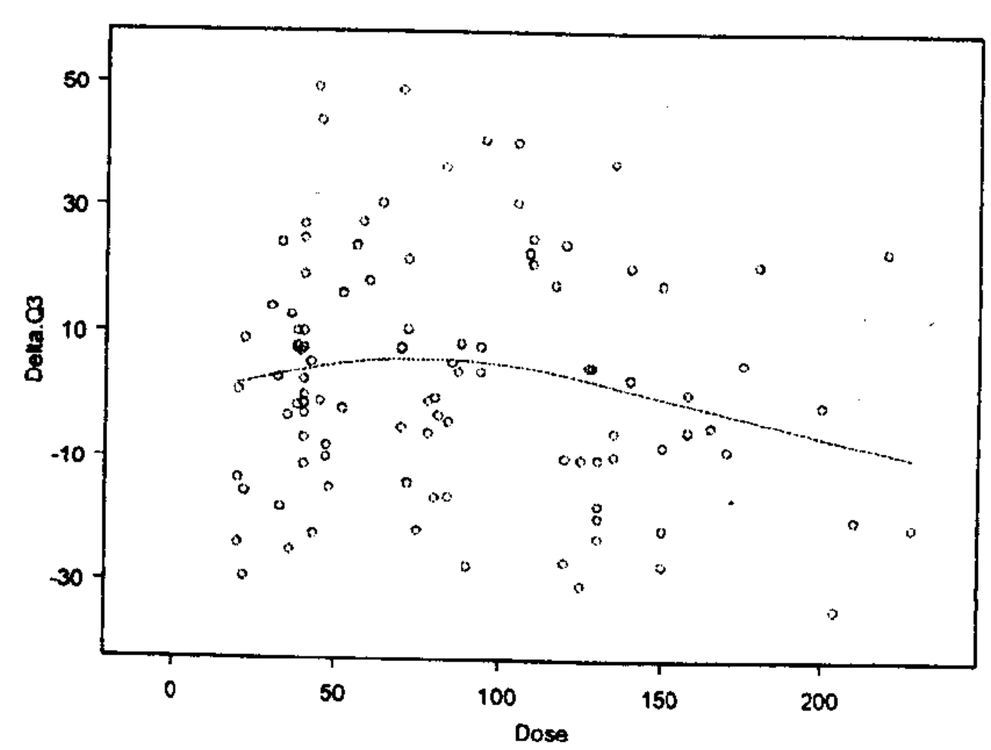


Table 3.5

Parameter estimates from basic, full and final model

		Basic		Full		Final	
		Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)
CL (L/h)	θ_1	28.0	5.32	20.1	8.06	74.9**	12.3
V_1 (L)	θ_2	9.88	8.77	9.93	8.87	9.99	8.66
Q_2 (L/h)	θ_3	29.0	14.0	29.1	14.0	29.5	13.8
V_2 (L)	θ_4	35.2	18.9	35.2	18.9	36.0	18.6
Q_3 (L/h)	θ_5	60.3	6.30	52.4	7.84	60.6	6.19
V_3 (L)	θ_6	780	6.50	791	6.52	790	6.38
Ln AST (CL)	θ_7	-		0.415	17.3	0.139	6.35
dose (CL)	θ_8	-		0.00367	30.0	-	-
BSA (CL)	θ_9	-		0.581	38.6	-	-
Lmets (CL)	θ_{10}	-		1.58	13.1	-	-
Ln dose (Q_3)	θ_{11}	-		0.182	32.6	-	-
Lmets (Q_3)	θ_{12}	-		1.31	7.60	-	-
ωCL (%)		53.3	16.5	36.9	16.9	42.5	15.4
ωQ_3 (%)		32.9	18.1	29.8	18.4	31.6	19.5
ωV_3 (%)		44.0	22.9	43.2	21.9	43.1	22.1
σ (%)		23.1	9.23	23.0	9.25	23.1	9.29

**CL=74.9-(74.9*0.139*LnAST)

Basic Model

TVCL=θ_1
TVV1=θ_2
TVQ2=θ_3
TVV2=θ_4
TVQ3=θ_5
TVV3=θ_6

Full Model

TVCL=θ_1*(1-θ_7*(LnAST-4.5)*(1+θ_8*(DOSE-78))*(1-θ_9*(BSA-1.7)) if liver metastases present*θ_{10}
TVV1=θ_2
TVQ2=θ_3
TVV2=θ_4
TVQ3=θ_5*(1+θ_{11}(LnDOSE-4.4)) if liver metastases present*θ_{12}
TVV3=θ_6
(N.B. covariates were centred around median values)

Final Model

TVCL=θ_1*(1-θ_7*(LnAST)
TVV1=θ_2
TVQ2=θ_3
TVV2=θ_4
TVQ3=θ_5
TVV3=θ_6

Estimates of parameters from the final model are given in Table 3.6. The relative standard errors (RSE) for the estimates of pharmacokinetic parameters and variability parameters ranged from 6 to 22 % indicating that the parameters were estimated from the model with a good degree of precision. The residual error was estimated to be approximately 23 %. Given the range of AST concentrations (7 – 815 units/L) in this group of patients, the CL is predicted to range from 5 to 55 L/h. The individual Bayesian estimates for CL ranged from 4 to 86 L/h.

3.3.6. Assessment of variance-covariance matrix

The OFV was lower (8035 versus 8089) following the inclusion of the off-diagonal terms in the variance-covariance matrix. The results of the model building with NONMEM are given in Table 3.6. The covariates selected for the full model were ln AST, dose, BSA and liver metastases for CL and were ln dose and liver metastases for Q_3 ie. the same covariates as when the variance-covariance was restricted to the diagonals. However, for V_3, height, bilirubin and albumin were also identified as statistically significant covariates. AST accounted for approximately 10 % of the inter-individual variability on CL, however, the remaining covariates each accounted for less than 2 % of the inter-individual variability of a pharmacokinetic parameter and therefore were not considered to be of clinical significance. The final model only included ln AST on CL:

$$CL = 72.9 \times (1 - (0.135 \times \ln AST))$$

Table 3.6

Summary of models using FOCE-INTER with full variance-covariance matrix

Model No.	CL Model	OFV	ΔOFV	cf. model No.
0	Base model	8034.60		
1	**Ln AST**	**7978.24**	**56.36**	**0**
2	**Dose**	**8007.49**	**27.11**	**0**
3	BSA	8032.10	2.50	0
4	WGT	8033.50	1.10	0
5	**LMETS**	**8016.10**	**18.50**	**0**
6	WHO$	8034.58	0.02	0
7	**WHO***	**8025.61**	**8.99**	**0**
8	Basic (103 patients)#	7874.54	-	
9	CrCL (103 patients)#	7873.51	1.03	8
10	Ln AST + Dose	7974.89	3.35	1
11	Ln AST + BSA	7975.75	2.49	1
12	Ln AST + WGT	7976.53	1.71	1
13	**Ln AST + LMETS**	**7972.70**	**5.54**	**1**
14	LnAST + WHO$	7977.24	1.00	1
15	LnAST and WHO*	7975.27	2.97	1
16	**Dose + BSA**	**7997.24**	**10.25**	**2**
17	**Dose + WGT**	**7999.89**	**7.60**	**2**
18	**Dose + LMETS**	**8002.01**	**5.48**	**2**
19	Dose + WHO*	8000.60	6.89	2
20	Lmets + WHO*	8011.88	4.22	5
21	**Ln AST + LMETS+Dose**	**7966.76**	**5.94**	**13**
22	Ln AST + LMETS+BSA	7971.02	1.68	13
23	Ln AST + LMETS+WHO*	7969.96	2.74	13
24	**Ln AST + LMETS+Dose+BSA**	**7962.12**	**4.64**	**21**
25	Ln AST + LMETS+Dose+WHO*	7962.57	4.19	21
26	Ln AST + LMETS+Dose+BSA+WHO*	7958.38	3.74	24
	V_3 Model			
27	AST	7959.53	2.59	24
28	Albumin	7960.63	1.49	24
29	**Height**	**7958.11**	**4.01**	**24**
30	**Bilirubin**	**7956.94**	**5.18**	**24**
31	**Bilirubin + Height**	**7953.01**	**3.93**	**30**
32	**Bilirubin + Height + albumin**	**7948.43**	**4.58**	**31**
33	Bilirubin + Height + AST	7952.98	0.03	31
34	Bilirubin + Height + albumin + AST	7947.38	1.05	32
	Q_3 Model			
35	**LMETS**	**7938.43**	**10.00**	**32**
36	WHO*	7945.82	2.61	32
37	WHO$	7947.80	0.63	32
38	LnDose	7946.77	1.66	32
39	Albumin	7946.56	1.87	32
40	**LMETS + LnDose**	**7930.64**	**7.79**	**35**
41	LMETS + WHO*	7945.82	-7.39	35
42	LMETS +WHO$	7937.24	1.19	35
43	LMETS + albumin	7938.37	0.06	35
44	LMETS + LnDose+albumin	7930.51	0.13	40
45	LMETS + LnDose+WHO$	7930.33	0.31	40

KEY. AST = Aspartate aminotransferase; BSA = Body surface area; CrCL = Creatinine clearance; LMETS = Liver metastases; WGT = Weight; WHO = World Health Organisation (performance status)
* = Fall in OFV of 7.81 required for statistical significance ($p<0.05$)
$ = Grades 0 and 1 combined, grades 2 and 3 combined
# = patients 223 and 228 were excluded due to missing creatinine data
Bold text indicates models that gave a statistically significant improvement

3.4 DISCUSSION

Following initial exploratory population analysis, estimation of peak concentrations using NONMEM was poor, i.e. predicted concentrations were very different to the measured concentrations. Difficulty in estimating epirubicin concentrations at early blood sampling times has previously been reported by other investigators (Camaggi et al. 1982). A very sharp peak was observed as plasma concentrations were seen to rise and fall several thousand ng/ml over the period of a few minutes. A minor inaccuracy in measurement of the actual blood sampling time in the first few minutes after administration of epirubicin could therefore result in a major error in the estimation of the epirubicin plasma concentration. Thus, minor deviations in measurement and/or recording of actual blood sampling times could be the cause of the poor estimations of the peak concentrations. In addition, compartmental analysis assumes that there is instantaneous and homogenous mixing of drug within the plasma or blood. However, there have previously been several reports of peak concentrations being observed up to 3 minutes after a rapid infusion of various drugs and it is thought that this could be due to the time for drug to reach the sampling site, mixing or other haemodynamic effects (Chiou 1979). The impact of the mixing phenomenon on early observations is greatest in drugs with very rapid distribution phases, such as epirubicin. Given the difficulty in accurately predicting peak plasma concentrations and the likely inaccuracy of the sampling times, it was considered appropriate that concentrations measured up to 6 minutes post-start of the infusion (end of the infusion or shortly after) should be excluded from further analysis.

Scatterplots of pharmacokinetic parameters versus covariates are a simple yet informative method of identifying parameter-covariate relationships and in particular the shape of any such relationships (Ette & Ludden 1995). However, the scatter plots only allow the effects of each covariate to be assessed individually and if some covariates are correlated, they may not all be required in the model. In addition, a covariate with a very strong influence on a parameter may mask a weaker influence of another covariate. Due to the larger number of parameters and covariates to be assessed in this analysis, the total number of possible models is very large and it would not be practical to test all potential models identified from the scatterplots due to the computing time required.

Generalised additive modelling (GAM) was used to identify combinations of covariates that influenced the pharmacokinetic parameters. The GAM analysis and the plots are dependent on the accuracy of the individual Bayesian estimates of the pharmacokinetic parameters. Parameter estimates using the FOCE are generally considered to be more reliable and less biased than those estimated by FO (Beal & Sheiner, 1992). However, the GAM analysis results using estimates from both methods were similar. From the GAM analysis, dose, AST, CrCL and BSA were identified as the combination of covariates that had the most influence on CL. Thus, although from the scatter plots, bilirubin and alkaline phosphatase appeared to be important covariates with respect to CL, they were not identified as important covariates by the GAM analysis. It is likely that their effect was over shadowed by the greater influence of AST, and hence, the addition of bilirubin and alkaline phosphatase to the model did not offer any further advantage over AST. A number of covariates were identified by GAM analysis, using FO estimates, as influencing V_1 and Q_2, however, the influence of these parameters was not tested with NONMEM as inter-individual variability of these parameters could not be estimated using FOCE. As graphical analysis did not find relationships between V_1 and Q_2, with any covariates, the influence of these parameters is likely to be of little clinical relevance.

Following analysis using NONMEM, dose was identified as influencing CL and Q_3. It was noted that following the inclusion of dose and AST separately in the CL model, there was a large fall in OFV, whereas when added into the model together there was only a marginal improvement in the OFV compared to inclusion of them singularly. Clinicians would expect patients with liver disease to have reduced epirubicin CL and hence are likely to have reduced the dose in these patients. Certainly, in 37 of the patients in the data set, the dose was administered according to the AST level to achieve a target AUC. This could explain the apparent increase in epirubicin CL with an increase in dose. Due to the interaction of dose with liver function, it is not possible to fully dissociate the individual influences of AST and dose on CL. The inclusion of AST in the model explained 11 % of the variability of CL but the addition of dose in the model only accounted for a further 1.7 % of the variability. From the model, the CL was estimated to change 2-fold over the entire dosage range whereas it was estimated to change 11-fold over the range of AST values, thus the inclusion of dose in the model was considered to be of much less clinical value than

AST. A study of 78 patients with metastatic breast cancer reported that the kinetics of epirubicin were linear over the dose range of 40 to 135 mg/m^2 (Jakobsen et al. 1991a). However, there was some suggestion of dose-dependent pharmacokinetics as AUC values increased in a slightly less than dose proportional manner, consistent with our findings, and statistical analysis found the terminal half-life at the 40 mg/m^2 dose level to be significantly longer compared to the 60 and 135 mg/m^2 dose levels. In contrast, a greater than dose proportional increase in AUC and C_{max} values was reported in a study of 19 patients with various malignancies (Tjuljandin et al. 1990). However, the pharmacokinetic parameters were calculated using a 2-compartment model and the results appeared to be inconsistent. Using a population approach, Wade and co-workers (1992) concluded that epirubicin has linear pharmacokinetics over the dose range of 25 to 100 mg/m^2. Thus overall, there is no consistent evidence to support dose-dependent pharmacokinetics for epirubicin.

The inter-individual variability in the basic model was estimated to be 53 %, however, using the full model that included 4 covariates on CL, only 16 % of the variability was explained. It is possible that the data set is too small to identify all the sources of variability and that some variability could be due to factors not available in this data set such as diet or genetic differences in metabolic enzymes. The inclusion of dose, BSA and liver metastases on CL and liver metastases and dose on Q_3 each accounted for 2 % or less of the interindividual variability. Thus, the influence of these covariates on the pharmacokinetics of epirubicin was deemed to be of little clinical significance and given the increased complexity of the model, and hence, increased complexity of any resulting dosage guidelines, were considered to be of marginal benefit. Hence, the final model included only AST as a natural log function on CL. Model building allowing a full covariance matrix resulted in the selection of the same final model i.e. CL as a natural log function of AST, as when the variance-covariance was restricted to be diagonal.

As data were available from each individual on one occasion only, it was not possible to account for the inter-occasion variability (intra-subject variability) in this data set. It is likely that the residual error (23 %) is artificially inflated and reflects inter-occasion variability in addition to the 'true' residual error (Karlsson & Sheiner 1993).

The current dosing guidelines in the UK recommend a reduction in dose of 50 % in patients with moderately elevated bilirubin levels and 75 % reduction in patients with severely elevated bilirubin levels (SPC, UK). However, bilirubin was not identified by the GAM analysis, whereas AST was, suggesting that AST is a better maker for epirubicin CL than bilirubin. It wàs noted that patients with liver metastases mostly had bilirubin levels within the reference range, whereas their AST levels were generally elevated. Thus, AST appeared to be a more sensitive marker than bilirubin of liver damage in patients with liver metastases. These results suggest that current dosage procedures in the UK, based on bilirubin, may not be ideal.

The current dosing procedures in the UK also recommend that dose is administered according to the body surface area of a patient. This method of dosing epirubicin was developed because of convention in oncology rather than by scientific research. A previous study in which 20 patients received 150 mg epirubicin regardless of body size, did not find a relationship between BSA and any pharmacokinetic parameter, despite the wide range of BSA (1.58 to 2.05 m^2) of the patients studied (Gurney et al. 1998). In the current analysis, a weak negative relationship was observed between CL and BSA, further indicating that the increase of epirubicin dose with increasing BSA does not offer any clinical advantage. The results from the population analysis in this Chapter are subsequently used to propose new guidelines for epirubicin (Chapter 5).

CHAPTER 4

VALIDATION OF POPULATION MODEL FOR EPIRUBICIN USING INTERNAL TECHNIQUES

4.1 INTRODUCTION TO CHAPTER

A population model is dependent on the quality of the data and by the decisions taken by the analyst during model building, which may be subjective. Therefore, the validity of the population model should be tested. In particular, if the results obtained are intended to define dosage guidelines, the predictive performance of the model should be assessed (Sun et al. 1999). A number of methods have been used to validate population pharmacokinetic models but there is currently no consensus on the correct approach. A universal validation method may never be available. Rather an appropriate validation method should be selected based on the objectives of the analysis (Mentre & Ebelin 1997). Perhaps the most compelling evidence for the validity of a population model is to measure the predictive performance of that model in a different data set (external validation). A limitation of this method is that if unsatisfactory predictive performance is attained, it is not possible to establish whether this is due to invalid model parameters or because of genuine differences between the data sets (Vozeh 1991). Despite this limitation, external validation is the most stringent test for validity of a population model and hence is the method of choice when possible (Sun et al. 1999).

The predictive performance of a population model can be determined by assessing the ability of the model to accurately predict concentrations in the validation data set. The measured concentrations in the validation data set are compared to those predicted by the population model given the clinical characteristics and dosing history of each patient. This method of validation may be of interest if a target concentration is desirable for optimum therapeutic outcome, however, it is unsuitable if more than one concentration is available per subject as concentrations within the same individual will be correlated. Although it is possible to use only one concentration from a patient, this would result in potentially valuable information being discarded. Vozeh and coworkers (1990) suggest that if more than one concentration per subject is available, a standardized mean prediction error for each patient is calculated (mean prediction error/standard deviation of prediction error). The standardized mean prediction errors should have a mean of zero and a standard deviation of one, if the model optimally describes the data. Alternatively, it may be that a desired pharmacokinetic parameter is of particular interest rather than individual concentration values. In this situation,

the population estimate of the parameter from the population model, given the clinical characteristics and dosing history of the patient is compared to the actual Bayesian estimate for that individual (Bruno et al. 1996). This approach overcomes the problems of multiple concentrations. However, this method assumes that the Bayesian estimate of the parameter is the 'true' value, and should only be used if one is confident that this estimate is reliable, hence, a rich data set is usually required.

In many cases, an external data set for validation purposes is not available so other internal techniques are used. One method is to split the data set (data-splitting) into a training data set and a validation data set before commencing analysis. The disadvantage of this method is that because the accuracy of the model is a function of sample size, removal of a proportion of the data (usually one third) may impact on the reliability of model parameter estimates. Furthermore, the results obtained from data-splitting are highly variable and dependent on the split of the data (Roecker 1991). One refinement is cross-validation which involves repeated data-splitting. This may be advantageous because the results are not dependent on a single data split and a larger proportion of patients can be included in the estimation process with each split. Furthermore, with cross-validation all subjects will contribute to both the estimation process and validation.

The bootstrap resampling method does not require the removal of a proportion of the data for validation purposes and thus, like cross-validation, has the advantage of utilising all the available data during model development. Bootstrap resampling can be used to test model stability in terms of covariate selection and to evaluate model performance (Ette 1997). A bootstrap sample is generated by random sampling from the original data set so that a new data set is formed that includes the same number of individuals as the original data set. A minimum of 200 bootstrap replicates is advised for model validation purposes (FDA guidelines, 1999). The extensive computation time required for model validation using hundreds of datasets in bootstrap validation methods is clearly a drawback of this approach, although with ever improving computer systems and the availability of automated model building software (Jonsson & Karlsson 1998), this is becoming less of a problem.

Sensitivity analysis can be used to determine the robustness of the model by changing values of covariates or plasma concentrations by a small amount to assess the sensitivity of the model to small changes in the data (Karlsson et al. 1998). Case

deletion diagnostics such as jacknifing can be used to identify outliers or influential individuals (Sadray et al. 1999). This could highlight that caution is required if the model parameters are highly dependent on only one or 2 unusual individuals.

Confidence intervals of parameters calculated using extended least squares, the algorithm used by NONMEM, were previously been shown to be too narrow, relative to the true values (Sheiner & Beal, 1987). Alternative methods to estimate standard errors or confidence intervals, such as the jacknife, are recommended to attain more reliable estimates of standard errors (FDA guidelines, 1999). The jacknife is a non-parametric method, which involves repeated estimation of population estimates following consecutive deletions of data from individuals (n-1) or a percentage of individuals (eg. n-10%) from the data set.

Although validation using an external data set is the preferred validation method, internal methods can identify an invalid model without the further need for testing with an external data set.

The aims of the analysis in this Chapter was to assess the model stability, the predictability of the model and the impact of some of the decisions taken in the development of the final population model, using internal validation techniques.

4.2 METHODS

4.2.1 The effect of removing concentration data collected before 0.1 h post-dose

In the population analysis (Chapter 3) a decision was taken to exclude all concentration data collected prior to 0.1 h post-dose. The impact of this decision on the results was evaluated. Population parameter estimates were calculated using all the concentration data available (i.e. including concentration data collected before 0.1 h post-dose) for the 105 subjects included in the final model using NONMEM as described previously. The effect of removing the early time-points was assessed by comparing the population parameter estimates and their precision on the exclusion of the early time-points, with those obtained on their inclusion.

4.2.2 Jacknife analysis

4.2.2.1 Calculation of standard errors

Standard errors for the population parameters were calculated by jacknife analysis according to the method described by Effron and Tibshirani (1993). From the original data set, 105 new data sets were produced so that each excluded the data from one patient (a different patient was excluded in each data set). These are termed jacknife samples. Each jacknife sample was analysed with NONMEM using the final population model. A population parameter estimated from a jacknife sample is termed $\hat{O}_i$ where i represents the jacknife sample in which the ith individual was deleted. Pseudo values ($\tilde{O}_i$) for each parameter were calculated as follows:

$$\tilde{O}_i = n\hat{O} - (n-1)\hat{O}_i$$

where $\hat{O}$ is population estimate of the parameter (estimated from all patients) and n is the total number of patients in the data set. The standard error for the population parameter ($\hat{O}$) was calculated as follows:

$$se(\hat{O}) = [\ (\sum (\tilde{O}_i - \tilde{O})^2) / n(n-1)\ ]^{1/2}$$

where $\tilde{O}$ is the average of the pseudo values ie. $\tilde{O} = \sum \tilde{O}_i/n$.

4.2.2.2 Identification of influential individuals

Population estimates from the jacknife samples were compared to the final population estimates to identify any individuals that had a large influence on the parameter values.

4.2.3 Likelihood based method for detection of individuals influencing covariate selection

Individuals influencing covariate (AST) selection were identified using the likelihood based method previously described by Sadray and coworkers (1999). The influence of an individual was assessed by comparing the difference in OFV between the basic and final models when calculated using all the data and when calculated with the individual in question removed:

$$OFV\Delta_{jacknife,i} = (OFV_{final,n} - OFV_{basic,n}) - (OFV_{final,n-i} - OFV_{basic,n-i})$$

Individuals that support the model have a negative $OFV\Delta_{jacknife,i}$, whereas individuals that do not support the model have a zero or positive value. Individuals with high negative values were termed 'driving' whereas those individuals with a high positive value were termed 'masking'.

For example, using all data, the OFV values for the final ($OFV_{final,n}$) and basic ($OFV_{basic,n}$) models are 7978 and 8034, respectively, and hence the first part of the equation ($OFV_{final,n} - OFV_{basic,n}$) is a constant (-56). If an individual supported the model (ie. they are 'driving'), on their removal from the data set, the fall in the objective function would be less than the fall observed when they were included, thus, the fall in the OFV ($OFV_{final,n-i} - OFV_{basic,n-i}$) may be, for example, reduced by only -30. Therefore, for a driving individual, $OFV\Delta_{jacknife,i}$ will be a negative number, as (-56) – (-30) = -26. Likewise, the opposite is true for a 'masking' individual.

The OFV for the basic and final models were available from previous analyses. Likewise the OFV's for the jacknife samples of the final model were available from the previous jacknife analysis. The OFV's for the jacknife samples of the basic model

were obtained by fitting the basic model to each of the jacknife samples using NONMEM.

4.2.4 Cross validation analysis

The data set was randomly split into 5 groups of 21 patients. A new data set was formed, referred to as 'Training dataset 1', comprising 4 of these groups, (i.e. 84 patients). This was used to estimate population parameters using the basic and final models as previously described. The remaining 21 patients were used for validation and referred to as 'Validation data set 1'. This process was repeated 4 times so that Training data sets 2 to 5 were formed, each with a different group removed for validation purposes (Validation data sets 2 to 5).

The basic and final CL model derived from each training data set were used to predict CL for patients in the corresponding validation data sets from their AST measurements. Individual Bayesian estimates of model parameters were estimated for the validation data sets. The ability of the basic and final population models derived from the training data sets to estimate CL in the corresponding validation data sets was assessed by calculating prediction errors as follows:

$$P_{ei}\ (\%) = (\text{Population CL} - \text{Bayesian CL}) / \text{Bayesian CL} \times 100$$

The root mean squared prediction error (rmse), which describes the precision of the population estimate relative to the Bayesian CL estimate, was calculated as described in Chapter 2. A paired t-test was performed on the squared prediction errors to assess whether differences in imprecision of CL estimated using the basic or final model (with AST) was statistically significant ($p<0.05$).

The prediction errors for CL estimates using the basic and final models were plotted against AST to assess whether each model performed equally well over the range of AST values.

4.2.5 Sensitivity of the model to 10 % errors in AST and plasma concentrations

The sensitivity of the model to errors in AST concentrations was assessed by performing a sensitivity analysis. AST concentrations were randomly changed by +/- 10 % of the measured value. Randomisation was performed by generating a column of random numbers in Excel using the 'random number generator' tool. The column of random number was inserted next to a column of patient ID numbers and both columns were sorted according to ascending order of the random numbers. The first 53 patients were assigned to be changed by + 10 % and the remaining 52 patients were assigned to be changed by – 10 %. Population analysis of the altered data was performed using NONMEM as previously described for the final population model. The population parameters and OFV using the altered dataset were compared to those attained using the original data. In a similar manner, a population analysis was performed using epirubicin plasma concentration data that were randomly changed by +/- 10 % to assess the sensitivity of the model to errors in plasma concentration measurements.

4.3 RESULTS

4.3.1 The effect of removing concentration data collected before 0.1 h post-dose

The population parameter estimates and corresponding precision (relative standard error) calculated with the early time points included and excluded are given in Table 4.1. The precision of the structural parameter estimates was markedly inferior, as measured by the relative standard errors (RSE) for all parameters when early time-points were included compared to when they were excluded. Parameters estimated using the early time-points, had an RSE ranging from 6.3 to 56 %, indicating that they were unreliable, compared to 4.5 to 16 % when these early time-points were excluded. The structural parameter estimates calculated when the early time-points were included were up to 67 % lower than those estimated when they were excluded. The parameters least affected by the inclusion/exclusion of the early time-points were THETA1 and THETA7, which represent CL and the influence of AST on CL, with differences of only 15 and 4 %, respectively. Estimates of inter-individual variability and their corresponding precisions were similar when calculated with and without the inclusion of early time-points. The residual error was higher following the inclusion of the early time points (31 %) compared to that estimated when they were excluded (23.0 %) indicating a poorer fit of the model to these data. This is a large increase in residual error given that the increase in the total number of data points was relatively small (from 1051 to 1120).

4.3.2 Jacknife analysis

4.3.2.1 Calculation of standard errors

The estimates of standard errors using the jacknife technique were very similar to those estimated by NONMEM, although the standard errors estimated by the jacknife method were generally higher than those estimated by NONMEM (Table 4.2). The mean population parameter estimates from the jacknife samples were almost identical to those estimated using NONMEM.

Table 4.1

Parameter estimates including and excluding concentration data before 0.1 h

	Early time-points Excluded	Early time-points Included	Early time-points Excluded	Early time-points Included
Pharmacokinetic parameter			RSE (%)	
θ_1	72.9	62.2	7.97	13.9
θ_2	10.3	4.97	8.18	34.2
θ_3	30.2	13.0	12.7	42.1
θ_4	35.7	11.9	16.1	56.4
θ_5	61.5	40.6	6.13	24.3
θ_6	772	583	5.45	18.5
θ_7	0.135	0.129	4.54	6.26
Inter-individual variability			RSE (%)	
ωCL (%)	39.4	35.9	14.3	16.0
ωQ_3 (%)	34.4	33.2	17.8	14.3
ωV_3 (%)	42.7	40.0	22.4	28.8
Residual error			RSE (%)	
σ (%)	23.2	30.7	9.27	17.1

Key
$TVCL=\theta_1*(1-\theta_7*(LnAST)$
$TVV_1=\theta_2$
$TVQ_2=\theta_3$
$TVV_2=\theta_4$
$TVQ_3=\theta_5$
$TVV_3=\theta_6$
where TV is typical value

ωCL, ωQ_3, and ωV_3 = interindividual variability (CV) for CL, V_3 and Q_3, respectively; σ = residual variability

Table 4.2

Estimates of population estimates and standard errors using NONMEM and Jacknife analysis

	Estimated from NONMEM		Estimated from Jacknife analysis	
	Population Estimate	se	Mean population Estimate	se
θ_1	72.9	5.8	72.9	5.9
θ_2	10.3	0.84	10.3	0.96
θ_3	30.2	3.8	30.2	4.0
θ_4	35.7	5.7	35.7	6.0
θ_5	61.5	3.8	61.5	3.7
θ_6	772	42	772	43
θ_7	0.135	0.0061	0.135	0.0066
$\eta 1$	0.155	0.022	0.155	0.023
$\eta 2$	0.118	0.021	0.118	0.022
$\eta 3$	0.182	0.041	0.182	0.044
$\eta 1$-2	0.0659	0.016	0.0660	0.017
$\eta 1$-3	0.0825	0.020	0.0825	0.020
$\eta 2$-3	0.0974	0.029	0.0975	0.030
σ	0.0537	0.0050	0.0537	0.0050

se = standard error

Model:

TVCL (l/h) = θ_1*(1-θ_7*(LnAST)
TVV_1 (l) = θ_2
TVQ_2 (l/h) = θ_3
TVV_2 (l) = θ_4
TVQ_3 (l/h) = θ_5
TVV_3 (l) = θ_6

CL = TVCL*EXP $\eta 1$
Q_3 = TVQ3*EXP $\eta 2$
V_3 = TVV3*EXP $\eta 3$

where TV is typical value

4.3.2.2 Identification of influential individuals

The estimates of the population parameters from the jacknife samples are illustrated in Figure 4.1. There was little variability in the population parameter estimates between jacknife samples with most estimates being within 5 % of the final population estimate. The structural parameter estimates from the jacknife samples were all within 5 % of the final population estimates with the exception of Theta 3 and Theta 4, which were 6 and 7.5 % higher, respectively, when patient 228 was removed. For the inter-individual variability parameters, only 1 or 2 jacknife samples varied from the final estimate by more than 5 %, with a maximum variation of –17 % for ETA3 when patient 402 was removed. The covariance parameters were most sensitive to removal of individual patients with up to 5 jacknife samples varying by more than 5 % from the final estimates. The maximum variation was 19 %, which was observed for covariance between ETA2 and ETA 3 when patient 143 was removed. All jacknife estimates of residual error were within 5 % of the final estimate.

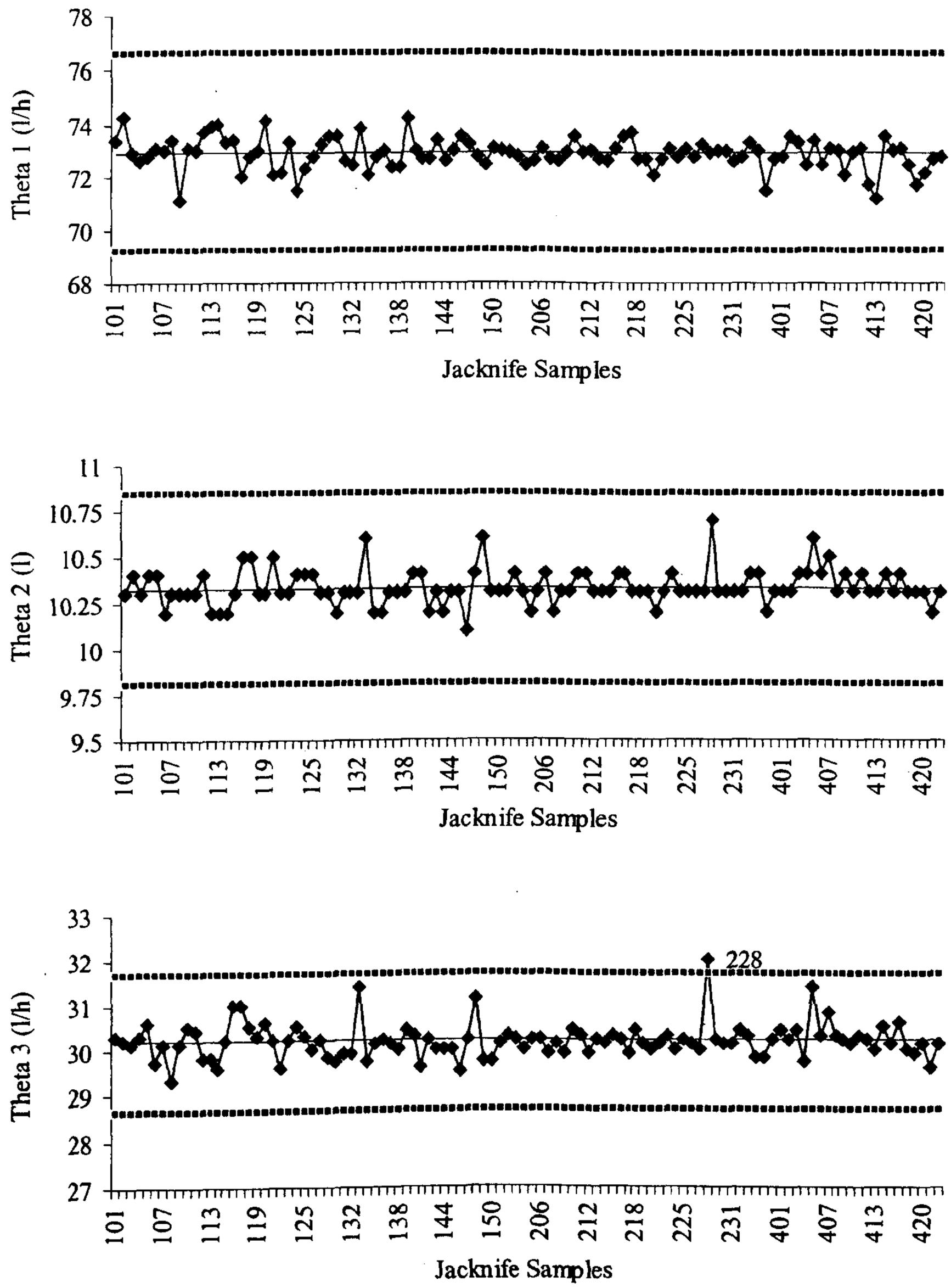
4.3.3 Likelihood based method for detection of individuals influencing covariate selection

The $OFV\Delta_{jacknife,i}$ values are illustrated in Figure 4.2. Patient 225 had a high positive value for $OFV\Delta_{jacknife,i}$ indicating that this patient was masking the relationship between AST concentration and CL. Patient 225 had a CL value of 30.5 L/h, which is is higher than expected from the final population model for CL based on the AST value of 179 U/L (21.8 L/h).

The individuals with the greatest positive influence on the inclusion of AST in the model were Patients 413, 228 and 419, who had highest AST concentrations in the data set at 815, 530 and 489 U/L respectively. Although these individuals had the greatest influence on the final covariate model, they were not outliers as they were not set apart from the other individuals (Figure 4.2). Data from 73 patients produced negative $OFV\Delta_{jacknife,i}$ values and thus were better described by the final model, whereas data from 32 patients generated positive values and consequently were better described by the basic model.

Figure 4.1 (1 of 5)

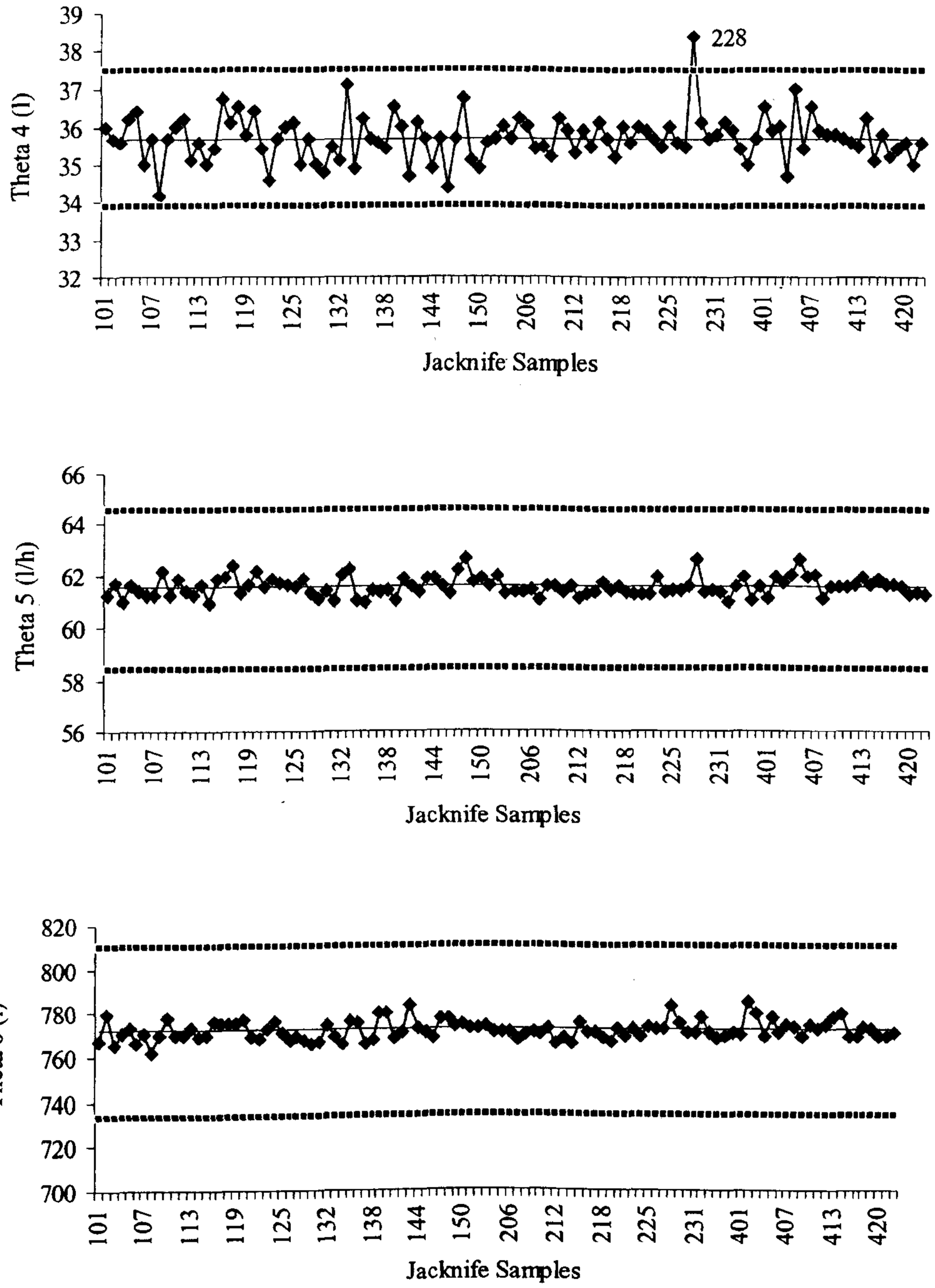
Population parameter estimates from jacknife samples



NB. Jacknife sample 101 was the data set excluding patient 101 etc
The solid line represents the population estimate from the final model.
The dotted line represents ± 5 % from the final population estimate.

Figure 4.1 (2 of 5)

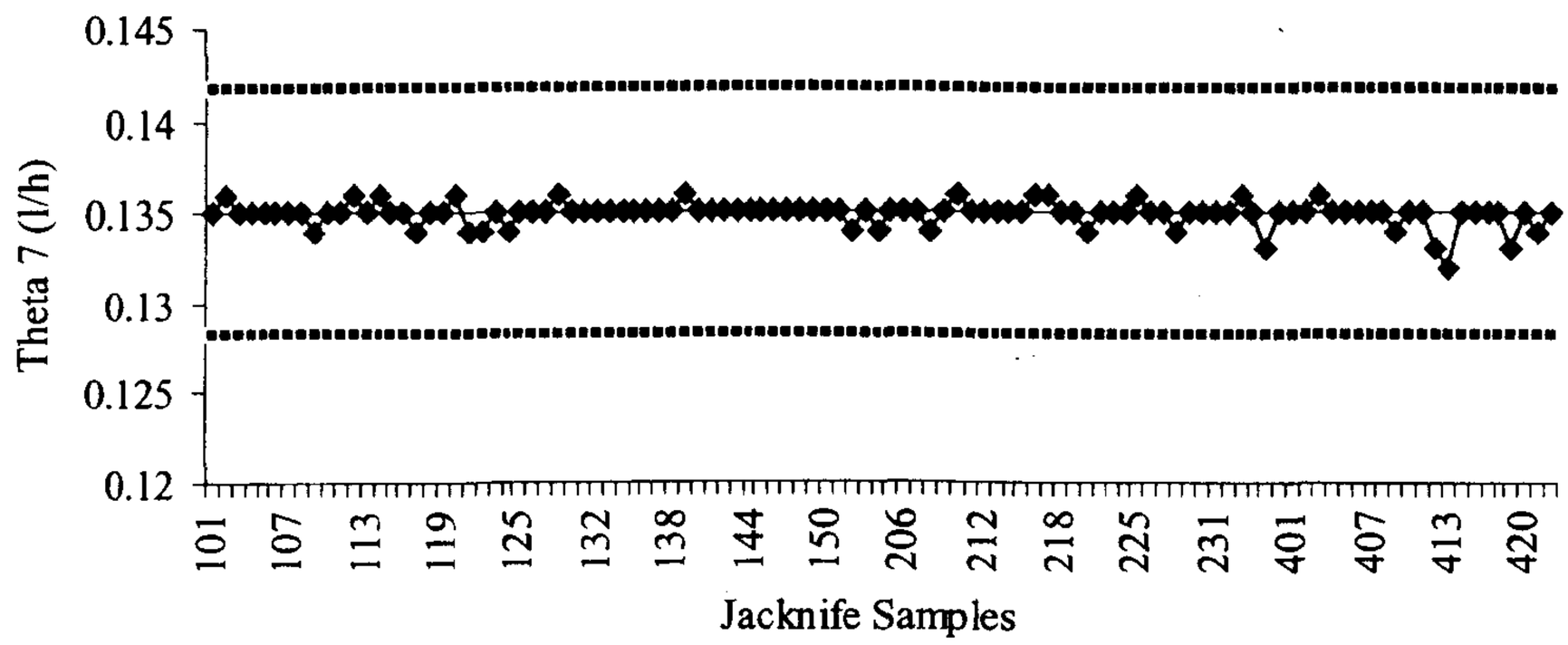
Population parameter estimates from jacknife samples

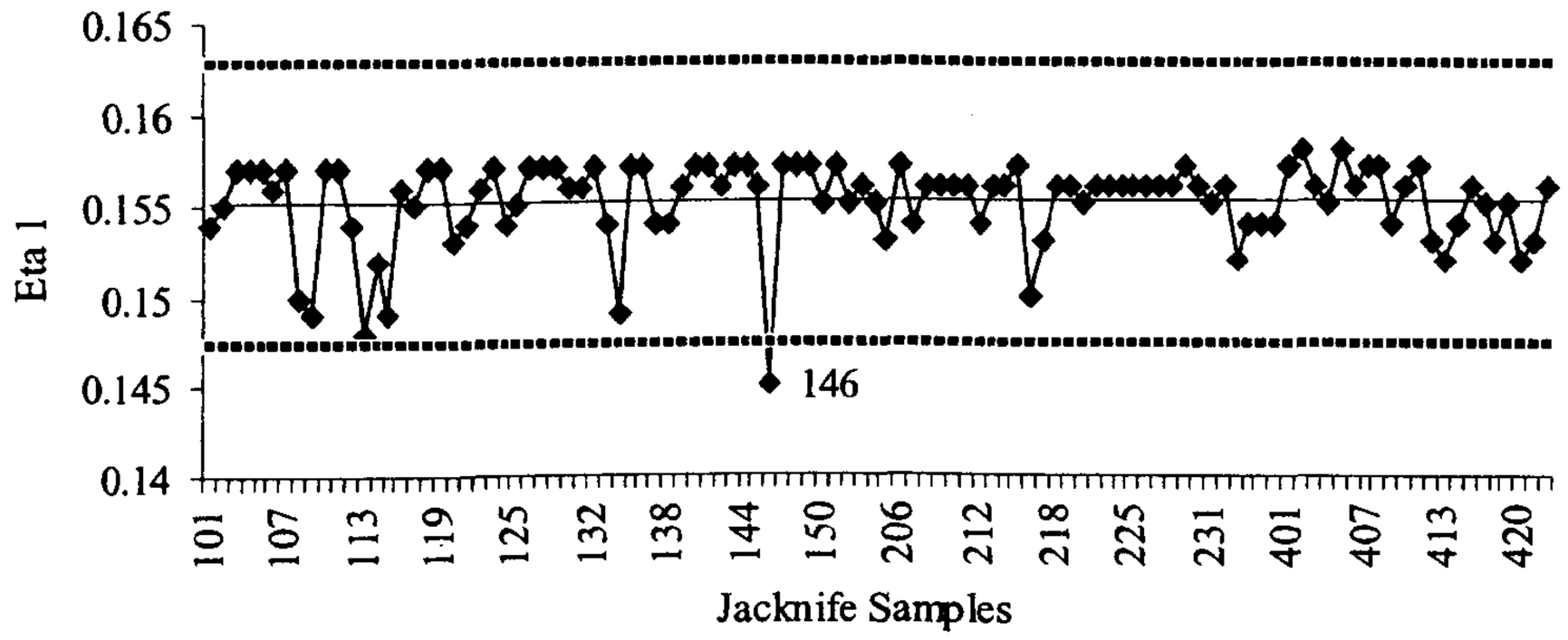


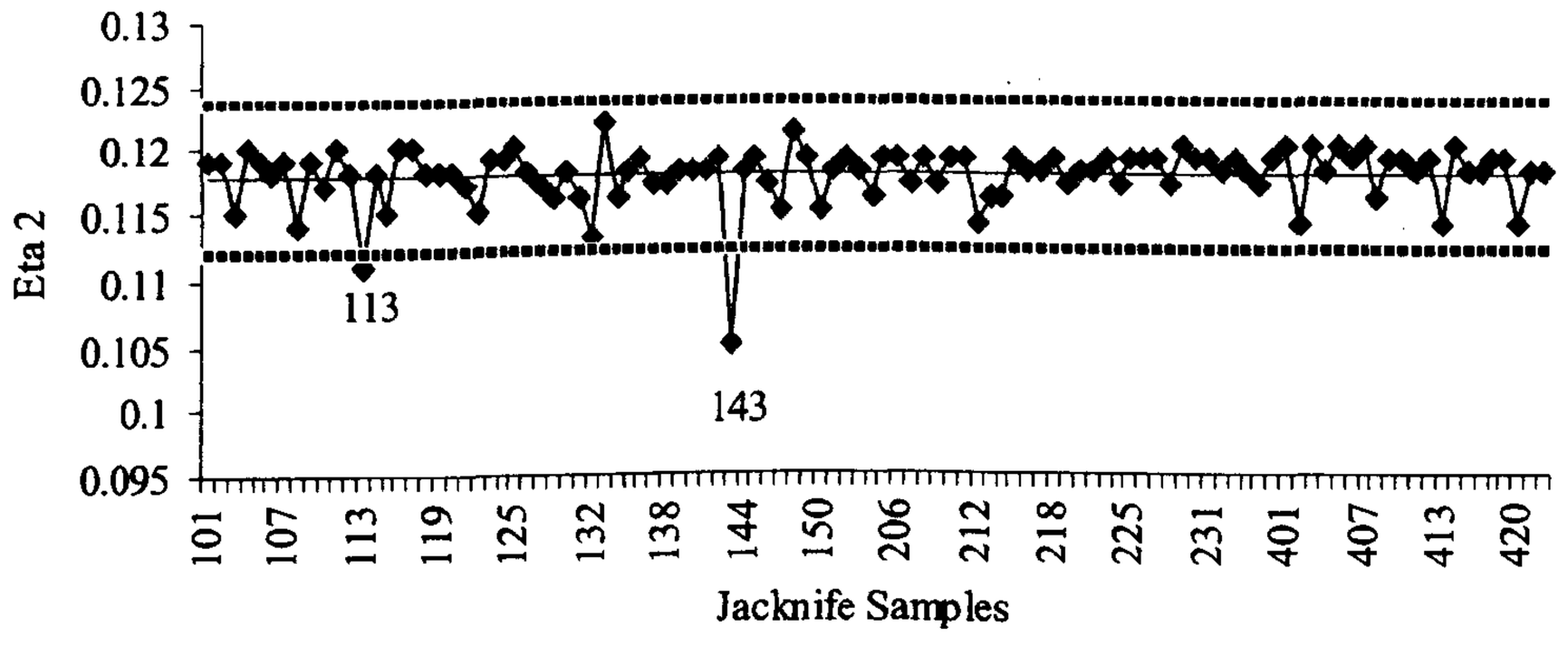
NB. Jacknife sample 101 was the data set excluding patient 101 etc
The solid line represents the population estimate from the final model.
The dotted line represents ± 5 % from the final population estimate.

Figure 4.1 (3 of 5)

Population parameter estimates from jacknife samples



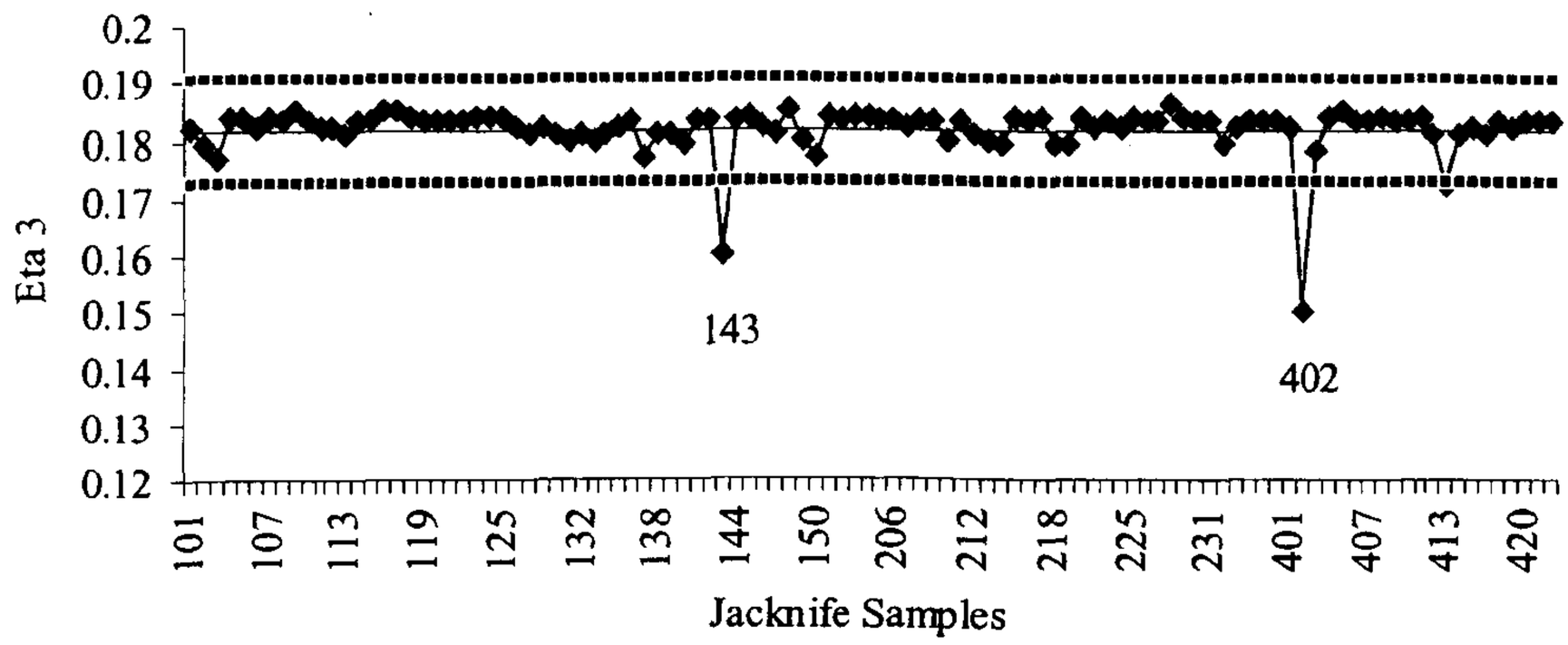


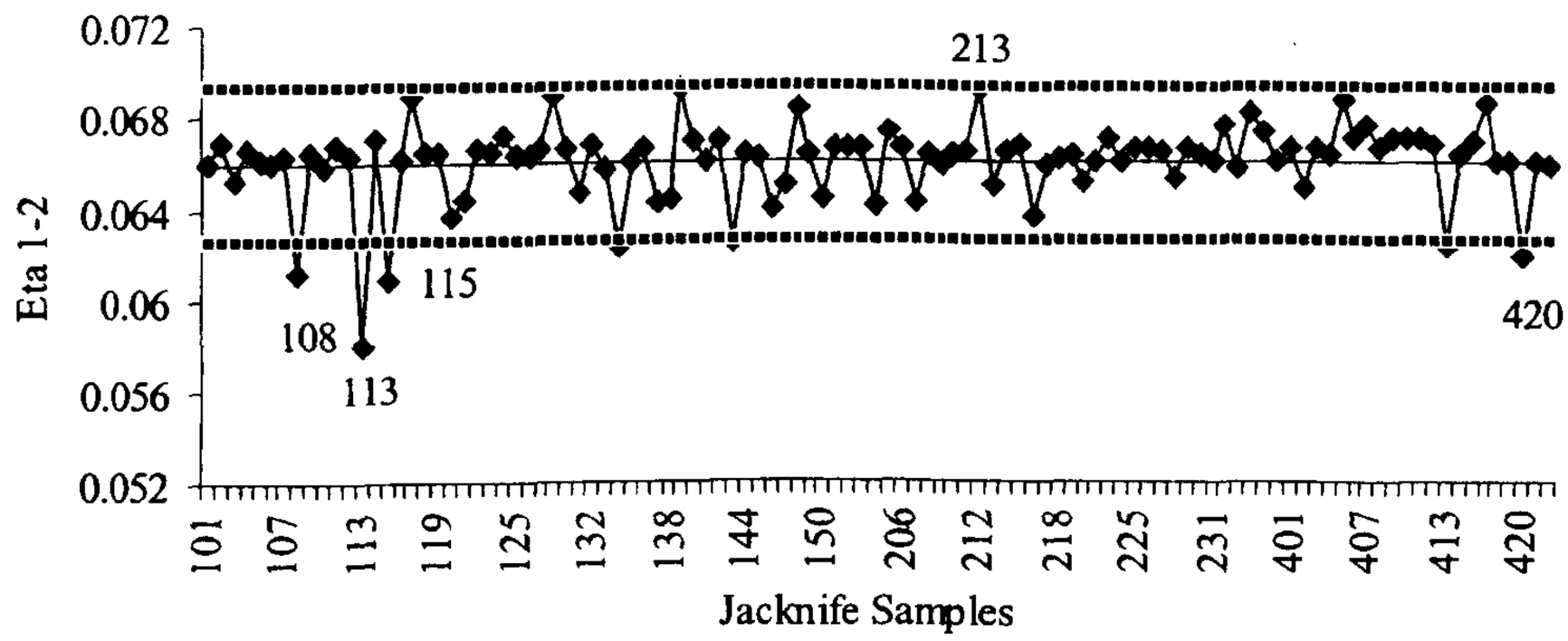


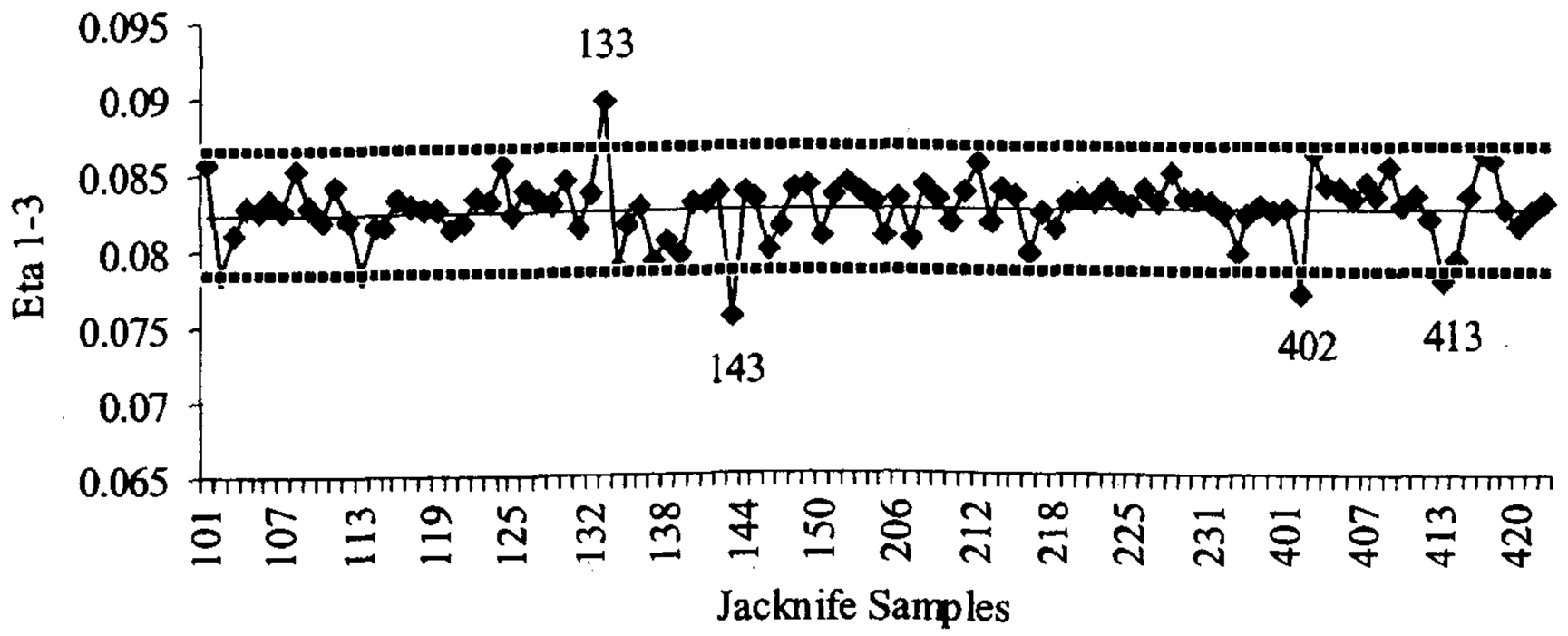
NB. Jacknife sample 101 was the data set excluding patient 101 etc
The solid line represents the population estimate from the final model.
The dotted line represents ± 5 % from the final population estimate.

Figure 4.1 (4 of 5)

Population parameter estimates from jacknife samples



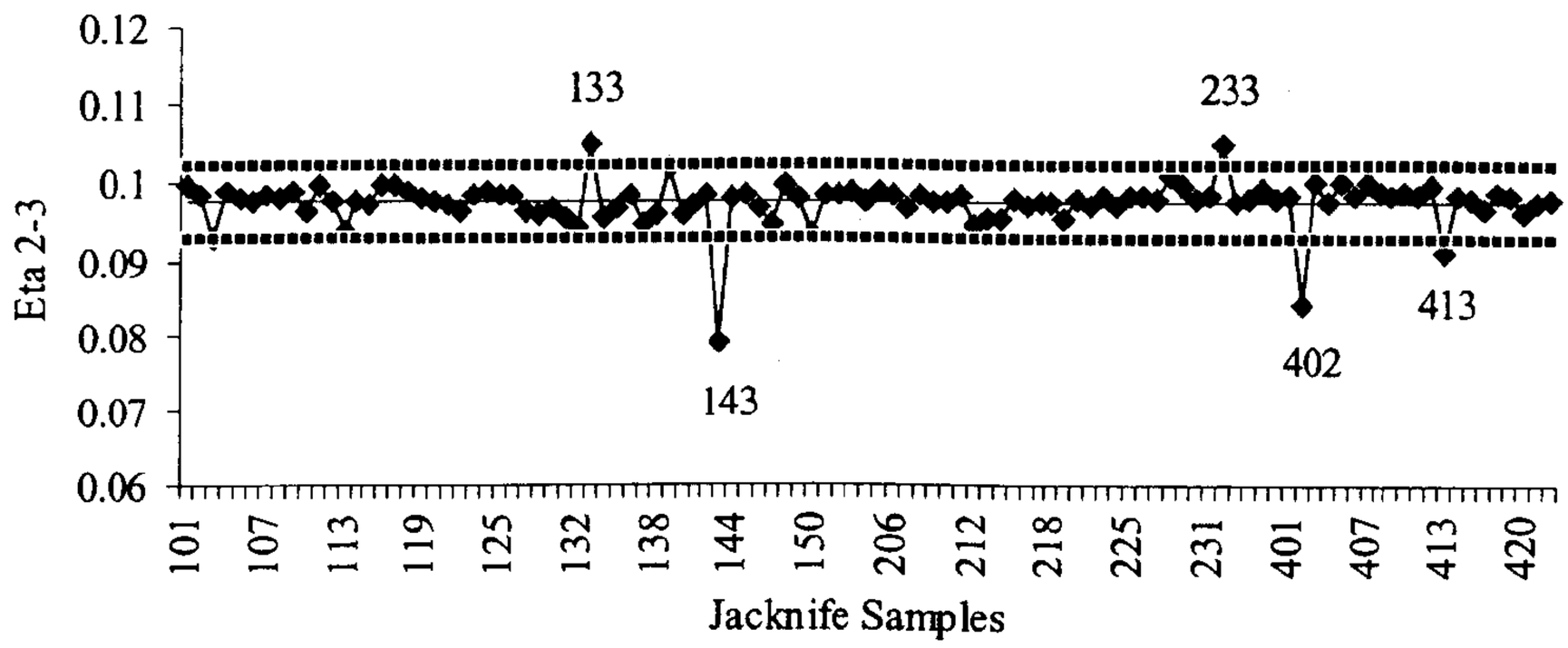


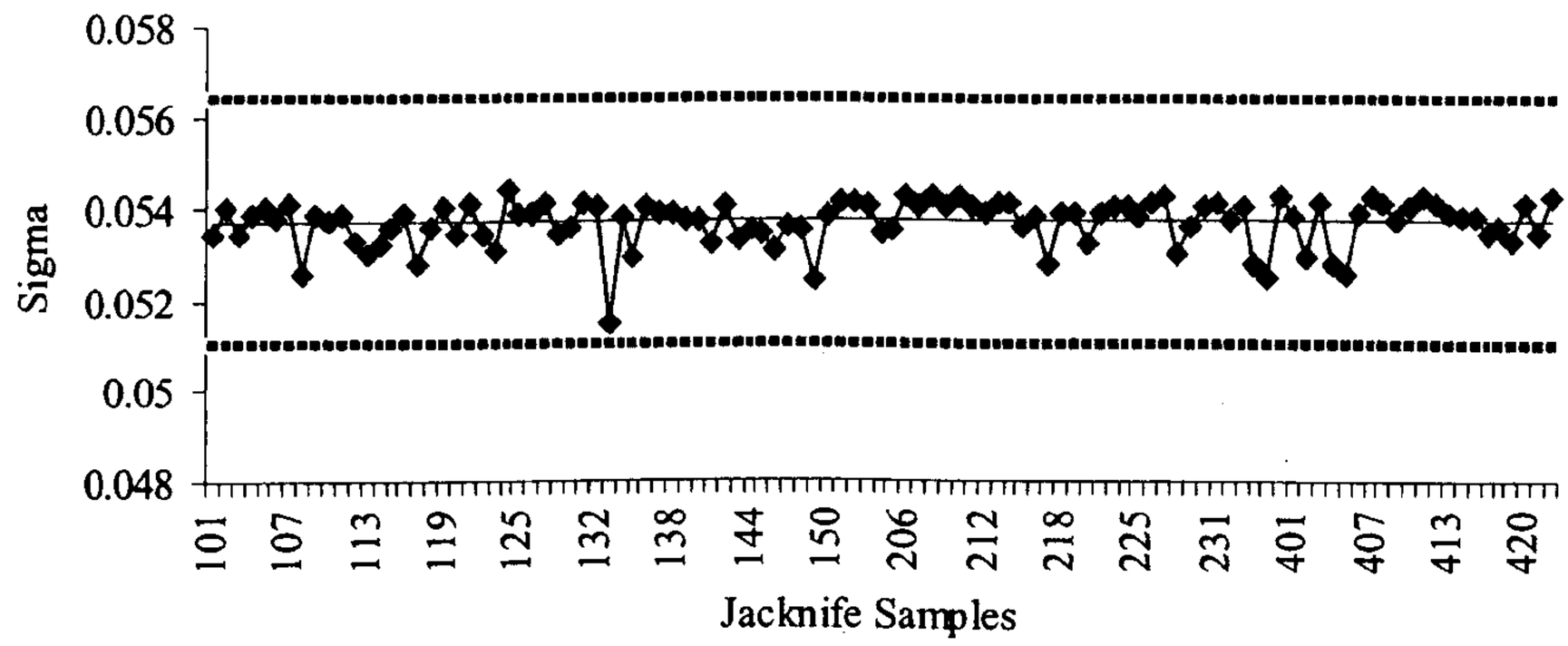


NB. Jacknife sample 101 was the data set excluding patient 101 etc
The solid line represents the population estimate from the final model.
The dotted line represents ± 5 % from the final population estimate.

Figure 4.1 (5 of 5)

Population parameter estimates from jacknife samples

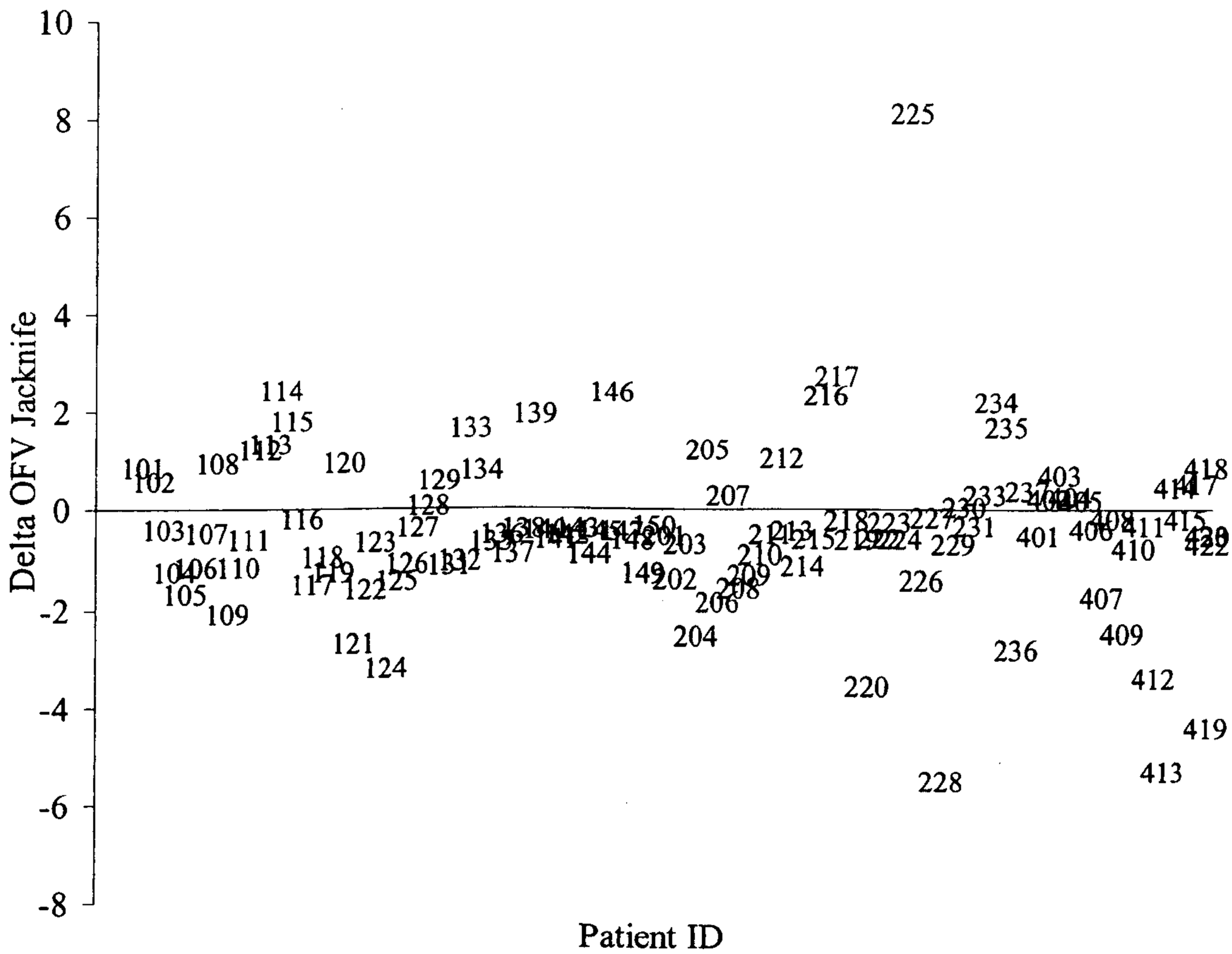




NB. Jacknife sample 101 was the data set excluding patient 101 etc
Solid line represents population estimate from final model.
Dotted line represents ± 5 % from final population estimate.

Figure 4.2

Likelihood-based method to identify individuals influencing covariate selection: change in OFV jacknife versus patient ID number



4.3.4 Cross validation analysis

Population estimates from the 5 training data sets were similar (within 10 %) to those attained using the complete dataset (Table 4.3). The rmse for CL values estimated using the basic model and using the final model based on AST values, showed that the imprecision of the CL estimates improved in 4 of the 5 validation data sets if AST was used to estimate CL compared to estimations using the basic model (Table 4.4). However, the observed improvements in rmse were not statistically significant in any of the validation data sets. As the lack of statistical significance could be due to the relatively small number of patients (21) in each validation data set, a t-test was performed on prediction errors combined from all datasets (n=105). Following combination of the validation data sets, there was an improvement in rmse from 66 % to 47 % with the inclusion of AST in the population model. Again, this was not statistically significant
($p = 0.062$).

When the prediction errors from the basic model for CL were plotted against AST, if the AST concentration was less than 150 U/L, they were generally within 50 % of the Bayesian estimate (Figure 4.3A). However, if the AST concentration was above 150 U/L, the prediction errors were up to 300 % and were generally much greater than 50 %, indicating that the basic model over predicted CL for patients with AST values above 150 U/L. As expected, prediction errors for CL at AST values above 150 U/L, improved following the inclusion of AST in to the final model and generally fell below 50 % (Figure 4.3B). However, it was noted that for AST values less than 150 U/L, CL was grossly overestimated by the final model for several patients. Prediction errors attained using the final model in only those patients with AST above 150 U/L showed a statistically significant improvement compared to the basic model ($p<0.01$). These results suggests that if AST values are above 150 U/L, AST can aid prediction of CL but if AST values are less than 150 U/L, AST is not a useful predictor of CL.

Table 4.3

Population estimates from complete data set and from training data sets

	THETA	Final		Training 1		Training 2		Training 3		Training 4		Training 5	
		Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)
**CL (L/h)	1	74.9	12.3	75.2	8.09	77.9	9.1	71.9	8.1	70.0	9.6	68.0	9.9
V_1 (L)	2	9.99	8.66	10.5	9.90	9.84	8.9	10.7	8.7	10.4	9.6	10.3	8.7
Q_2 (L/h)	3	29.5	13.8	30.6	14.8	29.4	14.6	30.3	13.7	29.1	15.0	31.4	13.3
V_2 (L)	4	36.0	18.6	36.1	19.1	35.9	18.3	35.9	17.4	33.1	18.9	37.6	17.0
Q_3 (L/h)	5	60.6	6.19	62.2	7.33	58.5	6.9	63.7	6.5	61.7	7.1	61.6	6.6
V_3 (L)	6	790	6.38	780	6.15	752	6.4	785	5.9	751	6.1	795	6.0
Ln AST (CL)	7	0.139	6.35	0.137	4.21	0.140	4.3	0.135	4.8	0.131	6.5	0.130	6.1
ωCL (%)		42.5	15.4	38.9	17.1	38.2	15.5	39.2	16.2	38.9	15.6	41.8	15.6
ωQ_3 (%)		31.6	19.5	34.8	19.3	34.8	20.3	35.4	21.2	34.8	19.8	32.7	17.8
ωV_3 (%)		43.1	22.1	39.7	23.8	43.8	25.3	43.6	25.7	45.8	22.7	39.0	27.9
σ (%)		23.1	9.29	22.8	10.1	23.4	10.6	23.3	10.1	23.2	10.5	23.1	10.5

**CL=74.9-(74.9*0.139*LnAST)

Table 4.4

Imprecision (rmse %) of population CL estimates calculated with basic and final models

Validation dataset	Basic Population CL model rmse (%)	Final Population CL model rmse (%)	delta rmse (%)	t-test p-value
1	45.1	43.6	-1.53	0.891
2	49.7	61.6	11.9	0.270
3	56.9	44.4	-12.5	0.505
4	94.1	47.1	-47.0	0.151
5	71.1	30.9	-40.2	0.120
Combined	65.8	46.6	-19.2	0.062

Figure 4.3A

Prediction errors from basic CL model versus AST

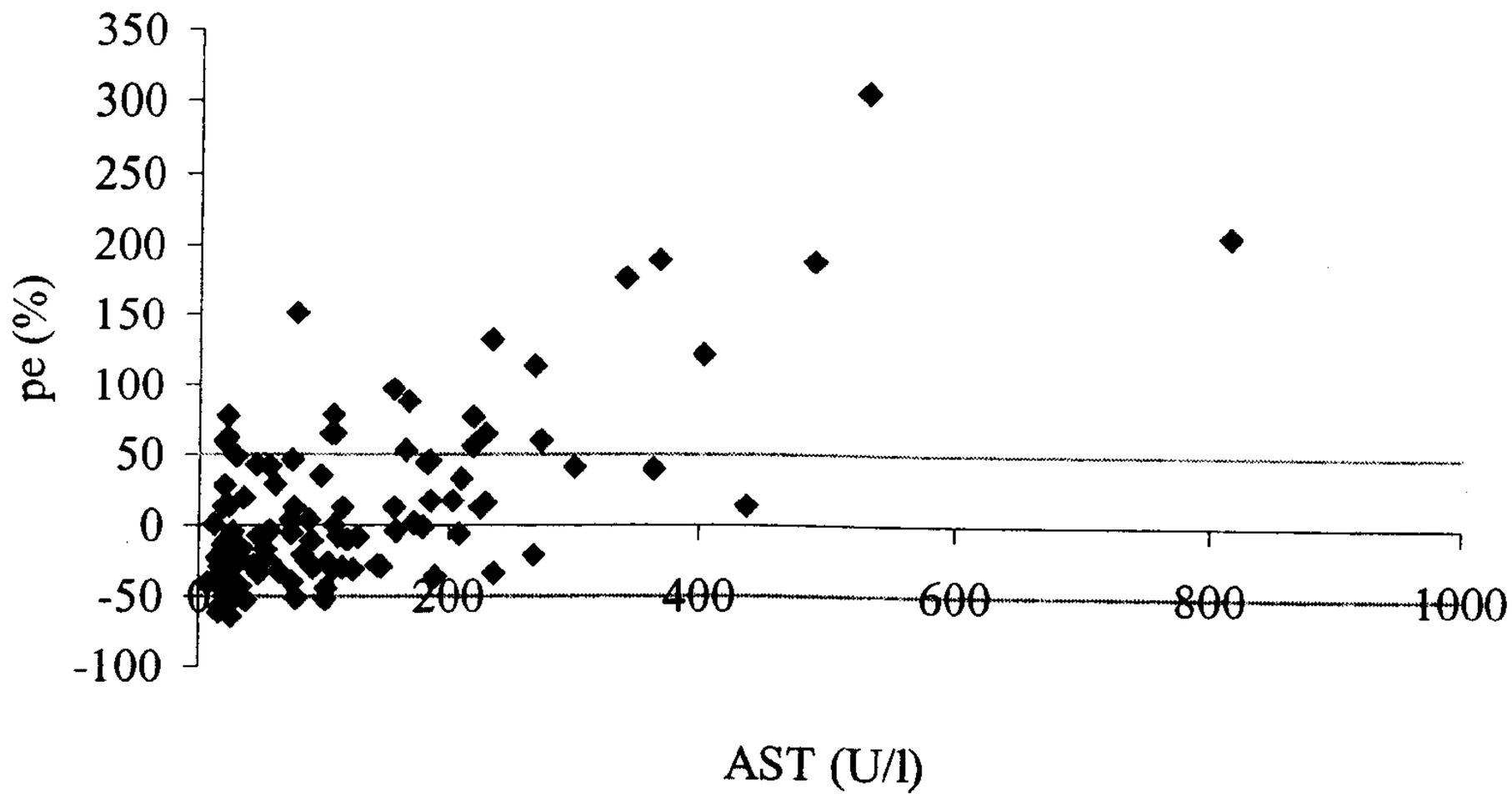
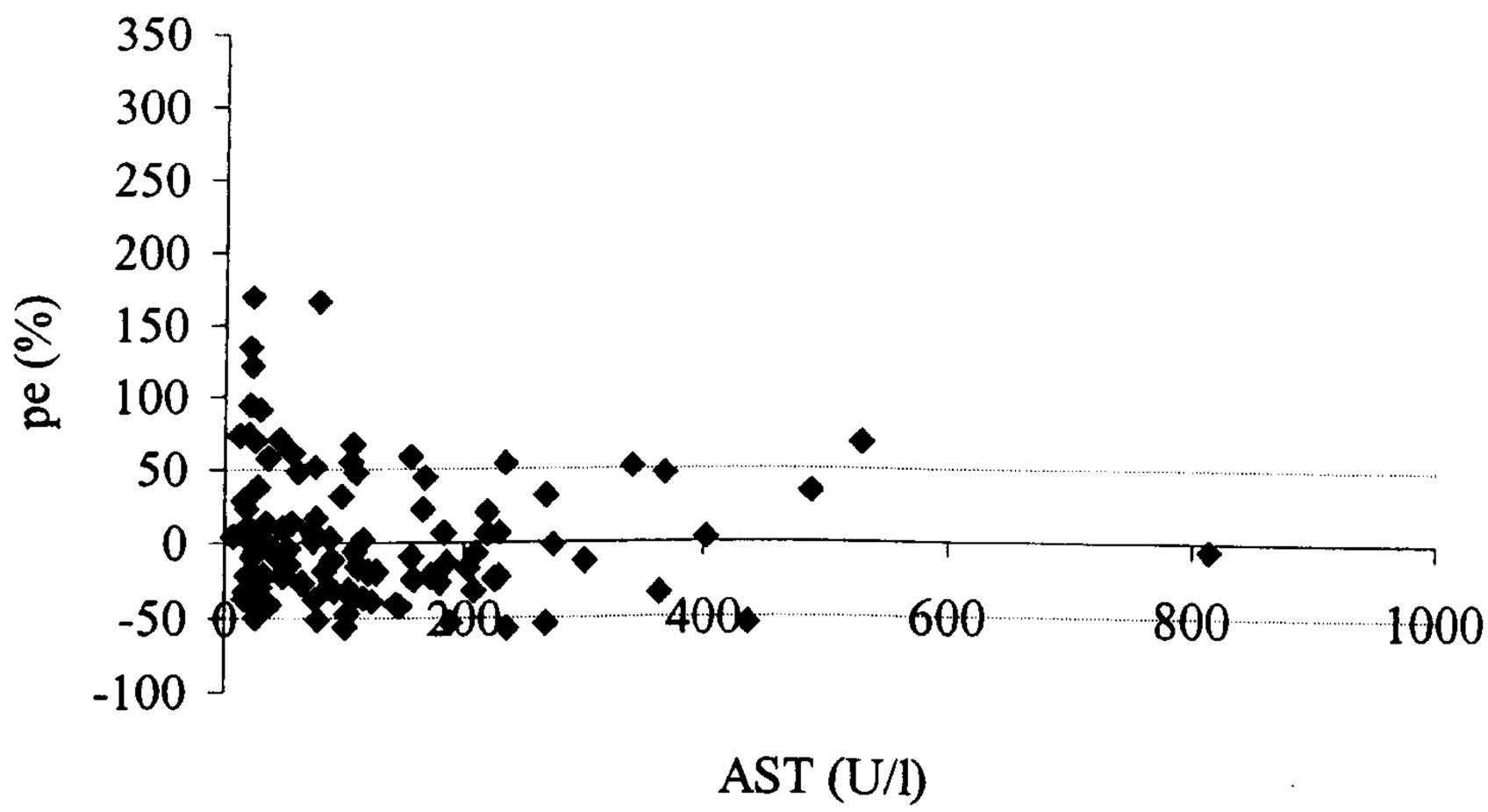


Figure 4.3B

Prediction errors from final CL model versus AST



4.3.5 Sensitivity of the model to 10 % errors in AST and plasma epirubicin concentrations

Following random alteration of AST concentrations by +/- 10 %, there was little change in OFV (7976) compared to that obtained with the original data (7978). The alteration of AST values by +/- 10 % had a negligible effect on the population parameter estimates and their imprecision (Table 4.5). Of particular interest, there was no change in Theta 7, which describes the influence of AST on CL. This indicates that the model is robust in terms of measurement errors in AST.

Following random alteration of epirubicin plasma concentrations by +/- 10 %, the OFV increased from 7978 to 8104, and the residual error increased from 23.2 to 24.9 % indicating a poorer fit of the data. However, there was little change in the population parameter estimates or their precision suggesting that the population model is robust in terms of measurement errors in concentrations (Table 4.5).

Table 4.5

Population parameter estimates following sensitivity analysis

	Original data		+/- 10 % error in AST		+/- 10 % error in concentrations	
	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)
Pharmacokinetic parameter						
θ_1	72.9	8.0	73.7	7.9	73.0	8.4
θ_2	10.3	8.2	10.3	8.2	10.5	9.0
θ_3	30.2	12.7	30.1	12.7	32.1	13.2
θ_4	35.7	16.1	35.6	16.0	39.8	17.1
θ_5	61.5	6.1	61.6	6.1	61.2	6.4
θ_6	772	5.5	772	5.5	782	6.1
θ_7	0.135	4.5	0.136	4.4	0.135	4.7
Inter-individual variability						
$\eta 1$	0.16	14.3	0.16	14.3	0.16	14.6
$\eta 2$	0.12	17.8	0.12	17.9	0.12	17.1
$\eta 3$	0.18	22.4	0.18	22.4	0.20	22.0
$\eta 1$-2	0.07	24.9	0.07	24.4	0.07	25.7
$\eta 1$-3	0.08	23.9	0.08	24.4	0.08	27.7
$\eta 2$-3	0.10	29.8	0.10	30.3	0.10	29.4
Residual Error						
σ	0.05	9.3	0.05	9.3	0.06	7.9

RSE = relative standard error

Model:

TVCL (l/h) = θ_1*(1-θ_7*(LnAST)
TVV_1 (l) = θ_2
TVQ_2 (l/h) = θ_3
TVV_2 (l) = θ_4
TVQ_3 (l/h) = θ_5
TVV_3 (l) = θ_6

CL = TVCL*EXP $\eta 1$
Q_3 = TVQ3*EXP $\eta 2$
V_3 = TVV3*EXP $\eta 3$

where TV is typical value

4.4 DISCUSSION

The population model for epirubicin determined in the previous chapter is intended for use in the development of new dosage guidelines for epirubicin, and therefore warrants validation (FDA guidelines, 1999). Since an external data set was not available for validation purposes at this stage, internal validation methods were used. The impact of some of the analytical decisions taken, the robustness of the model and the predictive performance of the model were assessed.

During model development, it was noted that the model provided a poor fit of early peak concentrations. The poor prediction of these early time-points could be due to an inadequate time for mixing in the circulation (Chiou 1979). Alternatively, minor inaccuracies in recording of blood sampling times could have had a substantial influence on predicted concentrations given their rapid change during the early part of the concentration-time profile. Therefore, time points up to 6 minutes post-start of the infusion were excluded from the analysis. The decision to remove these early time-points was subjective, so the impact of their removal was assessed. As expected, the residual error was substantially higher when the early time-points were included compared to when they were excluded indicating that the model provided a poor fit of these data. Inclusion of the early time-points resulted in parameter estimates that were highly unreliable, as judged by the large relative standard errors (up to 56 %), whereas on their removal, the relative standard errors were all below 17 % and therefore considered acceptable. This does not rule out the possibility that the inability of the model to fit the early time points was due to model mis-specification. However, it is widely accepted that the structural model for epirubicin is a 3-compartment model and the parameter values obtained following the removal of the early time-points are highly consistent with those reported elsewhere (Robert 1994). Furthermore, the removal of the early time-points had a minimal impact on the estimates of the parameters of most interest i.e. those influencing CL. Based on these results, the decision to remove the early time-points is justified.

The sensitivity analysis that involved +/-10 % changes in AST or epirubicin concentrations had a negligible impact on parameter estimates or their associated relative standard error. This suggests that the model is relatively robust and is not sensitive to minor measurement or recording errors. Likewise, the results of the

jacknife analysis showed that removal of any one individual did not result in any major changes to the population parameter estimates. The likelihood method, which was used to identify individuals influencing covariate selection, indicated that the selection of AST in the final model was not due to the presence of outliers. One individual, Patient 225 was identified as masking the relationship between AST and CL, as CL was much higher than expected for the measured AST concentration of 179 U/L. It is possible that this patient could have had an inaccurate AST measurement as both bilirubin and albumin levels were within the reference ranges. Three individuals, Patients 413, 228 and 419, were identified as having the greatest influence on the selection of AST in the model. The GAM analysis (Chapter 3) also previously identified Patient 413 as having a high influence on the selection of covariates for CL; however, the same covariates were selected in the GAM analysis on the exclusion of Patient 413. Although these individuals had the greatest influence on the final covariate model, they were not outliers. These results provide evidence that the model is stable and is not influenced by spurious data.

Given the relatively small size of the dataset, a split of the data into a training and a validation dataset was not performed as it may have compromised model development. Cross-validation was used to test the model for its ability to predict epirubicin CL, as AUC (Dose/CL) has previously been shown be a useful predictor of toxicity and therapeutic outcome (Jacobsen et al. 1991b; Hu et al. 1989). As the dataset contained rich data, the individual Bayesian CL estimates were likely to be accurate enough to be taken as the 'true' values. The results of the cross-validation were disappointing. Although the prediction of CL was better with the final model compared to the basic model, the improvement was not statistically significant. Further investigation revealed that a statistically significant improvement in CL predictions using the final model only occurred in patients with AST concentrations above 150 U/L. This suggests that the final model has improved predictive performance in patients with AST concentrations greater than 150 U/L but is not useful for those with a lower value.

The jacknife method has been recommended for the calculation of standard errors for parameter estimates (FDA guidelines, 1999) because unlike NONMEM estimations, this non-parametric method does not assume that parameters are normally distributed. In this analysis, the standard errors calculated using the jacknife technique

were, however, very similar to those attained from the NONMEM analysis. This is consistent with the analysis previously performed by Gibiansky and coworkers (2001), who found that standard errors estimated by NONMEM were in most cases reliable and rarely improved by more computer intensive methods such as bootstrap, jacknife and likelihood profile techniques. However, they found that standard errors were poorly estimated by NONMEM for parameters with non symmetrical distributions; for example, a parameter such as E_{max} that could have a value close to zero, bounded by zero and large error.

The true test for the usefulness of this model is its ability to predict CL, in a new patient group. However, internal validation methods can be useful to identify a model that is unstable or invalid, indicating that it would not be worthwhile performing further validation in a new dataset. The internal validation methods used in this analysis found the model to be stable, not based on outliers or spurious data and suggested favourable predictive performance of CL in patients with AST above 150 U/L. This provides evidence that further validation in an external data set is warranted.

CHAPTER 5

DEVELOPMENT OF NEW DOSAGE GUIDELINES

5.1 INTRODUCTION TO CHAPTER

The aim of this Chapter was to develop new dosage guidelines for epirubicin. In order to develop new dosage guidelines, the relationship between pharmacokinetics and pharmacodynamics should be clearly defined. In a study of patients with nasopharyngeal carcinoma treated with epirubicin (Hu et al. 1989), the mean AUC in 6 complete responders was approximately double that observed in 13 non-responders. In contrast, no statistically significant difference in C_{max} was observed between responders and non-responders. Furthermore, statistically significant relationships between epirubicin AUC and WBC nadir, in patients with advanced breast cancer, have also been identified (Dobbs & Twelves 1998; Jakobsen et al. 1991b). AUC, therefore, appears to be an appropriate PK target.

In this Chapter, further attempts were made to define the relationships between pharmacokinetic parameters and haematological toxicity. In Chapter 3, a population model was developed that identified AST as influencing epirubicin CL. As AUC can be calculated from the relationship: AUC = Dose/CL, the population model was used create dosage guidelines based on AST measurements that would achieve a target AUC. The accuracy and precision of these guidelines to achieve the target AUC was compared to that achieved using the current UK guidelines for epirubicin (SPC, UK), current USA guidelines for epirubicin (Product label, USA) and according to the equation developed previously by Dobbs and Twelves (2003). Finally, the proposed dosage guidelines were evaluated in an external data set.

5.2 METHODS

5.2.1 Investigation of PK/PD relationships

The ratio of nadir:pretreatment count and the ratio of the log nadir:log pretreatment count was calculated for haemoglobin, white blood cells (WBC), neutrophils and platelets. The ratios were plotted against dose (mg and mg/m^2) and the following pharmacokinetic parameters: AUC, C_{max} and time above a threshold concentration, where the threshold was set at 10, 25, 50, 100, 200, 500 and 1000 ng/ml. C_{max} values and time above threshold concentrations were determined were using WinNonlin version 3.0. The AUC values were calculated using the formula:

$AUC = Dose/CL_i$

where CL_i is the individual Bayesian estimate for CL determined from the previous NONMEM analysis (Chapter 3). Linear regression analysis was performed on the blood count ratios against the dose and against the pharmacokinetic parameters using Microsoft Excel. In addition, the published literature was examined for evidence of relationships between the PK and PD for epirubicin.

5.2.2 Development of new dosage guidelines

The standard dose for patients with normal liver function was set at 125 mg, calculated from the current recommended dose of 75 mg/m^2 multiplied by the median BSA of the patients (1.65 m^2). CL estimates that would require 100 %, 75 %, 50 % and 25 % of this standard dose, (i.e. 125 mg, 90 mg, 60 mg and 30 mg), to attain the target AUC were calculated using the formula:

$CL = Dose/AUC$

The AST concentrations associated with CL values in the middle of these ranges were calculated from the population model for CL:

$CL = 72.9 * (1 - (0.135 \times \ln AST))$

and were used to propose the new dosage guidelines.

5.2.3 Comparison of dosage guidelines

5.2.3.1 Prediction of AUC values

The expected AUC values for the 105 patients in the UK data set were determined from doses (mg) proposed in the new guidelines. The expected AUC values (AUC_i) were calculated for each patient using the following formula:

$AUC_i = Dose/CL_i$

where dose was entered according to the proposed guidelines and CL_i was the individual Bayesian estimate for CL estimated from the NONMEM analysis (Chapter 3). This was repeated but with doses adjusted for the BSA of the patients (mg/m^2). The expected AUC values were estimated in a similar manner using doses suggested by the current UK guidelines for epirubicin (SPC, UK), current USA guidelines for epirubicin (Product label, USA) and according to the equation developed previously by Dobbs and Twelves (2003).

The current UK guidelines suggest a standard dose of 60-90 mg/m^2 with a 50 % dose reduction if bilirubin concentrations are 24-51 μmol/L and a 75 % dose reduction if bilirubin concentrations are >51 μmol/L. For this analysis the standard UK dose was taken as 75 mg/m^2. The current USA guidelines suggest a standard dose of 100-120 mg/m^2 with a 50 % dose reduction if bilirubin concentrations are 20-51 μmol/L or AST concentrations are 86 to 172 U/L and a 75 % dose reduction if bilirubin concentrations are >51 μmol/L or AST concentrations are > 172 U/L. For this analysis the standard USA dose was taken as 110 mg/m^2. The Dobbs and Twelves equation (Dobbs, 2003) was recommended to be used in patients with AST concentrations > 2 x normal upper limit (>86 U/L) as follows:

Dose (mg/m^2) = Target AUC (97.5 – 34.2 x $\log_{10}$ AST)

In patients with AST levels ≤86 U/L a standard dose of 75 mg/m^2 was used.

5.2.3.2 Calculation of bias and imprecision

The prediction error (P_{ei}) of the AUC estimates relative to the target AUC was defined as

P_{ei} (%) = (AUC_i – target AUC) / target AUC x 100

where AUC_i is the individual estimate of AUC for the ith individual, given each set of dosage guidelines. The root mean squared prediction error (rmse) and the mean prediction error (me) with 95 % confidence intervals, which describes the imprecision and bias of the estimates, respectively were calculated as described in Chapter 2.

5.2.4 Validation of the population model using an external data set

5.2.4.1 Patients

Four patients were available from the original breast cancer patient data set who were previously excluded from the model building step of the population analysis due to missing covariates. A further data set was available from a trial conducted at Kings Hospital, London, which included 19 patients with hepatocellular carcinoma who were administered epirubicin as an intravenous infusion (n=11) or as an intra-arterial infusion (n=8). In 5 hepatocellular carcinoma patients unusual peaks occurred during the terminal concentration-time profiles so these patients were excluded from the

validation data set. In total, 18 patients were used to evaluate the proposed dosage guidelines.

5.2.4.2. Evaluation of new dosage guidelines

The data from the external data set was analysed using NONMEM (FOCE-INTER) by fitting a 3-compartment pharmacokinetic model with a proportional residual error model as described in Chapter 3. Interindividual variability was estimated for CL, V_3 and Q_3.

The expected AUC values (AUC_i) for each individual, given the proposed doses in the new guidelines, were calculated using the following formula:

$AUC_i = Dose/CL_i$

where dose was entered according to the proposed guidelines and CL_i was the individual Bayesian estimate for CL estimated from the NONMEM analysis. The precision and bias with 95 % CI of the expected AUC values relative to the target AUC were calculated as described above.

5.2.4.3. Validation of the population model for CL

CL values for each patient in the validation set were estimated from their recorded AST concentrations using the population model for CL:

$CL = 72.9 * (1- (0.135 \times \ln AST))$

The Bayesian estimates of CL estimated from NONMEM as described above, were considered to be the 'true' values for each individual. Estimates of CL from the AST values were compared to the 'true' estimates of CL using scatter plots and by calculating me and rmse, as described above.

5.3 RESULTS

5.3.1 Investigation of PK/PD relationships

The results of the linear regression of survival fraction for haemoglobin, WBC, neutrophils and platelets against AUC, C_{max}, dose (mg) and dose (mg/m^2) are given in Table 5.1A. A statistically significant relationship ($p<0.05$) was found between survival fractions of WBC, neutrophils and platelets all the explanatory variables: AUC, C_{max}, dose (mg and mg/m^2). Dose explained more of the variability in survival fraction than AUC or C_{max}, with dose expressed as mg/m^2 being the best explanatory variable as judged by the R^2 value. Dose (mg/m^2) accounted for 39.0 % and 40.6 % of the variability in WBC and neutrophil survival fractions, respectively. C_{max} was the only explanatory variable that was significantly correlated with haemoglobin survival fraction.

The results of linear regression of the log survival fraction were consistent with those using the survival fraction, however, in most cases more variability in the pharmacodynamic variables was explained when the log survival fraction was used (Table 5.1B). Dose (mg/m^2) accounted for 49.4 and 34.3 % of the variability in WBC and neutrophil log survival fractions, respectively. The relationships between log survival fraction of haemoglobin, WBC, neutrophils and platelets against AUC and dose (mg/m^2) are illustrated in Figures 5.1 and 5.2. It was noted that some blood cell counts increased following treatment; i.e. the survival fraction was greater than 1. However, at AUC values above 4000 ng.h/ml survival fractions were generally less than 1.

The strength of the relationship between the time above a threshold and the survival fraction generally increased as the threshold concentration increased (Table 5.2). The strongest relationship was observed between time above 500 ng/ml and platelet survival fraction with an R^2 value of 22.5 %, however, the amount of variability in platelet survival fraction explained by time above 500 ng/ml was still lower compared to that explained by dose.

Table 5.1A

Linear regression of survival fraction for haemoglobin, white blood cells, neutrophils and platelets against AUC, C_{max}, dose (mg) and dose (mg/m^2)

	AUC (ng.h/ml)		C_{max} (ng/ml)		Dose (mg)		Dose (mg/m^2)	
	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value
HB	3.47	0.104	5.10	0.0482*	4.39	0.0675	4.78	0.0560
WBC	13.1	0.00112*	15.3	0.000389*	35.5	p<0.0001*	39.0	p<0.0001*
Neutrophils	9.16	0.0193*	13.3	0.00161*	36.1	p<0.0001*	40.6	p<0.0001*
Platelets	15.1	0.000477*	12.0	0.00201*	21.5	p<0.0001*	23.8	p<0.0001*

Table 5.1B

Linear regression of log survival fraction for haemoglobin, white blood cells, neutrophils and platelets against AUC, C_{max}, dose (mg) and dose (mg/m^2)

	AUC (ng.h/ml)		C_{max} (ng/ml)		Dose (mg)		Dose (mg/m^2)	
	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value
HB	4.11	0.0771	5.56	0.0390*	4.77	0.0564	5.15	0.0471*
WBC	16.0	0.000281*	21.5	p<0.0001*	44.6	p<0.0001*	49.4	p<0.0001*
Neutrophils	12.1	0.00295*	12.9	0.00210*	30.5	p<0.0001*	34.3	p<0.0001*
Platelets	15.7	0.000368*	13.6	0.000972*	22.0	p<0.0001*	24.3	p<0.0001*

HB = Haemoglobin
WBC = White blood cells
* = Statistically significant (P<0.05)

Figure 5.1
Relationship between toxicity and AUC

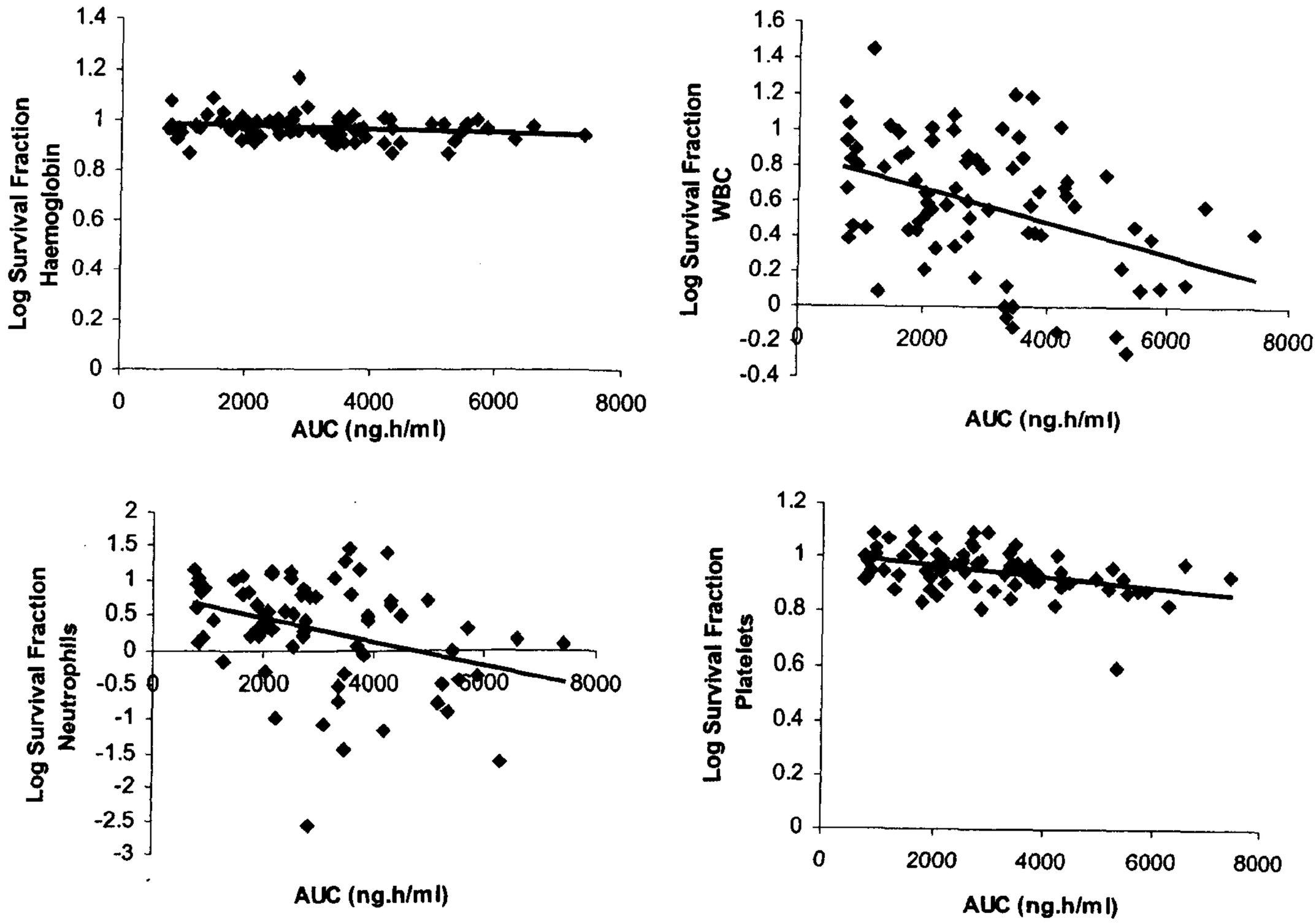


Figure 5.2

Relationship between toxicity and dose

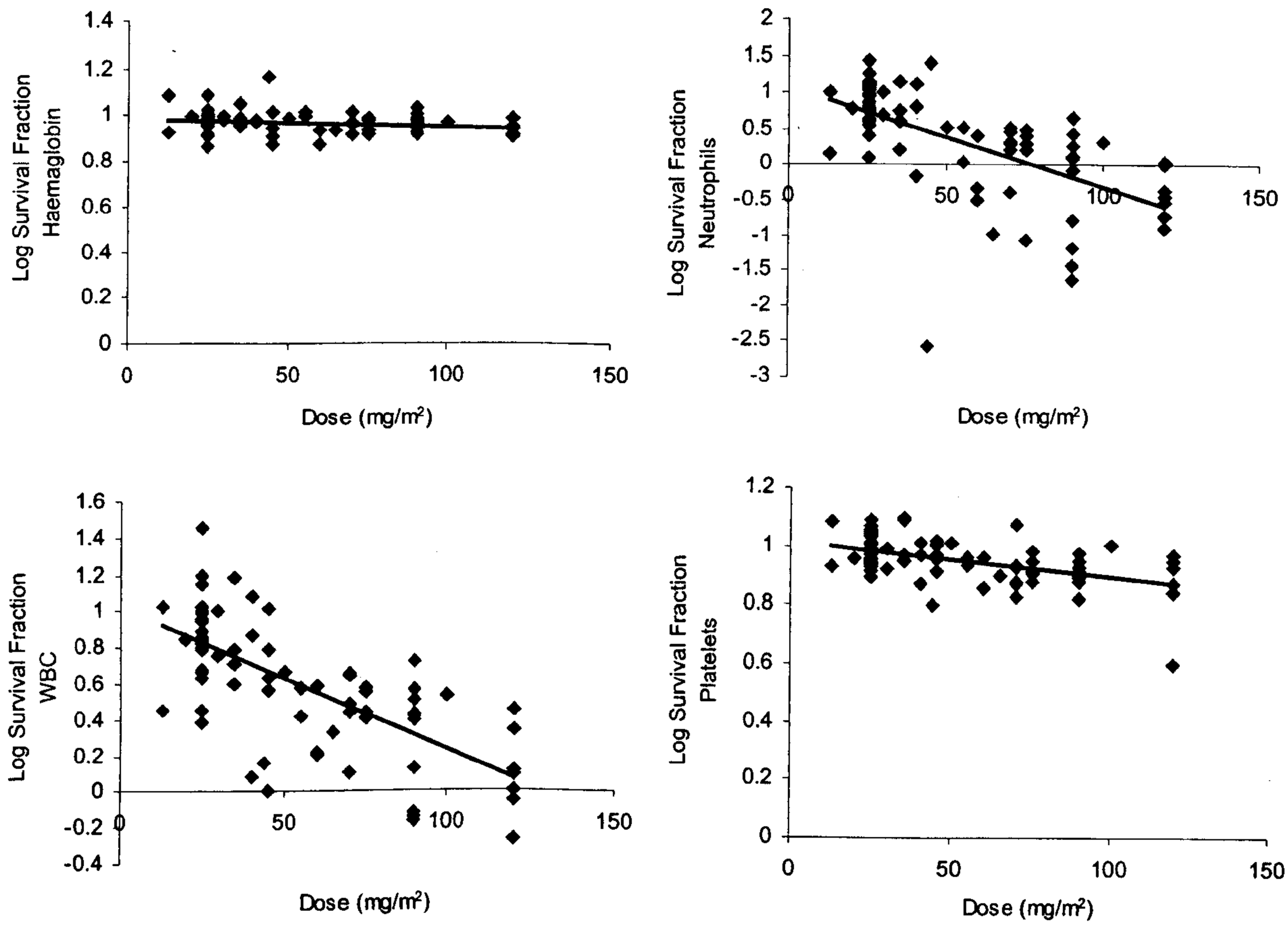


Table 5.2

Linear regression of survival fraction for haemoglobin, white blood cells, neutrophils and platelets against time above a threshold concentration

	Time above 10 ng/ml		Time above 25 ng/ml		Time above 50 ng/ml		Time above 100 ng/ml		Time above 200 ng/ml		Time above 500 ng/ml		Time above 1000 ng/ml	
	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value	R^2 (%)	p-value
HB	0.176	0.717	1.60	0.273	2.66	0.156	4.27	0.0713	3.33	0.112	5.71	0.0363	5.05	0.0501
WBC	4.90	0.0515	4.19	0.0721	11.2	0.00282	11.1	0.00282	12.7	0.00136	18.4	0	18.6	0
Neutrophils	1.52	0.306	0.830	0.450	11.1	0.00457	12.2	0.00289	10.6	0.00560	13.5	0.00159	16.5	0.000435
Platelets	3.10	0.126	5.04	0.0406	11.5	0.00259	8.22	0.0115	14.6	0.000615	22.5	0	21.4	0

HB = Haemoglobin
WBC = White blood cells

Due to the relatively weak relationships between PK and PD parameters in this study, the target AUC was ascertained from information available in the literature. Hu and coworkers (1989) found that the mean epirubicin AUC associated with complete response was 4002 ng.h/ml in a study of patients with nasopharyngeal carcinoma. Using the equation for the relationship between WBC and AUC previously described by Jakobsen et al. (1991) (survival fraction = -0.000115AUC – 0.147; r^2 = 30 %) and the average pre-treatment WBC count (5.6 x 10^9) observed in that study, AUCs of 2800-4400 ng.h/ml were associated with nadirs of 1-2 x 10^9 for WBC). Using this information, a target AUC of 4000 ng.h/ml was selected.

5.3.2 Development of new dosage guidelines

For the target AUC of 4000 ng.h/ml, the CL estimates associated with 125 mg, 90 mg, 60 mg and 30 mg were calculated to be 32 L/h, 22.5 L/h, 15 L/h and 7.5 L/h, respectively. CL values in the middle of these ranges were calculated to be 27.25 L/h, 18.75 L/h and 11.25 L/h. Using the population model, these CL values were associated with AST concentrations of 103, 245 and 525 Units/L. New dosage guidelines based on AST, for 50 % and 75 % dose reductions (as suggested by UK and USA guidelines), are given in Table 5.3A and likewise guidelines with additional adjustment for BSA are given in Table 5.3B.

5.3.3 Comparison of dosage guidelines

At the standard dose of epirubicin (75 mg/m^2) the median predicted AUC was 3730 ng.h/ml, in the UK data set in patients with normal liver function (bilirubin <23 μmol/L), which is similar to the proposed target AUC.

Figure 5.3 illustrates the predicted AUC values from the population model that would be achieved if patients received a standard dose of 125 mg or if doses were administered according to the new dosage guidelines. This figure shows that without dose-adjustments in patients with highly elevated AST concentrations, the expected AUC values could be up to 20,000 ng.h/ml, which is 5 times the target AUC, and therefore, likely to be associated with severe toxicity.

The imprecision (rmse) and bias (me) for the AUC estimates obtained using the different dosage guidelines, relative to the target AUC of 4000 ng.h/ml, are given in Table 5.4. The imprecision of the AUC estimates was lower when the dose was

Table 5.3A

New dosage guidelines based on AST concentration

AST Units/L	Dose (mg)	Dose (% normal)
< 250	125	100
250-500	60	50
>500	30	25

Table 5.3B

New dosage guidelines based on AST concentration and BSA

AST Units/L	Dose (mg/m^2)	Dose (% normal)
< 250	75	100
250-500	37.5	50
>500	18.75	25

Table 5.3C

New dosage guidelines based on AST concentration with additional 25 % dose reduction

AST Units/L	Dose (mg)	Dose (% normal)
< 150	125	100
150-250	90	75
250-500	60	50
>500	30	25

Figure 5.3

Predicted AUC values associated with AST values following no dose-adjustment or dose-adjustment according to new proposed dosage guidelines

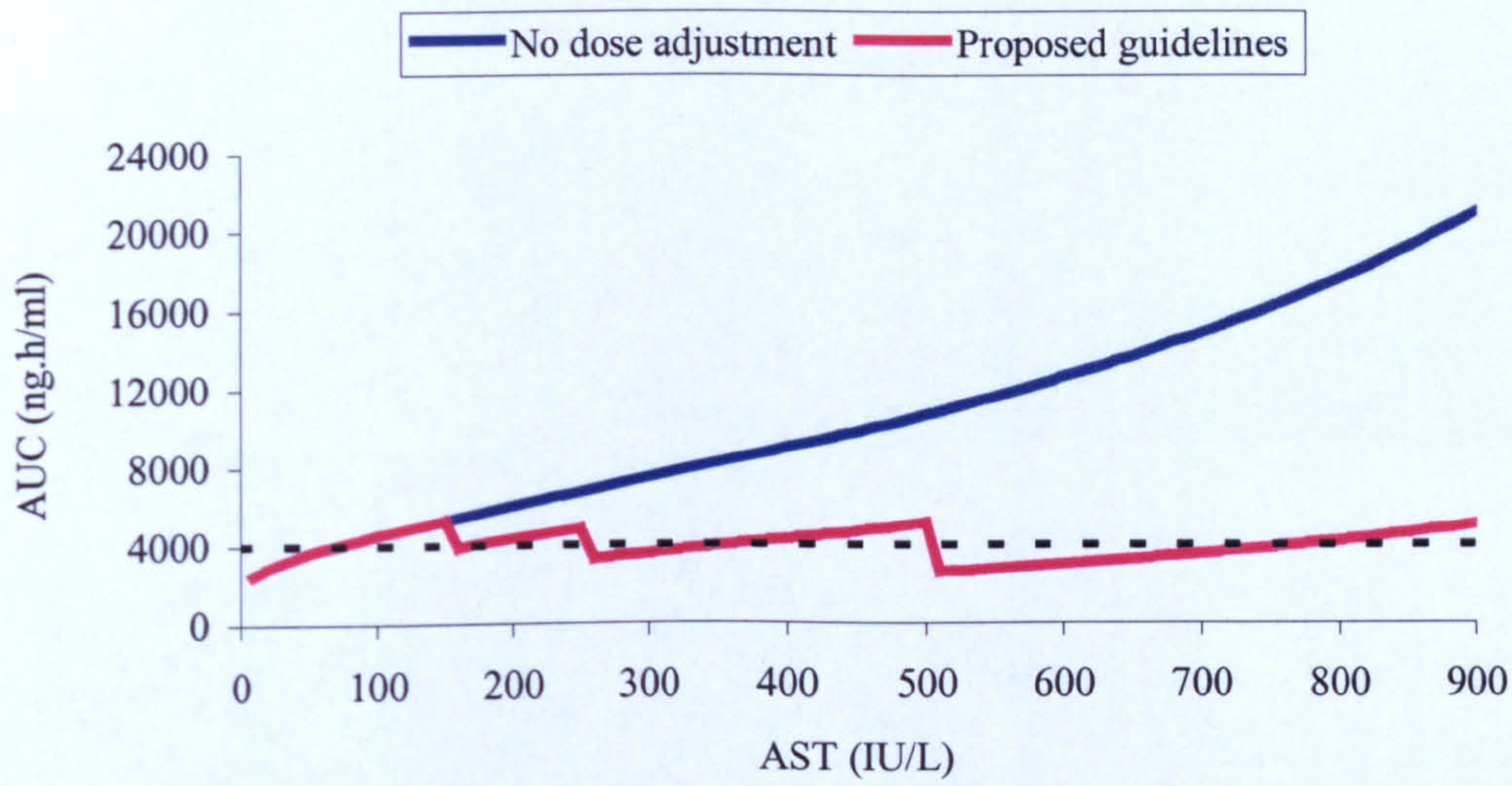


Table 5.4

Imprecision and bias of AUC estimates using different dosage guidelines

Dosage guidelines	Mean AUC (ng.h/ml)	CV (%)	Imprecision (rmse) (%)	95 % CI	Bias (me) (%)	95 % CI
New AST	4315	41.2	44.9	(37.0, 51.6)	7.87	(-0.626, 16.4)
New AST + BSA	4303	43.3	47.0	(39.8, 53.2)	7.56	(-1.34, 16.5)
Individualised doses based on AST	4298	39.0	42.4	(33.9, 49.4)	7.45	(-0.563, 15.5)
Current UK (Bilirubin and BSA)	4036	62.6	62.8	(39.0, 79.8)	0.890	(-11.2, 13.0)
Twelves' Equation	4755	46.9	58.7	(45.8, 69.2)	18.9	(8.16, 29.6)*
Current USA guidelines	4195	59.4	62.2	(46.5, 74.7)	4.88	(-7.07, 16.8)
New AST + 25 % reduction	3997	39.2	39.0	(31.3, 45.5)	-0.0703	(-7.57, 7.43)

* statistically significant

adjusted according to the proposed new guidelines (44.9 %) compared to dose adjustments based on the UK guidelines (62.8 %). For both dosage guidelines the AUC estimates were unbiased. There was no improvement in the imprecision of the AUC estimates if the new dosage guidelines were also adjusted for BSA (47 %) but a slight improvement was observed when the doses were individualised based on their individual AST concentration (42.4 %).

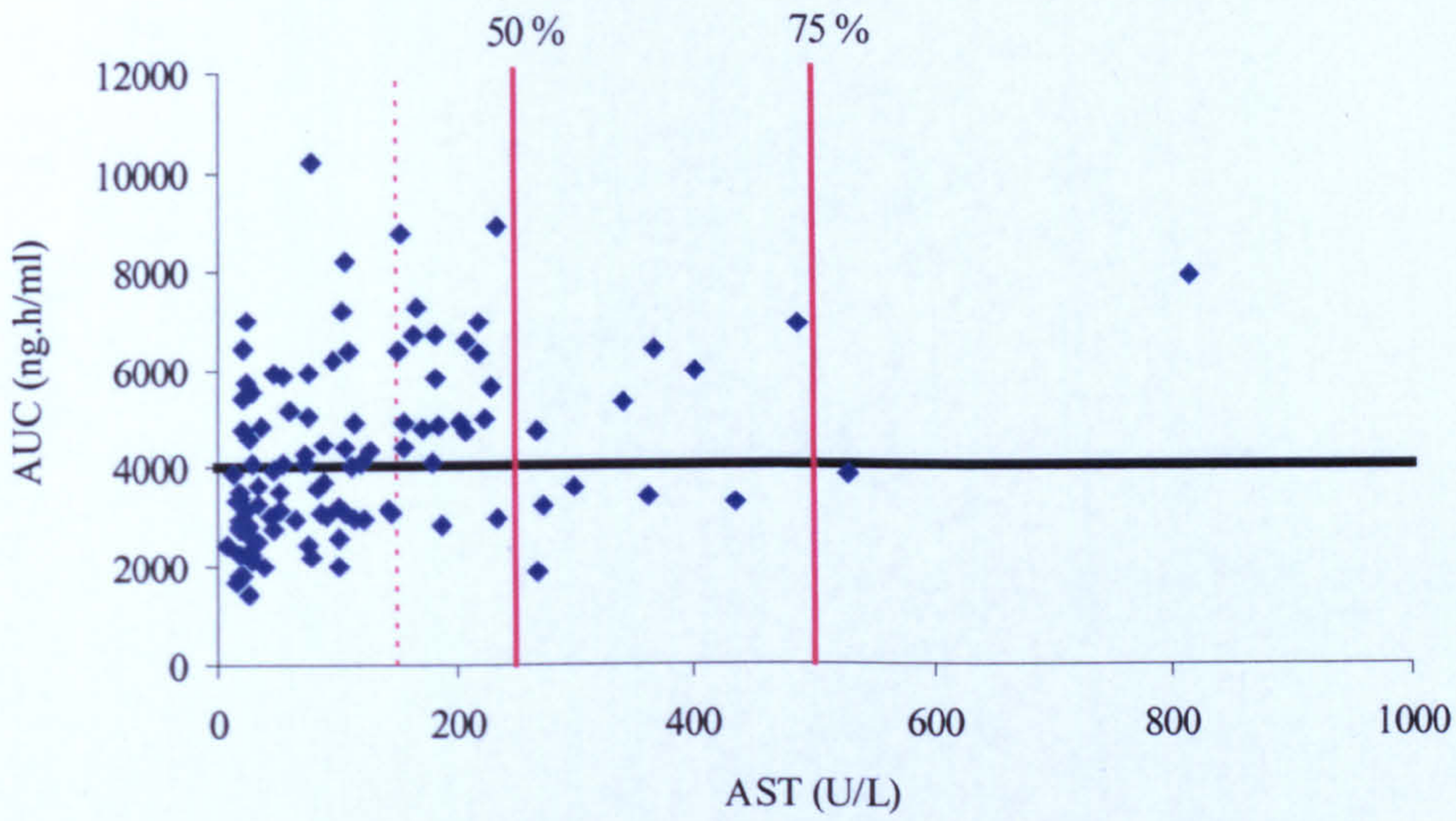
Predicted AUC values from the proposed new guidelines were plotted against AST in Figure 5.4A. It was noted that in patients with AST concentrations between 150 and 250 U/L, the predicted AUC values were generally greater than the target suggesting that the proposed dose for these patients is too high. An additional 25 % dose reduction was introduced for patients with AST values ranging from 150 to 250 U/L (Table 5.3C). Following the inclusion of the additional 25 % dose reduction criterion, a more random distribution of predicted AUC values around the target AUC was observed in the patients with AST values between 150 to 250 U/L (Figure 5.4B) and a further reduction in the imprecision to 39 % was observed (Table 5.4). The new proposed dosage guidelines were therefore amended to include the additional 25 % dose reduction (Table 5.3C).

The relationship between the AST and bilirubin concentrations measured in each individual is presented in Figure 5.5. This shows that many patients with elevated AST concentrations had bilirubin concentrations within the normal range. In contrast, there were no patients with elevated bilirubin levels who had normal AST concentrations. Figure 5.6 illustrates the similarities and differences in doses administered according to UK guidelines and the new proposed guidelines where the dose is adjusted according to bilirubin and AST, respectively. The dose in this figure is expressed as % standard dose for comparative purposes as the units of dose in the new dosage guidelines are mg whereas the units are mg/m^2 in the UK guidelines. The % of standard dose was the same in approximately two thirds of the patients (indicated by the black symbols). In the remaining patients the dose was different by up to 4-fold.

The doses selected for each individual using the new proposed guidelines, UK guidelines, USA guidelines and the Dobbs and Twelves equation are illustrated in Figure 5.7. It was noted that in patients with very high AST levels (>710 U/L) the equation proposed by Dobbs and Twelves, calculated a negative dose. One patient in

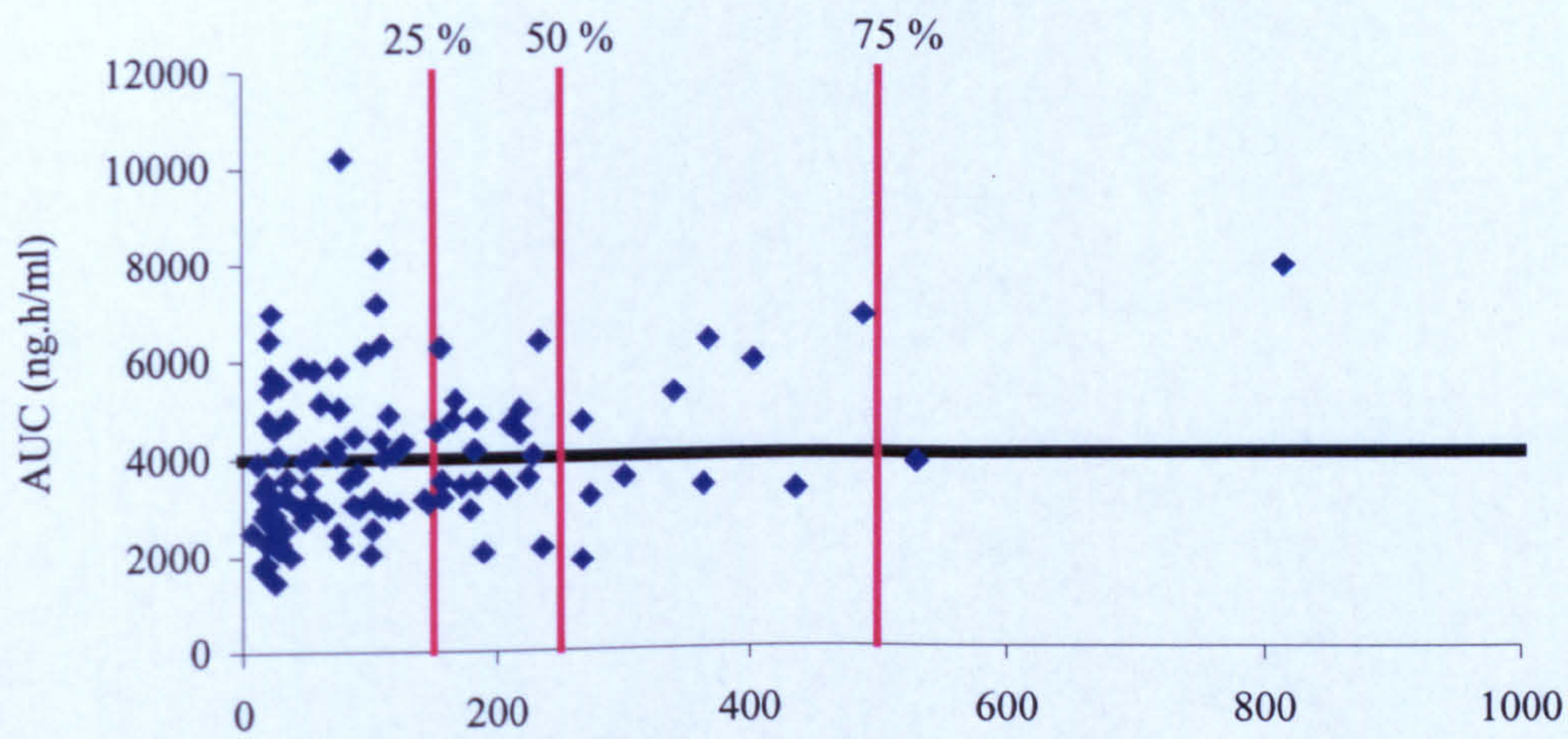
Figure 5.4

5.4A - Predicted AUC values for patients in UK data set administered a dose according to new AST guidelines



Solid red lines represent cut-offs for dose groups with recommended % dose reduction
Dotted line represents suggested cut-offs for new dose level
NB. Black horizontal line represents target AUC

5.4B - Predicted AUC values for patients in UK data set administered a dose according to new AST guidelines with additional 25 % dose reduction group



Solid red lines represent cut-offs for dose groups with recommended % dose reduction
NB. Black horizontal line represents target AUC

Figure 5.5

Comparison of AST and bilirubin concentrations

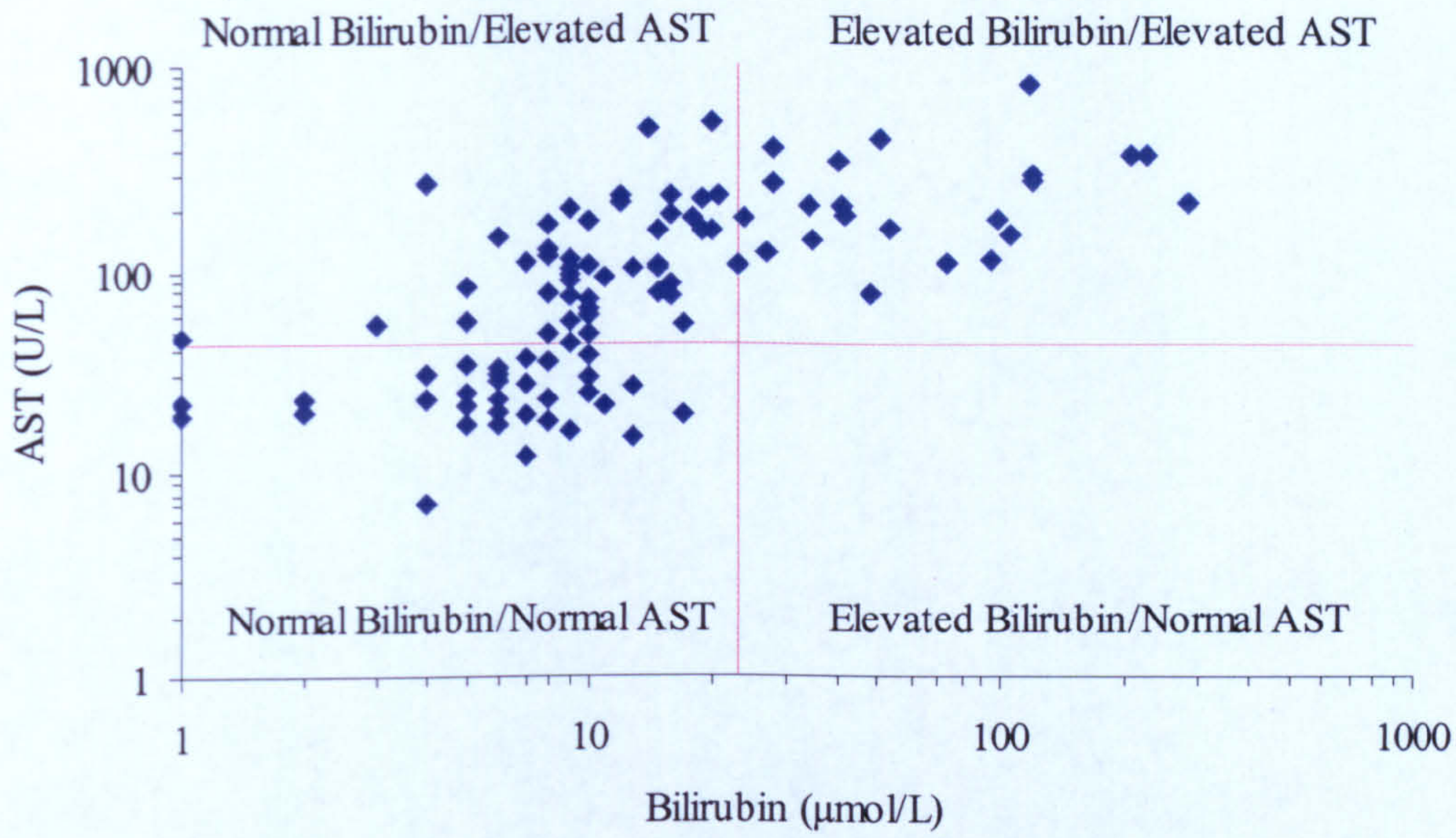
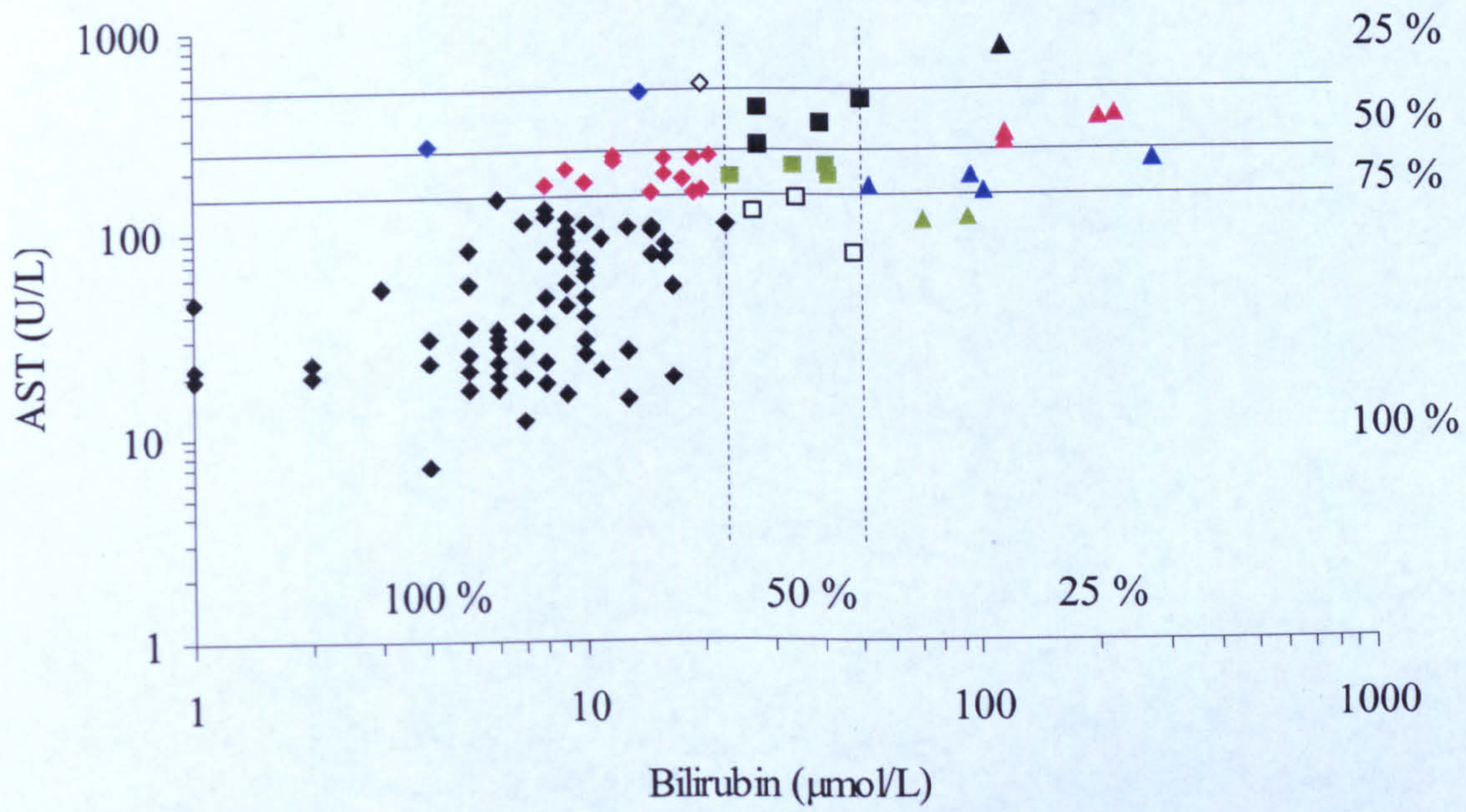


Figure 5.6

Comparison of AST and bilirubin concentrations and resulting doses according to current UK or new AST guidelines



Key

% of standard dose recommended by UK and new guidelines:

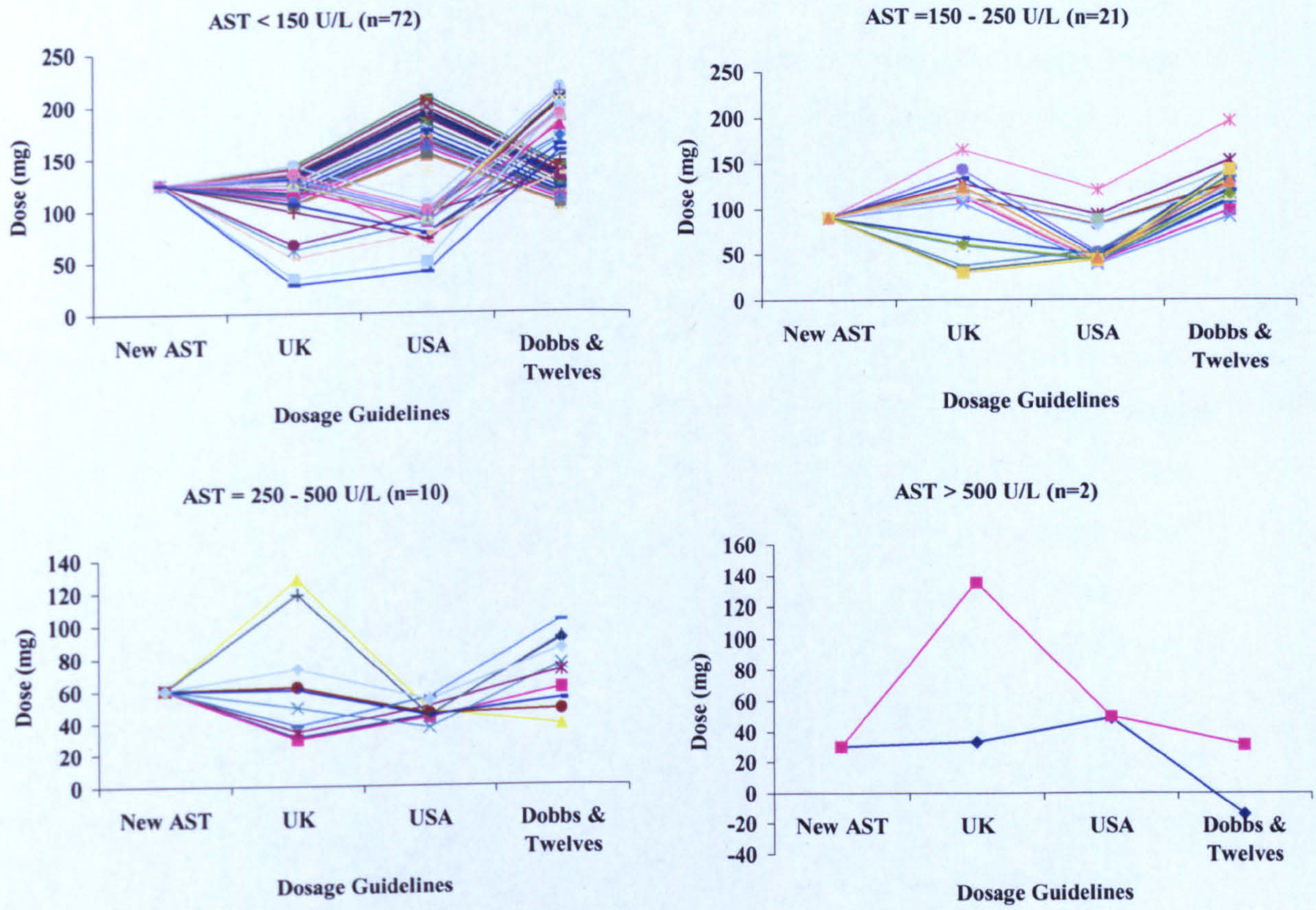
Black diamond = 100 % UK; 100 % new guidelines
Red diamond = 100 % UK; 75 % new guidelines
Blue diamond = 100 % UK; 50 % new guidelines
White diamond =100 % UK; 25 % new guidelines

Black Square = 50 % UK; 50 % new guidelines
White Square = 50 % UK; 100 % new guidelines
Green Square = 50 % UK; 75 % new guidelines

Black Triangle = 25 % UK, 25 % new guidelines
Green Triangle = 25 % UK, 100 % new guidelines
Blue Triangle = 25 % UK, 75 % new guidelines
Red Triangle = 25 % UK, 50 % new guidelines

Figure 5.7

Proposed doses using UK guidelines, USA guidelines, new AST guidelines and the Dobbs and Twelves equation



the data set who had an AST value of 815 U/L was therefore excluded from the evaluation of the Dobbs and Twelves equation. The imprecision in the predicted AUC values, using doses according to the USA guidelines and the Dobbs and Twelves equation, were similar to the current UK guidelines i.e. approximately 60 % (Table 5.4). However, using the Dobbs and Twelves equation, the predicted AUC values were, on average, 19 % greater than the target AUC, indicating that the dose recommended by these guidelines was too high for many patients. The AUC values estimated using doses calculated from the UK guidelines, the USA guidelines and using the equation proposed by Dobbs and Twelves are plotted against AST concentrations in Figure 5.8. Using the USA guidelines, AUC values tended to be greater than the target AUC in patients with normal AST concentrations and less than the target AUC in patients with elevated AST concentrations. Using the Dobbs and Twelves equation, AUC values tended to be greater than the target in patients with elevated AST concentrations. Out of the 105 patients in the group, the number estimated to achieve an AUC value between 3000 and 5000 ng.h/ml was 39 following the current UK dosage guidelines, 37 following the USA dosage guidelines and 35 using Dobbs and Twelves equation compared to 54 following the proposed new AST guidelines. If doses were administered according to current UK dosage guidelines the estimated AUC values ranged from 640 to 17200 ng.h/ml, whereas if doses were administered according to the new AST guidelines the estimated AUC values ranged from 1460 to 10200 ng.h/ml.

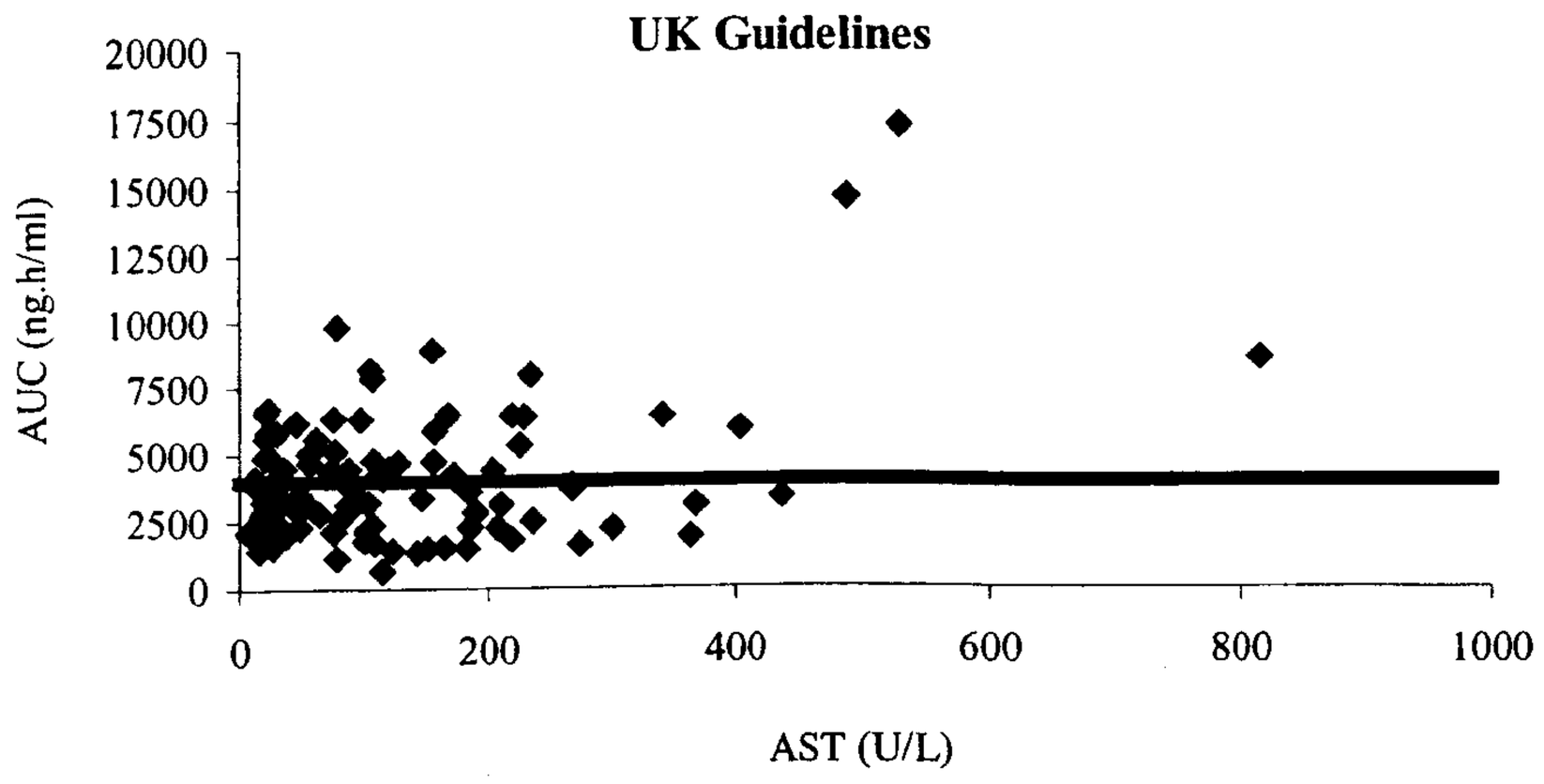
5.3.4 Validation of the population model using an external data set

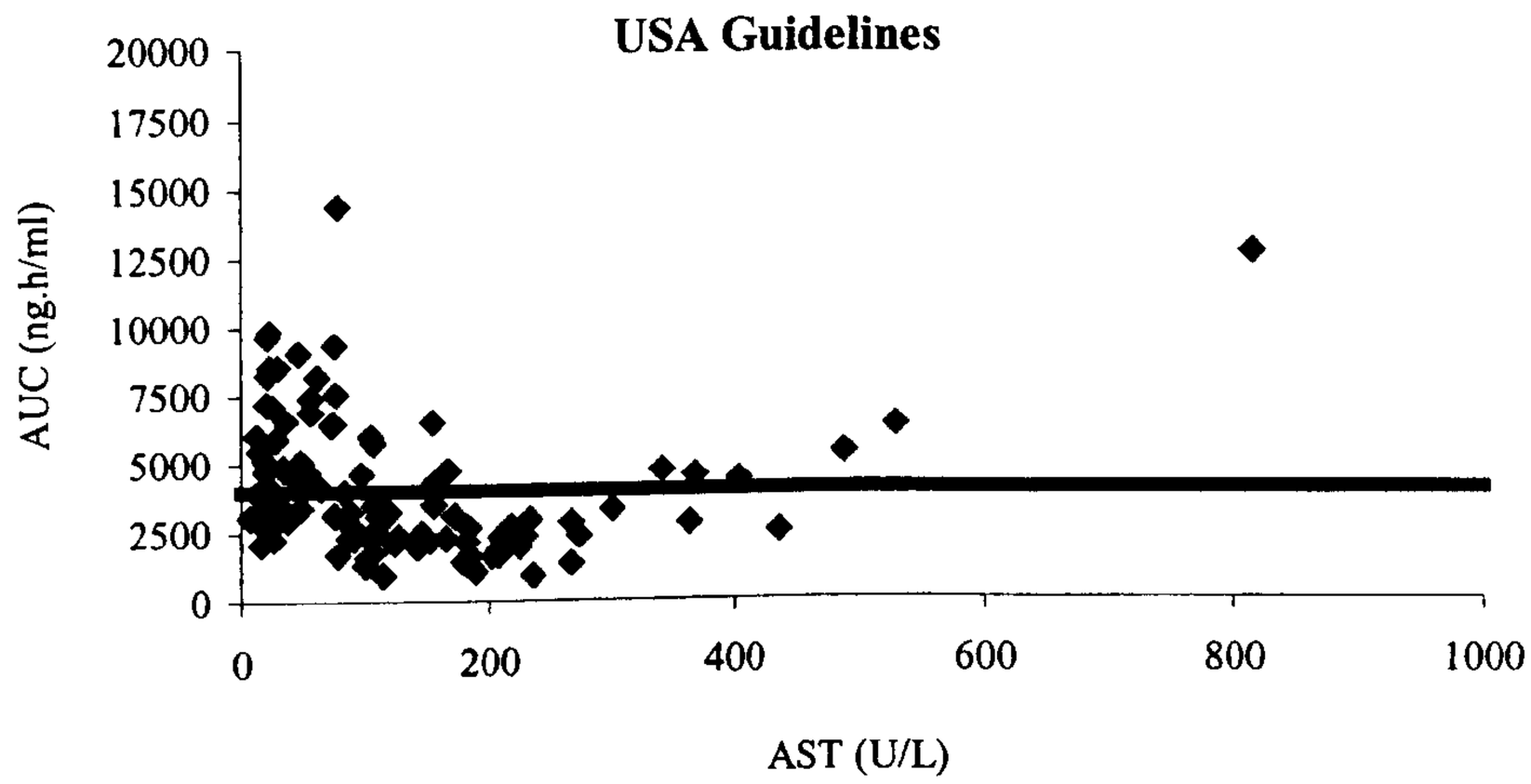
5.3.4.1 Patients

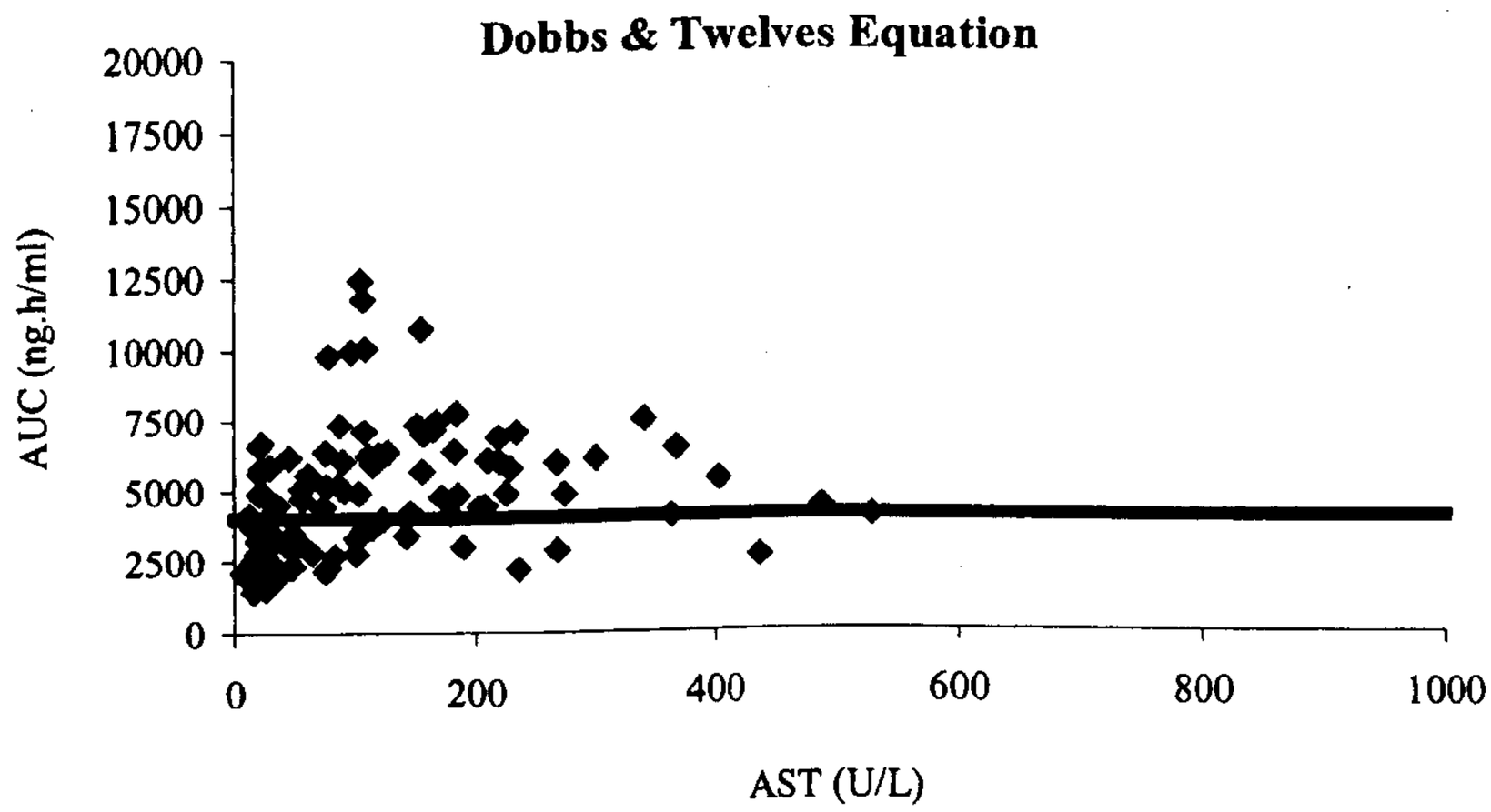
In the validation data set 5 patients received epirubicin as an intra-arterial infusion and 13 patients received epirubicin as an intravenous infusion. The length of infusion ranged from 2 to 15 minutes, with a median of approximately 5 minutes. The concentration time profile for each patient is illustrated in Figure 5.9. On average, 10 blood samples were collected post-dose from each patient, ranging from 8 to 17. Summary statistics of clinical characteristics available for the validation set are

Figure 5.8

Estimated AUC values for patients administered a dose according to the UK guidelines, USA guidelines or Twelves' equation



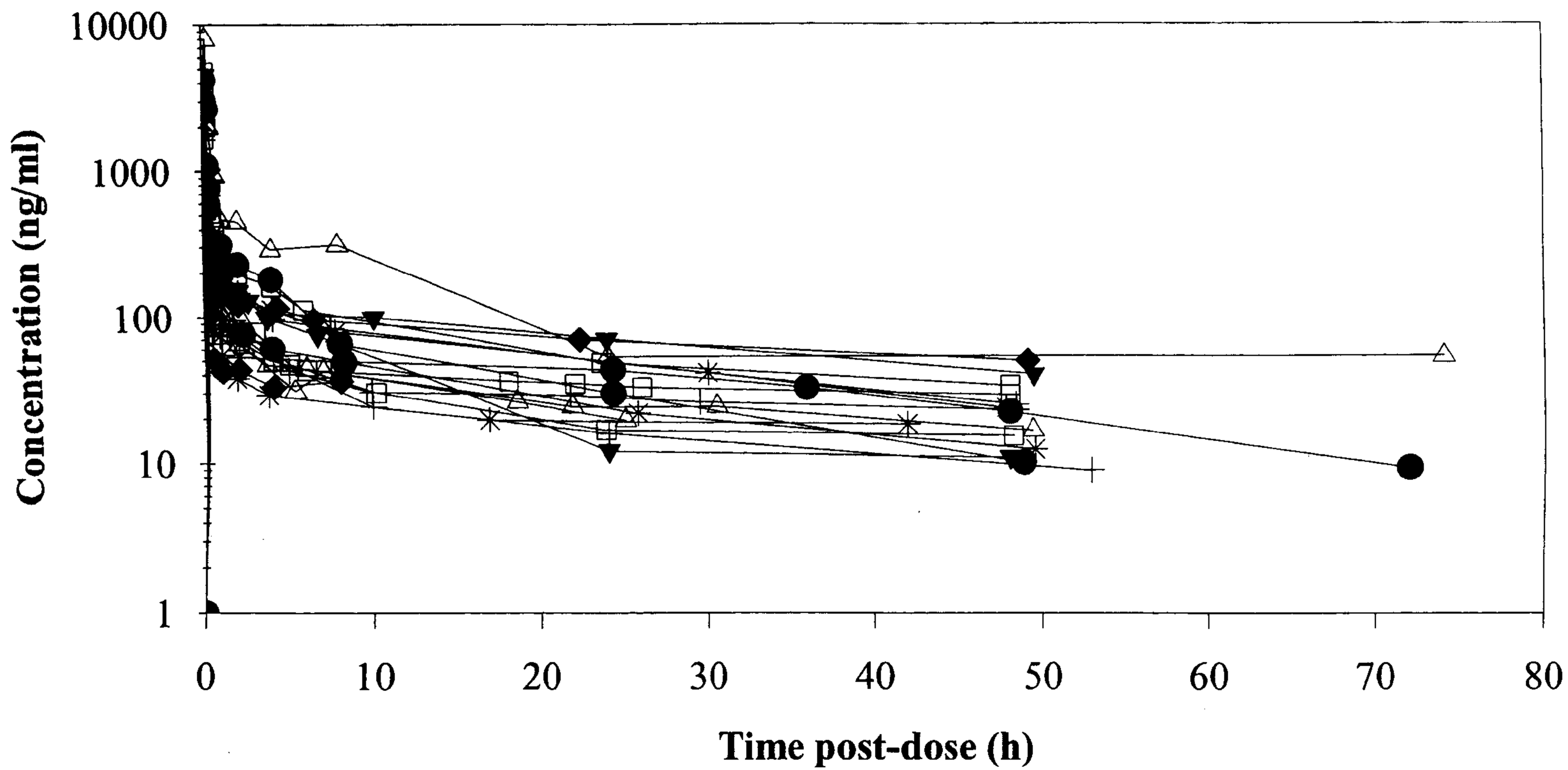




NB. Black horizontal line represents target AUC

Figure 5.9

Concentration-time profiles from validation data set



presented in Table 5.5. The ranges of bilirubin, AST and alkaline phosphatase levels extended to well above the reference range, whereas creatinine and albumin levels were generally within the reference range.

5.3.4.2. Evaluation of new dosage guidelines

The goodness of fit plots for the NONMEM analysis of the validation data set are given in Figure 5.10. The plot of IWRES against concentration shows that the IWRES were randomly distributed around the zero at all concentrations suggesting that the proportional residual error model was appropriate for this data set. In the plot of IWRES against time, the IWRES were generally randomly distributed around zero, although a slight positive bias was observed at 48 h post-dose. Overall, the individual predicted concentrations correlated well with the measured concentrations, thus the 3 compartment model with a proportional residual error appeared to be suitable for calculation of pharmacokinetic parameters for patients in the validation data set. The typical values of parameters for the validation data set were similar to those obtained for the main UK data set, although the relative standard errors of the parameter estimates were higher than those derived from the main UK data set (Table 5.6).

The imprecision (rmse) of the AUC estimates obtained using the proposed new guidelines, relative to the target AUC of 4000 ng.h/ml, was high at 72 % (95 % CI - 13.4, 103 %). The estimates of AUC were not biased relative to the target AUC (me = 16.8 %; 95 % CI -16.5, 50.1 %), however, it should be noted that the confidence interval were wide due to the high variability of the estimates and the small number of patients included (n=18).

5.3.4.3. Validation of the population model for CL

Estimates of CL obtained using the population model based on AST concentrations produced poor estimates of CL, as judged from the rmse and me values, which were 81.6 (95 % CI -33.1, 120) and 20.5 % (95 % CI -19.9, 60.9), respectively. The model appeared to overestimate low CL values (<20 L/h) and underestimate high CL values (>20 L/h) (Figure 5.11).

Table 5.5

Summary of clinical characteristics of patients in validation data set

Clinical characteristics	Reference range	Median	Range	n
Dose (mg)		125	(25-150)	18
Age (years)		57	(28-68)	18
Bilirubin ($\mu mol.l^{-1}$)	<23	25.5	(4-62)	18
AST ($units.l^{-1}$)	< 43	91.5	(29-560)	18
Creatinine ($\mu mol.L^{-1}$)	50-130	79	(59-187)	15
Albumin ($g.l^{-1}$)	30-46	34	(20-41)	15
Alkaline phoshatase ($units.l^{-1}$)	< 255	228	(104-1723)	17

Clinical characteristics	Count	n
WHO performance status		18
0	0	
1	13	
2	5	
3	0	
Liver metastases (yes)	17	18

Figure 5.10

Goodness of fit plots for validation data set

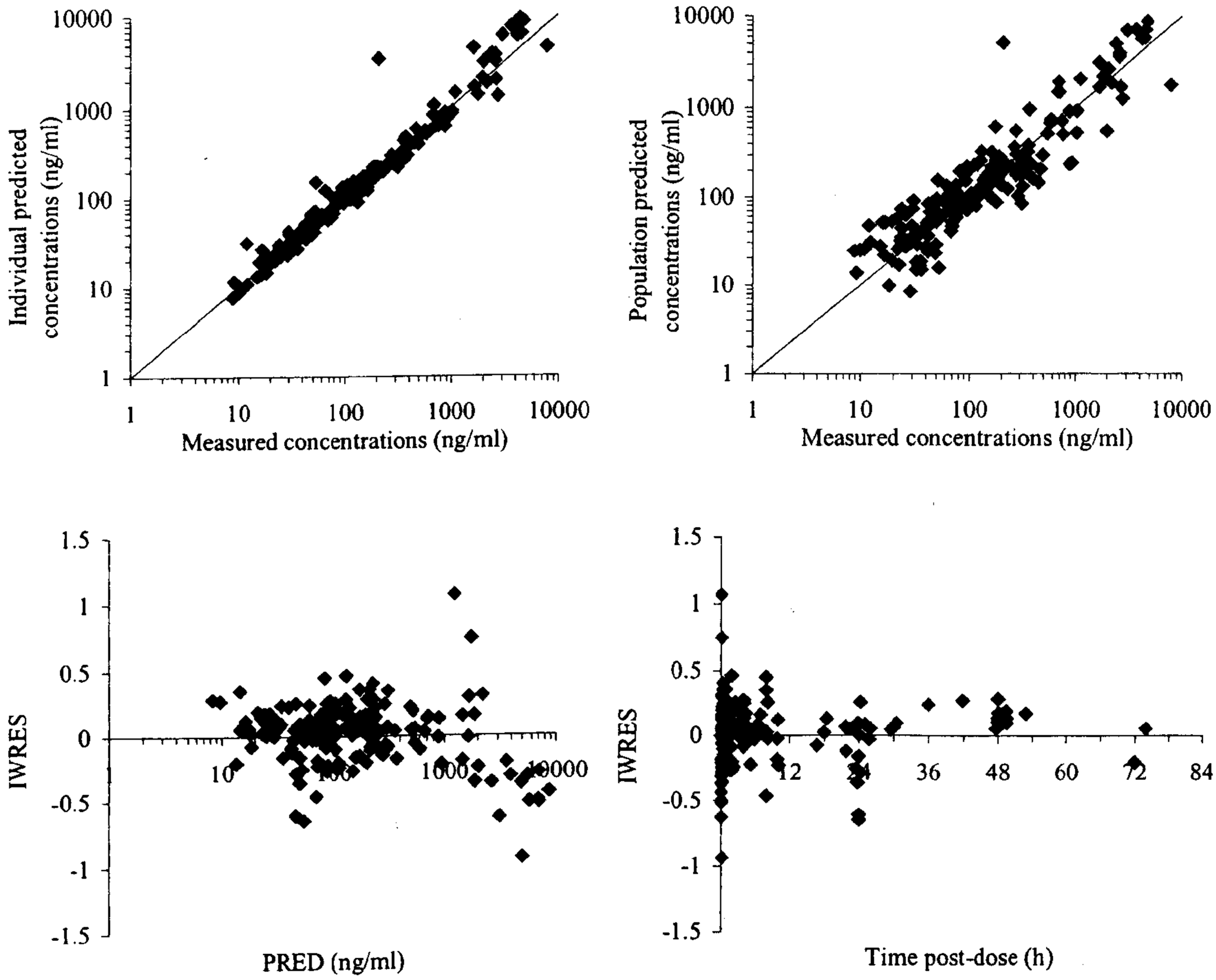


Table 5.6

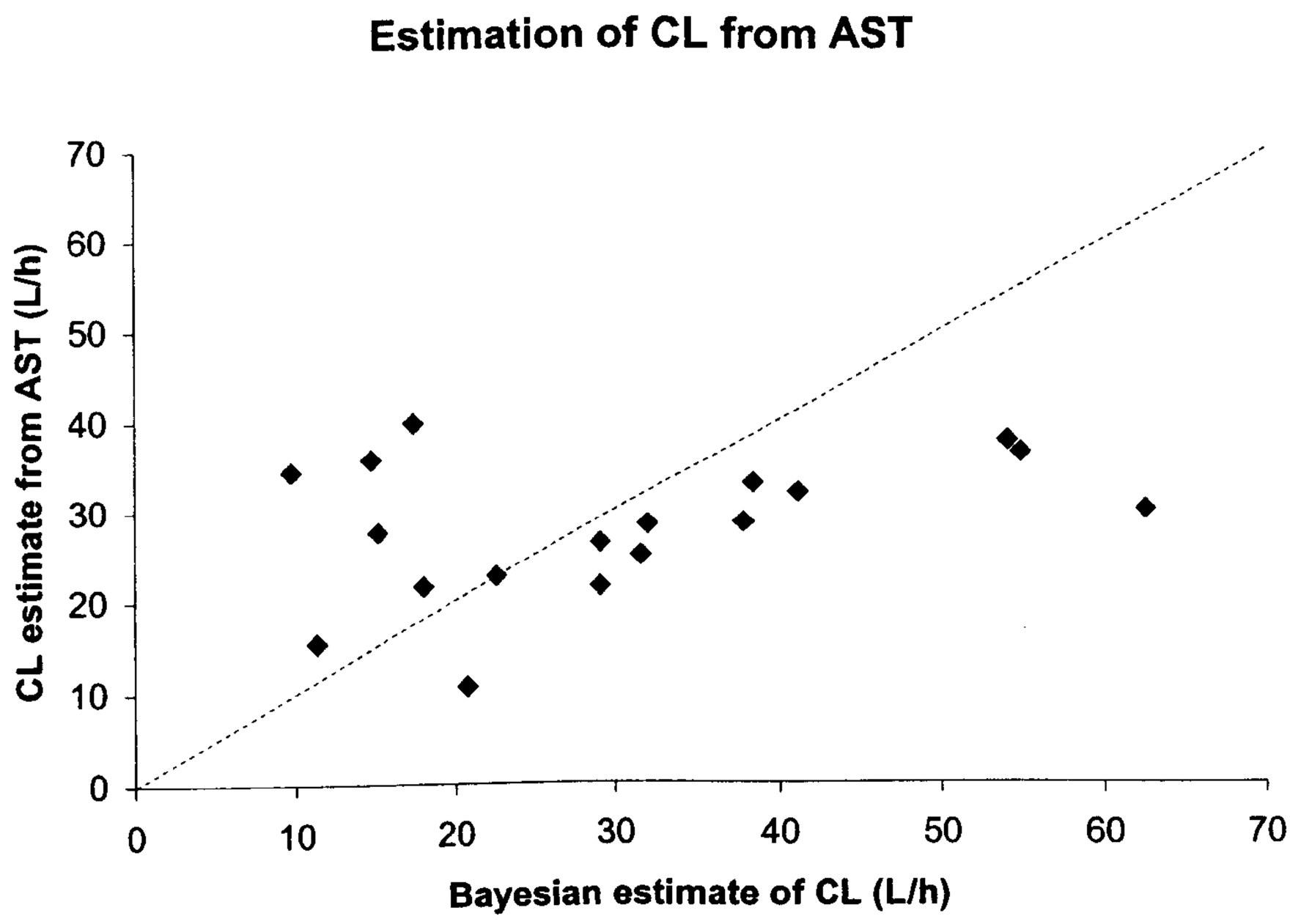
Population parameter estimates for the validation data set

	Validation data set		Original data set	
	Estimate	RSE (%)	Estimate	RSE (%)
CL (L/h)	25.8	15.0	28.0	5.32
V_1 (L)	8.34	28.8	9.88	8.77
Q_2 (L/h)	19.7	42.9	29.0	14.0
V_2 (L)	20.0	70.5	35.2	18.9
Q_3 (L/h)	62.2	19.9	60.3	6.30
V_3 (L)	695	15.0	780	6.50
ωCL (%)	56.7	26.1	53.3	16.5
ωQ_3 (%)	29.1	45.4	32.9	18.1
ωV_3 (%)	42.3	39.7	44.0	22.9
σ (%)	26.8	21.0	23.1	9.23

RSE = relative standard error

Figure 5.11

Scatter plot of the CL values estimated from AST values versus 'true' CL values in the validation data set



5.4 DISCUSSION

The results of the PK/PD analysis were disappointing, as although statistically significant relationships between PK parameters for epirubicin and WBC, neutrophils and platelets were identified, they only explained only up to 22.5% of the variability in the haematological toxicity. In addition, the PK parameters explained less of the variability in toxicity than dose, which was unexpected and inconsistent with the analysis performed by Dobbs and Twelves (1998) who found that AUC was more strongly correlated with WBC nadir or neutrophil nadir than dose. In the UK data set, a single blood sample to measure blood cell counts was taken between 7 and 14 days after dosing and therefore in many cases would not represent the true nadir, which generally occurs between days 10 and 14 (SPC, UK). The increase in blood cell counts post-dose observed in some patients is likely to be due to infection, and would distort any relationships between the PK and PD. Consequently, a target systemic exposure to epirubicin identified from the literature was considered a more reliable approach.

The new suggested dosage guidelines (based on AST concentration) had improved precision in attaining the target AUC compared to the current recommended UK dosage guidelines (based on bilirubin concentration and BSA). AST appears to be a more sensitive marker of liver dysfunction in this patient group than bilirubin as AST was elevated in many of the patients whose bilirubin levels were normal. The inclusion of an additional 25 % dose reduction for patients with AST concentrations of 150 to 250 Units/L resulted in further improvement of the precision. The USA guidelines and Dobbs and Twelves' equation also use AST concentrations to adjust epirubicin dose. However, the USA guidelines and Dobbs and Twelves equation did not achieve much improvement in the precision of AUC values compared to the UK guidelines and the predicted AUC values from the Dobbs and Twelves equation were on average 19 % greater than the target AUC. It was noted that the USA guidelines tended to achieve AUC values greater than the target in patients with normal AST concentrations but lower than the target in patients with moderately elevated AST concentrations. The USA guidelines recommend a 50 % dose reduction in patients with AST levels 2 to 4-fold the upper limit of reference levels, whereas the present analysis suggested that a reduction of only 0-25 % is required for these patients. Thus

it appears that the dose reductions recommended by the USA guidelines for patients with moderately elevated AST concentrations are too severe.

The Dobbs and Twelves equation and the current dosing guidelines in the UK and USA recommend that epirubicin doses are calculated from the BSA of the patient. This is a conventional approach used within oncology although the scientific basis with respect to epirubicin is unclear. This study found no improvement in the accuracy or precision of attaining the AUC target by including a dose-adjustment according to BSA. Administration of a dose in mg rather than mg/m^2 units, as proposed in the new guidelines, would be advantageous because adjusting dose according to BSA is time-consuming and prone to errors (Favier et al. 1994).

It should be noted that the accuracy and bias calculated for the AUC estimates in this analysis assume that the Bayesian estimates of CL are the 'true' values for each individual, when in fact the 'true' values are not known. It also assumes that the selected AUC target of 4000 ng.h/ml is correct. Furthermore, although the new proposed guidelines appear to be better than the other guidelines tested, they have been tested in the same data set in which they were developed. The real test of the dosage guidelines is to evaluate them in an external data set.

When the proposed new guidelines were evaluated using the validation patient group, the imprecision of the predicted AUC values was high at approximately 72 %. The validation set predominantly contained patients with hepatocellular carcinoma, unlike the main data set used to develop the population model which included only patients with advanced breast cancer. It is possible that AST is only a useful marker for epirubicin CL in patients with metastatic breast cancer and is not appropriate for patients with primary liver cancer. Another difference between the two data sets was that all patients in the main data set received epirubicin by intravenous infusion whereas 5 patients in the validation data set received epirubicin by intraarterial infusion. It was not possible to evaluate the other guidelines using this data set, as information on height and weight and thus BSA, was not available in these patients.

CHAPTER 6

VALIDATION OF UK POPULATION MODEL USING A SWEDISH DATA SET

6.1. INTRODUCTION TO CHAPTER

The aim of this Chapter was to perform an external validation of the UK population model developed in Chapter 3 using epirubicin data collected for a separate study in patients from Sweden (Sandstrom 2002). The ability of the UK population model to predict the measured concentrations and individual CL values in the Swedish data set was tested.

6.2. METHODS

6.2.1 Patient data

The data were derived from a previously published study and were provided in an Excel spreadsheet formatted for NONMEM (Sandstrom 2002). Data were available from 79 patients treated with epirubicin in combination with fluorouracil and cyclophosphamide (FEC) for early or advanced breast cancer. FEC was administered at doses of 600/60/600 mg/m^2 or 600/75/900 mg/m^2 of fluorouracil, epirubicin and cyclophosphamide, respectively. Generally, cyclophosphamide was administered as a 15 minute intravenous infusion, followed by a bolus dose of fluorouracil and then an intravenous infusion of epirubicin, median infusion time 1 h, range, 5 min to 2.3 h. A total of 235 plasma samples were available with a median of 3 samples per patient (range, 2 to 6). Blood samples were collected between 4 min and 25 h post-start of the infusion. Eighty seven blood samples were collected during the infusion and 148 after the end of the infusion. Blood samples collected more than 15 h post-dose were available from 41 patients.

Box-plots were produced to assess whether the data for each clinical characteristic was normally distributed. Clinical characteristics in UK and Swedish patients were compared using a 2-sided t-test. For characteristics that displayed skewed distributions, Box-plots were produced for the logged values and if the logged data was approximately normally distributed, logged values were used in the t-tests. Differences in mean values between UK and Swedish patients were considered to be statistically significant if $p<0.05$.

6.2.2 Prediction of concentrations in Swedish patients using UK population model

Using the final population model developed from the UK data set, predicted concentrations (PREDs) were calculated for each patient in the Swedish data set at the available blood sampling times, given each individual's dosage history and AST concentration. These predictions were obtained by entering the parameters of the structural model (including AST as a covariate) into NONMEM and fixing both inter-individual variability in the pharmacokinetic parameters and residual error to zero. The $ESTIMATION command was set as MAXEVAL=0 and NONMEM was then run. Prediction errors (Pe) were calculated for each PRED and expressed as a percentage of the measured value (DV) as follows:

Pe = (PRED-DV)/DV*100

A mean prediction error (MPE) was then calculated for each individual. Bias (me) and imprecision (rmse) of the MPEs was estimated with 95 % CI as described in Chapter 2.

6.2.3 Prediction of CL values in Swedish patients using UK population model

The UK population model was used to predict CL estimates (CL_{UK}) for the Swedish patients, given their measured AST concentration. NONMEM was then used to obtain Bayesian estimates of CL ($CL_{Bayesian}$) for the Swedish patients using all available concentration measurements and the UK population parameter estimates. The program was run with MAXEVAL = 0.

Prediction errors (Pe) were calculated for each predicted CL and expressed as a percentage of the Bayesian estimate as follows:

$Pe = (CL_{UK} - CL_{Bayesian}) / CL_{Bayesian} * 100$

The accuracy and precision of the CL values predicted from UK population model were assessed by calculation of rmse and me values (as described in Chapter 2).

6.3 RESULTS

6.3.1 Patient data

A summary of the clinical characteristics of the Swedish patients is given in Table 6.1. From the box-plots, all characteristics were approximately normally distributed with the exception of bilirubin, AST and creatinine. Taking logs of these parameters resulted in more normal distributions, and therefore, logged values were used for statistical comparisons of bilirubin, AST and creatinine. The mean dose in Swedish patients was 30 mg greater than the mean dose administered to UK patients. The Swedish patients were, on average (mean), 7 cm taller than UK patients and 5 kg heavier; these differences were statistically significant. The distributions of bilirubin and AST in the Swedish patients extended beyond their normal reference ranges. However, the mean AST and bilirubin values in the Swedish patients were statistically significantly lower than those in the UK patients. Approximately 6 % of Swedish patients had bilirubin levels above the upper reference limit and 20 % had AST concentrations above the upper reference limit. This is a smaller fraction compared to the UK data set where approximately 21 % and 69 % had bilirubin and AST concentrations above the normal range, respectively. The mean CrCL in Swedish patients was 11 L/h greater than the mean CrCL in UK patients. The mean ages of UK and Swedish patients were similar.

6.3.2 Prediction of concentrations in Swedish patients using UK population model

The epirubicin concentrations predicted in the Swedish patients using the UK population model are plotted against the measured concentrations in Figure 6.1. The UK population model poorly predicted the concentrations in the Swedish patients with imprecision (rmse) of 102 % (95 % CI: 96.4, 108 %). Concentrations were significantly overestimated (me) in the Swedish patients by 81.4 % (95 % CI: 68.5, 94.2 %). This over prediction was most evident at concentrations less than 200 ng/ml, which corresponded to the later time points in the concentration-time profile (elimination phase). The predicted and measured concentrations for the Swedish patients are plotted versus time in Figure 6.2.

Table 6.1

Summary of clinical characteristics in Swedish and UK patients

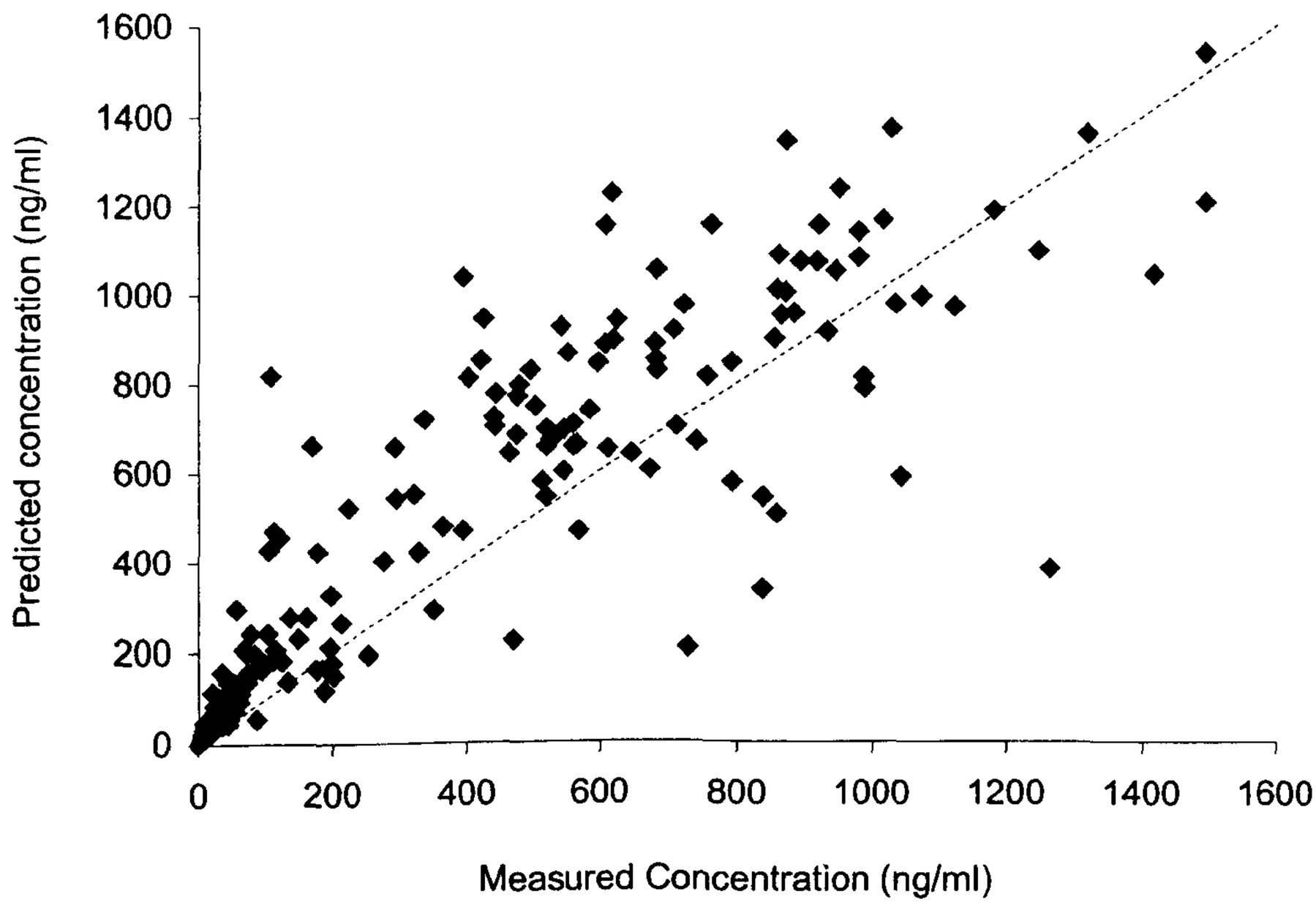
		Swedish (n=79)		UK (n=105)		
	Reference Range	Mean	Range	Mean	Range	Difference
Dose (mg)		118	(30-120)	87	(20-228)	*p<0.05
Age (years)		53	(32-83)	56	(35-79)	n.s.
Height (cm)		165	(154-177)	158	(132-175)	*p<0.05
Weight (kg)		68	(35-102)	63	(37-89)	*p<0.05
Body surface area (m^2)		1.74	(1.3-2.1)	1.64	(1.25-2.2)	*p<0.05
Bilirubin (µmol/L)	< 23	9	(3-63)	26	(1-282)	#*p<0.05
AST (U/L)	< 43	58	(11-511)	125	(7-815)	#*p<0.05
ALT (U/L)	< 34	42	(7-258)	-	-	-
Albumin (g/L)	30-46	40	(24-49)	37	(25-54)	*p<0.05
Creatinine (µmol/L)	50-130	79	(53-197)	82	(47-167)	#n.s.
Creatinine Clearance (ml/min)		77	(17-137)	66	(21-127)	*p<0.05

* = statisitically significant; n.s. = not statistically significant; # = t-test performed using logged data

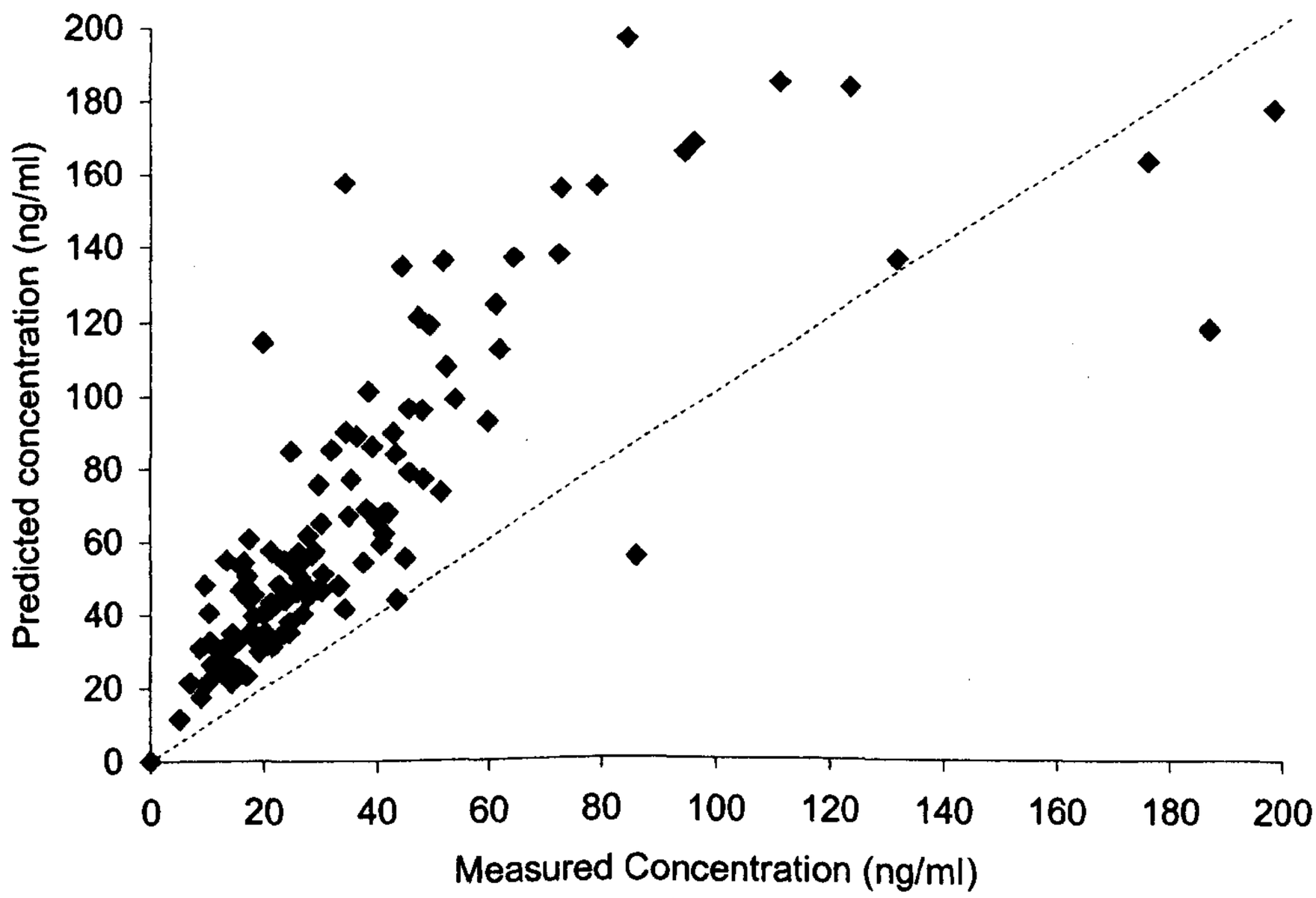
Figure 6.1

Concentrations predicted in Swedish patients using UK population model plotted versus measured concentrations

A. Concentration range 0-1600 ng/ml



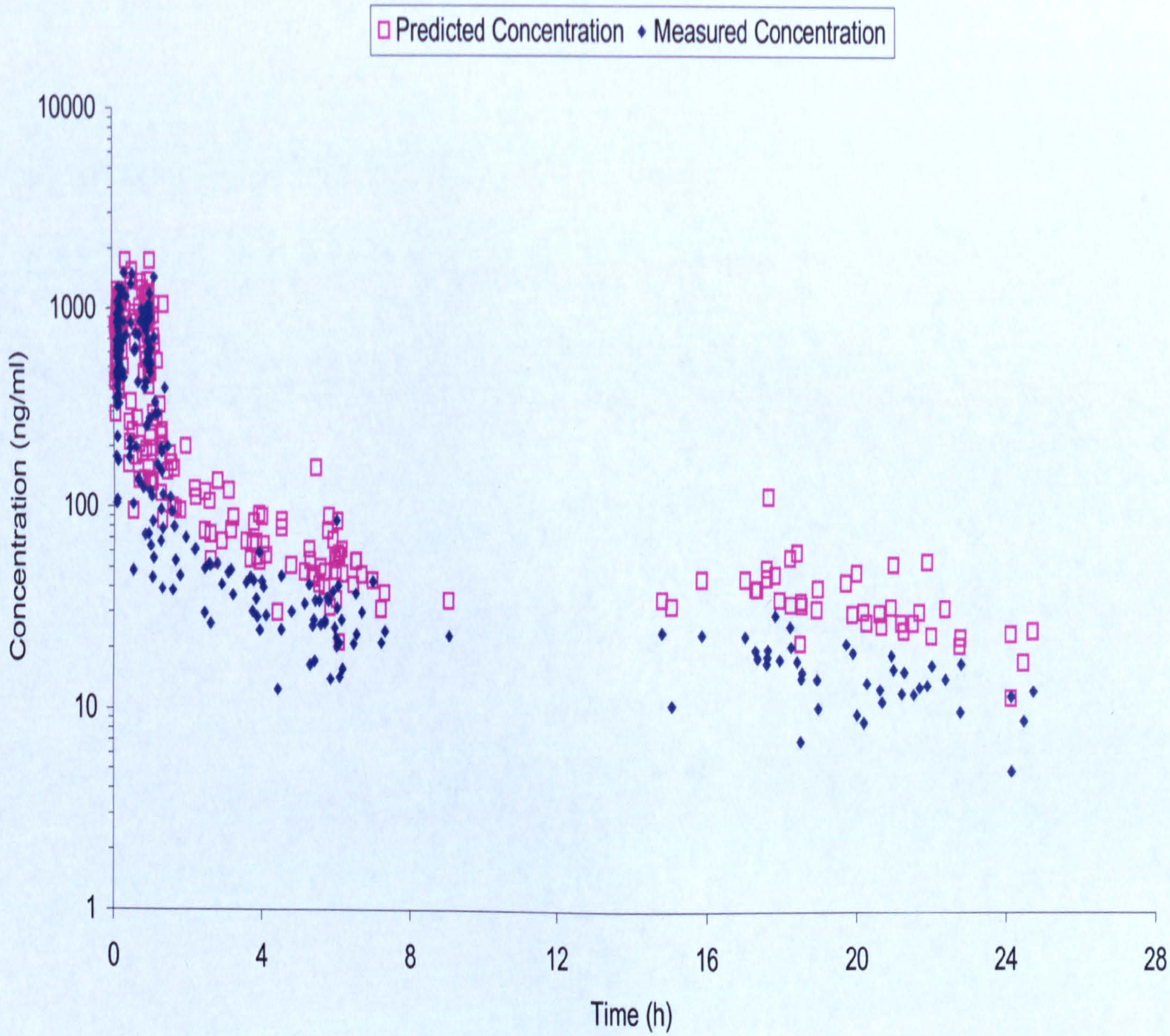
B. Concentration range 0-200 ng/ml



NB. dotted line represents the line of identity

Figure 6.2

Predicted concentrations obtained using the UK population model and measured concentrations in Swedish patients plotted versus time

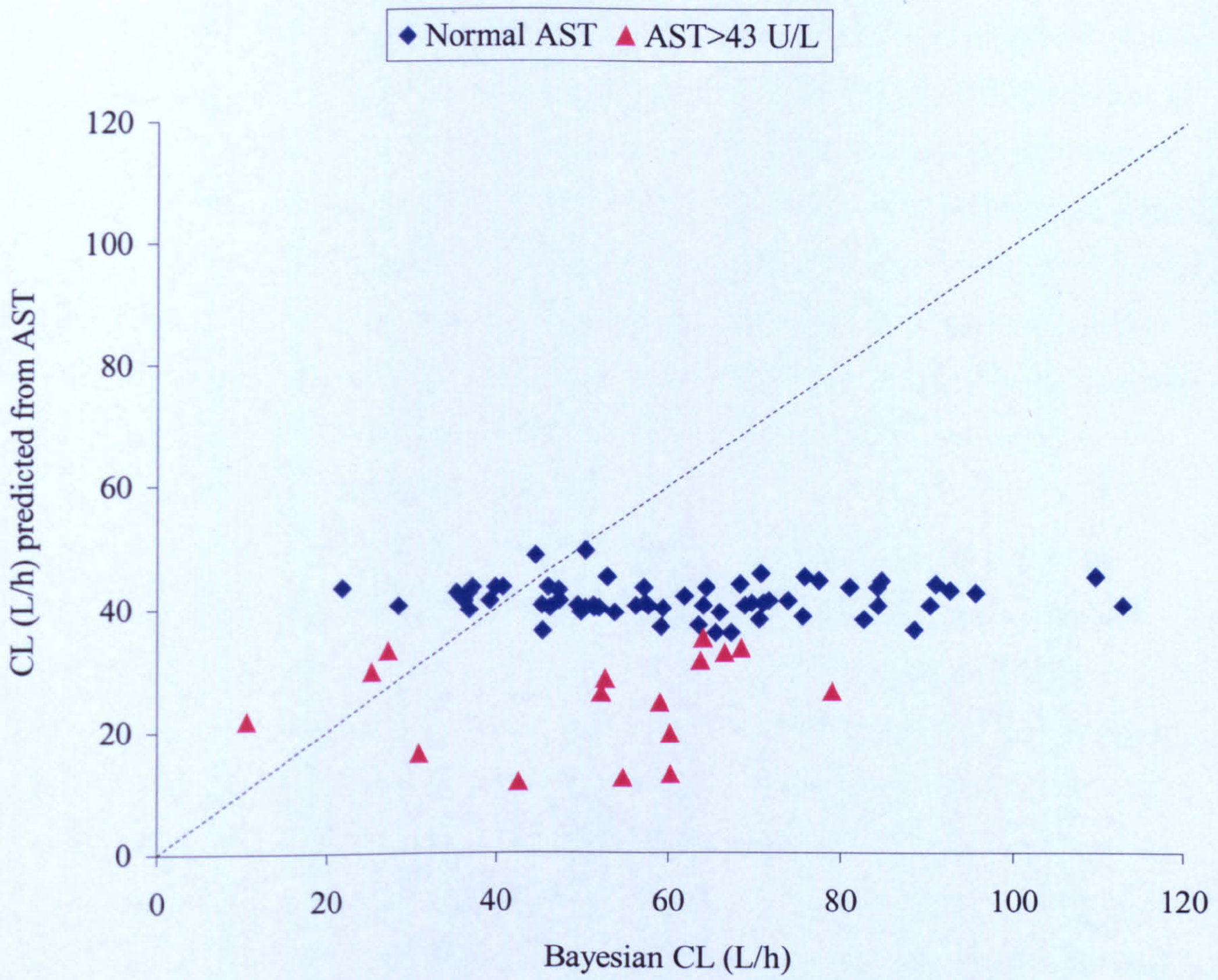


6.3.3 Prediction of CL values in Swedish patients using UK population model

Individual CL estimates were poorly predicted using the UK population model with imprecision (rmse) estimated to be 43 % (95 % CI: 37.8, 48.0). CL in the Swedish patients was underestimated by 27.9 % and this bias (me) was statistically significantly (95 % CI: -35.2, -20.5). CL values estimated from UK population model are plotted against Bayesian CL estimates in Swedish patients with normal and elevated AST levels in Figure 6.3.

Figure 6.3

CL estimates for Swedish patients obtained using the UK population model plotted against Bayesian CL estimates



6.4 DISCUSSION

The UK population model could not accurately or precisely predict CL or concentrations in the Swedish patients. Predicted concentrations in the Swedish data set were almost double the measured concentrations. Likewise, CL values in the Swedish patients, estimated using measured AST values and the UK population model, were not predictive of the Bayesian CL estimates. The question, therefore, arises as to whether the UK population model is invalid or whether there are genuine differences between the two populations. Results from the Chapter 4 indicated that the population model for CL only improved the precision of CL estimates in patients with AST above 150 U/L and as the Swedish data set contained only 6 patients with AST above 150 U/L, poor precision of the model was not unexpected. However, such pronounced bias was not anticipated and this suggests that there are genuine differences in the pharmacokinetics between the two data sets.

The two data sets differed in terms of the length of infusion, the dose, the number and timing of blood samples taken and the proportion of patients with liver dysfunction. Furthermore, the UK population model was developed in patients receiving single agent epirubicin, whereas the Swedish patients received epirubicin as part of a combination therapy including epirubicin, cyclophosphamide and 5-FU. Co-administration of verapamil (Kerr et al. 1986) or paclitaxel (Danesi et al. 1999), has previously been shown to affect the pharmacokinetics of anthracyclines. Although previously a relationship between systemic exposure of cyclophosphamide, 5-FU and epirubicin was not found (Sandstrom et al. 1996), the possibility of a drug interaction cannot be ruled out until a cross-over study has been performed. There could also be differences in the general health status of the patients. The UK data set included patients with advanced disease with poor prognosis and in general poor health. In contrast, in the Swedish data set approximately half of the patients were receiving epirubicin as adjuvant therapy and were therefore likely to be in better health overall. Some small differences in height, weight, albumin and CrCL were observed between the UK and Swedish patients, but such small differences would not be expected to result in such marked differences in the PK of epirubicin. Other physiological differences due to genetics, diet or other environmental factors in Swedish and UK patients would appear unlikely but cannot be excluded.

The true CL values in the Swedish patients were not known, and as the 'true' CL values were estimated from sparse data, they are likely to be unreliable. However, potential inaccuracies of the Bayesian CL estimates in the Swedish data set does not account for the over predictions observed in the concentration data.

The validation of the UK population model may have been clearer if the Swedish data set had included patients with single agent epirubicin therapy and a similar health status, liver function and dosage regimen as observed in the UK data set. However, a favourable outcome with such a validation data set may have mislead the reader into believing that the UK population model was valid in all patients despite the reality of great heterogeneity between patients and treatment schedules. This study has highlighted some of the difficulties in using an external data set to validate a population model and also the limitations of extrapolating results from a relatively small patient group to the population as a whole. Clearly, more work is required to identify the many factors that influence the pharmacokinetics of epirubicin so that more safe and effective dosage guidelines can be developed.

CHAPTER 7

INVESTIGATION INTO THE DIFFERENCES IN PHARMACOKINETICS BETWEEN THE UK AND SWEDISH DATA SETS

7 INTRODUCTION TO CHAPTER

This Chapter explores the possible reasons into why the population model developed from the UK data set poorly predicted concentrations and CL values in the Swedish patients. Section A compares epirubicin population pharmacokinetic parameters and distributions of individual epirubicin CL estimates in the UK data set to those in the Swedish data set. Section B evaluates the differences in clinical characteristics and dosage regimens between the two data sets. Section C assesses the impact of the limited number of blood samples included in the Swedish data set and assesses how the estimates of CL vary depending on the method used to estimate CL.

7A COMPARISON OF EPIRUBICIN PHARMACOKINETICS IN UK AND SWEDISH DATA SETS

7A.1 METHODS

7A.1.1 Estimation of population parameters for Swedish data set

Development of a population model for epirubicin using the Swedish data set was performed using NONMEM with FOCE-INTER. For the structural model, the fit of 2 and 3 compartment models to the data were compared and for the residual error model additive, proportional and combined models were tested. Inter-individual variability terms for parameters were added as judged appropriate based on the OFV and the ability of the model to converge without termination errors. The inclusion of a full covariance matrix was included if variance-covariance terms could be reliably determined and if judged appropriate from the OFV. The goodness of fit of the models was assessed using plots, as described in Chapter 2. The population parameters from the Swedish data set were compared to the population parameters obtained from the UK data set.

7A.1.2 Estimation of population parameters for the combined data set (UK and Swedish patients)

The Swedish data set and UK data set were combined and population analysis was performed using NONMEM with FOCE-INTER. A 3 compartment model with proportional residual error was fitted to the data. Inter-individual variability terms were included as judged appropriate based on the OFV and the ability of the model to converge without termination errors. Population pharmacokinetics parameters were compared to those obtained from analysis of the UK and Swedish data sets separately.

7A.1.3 Comparison of individual Bayesian CL estimates in UK and Swedish patients

Individual Bayesian CL estimates for the UK and Swedish patients were obtained from the population analysis of the combined data set. The distributions of Bayesian CL estimates in UK and Swedish patients were compared using Box and Whisker plots. A t-test was performed and differences in mean values between UK and Swedish patients were considered to be statistically significant if $p<0.05$.

7A.2 RESULTS

7A.2.1 Estimation of population parameters for Swedish data set

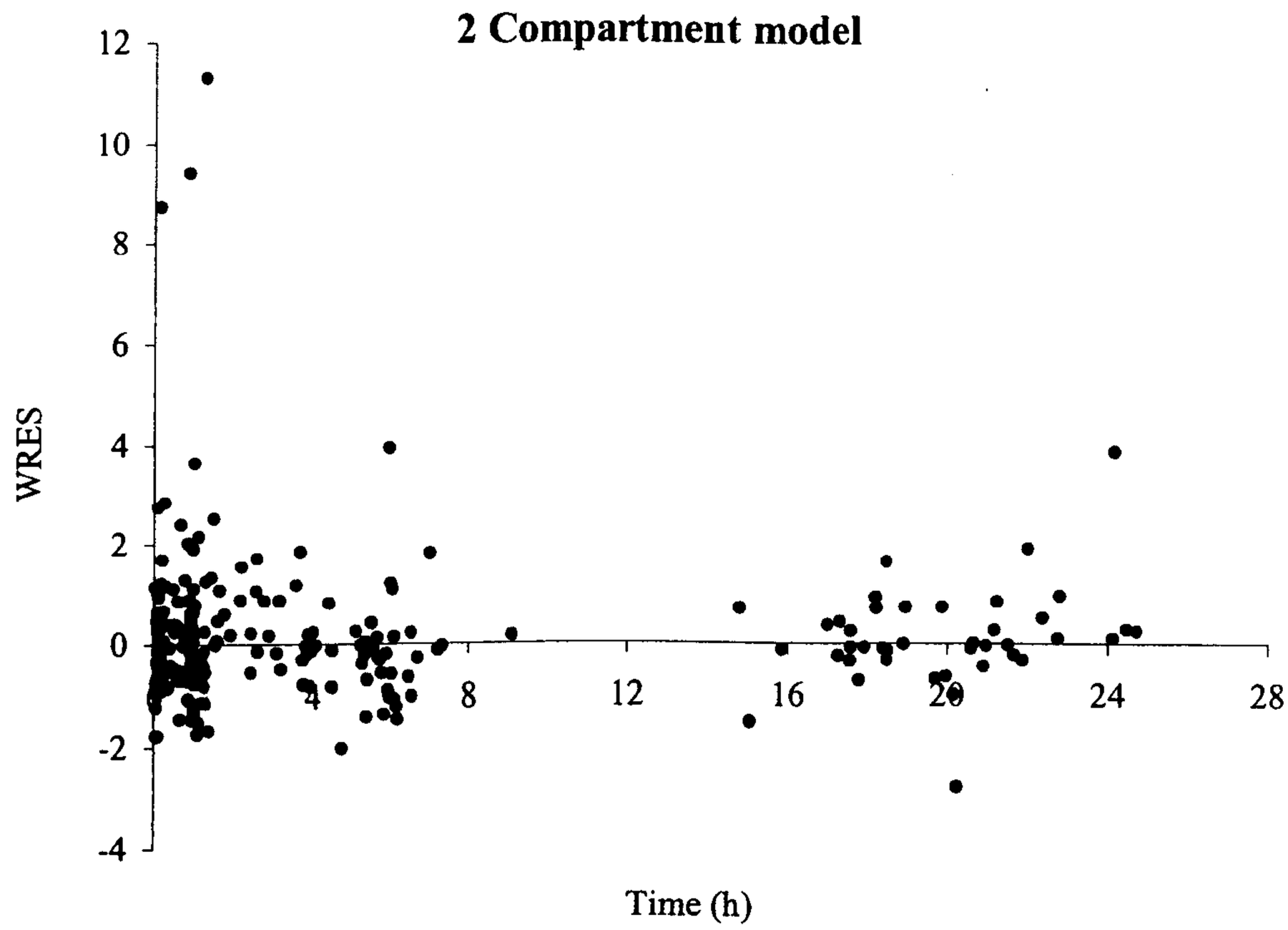
The additive residual error model did not successfully converge for either the 2 or 3-compartment models. The proportional and combined residual error models did converge successfully. The additive component of the combined error model was, however, negligible and there was no improvement in the OFV of the combined error model compared to the proportional error model. The OFV for the 3-compartment model (2043) was lower by 25 units than the OFV for the 2-compartment model (2068). On close examination of the weighted residual (WRES) versus time plots, a slight U-shaped pattern is observed for the 2-compartment model ie. WRES tend to be positive at approximately 2 h, negative at approximately 6 h and positive around 22 h (Figure 7.1). A more random distribution of WRES is observed for the 3-compartment model. Thus, the 3-compartment model with proportional error was selected.

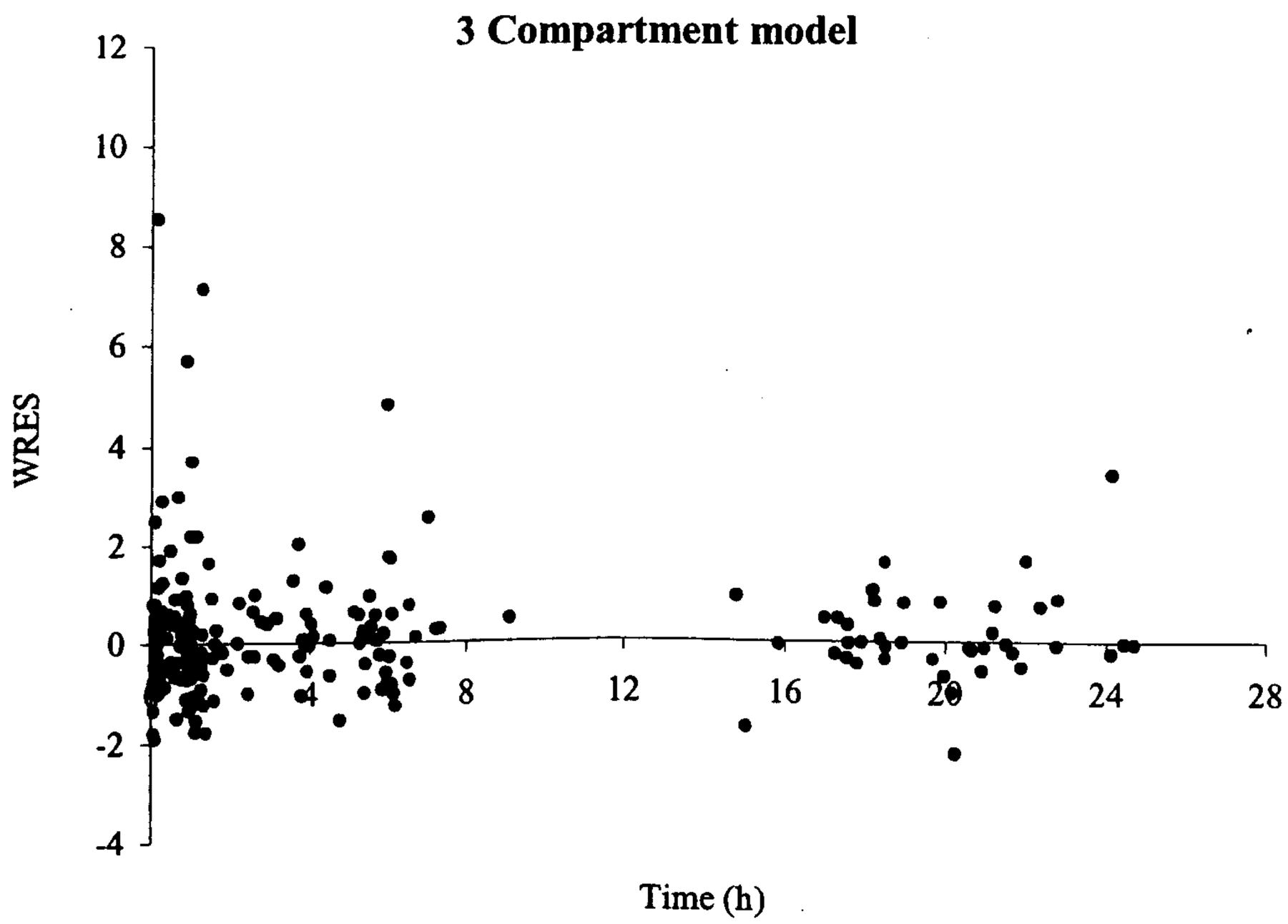
Figure 7.2 illustrates the population and individual predicted concentrations plotted against the measured concentrations for the Swedish data set. The predicted concentrations are in good agreement with the measured concentrations and without any obvious bias, suggesting that the model fitted the data well.

The population model parameters for the Swedish data set are given in Table 7.1. When inter-individual variability terms were included on all PK parameters, estimates of inter-individual variability on V_2 and V_3 were indeterminate and consequently they were removed from the model. The inter-individual variability term for Q_2 was also removed from the model as its inclusion resulted in termination errors. Therefore, the final model included inter-individual variability terms on CL, V_1 and Q_3. Inclusion of a full variance-covariance matrix resulted in a fall in OFV of 28. However, the relative standard error associated with the additional variance-covariance terms was high (44-76 %), indicating that they could not be reliably estimated, and therefore, covariance was restricted to diagonals. Population estimates of V_1, Q_3 and V_3 and residual error were similar to those observed in the UK epirubicin data set. However, the typical value of CL of 71 L/h was much higher than that estimated with the UK data set (28 L/h) (Table 7.1).

Figure 7.1

Swedish data set population analysis: WRES versus time for 2- and 3-compartment models

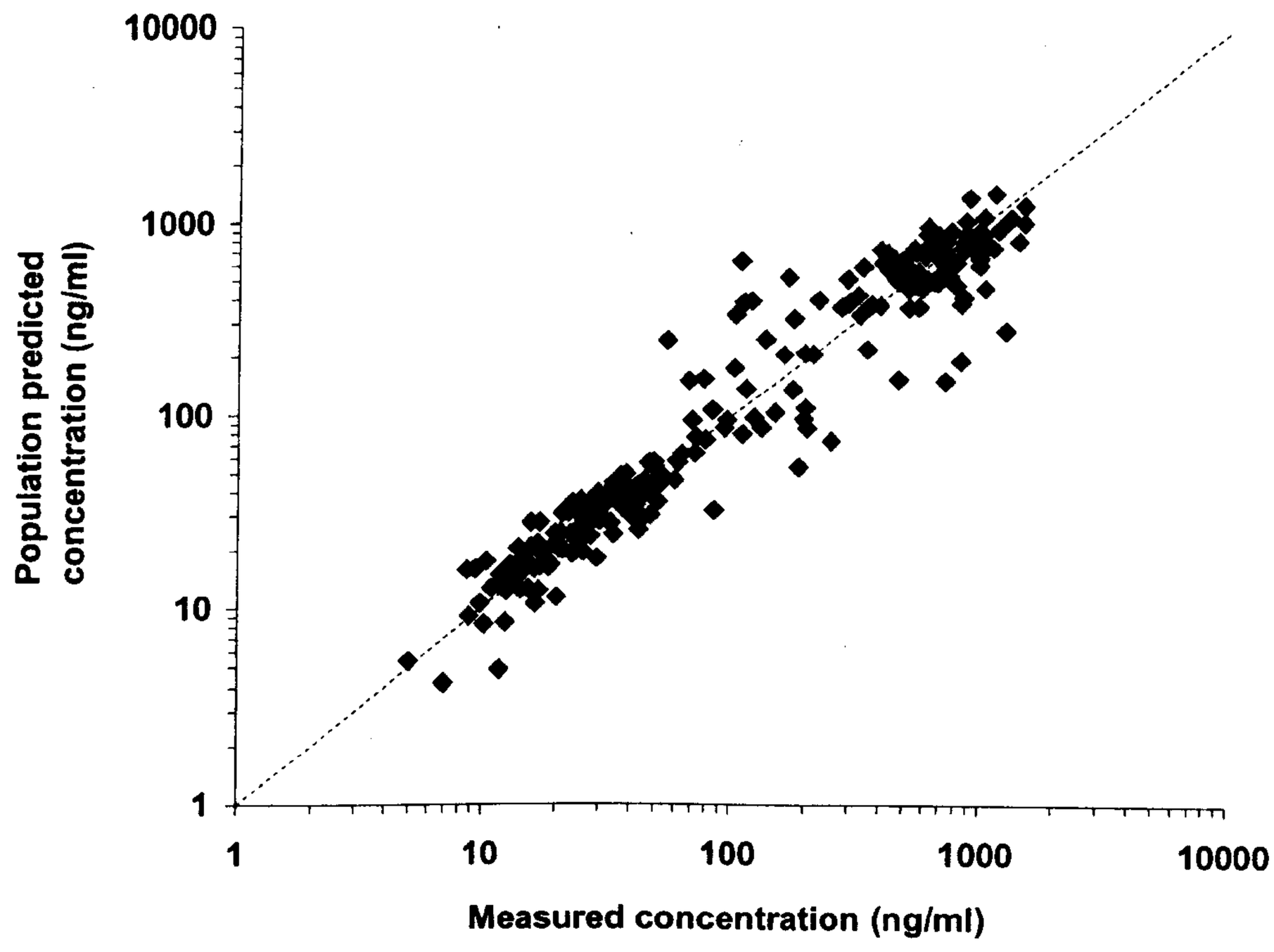


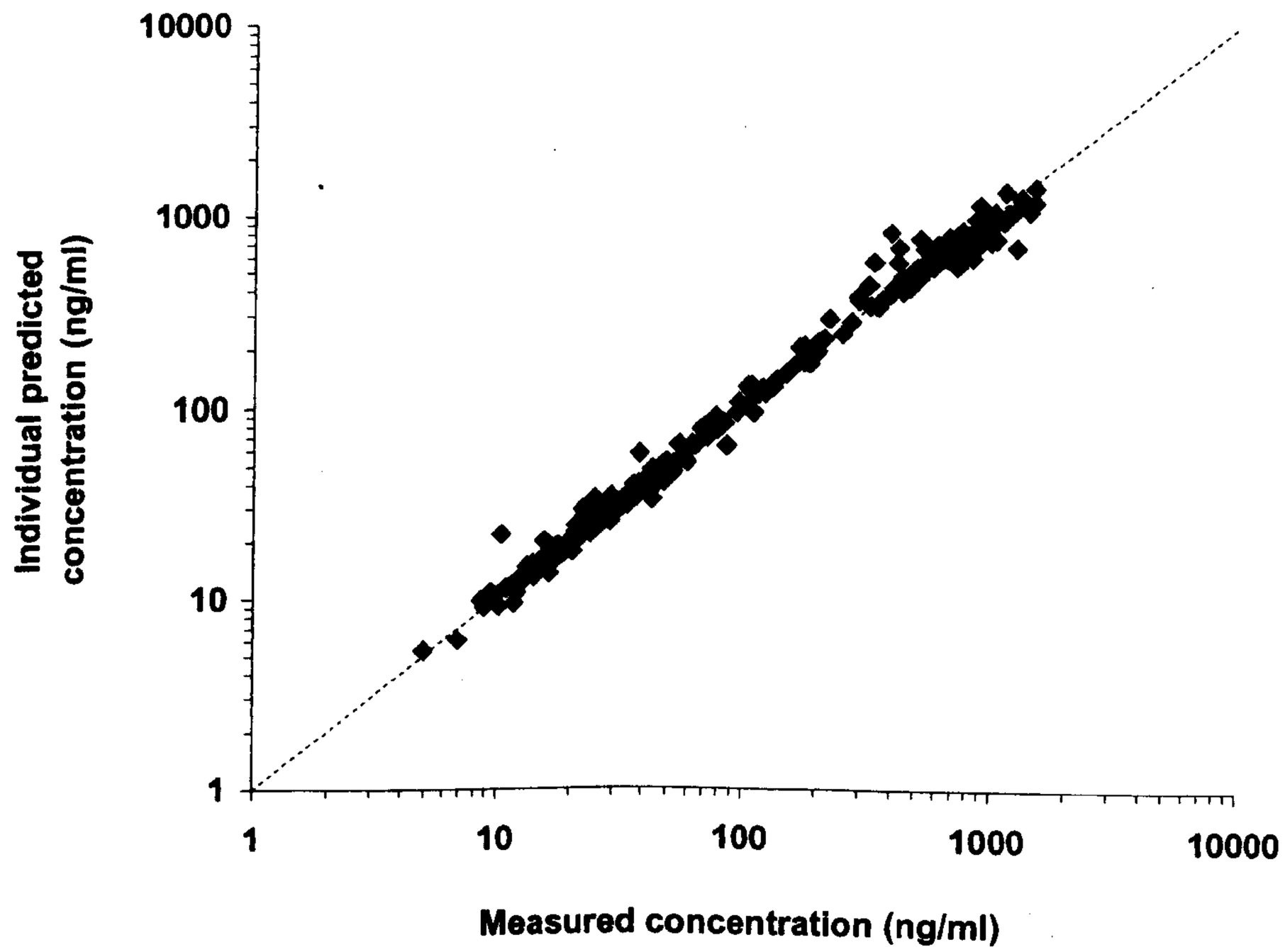


WRES = weighted residuals

Figure 7.2

Population predicted and individual predicted concentrations plotted against measured concentrations for Swedish data set





Dotted line = line of unity

Table 7.1

Population parameter estimates for the UK data set, the Swedish data set and the combined data set

	UK data set		Swedish data set		Combined data set	
Parameter	Estimate	RSE (%)	Estimate	RSE (%)	Estimate	RSE (%)
CL (L/h)	28.0	5.32	71.3	3.62	38.0	5.50
V_1 (L)	9.88	8.77	13.8	11.7	8.72	8.66
Q_2 (L/h)	29.0	14.0	13.7	19.2	28.2	10.8
V_2 (L)	35.2	18.9	15.0	31.7	41.1	14.5
Q_3 (L/h)	60.3	6.30	69.4	6.24	49.8	6.08
V_3 (L)	780	6.50	762	8.06	782	5.86
ωCL (%)	53.3	16.5	19.8	43.3	74.2	13.4
ωV_1 (%)	-	-	72.5	28.4	46.0	29.6
ωQ_3 (%)	32.9	18.1	33.3	33.9	-	-
ωV_3 (%)	44.0	22.9	-	-	-	-
σ (%)	23.1	9.23	20.5	22.6	28.3	8.63

Key: RSE = relative standard error

The estimate of inter-individual variability in CL (ωCL) was much lower in the Swedish data set (20 %) than in the UK data set (53 %), although the RSE for ωCL was high indicating that it could not be reliably estimated in the Swedish data set. Estimates of Q_2 and V_2 for the Swedish data set were approximately half of those estimated in the UK data set. Again, it should be noted that RSE for estimates of Q_2 and V_2 for both the Swedish data set and the UK data set were higher than for the other PK parameters indicating that these estimates were determined with the least confidence.

7A.2.2 Estimation of population parameters for the combined data set (UK and Swedish patients)

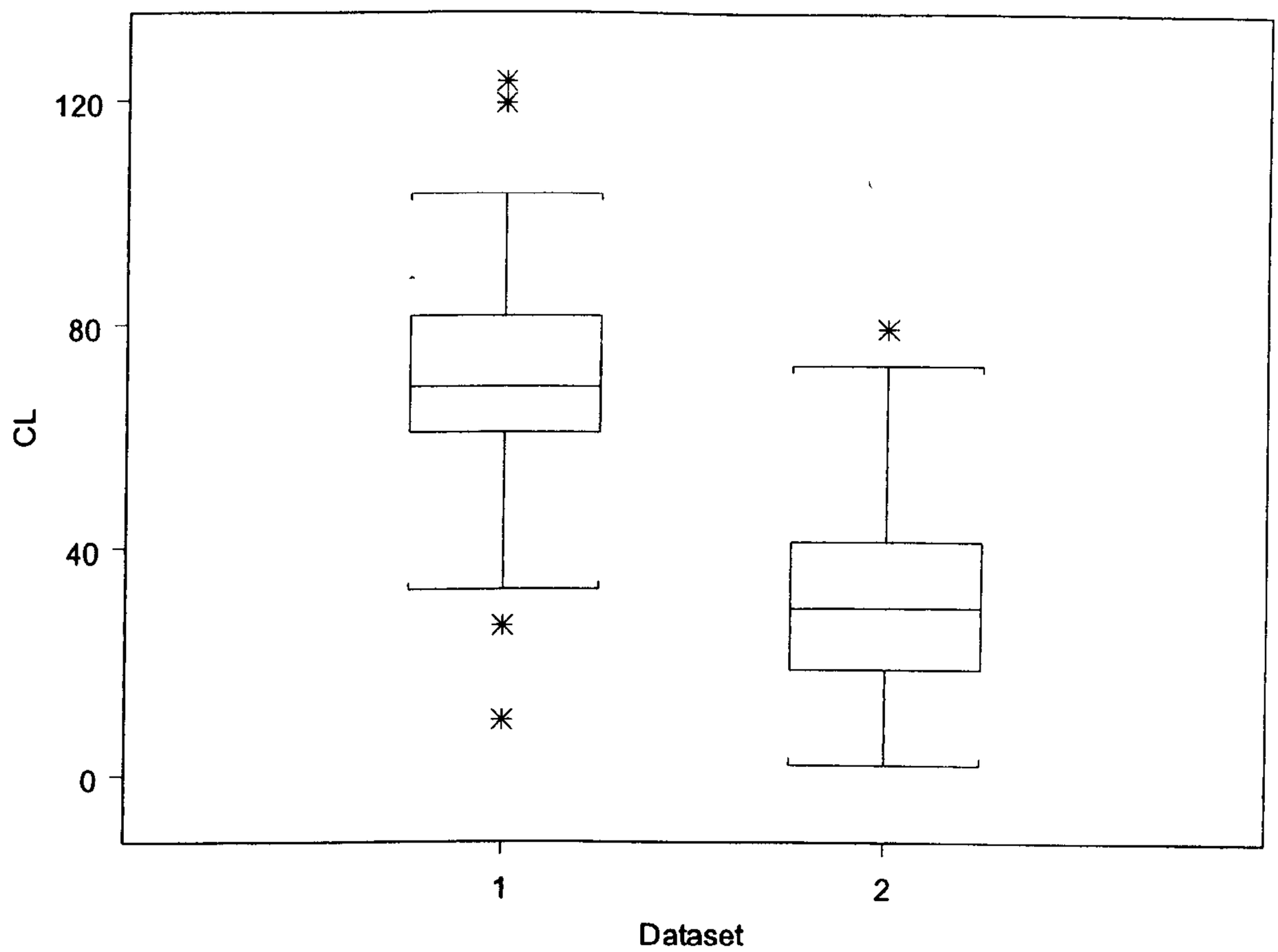
A 3 compartment model with proportional error fitted the combined UK and Swedish data well as judged from the goodness of fit plots (data not shown). Termination errors occurred if inter-individual error terms were included on all parameters. Successful convergence was achieved if only 2 inter-individual error terms were included. Inter-individual error terms were therefore included on CL, as this is the parameter of most interest, and on V_1 as this gave the lowest OFV compared to inclusion of other terms. The population parameter estimates obtained with the combined data set are given in Table 7.1 and were similar to those obtained by analysis of the UK data set alone. Although the population estimate of CL increased from 28 L/h for the UK data set to 38 L/h for the combined data set, this is still much lower than that estimated from the Swedish data set alone (71 L/h). Inter-individual variability of CL increased from 53 % and 18 % in the UK and Swedish data sets, respectively, to 74 %, reflecting the greater spread of CL values in the combined data set.

7A.2.3 Comparison of individual Bayesian CL estimates in UK and Swedish patients

The distributions of the individual Bayesian CL estimates for UK and Swedish patients are illustrated in Box and Whisker plots in Figure 7.3. The median CL value in the Swedish data set was 69 L/h compared with only 30 L/h in the UK data set. The difference in CL between the two data sets was found to be statistically significant ($p<0.000001$).

Figure 7.3

Box plots of individual CL estimates in Swedish and UK data sets estimated from population analysis of the combined data sets



Key: 1 = Swedish data set, 2 = UK data set

7A.3 DISCUSSION

A population analysis of the Swedish data set estimated the typical value of CL to be 71 L/h, which was significantly higher than that estimated in the UK data set (28 L/h). Epirubicin CL has previously been reported in the literature to range from 33 to 89 L/h (Plosker & Faulds 1993). The relatively lower CL in the UK data set is likely to reflect, at least in part, the high proportion of patients with liver metastases and/or abnormal liver function in this data set.

Inter-patient variability in CL (ωCL) estimated in the Swedish data set was only a third of that estimated in the UK data set, which could also reflect the smaller proportion of patients with abnormal liver function in the Swedish data set. In addition, as the blood samples were sparse in the Swedish data set, it is likely that there was shrinkage towards the typical population value for CL, and hence, an underestimation of inter-individual variability.

Estimates of Q_2 and V_2 for the Swedish data set were approximately half of the values obtained with the UK data set. These differences may also be due to the sparseness of the blood samples in the Swedish data set, resulting in difficulties in defining all phases of the concentration time profile. With both data sets, the relative standard errors for V_2 and Q_2 were higher than those for other parameters, indicating that they were estimated with less certainty. The evidence supporting selection of a 3-compartment model over a 2-compartment model was less convincing with the Swedish data set compared to the UK data set.

Following population analysis of the Swedish data set, inter-individual variability terms could be determined for CL, Q_3 and V_1. By contrast, population analysis of the UK data set enabled inter-individual variability terms to be determined for CL, Q_3 and V_3 but not V_1. The different infusion durations and sampling designs may influence the ability to define different PK parameters. The limited number of samples available in the Swedish patients during the terminal phase of the concentration-time profile, is likely to have resulted in difficulties in defining CL. It should be noted that although inter-individual variability could not be estimated for 3 parameters in the analysis of both data sets, this does not mean that there was no inter-individual variability on these parameters. Inter-individual variability in the pharmacokinetics appear to be 're-partitioned' on to the other parameters that were

more readily defined. For example, in the Swedish data set inter-individual variability on V_1 was very high, whereas it was very low for CL. In contrast, in the UK data set inter-individual variability on V_1 was apparently zero, whereas it was very high for CL. It might be expected that on combination of the two data sets that inter-individual variability could be more readily characterised on an increased number of parameters. In practice, on combination of the two data sets, inter-individual variability could only be defined for 2 pharmacokinetic parameters, CL and V_1. The model became very unstable on the combination of the two data sets and difficulties were experienced in achieving successful convergence, which may be due to the apparently different CL distributions for the two data sets.

7B: INVESTIGATION INTO THE DIFFERENCES IN LIVER FUNCTION AND DOSAGE REGIMENS BETWEEN THE UK AND SWEDISH DATA SETS

7B.1 INTRODUCTION

The aim of this section was to explore the possible reasons why the population model developed in the UK data set produced poor predictions of CL values and concentrations in the Swedish data set. There were various differences between the two data sets including length of infusion, the number and timing of blood samples taken and differences in the proportion of patients with liver dysfunction. The differences in the number and timing of blood samples is explored in Section C. This section explores the differences in liver dysfunction and dosage regimen between the two data sets and their influence on the pharmacokinetics of epirubicin as follows:

1) The differences in mean CL between the two data sets could be due to differences in the proportion of patients with liver dysfunction. The distributions of CL and dose-adjusted concentration time-profiles in the two data sets were compared in patients with normal liver function only.
2) It is possible that AST is not a good marker for epirubicin CL in the Swedish patients. The relationship between epirubicin CL and AST in the Swedish patients was explored using NONMEM.
3) The average length of infusion in the UK data set was 3 min compared with 1 h in the Swedish data set. A difference in the infusion rate will result in concentration-time profiles with different shapes. Assuming identical (schedule-independent) pharmacokinetics, the shape of the concentration time profiles were predicted following either a 3 min or a 1 h infusion of the same epirubicin dose. This could indicate if the differing concentrations at the later time-points are simply due to the differing lengths of infusion.

7B.2 METHODS

7B.2.1 Comparison of CL distributions and concentrations in Swedish and UK data sets in patients with normal liver function

Box and Whisker plots were produced to compare the CL distributions of the UK and Swedish patients with normal liver function. Patients were considered to have normal liver function if their AST concentration was less than 43 U/L and their bilirubin concentration was less than 23 μmol/L. Bayesian CL estimates from the combined population analysis were used. A t-test was performed on CL values and differences in mean values between UK and Swedish patients were considered to be statistically significant if $p<0.05$.

Concentrations in all patients with normal liver function were adjusted to a "standard" dose of 100 mg and the concentration time-profiles for the Swedish and UK patients were compared graphically.

7B.2.2 Assessment of the relationship between AST and CL in the Swedish data set

AST was included as a natural log function in the population model for CL in the Swedish data set and the resultant OFV was compared to that attained from the basic Swedish population model with no covariates included.

7B.2.3 The effect of varying the duration of infusion on concentration time profiles

The effect of the different infusion durations between the Swedish and UK data sets on the shape of concentration-time profiles was illustrated by predicting the concentrations in a typical individual, administered epirubicin as either a 3 min or 1 h infusion. Population parameter estimates for epirubicin from the combined data set analysis were entered into NONMEM. Residual error and inter-individual variability were fixed to zero. The $Estimation step was entered as MAXEVAL=0. Population predicted concentrations (PREDS) were generated up to 48 h assuming an intravenous dose of 100 mg administered over either 3 min or 1 h, to represent the UK and Swedish dosage regimens, respectively. The predicted concentration-time profiles for each dosage regimen were compared graphically.

7B.3 RESULTS

7B.3.1 Comparison of CL distributions and concentrations in Swedish and UK data sets in patients with normal liver function

Thirty-three patients in the UK data set and 62 patients in the Swedish data set were considered to have normal liver function. The distributions of Bayesian CL estimates are illustrated in Figure 7.4. Median CL values in the Swedish patients with normal liver function were markedly higher (62 L/h) that those in the UK patients (38 L/h). A t-test of CL values found the difference in CL values between the two data sets to be statistically significant ($p<0.00001$).

The dose-adjusted concentration time-profiles in the Swedish and UK patients with normal liver function are illustrated in Figure 7.5. Although there is some overlap in concentrations between the two data sets, the concentrations in Swedish patients tended to be lower than those in the UK patients.

7B.3.2 Assessment of the relationship between AST and CL in the Swedish data set

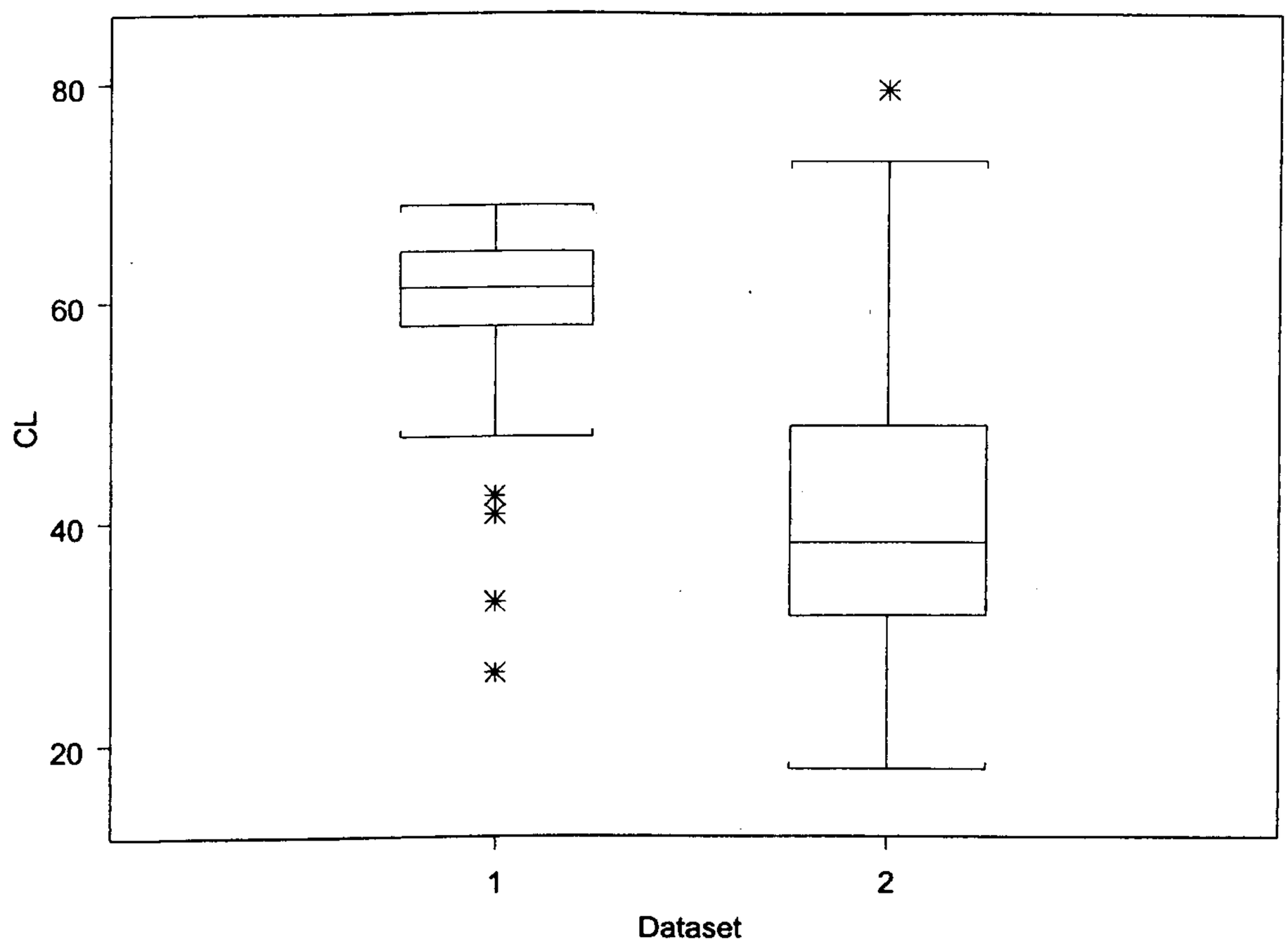
Inclusion of AST as a $\log_e$ function in the CL model for the Swedish data set resulted in a fall in the OFV of 11.9, which is a statistically significant improvement in the model ($p<0.001$). Following inclusion of AST in the model, inter-individual variability in CL fell from 19.8 % to 17.3 %.

7B.3.3 The effect of varying the duration of infusion on concentration time profiles

The effect of varying the duration of infusion by 3 to 60 minutes on the concentration time profile for a typical individual is illustrated in Figure 7.6. The predicted concentration-time profiles for a patient with typical pharmacokinetics show that following administration of epirubicin as a 1 hour infusion, the concentrations are markedly higher for 20 to 60 minutes post-start of the infusion compared to following administration of a 3 minute infusion. After the end of the 1 hour infusion

Figure 7.4

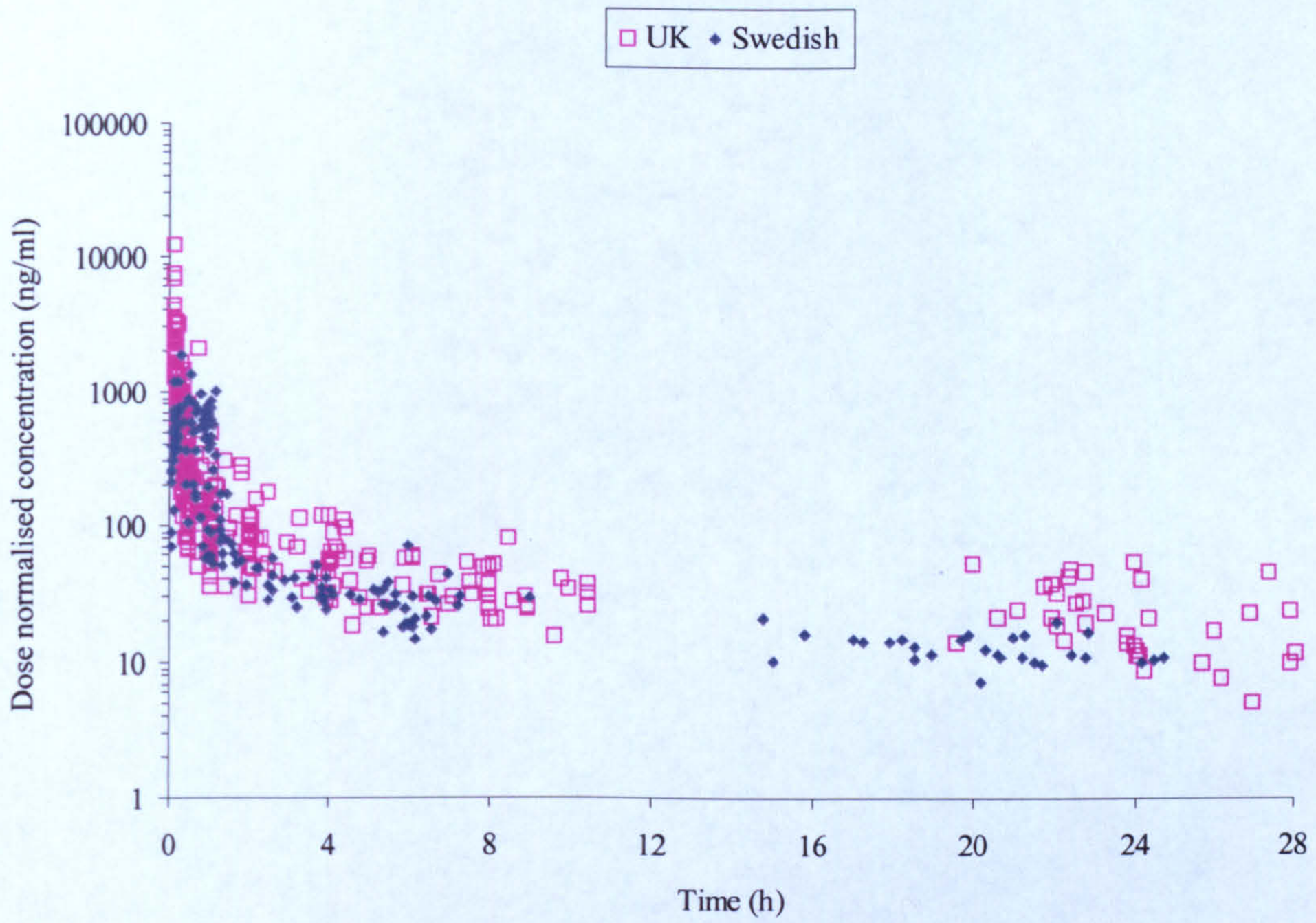
Comparison of CL distributions in Swedish and UK data sets in patients with normal liver function



Data set 1 = Swedish
Data set 2 = UK

Figure 7.5

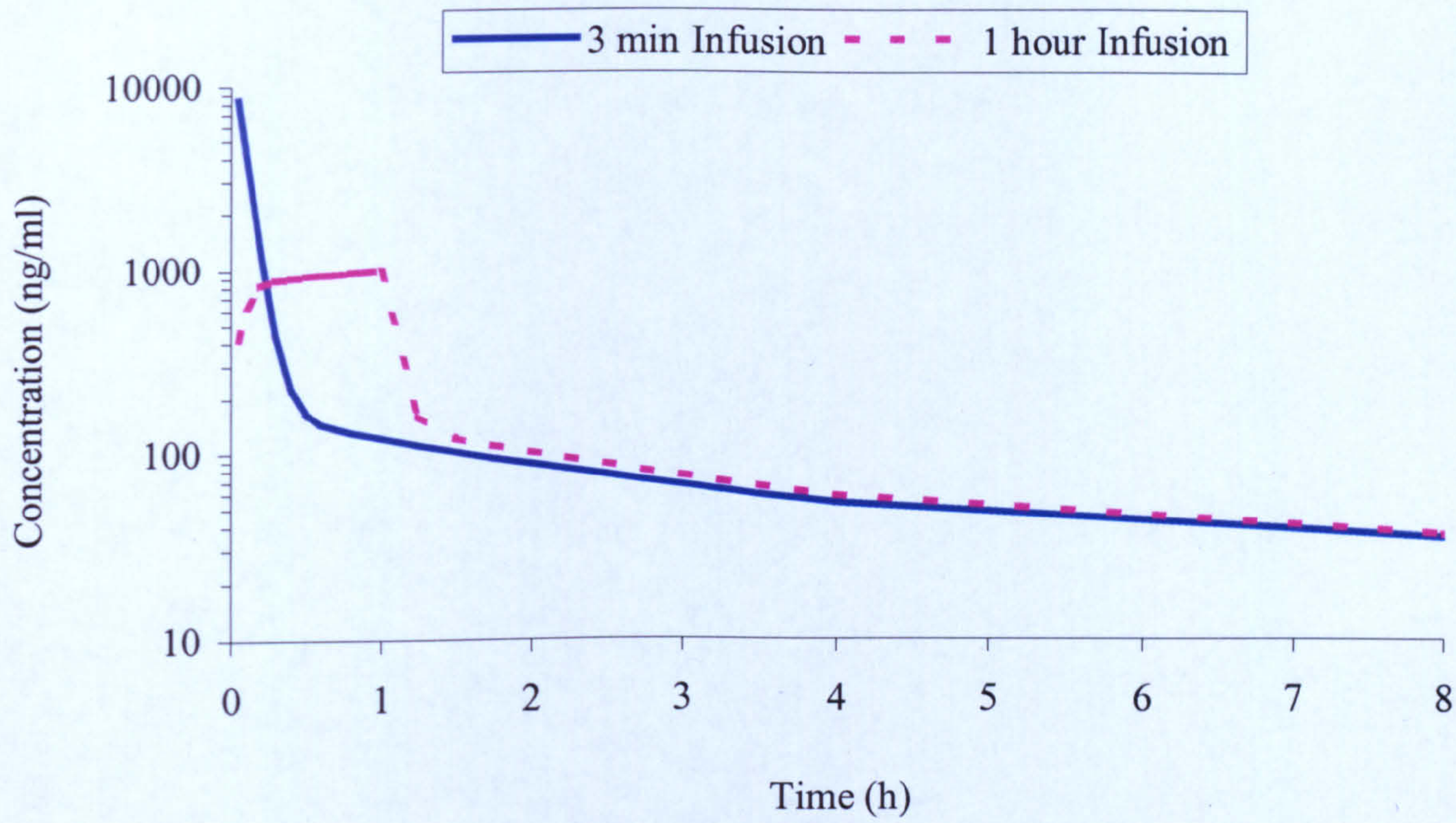
Dose-adjusted concentration time profiles in Swedish patients and UK patients with normal liver function



N.B. = Concentrations adjusted for a dose of 100 mg

Figure 7.6

Predicted epirubicin concentration-time profiles in a typical individual assuming a 100 mg dose administered as a 3 min or a 1 hour infusion



concentrations fall rapidly, approaching those following a 3 minute infusion, and by 8 h post-start of dosing, concentrations following a 1 h infusion are within 3 % of those following a 3 minute infusion.

7B.4 DISCUSSION

This section has investigated some of the potential reasons for the poor prediction of CL in the Swedish patients when the UK population model was used. The UK population model included CL as a function of $\log_e$ AST. Investigations in the Swedish data set also identified a statistically significant relationship between CL and $\log_e$ AST. Thus AST also appeared to be a suitable marker for epirubicin CL in the Swedish patients. However, on the inclusion of AST in the model for CL, the fall in inter-individual variability was smaller in the Swedish patients (-2.5 %) than that observed in the UK patients (-11 %). This is likely to be due to the smaller proportion of patients with abnormal liver function in the Swedish data set.

The population analysis in Section A showed the typical estimate of CL in the Swedish data set to be more than double that in the UK data set. It was suggested that this difference may be due to differences in the number of patients with liver dysfunction between the data sets. However, comparison of CL values in patients with normal liver function only also showed that median CL in Swedish patients was nearly 2-fold that in UK patients. As previously discussed, there is uncertainty associated with the accuracy of the individual CL values in the Swedish patients due to the sparseness of the blood samples taken. Hence, comparison of dose-adjusted concentration time data in Swedish and UK patients with normal liver function was also compared. Consistent with the analysis of CL values, graphics showed that dose-adjusted epirubicin concentrations in Swedish patients were lower than those in UK patients. This suggests that there are genuine differences in the pharmacokinetics between the two patient groups that cannot be attributed to differences in liver function alone.

A potential problem with comparing dose-adjusted concentration-time profiles is that two groups of patients were administered epirubicin for different infusion durations and this difference in itself could influence the concentration-time profiles. The median infusion time in the Swedish data sets was 1 h compared to 3 min in the UK data set. Simulation of the concentration-time profiles following a 3 minute infusion or a 1 h infusion indicated that the concentrations following either dosage regimen are

expected to be similar by 8 h post-start of infusion. Therefore, the lower concentrations measured at later time points in the Swedish patients could not be attributed to different dosage schedules *per se*.

7C ASSESSMENT OF CL ESTIMATES IN THE SWEDISH DATA SET

7C.1 INTRODUCTION

Only sparse blood samples were available in the Swedish data set (range, 2 to 6 per patient) and in 38 patients no samples were collected after 15 h post-dose. Therefore, it is likely that the estimates of CL in the Swedish data set are unreliable. In this Section, individual CL estimates were calculated from 3 different methods to assess whether the estimates were highly influenced by the method used. The 3 methods used to estimate epirubicin CL were as follows:

1) Estimated from the population analysis of the Swedish data set alone without external prior information.
2) Estimated by fixing the population parameters to those estimated from the UK data set using "Maxeval=0" option.
3) Estimated from the population analysis of the combined data set (Swedish and UK) without external prior information.

Next, in an attempt to determine which method most reliably estimated CL, a data set was simulated that mimicked the sampling times in the Swedish data set and the CL estimates from this data set using each method were compared to the true CL values. Finally, by performing another simulation analysis, the ability of each method to estimate CL if an additional 48 h blood sample had been available, as in the UK data set, was evaluated.

7C.2 METHODS

7C.2.1 Comparison of Bayesian CL estimates in Swedish data set obtained by 3 different methods

Individual Bayesian CL estimates estimated by methods 1, 2 and 3 described above were available from previous analysis performed in Section 7A.1.1, 6.2.3 and 7A.1.2, respectively. Individual Bayesian CL estimates from methods 1, 2 and 3 were compared using graphics.

7C.2.2 Simulation analysis to assess 3 methods of attaining Bayesian estimates of CL

5 new data sets containing 200 patients were simulated. The simulated data sets were intended to imitate the blood sampling times and dosage regimens used in the Swedish data set. A resampling method was used to determine blood sampling times and dosage regimens for the 200 simulated patients based on those observed in the Swedish data set. The patient identification (ID) numbers of the Swedish data set (n=79) were listed in Microsoft Excel and using the resampling option in Microsoft Excel, a new list of 200 patient ID numbers generated. For example, the new list of ID numbers could be as follows: 1, 1, 1, 2, 4, 4, 4, 4, 5, 5, 6, 6, 6, 7, 7, 7, 7, 8, 8, 9, 10, 10... etc. and was used to generate the simulated data set so that the blood sampling times with corresponding dosage history for patient 1 would be included three times, whereas patient 2 would be included once and patient 4 included four times, etc. This process was performed 5 times to generate 5 simulated data sets (called data sets A-E). Population parameter estimates attained from the previous analysis of the combined UK and Swedish data sets were entered into NONMEM and used to simulate individual pharmacokinetic parameters and concentration data for each patient in the simulated data sets using the ONLY SIM option (details are provided in Chapter 2). The simulated individual pharmacokinetic parameters were designated the 'true' pharmacokinetic parameters. The concentration data generated for each subject was transferred to a new NONMEM data file and was used to estimate CL for each subject using three different methods, i.e.

1) population analysis of the simulated data set alone using FOCE-INTER with variability terms included on CL, V_1 and Q_3 (mimics the analysis of the Swedish data set without external prior information);
2) Bayesian estimation of CL using prior information from the UK population parameters with the MAXEVAL=0 option (mimics the analysis of the Swedish data set with prior information taken as the UK population estimates);
3) combination of the simulated data set with the UK data set and population analysis of this combined data set using FOCE-INTER with variability terms included on CL and V_1 (mimics the analysis of the Swedish data set when combined with the UK data set).

The imprecision (rmse) and bias (me) for CL estimates relative to the true CL values were calculated for the 3 different methods for each of the 5 simulated data sets.

7C.2.3 Simulation analysis to assess whether inclusion of a 48 h blood sample affects the accuracy and precision of CL values estimated by 3 methods

The 5 data sets generated above (A-E) were modified to include an extra blood sample taken at 48 h post-dose in each patient. Individual PK parameters and corresponding concentrations for patients in each data set were then simulated as described previously and Bayesian CL estimates were obtained by the 3 methods described above. The imprecision (rmse) and bias (me) for the CL estimates relative to the true CL values were calculated for the 3 estimation methods for each of the 5 data sets.

7C.3 RESULTS

7C.3.1 Comparison of Bayesian CL estimates in Swedish patients obtained by 3 different methods

The individual Bayesian CL estimates in Swedish patients obtained by the 3 different methods are illustrated in Figure 7.7. There was a marked increase in the spread of the Swedish CL estimates when analysed in combination with the UK data (10-123 L/h, CV = 33 %) or when estimated using priors from the UK population model (11-113 L/h, CV = 28 %), compared to when the Swedish data set was analysed alone without external prior information on population estimates (40-92 L/h, CV = 14 %).

The median of the Bayesian CL estimates for the Swedish patients was similar when the data set was analysed separately (73 L/h) and when combined with the UK data set (69 L/h). In contrast, the median of Bayesian CL estimates in Swedish patients obtained using UK priors, was lower at 59 L/h.

7C.3.2 The accuracy and imprecision of Bayesian CL estimates attained by 3 different methods

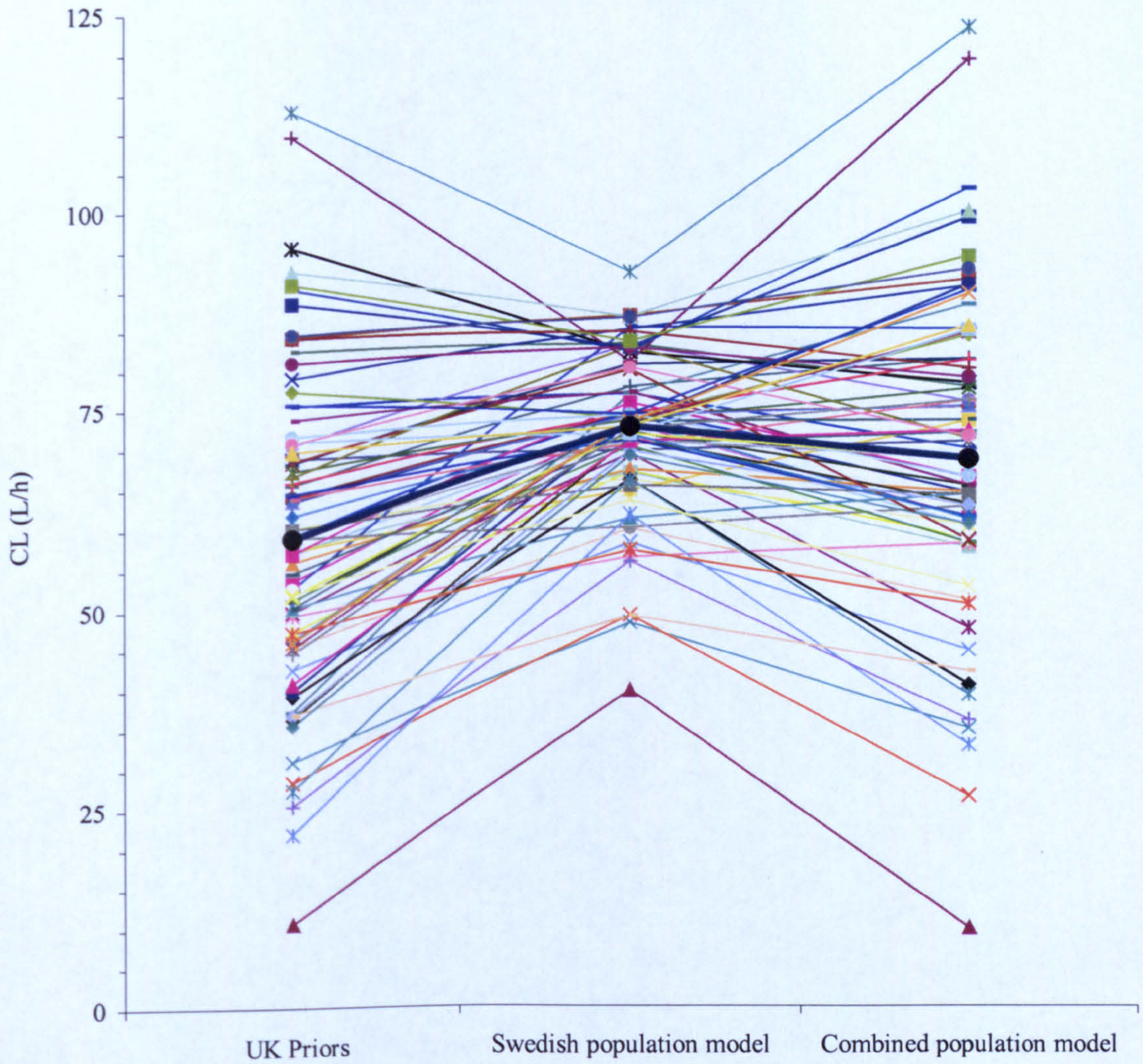
Table 7.2 lists the accuracy and imprecision of individual Bayesian CL values for the 5 simulated data sets (A-E) following estimation by 3 different methods:

1) analysis of the simulated data set alone
2) analysis using fixed external priors from the UK population model
3) analysis of the simulated data set combined with the UK data set

Individual CL values estimated by the 3 different methods are plotted against the true values in Figure 7.8. True individual CL values ranged from 5 to 475 L/h. However, to optimise visual interpretation of the data, the axis was restricted to a maximum of 200 L/h; this resulted in the exclusion of data from 10 individuals out of a total of 1000. It would seem unlikely that in practice patients would have an epirubicin CL value of greater than 200 L/h, given that the maximum CL value estimated in the Swedish data set was 123 L/h.

Figure 7.7

Bayesian CL estimates in Swedish patients obtained by 3 different methods



Key: = Median CL (L/h) for each estimation method

UK priors = analysis of Swedish data set alone using priors from the UK population model
Swedish population model = analysis of Swedish data set alone without external priors
Combined population model = analysis of Swedish data set combined with the UK data set

Table 7.2

Bias and imprecision of Bayesian CL values in simulated data sets estimated using 3 different methods

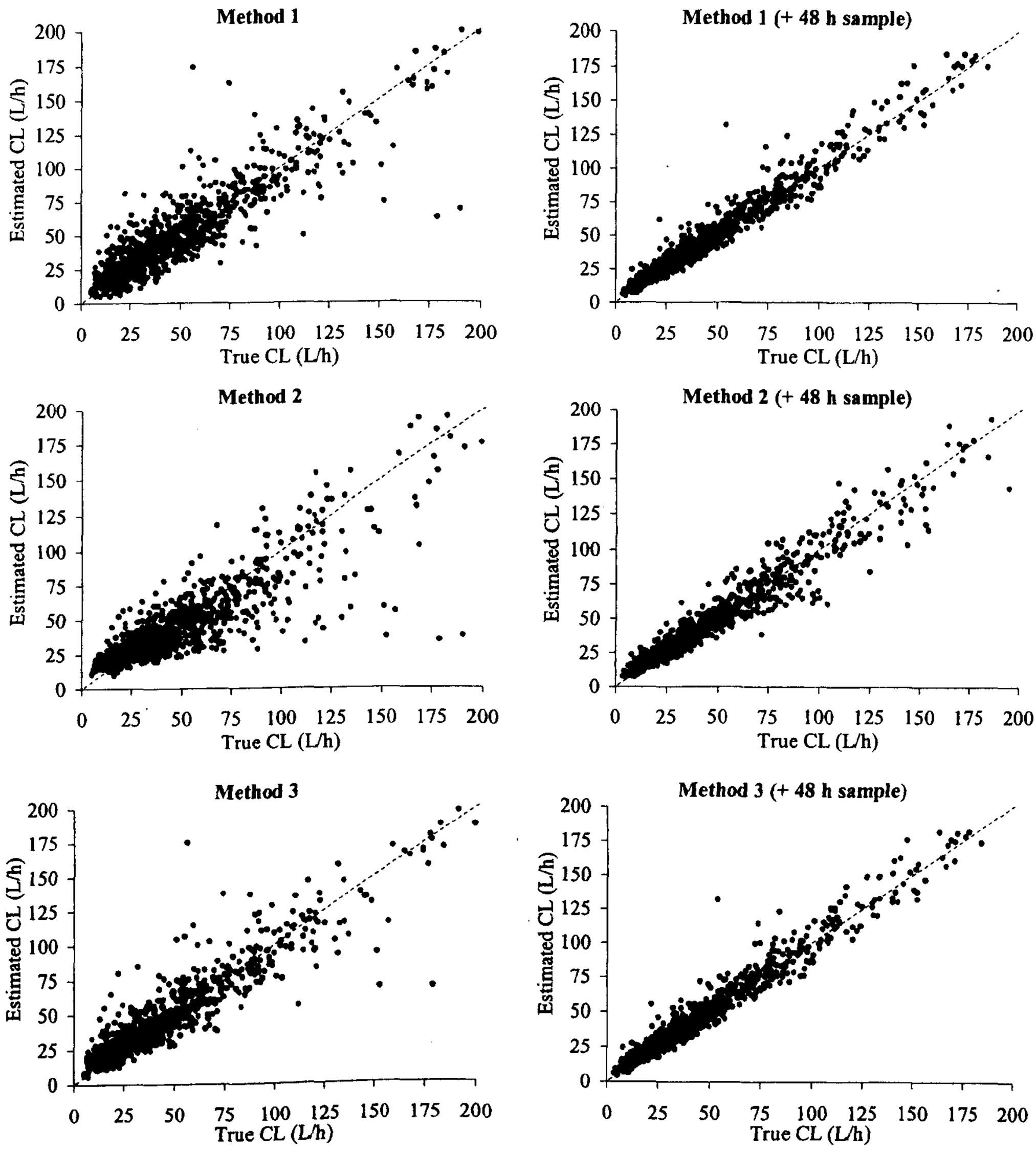
Data set No.	Method 1				Method 2				Method 3			
	me	95 % CI	rmse	95 % CI	me	95 % CI	rmse	95 % CI	me	95 % CI	rmse	95 % CI
A	26.8	(21.5, 32.1)	46.5	(39.8, 52.4)	9.82	(4.42, 15.8)	40.1	(33.4, 45.8)	12.6	(8.13, 17.0)	34.3	(28.9, 39.0)
B	-7.43	(-23.4, 8.51)	115	(98.2, 190)	9.05	(3.45, 14.7)	41.3	(32.4, 48.6)	21.2	(5.60, 36.7)	114	(94.5, 187)
C	23.3	(16.7, 29.9)	52.8	(38.3, 64.1)	7.48	(1.56, 13.4)	43.3	(34.4, 50.6)	15.5	(9.29, 21.6)	46.9	(32.4, 57.9)
D	16.4	(11.2, 21.7)	41.1	(33.3, 47.7)	3.06	(-2.02, 8.15)	36.7	(30.8, 41.8)	nc	nc	nc	nc
E	24.3	(17.1, 31.4)	56.7	(40.1, 69.4)	9.72	(3.68, 15.8)	44.5	(36.4, 51.4)	16.2	(9.81, 22.5)	48.6	(34.1, 59.7)

Key:

Method 1 = analysis of the simulated data set alone without external priors
Method 2 = analysis of the simulated data set alone using external priors from the UK population model
Method 3 = analysis of the simulated data sets when combined with the UK data sets (without external priors)
nc = not calculated; termination error
me = mean error
rmse = root mean squared error

Figure 7.8

Bayesian CL estimates for simulated data sets estimated using 3 different methods plotted against true CL values (with and without additional 48 h sample)



Key:
Method 1 = analysis of the simulated data set alone
Method 2 = analysis of the simulated data set alone using external priors from the UK population model
Method 3 = analysis of the simulated data sets when combined with the UK data set
Dotted line represents line of unity

Estimation of CL using Method 1 resulted in estimates of CL that were positively biased (me) by approximately 20 % in 4 out of 5 simulations. The imprecision was high with a median rmse of 53 %. Estimation of CL using Method 3 resulted in only a slight improvement in the median bias (16 %) and imprecision (48 %); however, one of the data sets did not successfully converge using this method. CL was poorly estimated for data set B using both method 1 and method 3, with imprecisions of 115 and 114 %, respectively. This unusually high imprecision was due to a poor estimation of CL in one individual. Removal of this individual reduced the imprecision to 39 % and 43 % respectively.

Method 2 resulted in CL estimates with a median bias of 9 % and median imprecision of 41 %. These results were more consistent than those obtained with the other methods as no unusually high imprecision values were observed. However, examination of the plots estimated CL against 'true' CL suggested that Method 2 overestimated low CL values and underestimated high CL values (Figure 7.8).

7C.3.3 Simulation analysis to assess whether inclusion of a 48 h blood sample affects the accuracy and precision of CL values estimated by 3 methods

Table 7.3 shows the accuracy (me) and imprecision (rmse) of CL values when estimated using the 3 different methods. On the inclusion of the 48 h sample, all 3 methods gave CL estimates with similar bias and imprecision. Median bias in the CL estimates was approximately 6-8 % and the imprecision was 22-24 %, which is approximately half that observed without the 48 h value.

Table 7.3

Bias and imprecision of Bayesian CL estimates obtained using 3 different methods when an additional 48 h sample was included

Data set No.	Method 1				Method 2				Method 3			
	me	95 % CI	rmse	95 % CI	me	95 % CI	rmse	95 % CI	me	95 % CI	rmse	95 % CI
A	6.21	(3.65, 8.78)	19.5	(16.2, 22.3)	5.98	(2.81, 9.14)	23.6	(18.5, 27.7)	4.86	(2.34, 7.37)	18.8	(15.6, 21.5)
B	9.40	(6.27, 12.5)	24.4	(15.3, 30.9)	6.49	(3.51, 9.46)	22.4	(17.8, 26.1)	7.41	(4.45, 10.4)	22.5	(15.5, 27.8)
C	7.73	(4.67, 10.8)	23.4	(10.2, 31.5)	6.01	(2.25, 9.77)	27.7	(17.6, 35.0)	6.49	(3.52, 9.46)	22.3	(8.52, 30.4)
D	6.17	(3.41, 8.92)	20.7	(15.8, 24.7)	5.08	(1.87, 8.29)	23.6	(16.9, 28.8)	5.25	(2.58, 7.92)	19.9	(15.3, 23.6)
E	10.5	(7.09, 13.8)	26.4	(19.4, 31.9)	10.1	(6.61, 13.5)	26.9	(21.3, 31.5)	9.12	(5.78, 12.5)	25.7	(18.6, 31.2)

Method 1 = analysis of the simulated data set alone without external priors
Method 2 = analysis of the simulated data set alone using external priors from the UK population model
Method 3 = analysis of the simulated data sets when combined with the UK data sets
me = mean error
rmse = root mean squared error

7C.4 DISCUSSION

Bayesian estimation of individual CL values in Swedish patients, estimated using prior information from the UK population model or when the data set was combined with the UK data set resulted in CL estimates with a CV % that was approximately double that obtained when estimated without prior information. This suggests that when the Swedish data set was analysed alone without external prior information on the population estimates, due to the sparseness of the blood samples per patient, the individual estimates of CL shrank towards the population estimate. However, Bayesian CL estimates calculated using prior UK estimates were, on average, lower than estimates attained using the other two methods. An evaluation of vancomycin dosage guidelines found that individual CL estimates of vancomycin varied considerably depending on the population parameters taken as priors in the analysis (Thomson & Sie; 2003). Thus, using the PK parameter estimates from the UK data set may have resulted in underestimates of CL as the population estimate for CL from the UK data set was low relative to values reported in the literature (Plosker & Faulds 1993).

A simulation analysis showed that all three methods gave poor results in terms of bias and precision of the CL estimates. The least biased (9 %) and least imprecise (41 %) results were obtained when CL was estimated by fixing the population values to those estimated for the UK data set, and it was this method of CL estimation that was used in the validation of the UK model (Chapter 6). Further simulation analysis confirmed that the imprecision and bias would have been greatly reduced if an additional blood sample had been taken at 48 h. If a 48 h sample had been taken, the accuracy and precision of the 3 methods in the estimation of epirubicin CL was similar. Thus it appears that the difficulties in attaining accurate and precise estimates of CL for individual patients are more greatly affected by the timing of the blood samples in those individuals than the method used to attain the estimates. It was noted that all methods over estimated CL in the Swedish patients, however, the 2 fold differences in CL observed between the two data sets can not be accounted for the 9 % bias observed in the CL estimates for the Swedish patients.

CHAPTER 8

POPULATION ANALYSIS OF COMBINED UK AND SWEDISH DATA SETS

8.1 INTRODUCTION TO CHAPTER

The aim of this Chapter was to perform a population analysis on the combined UK and Swedish data sets to investigate whether the increase in patient numbers enables identification of any further covariates that influence epirubicin pharmacokinetics.

8.2 METHODS

8.2.1 Generation of the data set

The UK and Swedish data sets were combined and included all covariates that were available for both data sets i.e.: bilirubin, AST, albumin, height, weight, dose, age, creatinine clearance and BSA. An additional covariate, 'study', was included to identify the source of the patient data: patients from the UK study were coded as '0' and patients from the Swedish study were coded as '1'.

8.2.2 Model building

A 3 compartment model was fitted to the data and analysis was performed using NONMEM with the first order method. This approach maximised the number PK parameters for which inter-individual variability could be estimated. The inclusion of a full covariance matrix was included if variance-covariance terms could be reliably determined and if judged appropriate from the OFV. Proportional, additive and combined residual error models were evaluated using OFVs and graphics.

Covariates were plotted against individual pharmacokinetic parameters. Covariates that appeared to have a relationship with a PK parameter were subsequently included in the model building analysis with NONMEM. Covariates were considered to have a statistically significant influence on a PK parameter if the fall in the OFV was greater than 10.83, which corresponds to a p value of < 0.001 for one degree of freedom.

8.3 RESULTS

8.3.1 Selection of the basic model

The combined data set included 105 patients from the UK data set and 79 patients from the Swedish data set. Inter-individual variability on V_2 was indeterminate and consequently this parameter was removed from the model. Covariance was restricted to diagonals as the relative standard error associated with variance-covariance terms was high (60-240 %), indicating that they could not be reliably estimated. The best residual error model was the combined error model, which resulted in a fall in the OFV of 16 relative to the proportional error model. The additive residual error model did not converge successfully. When model predicted concentrations were plotted against measured concentrations, they were randomly and closely distributed around the line of unity suggesting that the model adequately fitted the data (Figure 8.1). However, a slight positive bias was observed at 48 h post-dose when weighted residuals were plotted versus time indicating that the model slightly under predicted concentrations at later time points. Weighted residuals were equally and randomly scattered around zero when plotted against predicted concentrations, indicating that the residual error model fitted the data well. The parameter estimates for the basic model are given in Table 8.1. The RSEs for the PK parameters ranged from 5 to 15 % indicating that they were estimated with a good degree of precision. The RSEs for the estimates of inter-individual variability terms were generally less than 30 %, with the exception of ωV_1 for which the RSE was 66 %, indicating that this parameter was estimated with less certainty.

8.3.2 Model building with covariates

Scatter plots of CL versus covariates are illustrated in Figure 8.2. The following covariates appeared to have a positive correlation with CL: albumin; height; and creatinine clearance. The following covariates appeared to have a negative correlation with CL: bilirubin; AST; and age. For the other pharmacokinetic parameters (V_1, Q_2, Q_3 and V_3), there were no parameter-covariate relationships identified from the scatter plots (data not shown). Box plots of CL, V_1, Q_2, Q_3 and V_3 for the UK and Swedish data sets are illustrated in Figure 8.3. The median CL in Swedish patients was higher than the median CL in UK patients, consistent with that observed in Chapter 7 using

Figure 8.1

Goodness of fit plots for combined UK and Swedish data sets using the first order method

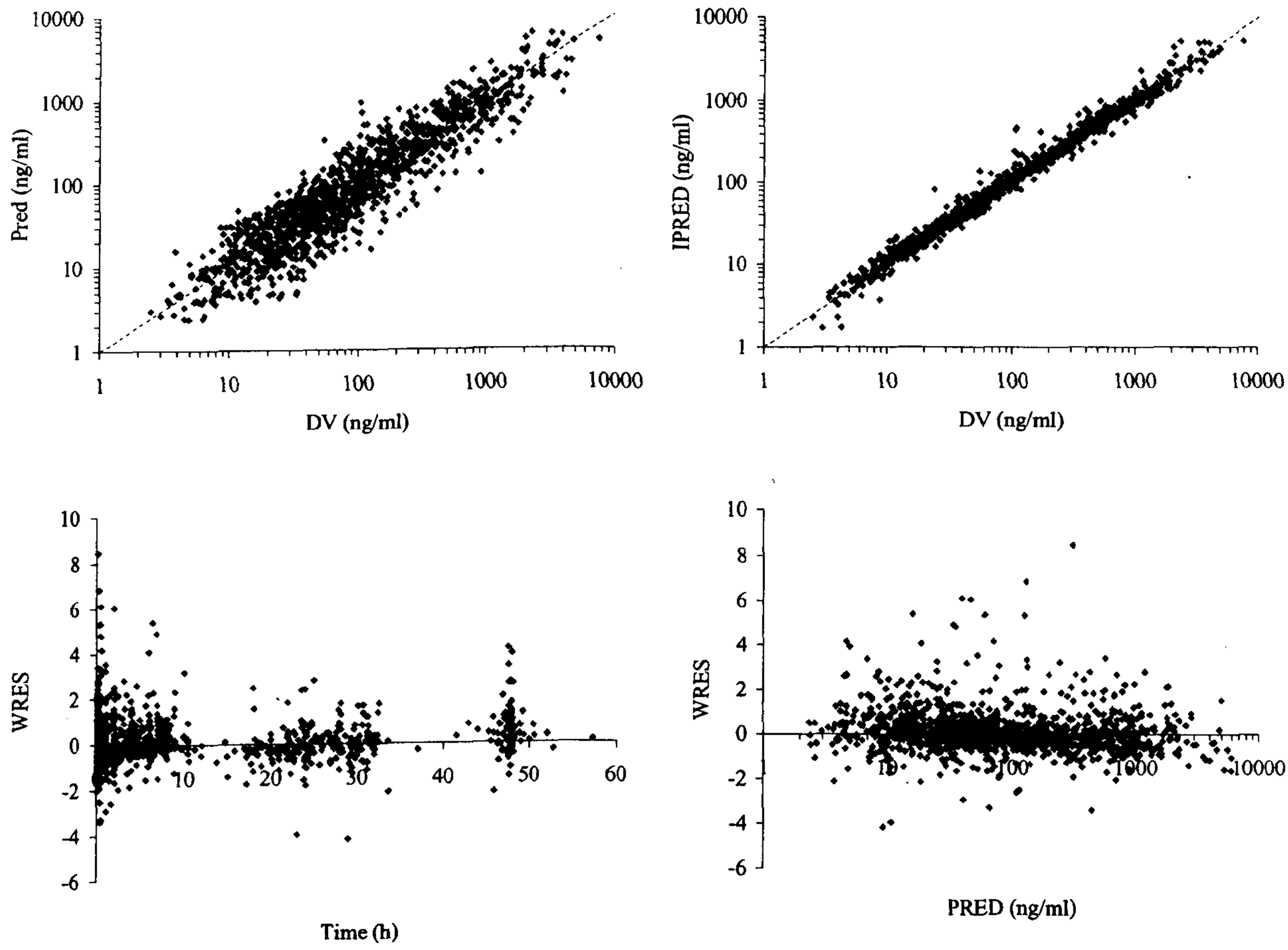


Table 8.1

Population parameter estimates for the combined UK and Swedish data sets calculated using the first order method

Parameter	Estimate	RSE (%)
CL (L/h)	40.0	6.88
V_1 (L)	12.3	9.67
Q_2 (L/h)	38.5	12.0
V_2 (L)	41.8	15.0
Q_3 (L/h)	65.7	5.27
V_3 (L)	953	10.4
ωCL (%)	85.0	23.8
ωV_1 (%)	41.2	66.5
ωQ_2 (%)	60.4	27.7
ωV_2 (%)	-	-
ωQ_3 (%)	40.2	21.5
ωV_3 (%)	81.9	29.2
Additive σ	2.62	33.4
Proportional σ (%)	29.0	9.41

Figure 8.2

Scatter plots of individual estimates of CL versus clinical characteristics

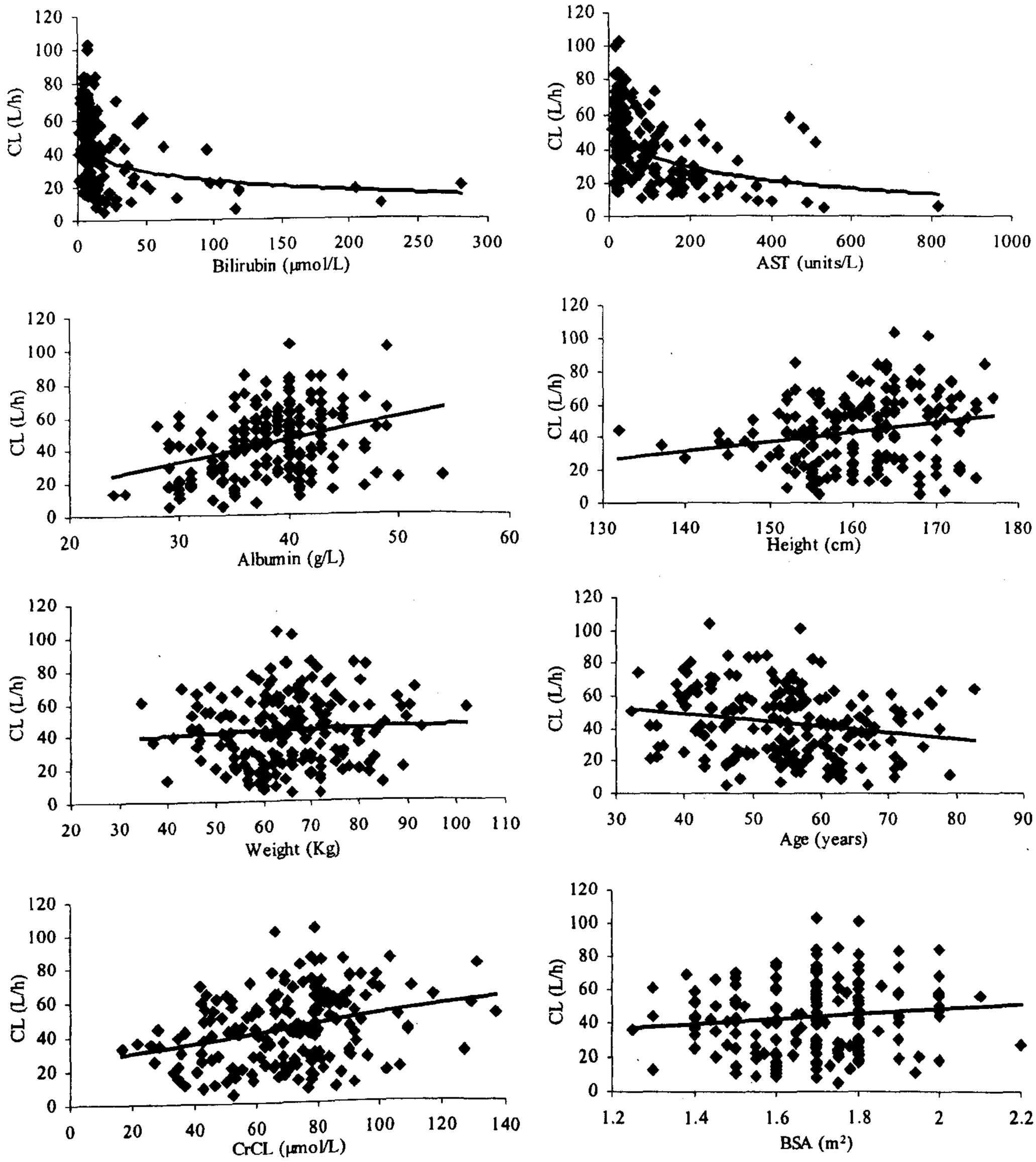
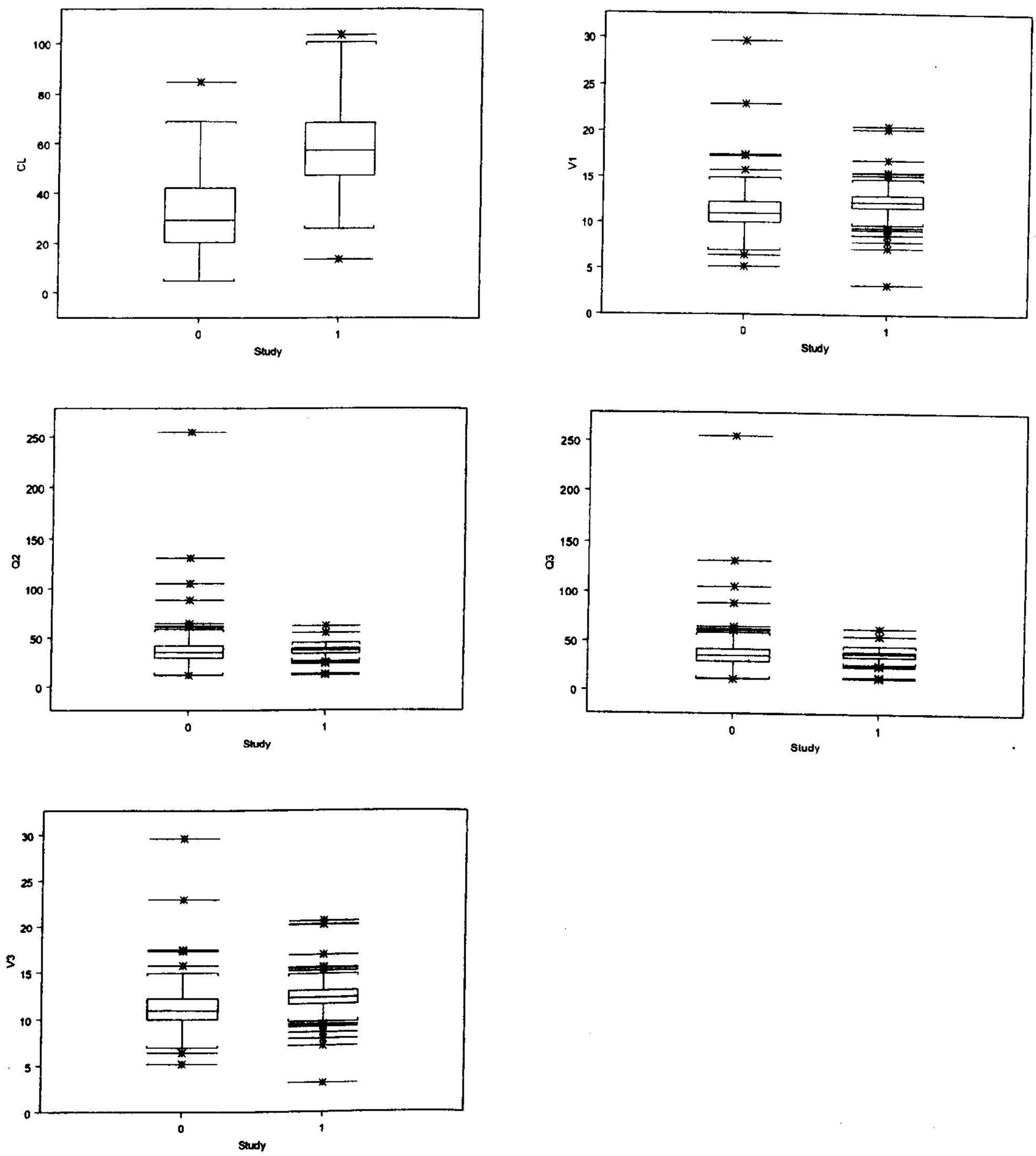


Figure 8.3

Box-plots of CL, V_1, Q_2, Q_3, and V_3 in UK and Swedish patients



Key: UK study = 0; Swedish study = 1

FOCE-INTER. Median values of Q_2 and Q_3 were similar in the UK and Swedish data sets, whereas median values of V_1 and V_3 were slightly higher in the patients from the Swedish data set.

A summary of the OFVs obtained for the models tested for CL using NONMEM is given in Table 8.2. Creatinine clearance (CrCL) data were not available for patients 223 and 228 (UK data set), thus the influence of CrCL was tested in a reduced data set in which these patients were removed. Inclusion of ln AST, study, height, ln bilirubin and CrCL in the model for CL resulted in a statistically significant improvement in the model as determined by the OFV.

Inclusion of study into models V_1 and V_3 resulted in a statistically significant fall in the OFV, with the largest improvement observed for V_1 (Table 8.3). Inclusion of study on both V_1 and V_3 did not result in a statistically significant fall in the OFV compare to inclusion on V_1 only.

The full model included CL as a function of ln AST, study, height, ln bilirubin and CrCL and V_1 as a function of study. The inclusion of AST and study in the model reduced inter-individual variability in CL from 85 % to 54 % and 41 %, respectively. In contrast, the inclusion of height, bilirubin and CrCL in the CL model accounted for no variability in CL and the inclusion of study in the model for V_1 reduced the inter-individual variability by only 1 %. The influence of these covariates in the population model for epirubicin was therefore deemed to be of little clinical significance. Thus, the final model included CL as a function of study and a natural log function of AST:

CL=75.9*(1-(0.132*LnAST)) and if study is Swedish * 1.57

Estimates of parameters from the final model are given in Table 8.4. Figure 8.4 illustrates the fit of the final population model to the individual CL estimates.

Table 8.2

Summary of clearance models tested using the combined data set

Model No.	CL Model	OFV	Delta OFV	cf model No.
No Covariates				
1	Basic model	11000.43		-
1 Covariate				
2	**Ln AST**	**10793.40**	**-207.03**	**1**
3	**Ln Bili**	**10936.61**	**-63.82**	**1**
4	**Study**	**10937.19**	**-63.24**	**1**
5	**HGT**	**10973.48**	**-26.94**	**1**
6	**Alb**	**10977.44**	**-22.99**	**1**
7	Basic model#	10803.09	-	-
8	**CrCL#**	**10788.64**	**-14.45**	**7**
9	**Age**	**10987.24**	**-13.19**	**1**
10	BSA	10992.88	-7.55	1
2 Covariates				
11	**Ln AST + Study**	**10754.76**	**-38.64**	**2**
12	**Ln AST + Alb**	**10764.16**	**-29.24**	**2**
13	**ln AST + Ln Bili**	**10769.73**	**-23.67**	**2**
14	**Ln AST + HGT**	**10776.26**	**-17.14**	**2**
15	Ln AST#	10793.40	-	-
16	Ln AST + CrCL#	10785.44	-7.96	15
17	Ln AST + BSA	10785.73	-7.68	2
18	Ln AST + Age	10787.64	-5.76	2
3 Covariates				
19	**Ln AST + Study + Hgt**	**10712.47**	**-42.29**	**11**
20	**Ln AST + Study + Ln Bili**	**10727.79**	**-26.97**	**11**
21	**Ln AST + Study + Alb**	**10731.35**	**-23.41**	**11**
22	**Ln AST + Study + BSA**	**10738.91**	**-15.85**	**11**
23	Ln AST + Study#	10591.22	-	-
24	Ln AST + Study + CrCL#	10587.86	-3.36	23
25	Ln AST + Study + Age	10753.68	-1.08	11
4 Covariates				
26	**Ln AST + Study + Hgt + Ln Bili**	**10692.63**	**-19.85**	**19**
27	Ln AST + Study + Hgt#	10550.01	-	-
28	**Ln AST + Study + Hgt + CrCL#**	**10531.89**	**-18.12**	**27**
29	**Ln AST + Study + Hgt + Alb**	**10698.87**	**-13.60**	**19**
30	Ln AST + Study + Hgt + Age	10704.04	-8.43	19
31	Ln AST + Study + Hgt + BSA	10712.32	-0.16	19
5 Covariates				
32	Ln AST + Study + Hgt + Ln Bili#	10533.70	-	-
33	**Ln AST + Study + Hgt + CrCL + Ln Bili#**	**10514.05**	**-19.65**	**32**
34	**Ln AST + Study + Hgt + Ln Bili + Age**	**10678.69**	**-13.94**	**26**
35	Ln AST + Study + Hgt + Ln Bili + Alb	10681.85	-10.77	26
36	Ln AST + Study + Hgt + Ln Bili + BSA	10692.44	-0.19	26
6 Covariates				
37	Ln AST + Study + Hgt + CrCL + Ln Bili + Alb	10504.52	-9.53	33
38	Ln AST + Study + Hgt + CrCL + Ln Bili + BSA	10507.77	-6.28	33
39	Ln AST + Study + Hgt + CrCL + Ln Bili + Age	10512.78	-1.27	33

KEY. Alb = Albumin; AST = Aspartate aminotransferase; Bili = Bilirubin; BSA = Body surface area; CrCL = Creatinine clearance; Hgt = Height;
= patients 223 and 228 were excluded due to missing creatinine data
Bold text indicates models that gave a statistically significant improvement

Table 8.3

Summary of volume models tested using the combined data set

Model No.	Model	OFV	Delta OFV	cf model No.
V_1 model				
40	**Study**	**10479.16**	**-34.89**	**33**
V_3 model				
41	**Study**	**10490.41**	**-23.64**	**33**
V_1 + V_3 model				
42	V_1 + Study and V_3 + Study	10474.09	-5.07	40

Table 8.4

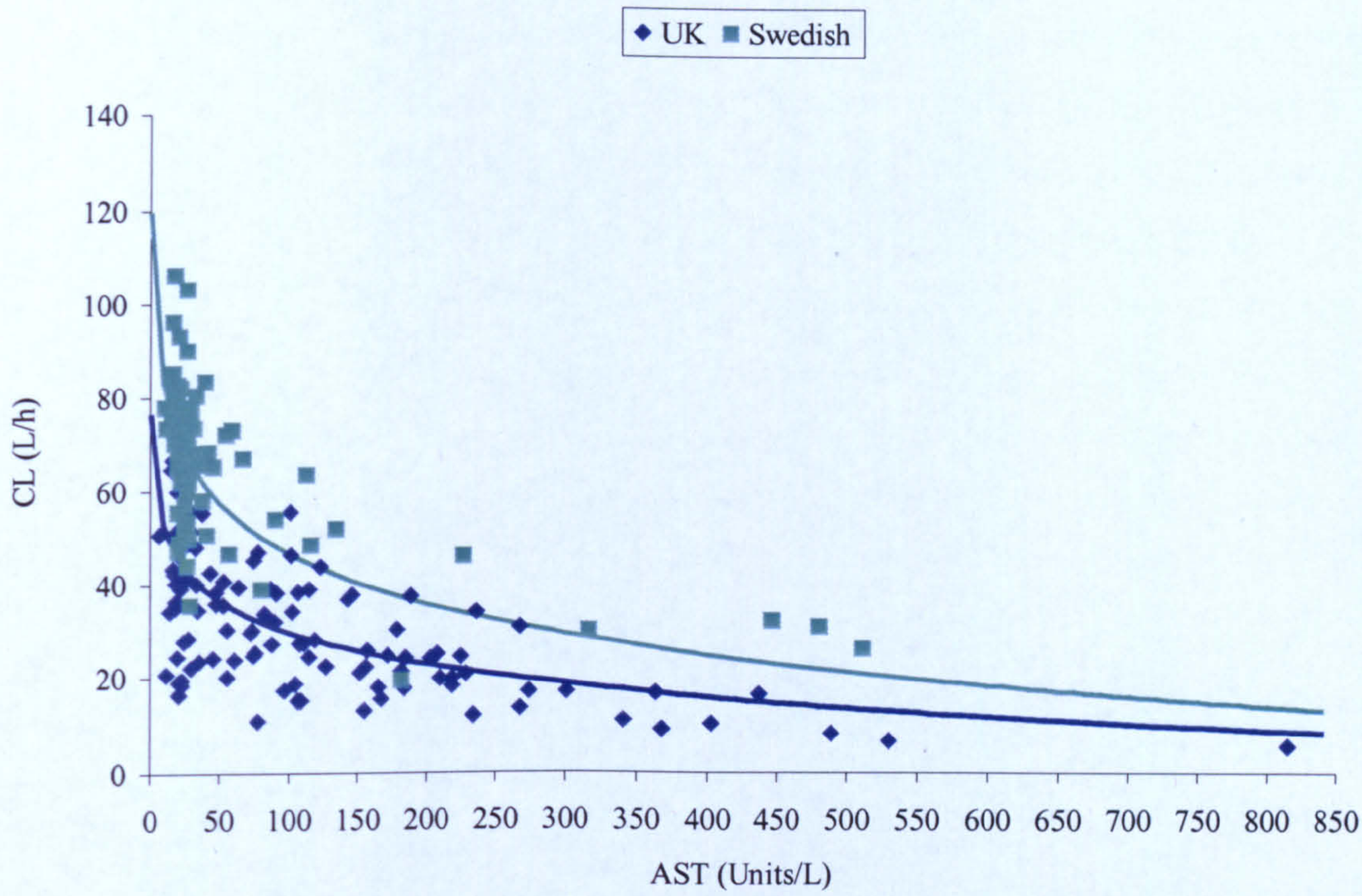
Population parameter estimates from final model

		Final	
		Estimate	RSE (%)
CL **(L/h)	θ_1	75.9	10.7
V_1 (L)	θ_2	11.5	16.5
Q_2 (L/h)	θ_3	38.2	11.6
V_2 (L)	θ_4	43.1	17.7
Q_3 (L/h)	θ_5	58.5	5.06
V_3 (L)	θ_6	833	10.7
Ln AST (CL)	θ_7	0.132	6.40
Study (CL)	θ_8	1.57	12.1
ωCL (%)		41.2	23.9
ωV_1 (%)		61.5	76.7
ωQ_2 (%)		52.6	33.4
ωQ_3 (%)		36.7	22.8
ωV_3 (%)		67.6	20.5
σ additive		1.15	76.0
σ proportional (%)		24.6	11.1

** CL=75.9*(1-(0.132*LnAST)) if Study = Swedish * 1.57

Figure 8.4

Individual CL estimates fitted with the final population model



8.4 DISCUSSION

A population analysis was performed using the combined UK and Swedish data sets to investigate whether any further covariates could be identified as influencing epirubicin pharmacokinetics following the increase in patient numbers. Previously, (Chapter 7) CL was shown to be higher in the Swedish patients than the UK patients. In Chapter 7, population analysis was performed using FOCE-INTER, however, using this method of estimation, inter-individual variability could only be estimated for CL and V_1. As it was of interest to see whether other PK parameters differed between the two data sets, in this Chapter the FO method was used as convergence problems were less frequent enabling estimates of inter-individual variability to be obtained for CL, V_1, Q_2, Q_3 and V_3.

The population value of CL in the Swedish data set was estimated to be 1.57-fold greater than the population CL value in the UK data set. No differences in other PK parameters between the two data sets were observed. Despite the increase in number of patients from 105 to 184, analysis of the combined data set identified AST as the only clinical factor that significantly influenced CL. The relationship between CL and AST identified from the combined analysis was:

CL = 75.9*(1 - (0.132*LnAST)) if study = Swedish * 1.57

which is very similar to that identified from analysis of the UK data set alone ie.

CL = 72.9*(1 - (0.135*LnAST)).

Another advantage of the FO method is that the run times for each model are substantially less than those using the FOCE method eg. approximately 2 minutes compared to 1 hour. The number of covariates tested in the analysis of the combined data sets was less than in the original analysis of the UK data set alone, as the Swedish data set did not contain information on presence/absence of liver metastases, bone metastases, prior chemotherapy or endocrine treatment, performance status and alkaline phosphatase. The reduced number of covariates and reduced run times meant that screening of parameters of interest to reduce NONMEM analysis time was less crucial, and therefore, all covariates that appeared to have any relationship (even a very weak one) with a PK parameter from the plots were tested in the model building analysis with NONMEM. This is advantageous over screening methods which could result in the loss of potentially significant covariates. A disadvantage of using FO is

that parameter estimates have previously been shown, in some cases, to be more biased than those estimated using FOCE (Beal & Sheiner 1992). The parameter estimates using FO were higher than those previously estimated using FOCE-INTER (Tables 7.1 and 8.1), with the largest differences in Q_2, Q_3 and V_3.

A simulation analysis that compared the change in OFV on the addition of covariates, hence the apparent significance level to the actual significance level, indicated that the FO method tends to over estimate the significance level of covariates, and therefore, is more likely to result in false covariates being included in the model than if FOCE is used (Wählby et al. 2001). The apparent significant level was increased to 0.001 (corresponding to a change in the OFV of 10.83) in this analysis to reduce the risks of inclusion of false covariates. However, despite this increase in the stringency of the inclusion criteria, 5 of the 8 covariates were found to have a statistically significant relationship with CL whereas examination of inter-individual variability accounted for by each covariate showed that only 2 of these covariates appeared to be of any clinical relevance. Despite the disadvantages of using the FO method, the conclusions drawn from this analysis were entirely consistent with those from the previous analysis of the UK data set using FOCE.

In contrast, a covariate analysis of the Swedish data set previously included albumin and bilirubin in the CL model but not AST (Sandstrom 2002). In Chapter 3, AST was found to be correlated with both albumin and bilirubin. It has previously been reported that if covariates 'A' and 'B' are correlated with each other, one study can identify covariate 'A' as being important whereas a study with a different data set may identify covariate 'B' as being important (Bonate 1999). Both albumin and bilirubin were identified as influencing epirubicin CL in the combined analysis, but the relationship was weaker than that observed for AST and once AST was included in the model, the addition of bilirubin and albumin offered no further improvement.

CHAPTER 9

DEVELOPMENT OF A LIMITED SAMPLING STRATEGY

9.1 INTRODUCTION TO CHAPTER

The previous population analysis showed that there is a large amount of inter-patient variability in CL of epirubicin and identified AST as a covariate that influenced CL (Chapter 3). However, only a small proportion of the variability in CL was accounted for by AST and the model poorly predicted CL in two external data sets. Thus, it appears that investigation of an alternative approach for dose-individualisation is warranted. *A posteriori* dose adjustment requires blood samples to be taken from a patient so that individual pharmacokinetic parameters can be determined and used to adjust the next dose to achieve the target systemic exposure. In practice, taking a large number of blood samples would not be feasible, therefore, this Chapter investigates whether epirubicin CL can be adequately calculated from a small number of blood samples.

This Chapter is split into 4 sections. The aim of Section 9A was to identify a limited sampling design that would achieve good estimates of epirubicin CL. D-optimality was used to identify 6 optimum blood sampling times for characterisation of epirubicin pharmacokinetics. Limited sampling designs including 2 or 3 blood samples in conjunction with MAP Bayesian estimation were then investigated, giving particular attention to sampling times that would be practical in a routine clinical setting. Predictive performances were assessed by comparing MAP Bayesian estimates of CL from the limited sampling designs with estimates of CL that were obtained using all the available concentration data. In addition an external data set was used to validate the limited sampling designs.

In Section 9B a simulation analysis was performed to assess the sensitivity of the best limited sampling designs to sample time recording errors of 0-10 % or 10-20 %.

As Section 9B indicated that the Bayesian estimates of epirubicin CL were biased, Section 9C investigates the potential reasons for the observed bias.

A second simulation analysis was performed in Section 9D to assess the imprecision and bias attained using the limited sampling designs previously developed using multiple linear regression (Jakobsen et al. 1991a).

9A. DEVELOPMENT AND VALIDATION OF LIMITED SAMPLING DESIGNS TO ESTIMATE EPIRUBICIN CL

9A.1. METHODS

9A.1.1. Identification of optimum sampling times

Six optimal sampling times that could define the pharmacokinetics of epirubicin were identified by D-optimality using Adapt II (D'Argenio & Schumitzky 1997). The pharmacokinetic parameters entered were those previously identified by population analysis of the UK data set using the basic model (no covariates). The intravenous input was entered as a bolus or as an infusion lasting either 3 or 19 minutes. These inputs were chosen as they represent the median infusion time (3 minutes) and the minimum and maximum infusion times for the data set. The slope was entered as 0.2 as residual error was previously estimated to be approximately 20 %. The blood sampling times were restricted to be between the end of infusion and 48 h post-dose.

9A.1.2 Assessment of limited sampling designs

10 limited sampling designs labelled A to J consisting of 2 or 3 blood samples were selected based on the times identified from Adapt II and practical issues. The UK data set (105 patients) was truncated to include the sampling times for each of the designs. Blood samples closest to the selected time were used and if two blood samples were equally close to the selected time, the earlier sampling time was used. If a patient did not have a blood sample taken within 33 % of the selected sampling time, their data were excluded from the analysis for that design. Bayesian estimates of CL for individuals in each of the truncated data sets were estimated with NONMEM using the MAXEVAL=0 option in the $ESTIMATION step. The population estimates for the PK parameters, interindividual variability and residual error determined previously with the basic model (Chapter 3) were used in the estimation. The 'true' CL values were taken as the individual estimates obtained from the NONMEM FOCE-INTER analysis that was conducted using the full data set. CL estimates from the truncated data sets were compared to the 'true' estimates of CL using scatter plots and by calculating the relative me and rmse with 95 % CI (previously described in Chapter 2).

9A.1.3 Selection of optimum sampling times using the minimum and maximum CL estimates in the data set

The pharmacokinetic parameter values estimated for the individuals with the minimum and maximum CL values in the data set were used in Adapt II to identify six optimal sampling times for each patient. The intravenous input was entered as an infusion lasting 3 minutes. The slope was entered as 0.2 and blood sampling times were restricted to be between the end of infusion and 48 h post-dose, as previous.

9A.1.4 Validation of limited sampling designs using a new patient group

A data set containing 18 patients with hepatocelluar carcinoma or breast cancer, described in Chapter 5, was used to validate the 10 proposed limited sampling designs. Ten new truncated data sets were produced that included the sampling times described for Designs A to J. Bayesian estimates of CL were obtained for each of the truncated data sets with NONMEM using the MAXEVAL = 0 option, with PK parameters fixed to those previously estimated for the UK population model. The 'true' CL values were taken as the individual estimates obtained from the NONMEM FOCE-INTER analysis that was conducted using the full data set in Chapter 5. Bayesian estimates of CL estimated using the limited sampling designs were compared to the 'true' estimates of CL using scatter plots and by calculating the relative me and rmse with 95 % CI, as described previously.

9A.2. RESULTS

9A.2.1 Identification of optimum sampling times

The 6 optimal sampling times identified following administration of epirubicin as a bolus injection, as a 3 minute infusion and as a 19 minute infusion are given in Table 9.1. The duration of the infusion had very little effect on the later blood sample times and only a modest influence on the earlier optimal times, so the limited sampling designs were based on the optimum times identified for a 3 minute (0.05h) infusion. The same times post-start of infusion were therefore selected in each patient regardless of the length of infusion received. As the first blood samples available in each patient ranged from between 0.1 and 0.4 hours post-dose, the first 2 optimum times identified (end of infusion and 0.3 h) were combined and represented by the first available blood sampling time in the patient. Ten limited sampling designs (2 or 3 blood samples) were proposed using different combinations of the first available sample and the 0.7, 3, 10 and 48 h samples. These are given in Table 9.2. Designs A and B were selected as they include blood samples taken within the first 3 hours and thus would cause minimum inconvenience to the patient. Designs C to G included a 48 h time point to test if a later time point would improve the estimates of CL. Design H was proposed because if the 48 h sample fell at the weekend it may be more convenient to take a 24 h sample. The 10 h sample would cause the most inconvenience to patients and hospital staff as this would be required to be taken during the evening or night; consequently, designs I and J were proposed to test if a 10 h sample offered any improvement in the estimates of CL.

9A.2.2. Assessment of limited sampling designs

Scatter plots of the CL values estimated from the truncated data sets were plotted against the estimates from the full data set (Figure 9.1). The me and rmse estimates with corresponding 95 % confidence intervals for each of the sampling designs are given in Table 9.3. Designs C, D, E and J were unbiased and had the lowest rmse values (9.06 to 10.7 %) indicating that the CL estimates were the most similar to those obtained from the full data set. These designs all contained 3 blood samples, including an early sample (either the first available sample or 0.7 h) and a 48 h sample.

Table 9.1

Optimum sampling times (h) selected following administration of epirubicin as a bolus injection, as a 3 minute infusion and as a 19 minute infusion

sample number	bolus	3 minute infusion	19 minute infusion
1	EOI	EOI	EOI
2	0.3	0.3	0.5
3	0.6	0.7	0.9
4	3.0	3.0	3.2
5	10.4	10.4	10.6
6	48.0	48.0	48.0

EOI = End of infusion

Table 9.2

Limited sampling designs

Design	Selected Times		
A	1st	3 h	
B	1st	0.7 h	3 h
C	1st	3 h	48 h
D	1st	0.7 h	48 h
E	0.7 h	3 h	48 h
F	1st	48 h	
G	3 h	48 h	
H	1st	3 h	24 h
I	1st	3 h	10 h
J	1st	10 h	48 h

1st = first available blood sample

Figure 9.1 (1 of 3)

Scatter plots of the CL estimates from the truncated data sets versus CL estimates from full data set

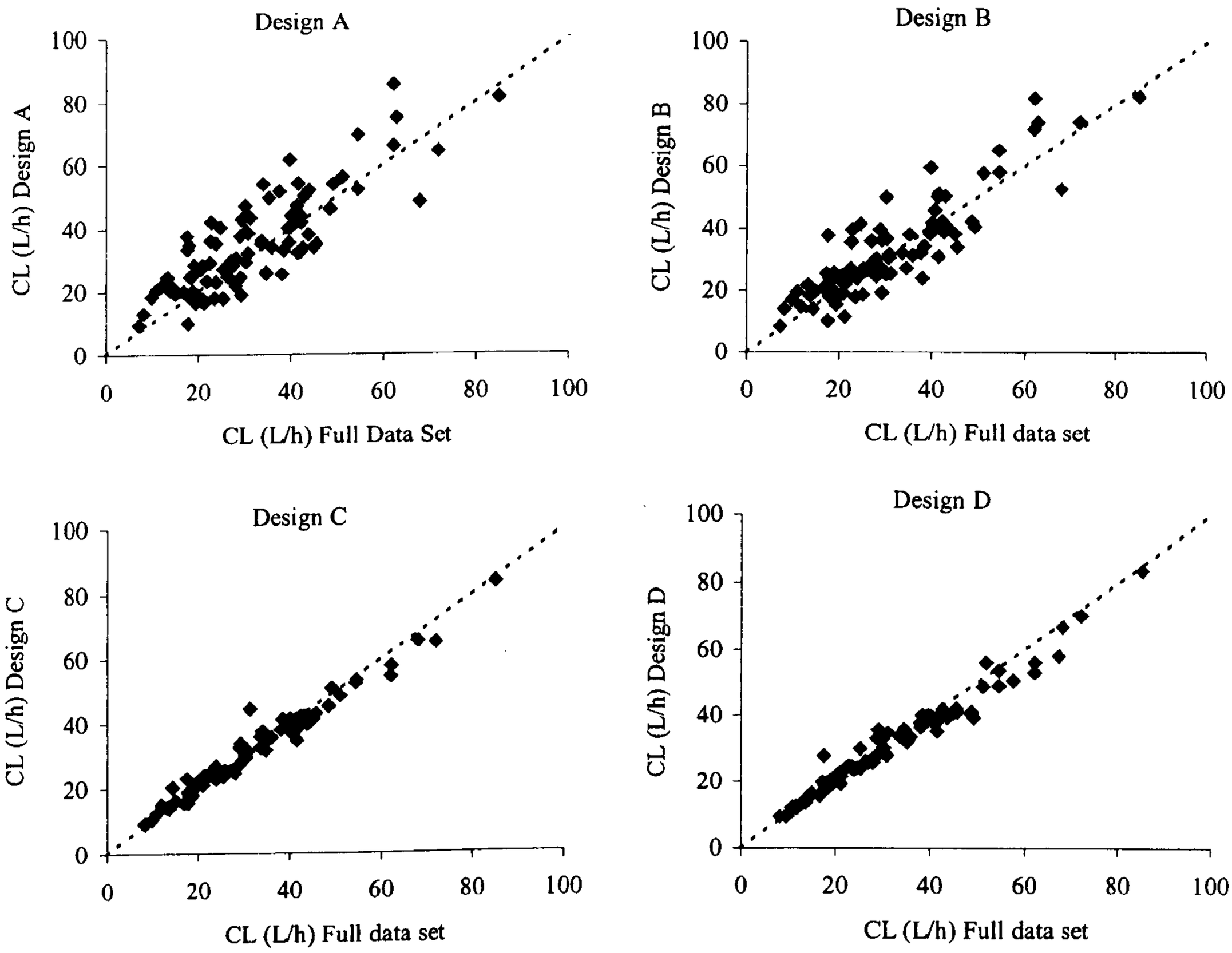


Figure 9.1 (2 of 3)

Scatter plots of the CL estimates from the truncated data sets versus CL estimates from full data set

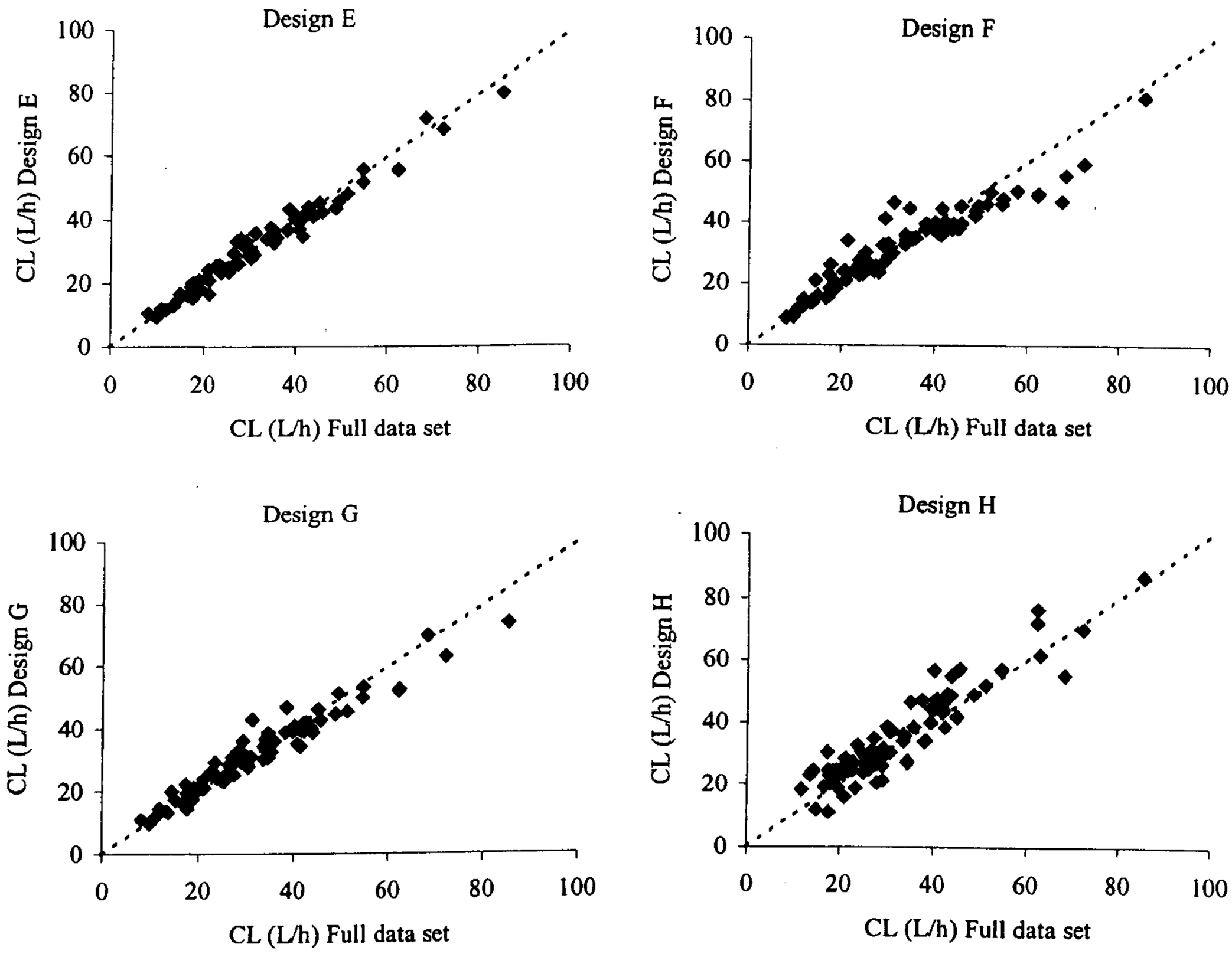
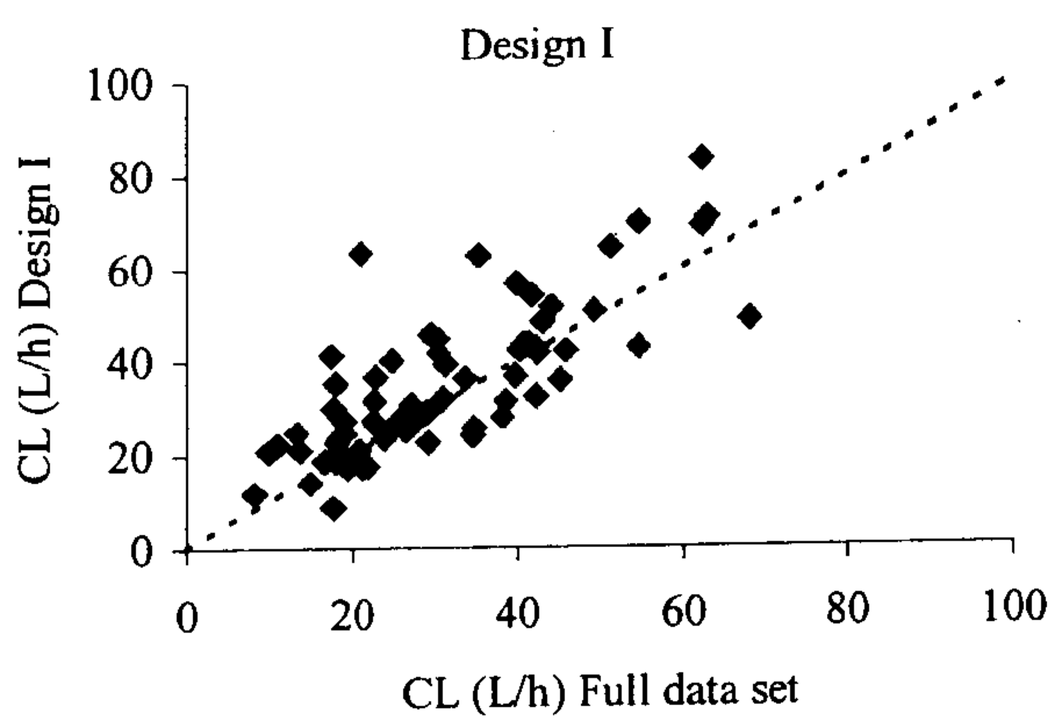


Figure 9.1 (3 of 3)

Scatter plots of the CL estimates from the truncated data sets versus CL estimates from full data set



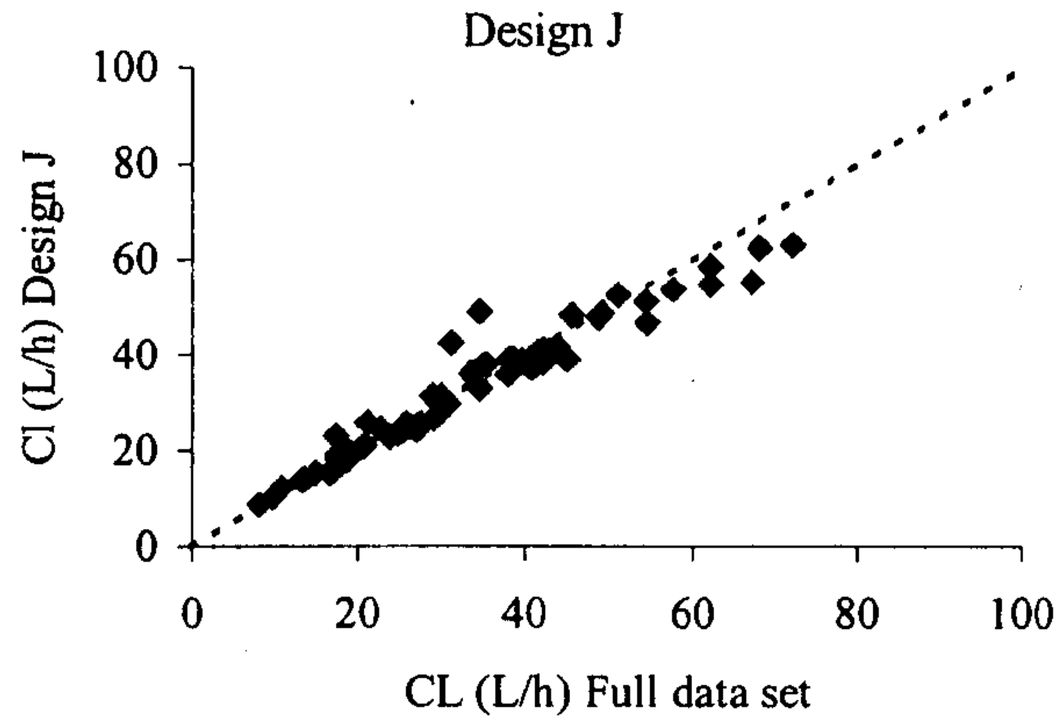


Table 9.3

Me and rmse estimates with 95 % confidence intervals for each limited sampling design

Design	Sampling Times			n	me (%)		rmse (%)	
					estimate	95 % CI	estimate	95 % CI
A	1st	3 h		93	16.2	(9.3, 23.2)*	37.7	(30.7, 43.5)
B	1st	0.7 h	3 h	86	9.4	(3.1, 15.6)*	30.9	(23.7, 36.6)
C	1st	3 h	48 h	77	0.6	(-1.8, 2.9)	10.6	(6.7, 13.3)
D	1st	0.7 h	48 h	80	-1.7	(-3.9, 0.6)	10.3	(5.6, 13.5)
E	0.7 h	3 h	48 h	70	-0.3	(-2.4, 1.9)	9.1	(7.3, 10.5)
F	1st	48 h		87	0.7	(-2.7, 4.1)	15.9	(11.5, 19.4)
G	3 h	48 h		77	0.9	(-1.9, 3.7)	12.4	(9.6, 14.6)
H	1st	3 h	24 h	83	10.0	(5.5, 14.5)*	22.9	(17.8, 27.1)
I	1st	3 h	10 h	67	20.1	(9.9, 30.4)*	47.0	(29.0, 59.8)
J	1st	10 h	48 h	62	-0.6	(-3.3, 2.1)	10.7	(6.6, 13.6)

* = statistically significant, $p<0.05$

Design H, which included the first available sample, a 3 h sample and a 24 h sample (rather than a 48 h sample) gave less precise estimates of CL (rmse = 22.9 %) than if a 48 h sample was used (rmse = 10.6 %). The worst estimates of CL were attained when a late sample i.e. 48 h or 24 h was not included (Designs A, B and I) with rmse values ranging from 30.9 to 47.0 % and bias (me) ranging from 9.4 to 20.1 %. Of the 2 sample designs, Design G, which included the 3 h sample and the 48 h sample gave estimates of CL that were not biased and had the lowest rmse (12.4 %).

9A.2.3 Selection of optimum sampling times using the minimum and maximum CL estimates in the data set

The pharmacokinetic parameter values estimated for the individuals with the minimum and maximum CL (7 and 85 L/h, respectively) along with the population pharmacokinetic parameters are given in Table 9.4. The optimum sampling times identified using all three sets of PK parameters were the same or within 33 % of each other (Table 9.4).

9A.2.4 Validation of limited sampling designs using a new patient group

Scatter plots of the CL estimates obtained using the truncated data sets were plotted against the 'true' CL values (Figure 9.2). The me and rmse estimates with corresponding 95 % confidence intervals for each of the sampling designs are given in Table 9.5. Designs C, D, E and G had the lowest rmse values (4.2 to 5.6 %) and were not significantly biased. Designs C, D and E contained 3 blood samples, including an early sample (either the 1st available sample or 0.7 h) and a 48 h sample. However, Design G included only 2 blood samples, 3 h and 48 h. The worst estimates of CL were attained when a 48 h sample was not included (Designs A, B, H and I) with rmse values ranging from 27.1 to 37.2 %. Designs A, B and H were significantly biased, with me values ranging from 15.5 to 21.3 %.

Table 9.4

Comparison of optimal sampling times selected using population PK parameters to those selected using the PK parameters of individuals with minimum and maximum CL

Pharmacokinetic Parameter	Population Estimates	Minimum CL	Maximum CL
CL (L/h)	28	7.2	85
V_1 (L)	9.9	9.9	9.9
Q_2 (L/h)	29	29	29
V_2 (L)	35	35	35
Q_3 (L/h)	60	32	71
V_3 (L)	780	237	852
Sample Time (h)			
1	0.05	0.05	0.05
2	0.3	0.4	0.2
3	0.7	0.9	0.5
4	3.0	3.5	2.7
5	10	12	10
6	48	48	48

Figure 9.2 (1 of 3)

Scatter plots of the CL estimates from the validation truncated data sets versus CL estimates from full validation data set

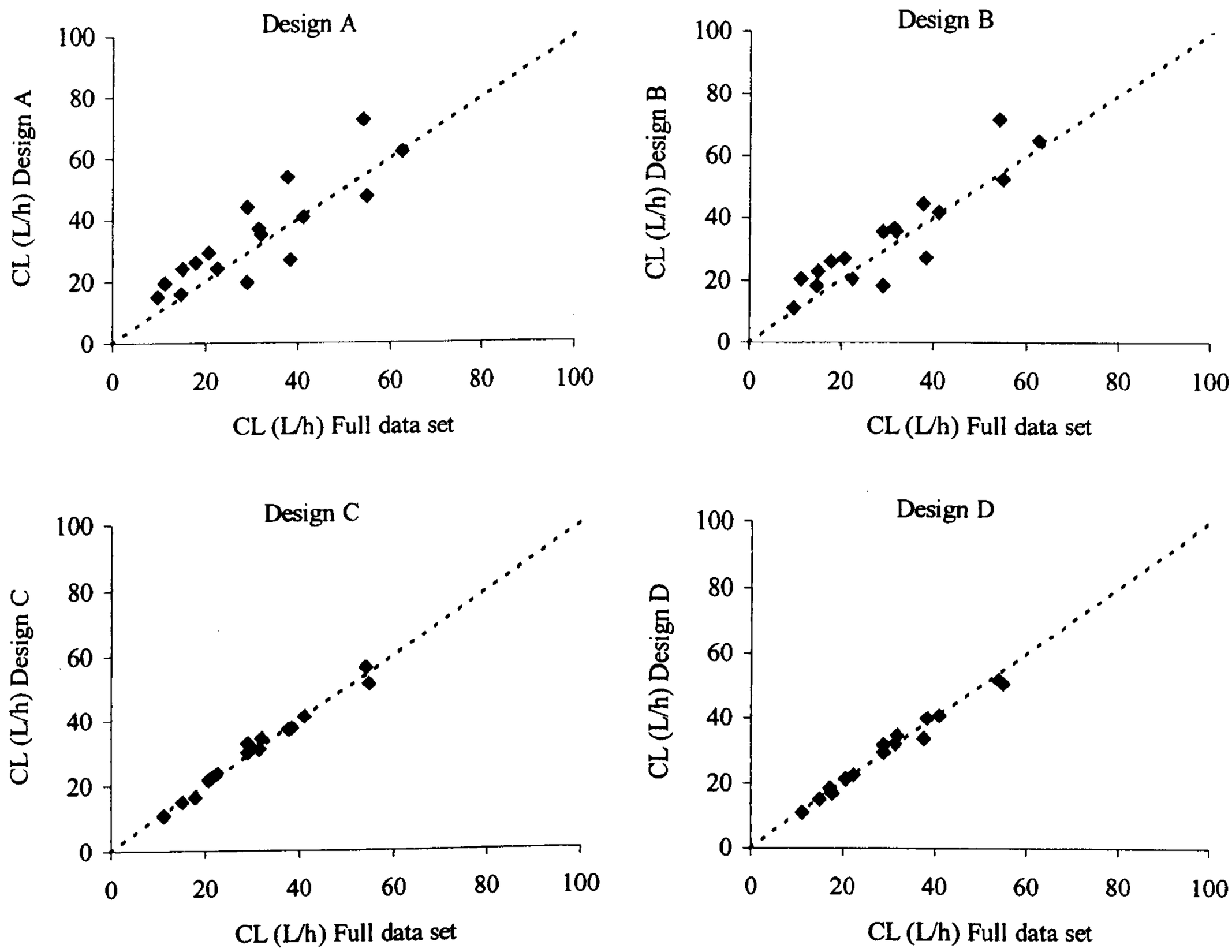
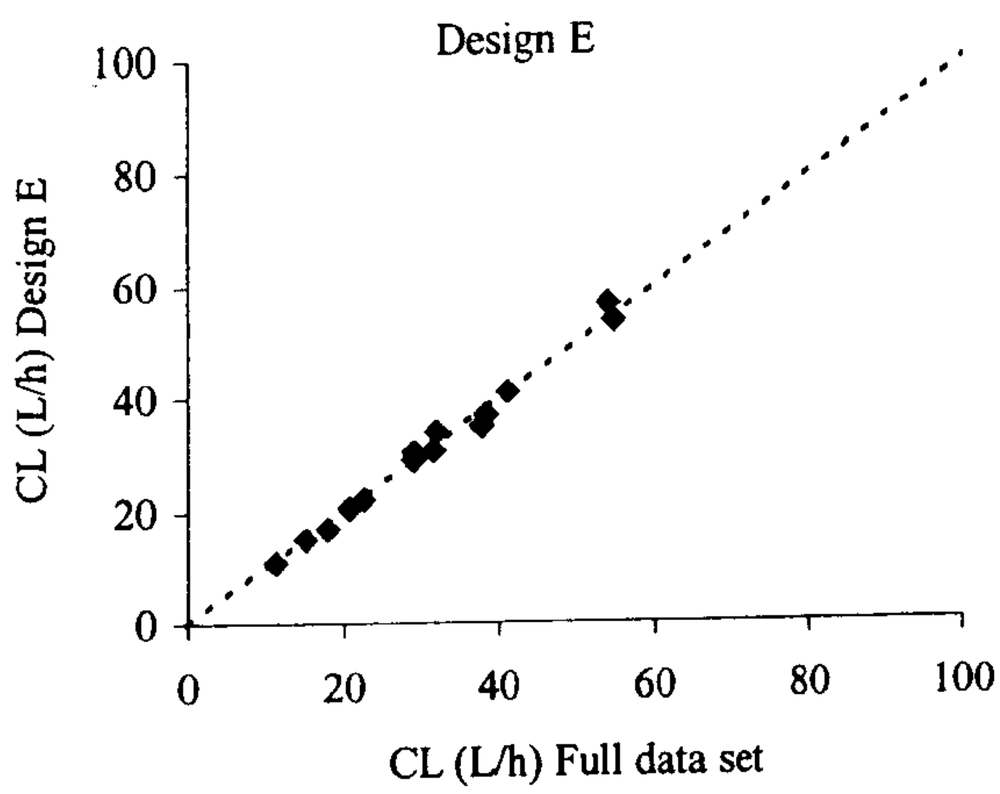
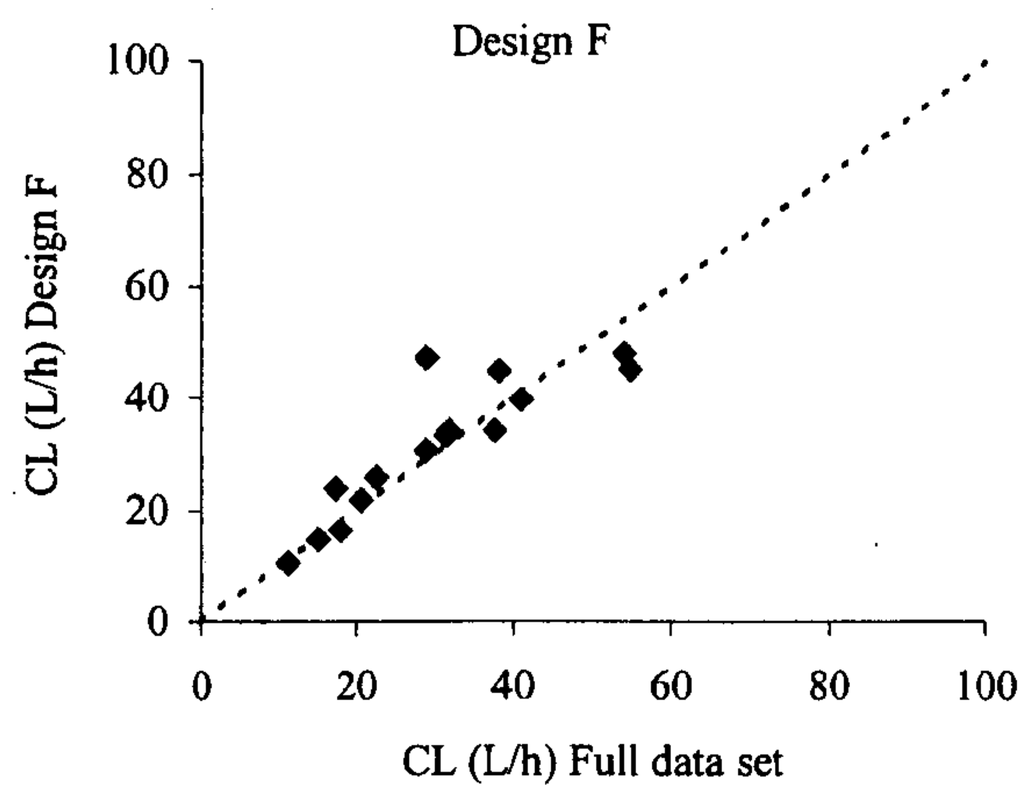
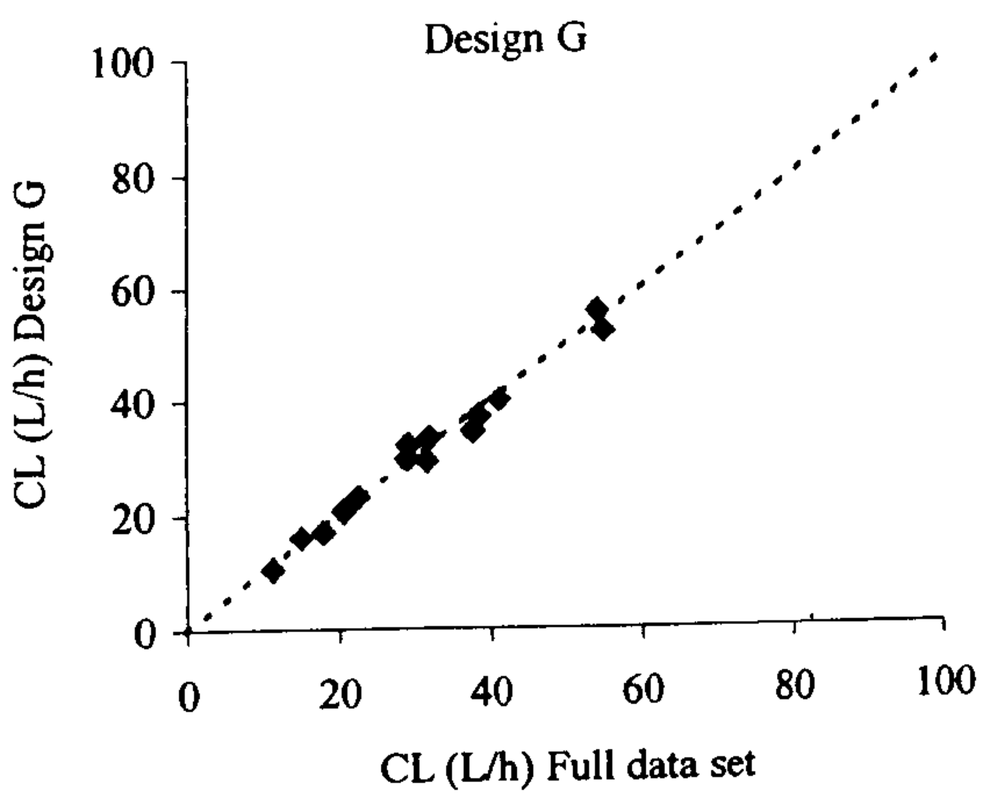


Figure 9.2 (2 of 3)

Scatter plots of the CL estimates from the validation truncated data sets versus CL estimates from full validation data set







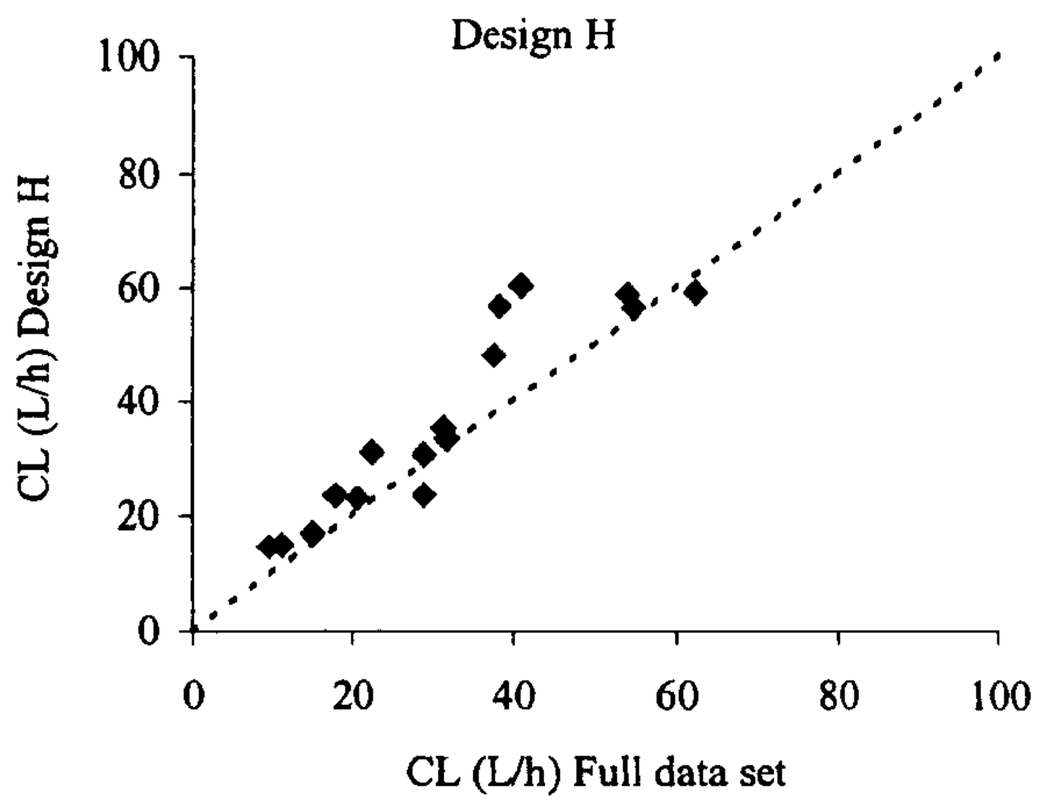
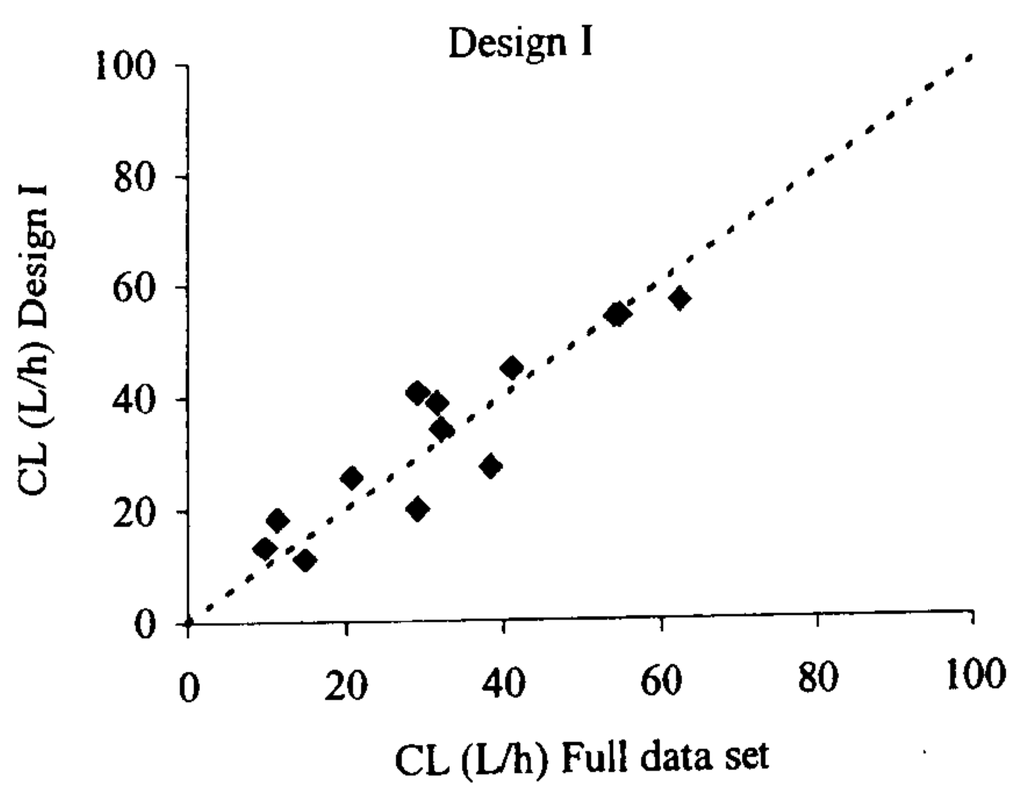


Figure 9.2 (3 of 3)

Scatter plots of the CL estimates from the validation truncated data sets versus CL estimates from full validation data set



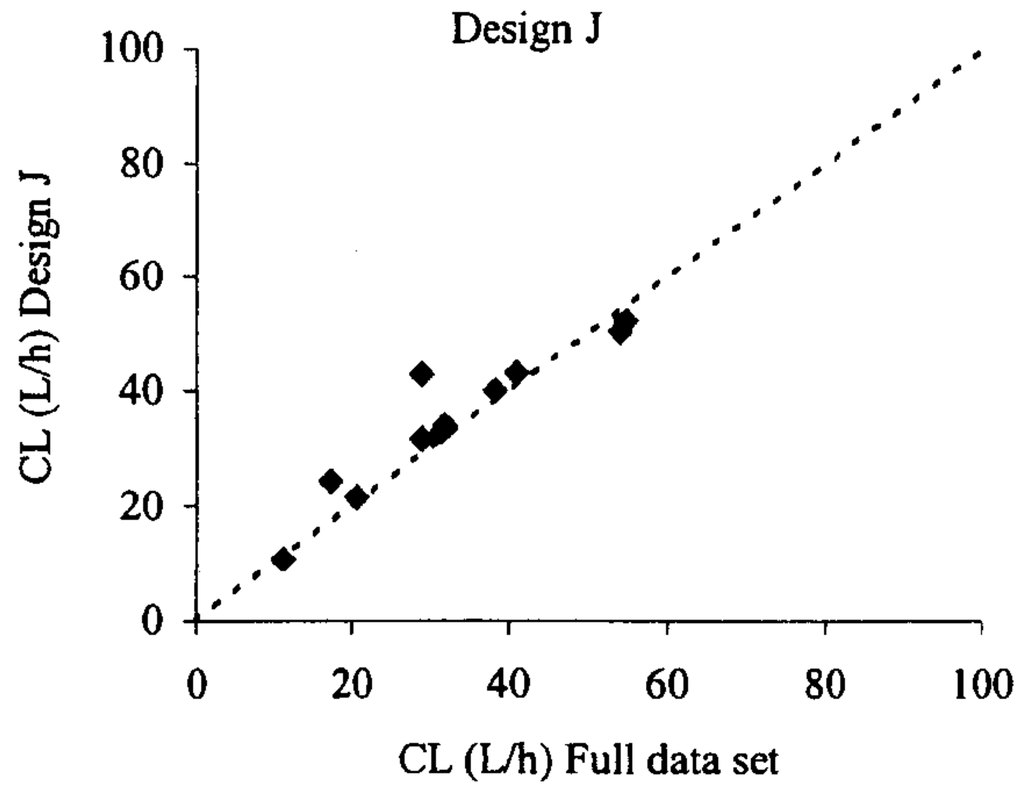


Table 9.5

Me and rmse estimates with 95 % confidence intervals for each limited sampling design using the validation data set

Design	Sampling Times			n	me (%)		rmse (%)	
					estimate	95 % CI	estimate	95 % CI
A	1st	3 h		17	21.3	(5.1, 37.5)*	37.2	(24.8, 46.5)
B	1st	0.7 h	3 h	17	15.5	(0.7, 30.2)*	31.8	(14.1, 42.7)
C	1st	3 h	48 h	14	0.34	(-3.0, 3.7)	5.6	(2.8, 7.5)
D	1st	0.7 h	48 h	15	-0.7	(-3.9, 2.5)	5.6	(3.3, 7.3)
E	0.7 h	3 h	48 h	14	-1.2	(-3.5, 1.3)	4.2	(2.6, 5.3)
F	1st	48 h		15	6.3	(-5.2, 17.7)	20.9	(-11.1, 31.6)
G	3 h	48 h		14	-1.1	(-4.3, 2.2)	5.6	(3.7, 7.0)
H	1st	3 h	24 h	16	18.6	(7.8, 29.5)*	27.1	(16.5, 34.6)
I	1st	3 h	10 h	13	7.8	(-9.2, 24.8)	28.2	(13.5, 37.5)
J	1st	10 h	48 h	11	9.0	(-3.2, 21.1)	19.4	(-12.2, 30.1)

* statistically significant bias, $p<0.05$

9A.3. DISCUSSION

Without time constraints, D-optimality will select an optimum blood sampling time that is essentially zero. This is because it is assumed that volume will be most accurately determined if a blood sample is taken at the earliest possible time before any elimination has occurred. In addition, for a proportional error model the smallest error is associated with the lowest concentration. However, in practice, it would be difficult to take a blood sample at this time and it is likely that the concentration would be below the limit of quantification of the assay. Moreover, the computer software will assume that there is instantaneous mixing within the central compartment, whereas it has previously been shown that mixing in the blood can take up to 3 minutes (Chiou 1979). For these reasons the optimal blood sampling times were restricted to be after the end of the infusion.

The most convenient times to take a blood sample would be within the first three hours as this would allow the patient to leave the hospital within a relatively short period of time after dosing. Design B included 3 samples taken within the first 3 hours and although this design offers practical advantages, the imprecision was approximately three-fold greater than that of other designs which included later blood samples. A 10 h sample may require the patient to remain in hospital until the evening or overnight. Alternatively, it would require hospital staff to visit the patient at home outside normal working hours. Thus, taking a 10 h sample would be costly and impractical and as it did not appear to offer any advantages in terms of accuracy and precision of CL estimates over other blood sampling times, the 10 h sample was excluded from further consideration. The most precise estimates of CL were obtained from Designs C, D, E and J, which required 3 blood samples to be taken, including an early blood sample and a 48 h sample. However, these designs would require the patient to return to the clinic two days after dosing.

Blood samples were not available in most patients at the exact recommended sampling times and so samples taken within 33 % of the optimum time were allowed. This allowance of variation round the selected times and variability in the infusion times between patients is consistent with what is likely to happen in the clinic. Despite this variability in the actual blood sampling time from the optimum blood sampling time, the estimates of CL were still estimated well, and thus, suggests that the sampling designs will still work well in a clinical setting where it may often not be practical to take a blood sample exactly at the optimum blood sampling time. The least inconvenient design of the best four designs is Design D as this design would enable the patient to leave the clinic within 45 minutes after dosing. Given the long half-life of the drug, it may be that

concentrations are measurable 7 days after dosing, so if a patient was receiving weekly treatment with epirubicin, the blood sample could be taken before dosing on day 7. Of course, additional practical problems such as assay turn-around time may not make this approach feasible.

Despite the large range in individual CL estimates (7 to 85 L/h), the sampling times selected using the PK parameters in the individuals with the minimum and maximum CL were similar to those estimated using the population estimates of PK parameters. This indicates that the optimum sampling times selected in this analysis are suitable for all the patients in this data set.

It is recognised that the above testing of the truncated data sets does not represent a true Bayesian analysis, as each individual was present in the original analysis from which the population parameters were determined. To test this approach properly, Bayesian estimates using a new data set were used. Like the original training data set, Design E was also confirmed as the best limited sampling design using the validation data set. However, unlike the training data set, in which all patients had breast cancer and epirubicin was administered intravenously, the validation data set mainly included patients with hepatocellular carcinoma and included 5 patients in whom epirubicin was administered by intra-arterial administration. Despite these differences between the data sets, the best limited sampling designs achieved precise and accurate predictions of epirubicin CL for all patients in the validation data set.

Estimation of CL by using a limited sampling strategy gave substantially better estimates of CL compared to using AST concentrations to estimate CL. Using the best limited sampling strategy, the imprecision of CL estimates was approximately 4 % whereas when the population model was used to calculate CL from AST concentrations, the imprecision of CL estimates was approximately 82 %. Estimates of CL using the limited sampling strategy (Design E) were not significantly biased (me = –1.2 %; 95% CI –3.5 %, 1.3 %) and although the bias of CL estimated from AST concentrations was also not found to be statistically significant, it should be noted that the confidence interval were wide (me = 20.5 %; 95% CI -19.9 %, 60.9 %). Thus, although *a posteriori* dose-adjustment has a number of disadvantages, including inconvenience to patients and addition cost and time involved for the health service, it appears to be a far superior method to estimate epirubicin CL, and hence achieve a target AUC, than *a priori* methods.

There are a number of limitations of the analysis presented here. The first is that the accuracy and bias for the CL estimates calculated in this analysis assume that the

Bayesian estimates from the full validation data set are the 'true' values of CL for each individual. In addition, although the estimates of CL from the limited sampling strategy were on average only 4 % different from the 'true' estimate for CL, if a dose was based on an individual's CL estimate, it is unlikely that the systemic exposure would be attained with such precision, as an individual's CL may vary between occasions. It has not been possible to determine the interoccasional variability of epirubicin in this analysis as blood samples were available on one occasion only. Clearly, the lower the interoccasional variability, the greater the usefulness of this approach.

9B SENSITIVITY OF LIMITED SAMPLING DESIGNS TO RECORDING ERRORS OF BLOOD SAMPLING TIMES

9B.1 METHODS

9B.1.1 Simulation of reference data sets and estimation of PK parameters

Using NONMEM, a data set (reference data set) containing 200 patients was simulated using population model parameters previously estimated for epirubicin. These parameters were obtained using FOCE-INTER, with the variance-covariance matrix restricted to a diagonal format and with no covariates included in the model. The dose was 125 mg given as an intravenous infusion over 3 minutes. Concentrations were simulated for the following sampling times: pre-dose, 3 (end of infusion), 6, 10, 20 and 40 min and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h post-dose using a seed value of 3396450. The individual PK parameters were generated with NONMEM using the 'ONLY SIM' option (as described in Chapter 2) and were designated the 'true' individual PK parameters.

The reference data set was truncated four times to include only the sampling times for designs C, D, E and G. Individual PK parameters for each truncated data set and the full reference data set were estimated by Bayesian analysis with NONMEM using the MAXEVAL=0 option. The precisions (rmse) and biases (me) for CL, V_3 and Q_3 estimates using the truncated data sets and full data set relative to the 'true' parameter values were calculated.

9B.1.2 Simulation of data sets with recording errors and estimation of CL

Designs C, D, E and G include the following times: end of infusion, 40 min, 3 h and 48 h. The random number generator function in Excel was used to create 200 new times for each of the nominal times. These new times differed by up to ±10 % (uniformly distributed) from the nominal time and were intended to mimic errors in the recording of the sample time. Four new data sets based on designs C, D, E and G were created from the truncated reference data sets described above but modified to include the new simulated recording error times. The individual CL values for each truncated recording error data set were estimated by Bayesian analysis with NONMEM using the

MAXEVAL=0 option. The precision (rmse) and bias (me) of the truncated recording error data sets, relative to the 'true' CL values were calculated.

Four additional data sets were generated using a similar approach but in this case the nominal blood sampling times were changed by ±10-20 %. Individual CL values were estimated as before and precisions and biases relative to the 'true' CL values were calculated using the methodology described previously.

9B.2 RESULTS

9B.2.1 Estimation of PK parameters from reference data sets

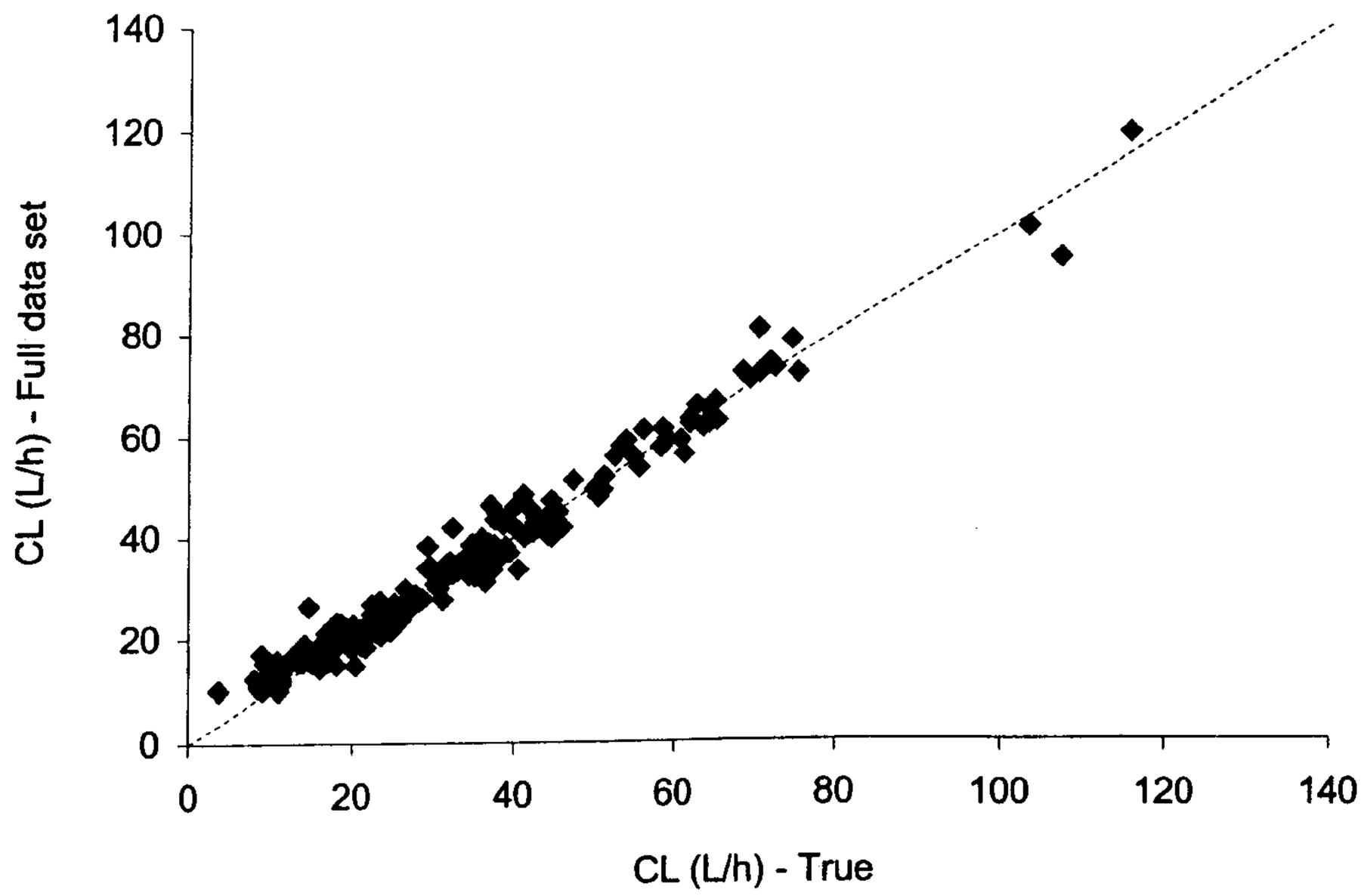
Estimates of CL using the full data set with the correct blood sampling times were plotted against the 'true' CL estimates and are shown in Figure 9.3. The rmse and me values calculated for CL from the full data set relative to the true simulated estimates are given in Table 9.6. The full data set gave estimates of CL that were significantly biased (5.6 %) with an imprecision of 19.6 (Table 9.6).

The CL values estimated from truncated designs C, D, E and G using the data set with correct blood sampling times, were plotted against the 'true' CL estimates in Figure 9.4. The figures show that all designs gave estimates of CL that were in close agreement with the true CL estimates. All four limited sampling designs, without recording errors, gave estimates of CL that were upwardly biased (me) by approximately 10 to 11 % (Table 9.6). As none of the 95 % CI's for these designs included zero, this was a statistically significant overestimation. The imprecision (rmse) was on average 29 % in the truncated designs without error.

Estimates of V_3 and Q_3 from the full data set with the correct blood sampling times were plotted against the 'true' estimates in Figure 9.5 and rmse and me values are given in Table 9.7. The full data set gave estimates of V_3 and Q_3 that were in good agreement with the true values with imprecision of 15 and 10 %, respectively. Estimates of V_3 were unbiased and Q_3 was only slightly biased (1.5 %). In contrast, the limited sampling designs did not achieve good estimates of V_3 and Q_3 (Table 9.7). For Designs C, E and G, the imprecision of V_3 estimates was approximately 29 %. The figures show that for these designs the estimates of V_3 were most poorly estimated at values greater than 1000 L, where estimates were shrunk towards the population value (Figure 9.6). The poorest estimates of V_3 were attained using Design D, in which the imprecision was 44 %. The figure for Design D illustrates that estimates at all values of V_3 were shrunk towards the population value. For Q_3, the best estimates were attained using Designs D and E, with an imprecision of approximately 26 %. The figures show that for these designs the estimates of Q_3 were most poorly estimated at values greater than 80 L/h, where estimates were shrunk towards the population value (Figure 9.7). The poorest estimates of Q_3 were attained using Designs C and G, in

Figure 9.3

CL estimates using full data set against 'true' CL values



Dotted line represents line of identity

Table 9.6

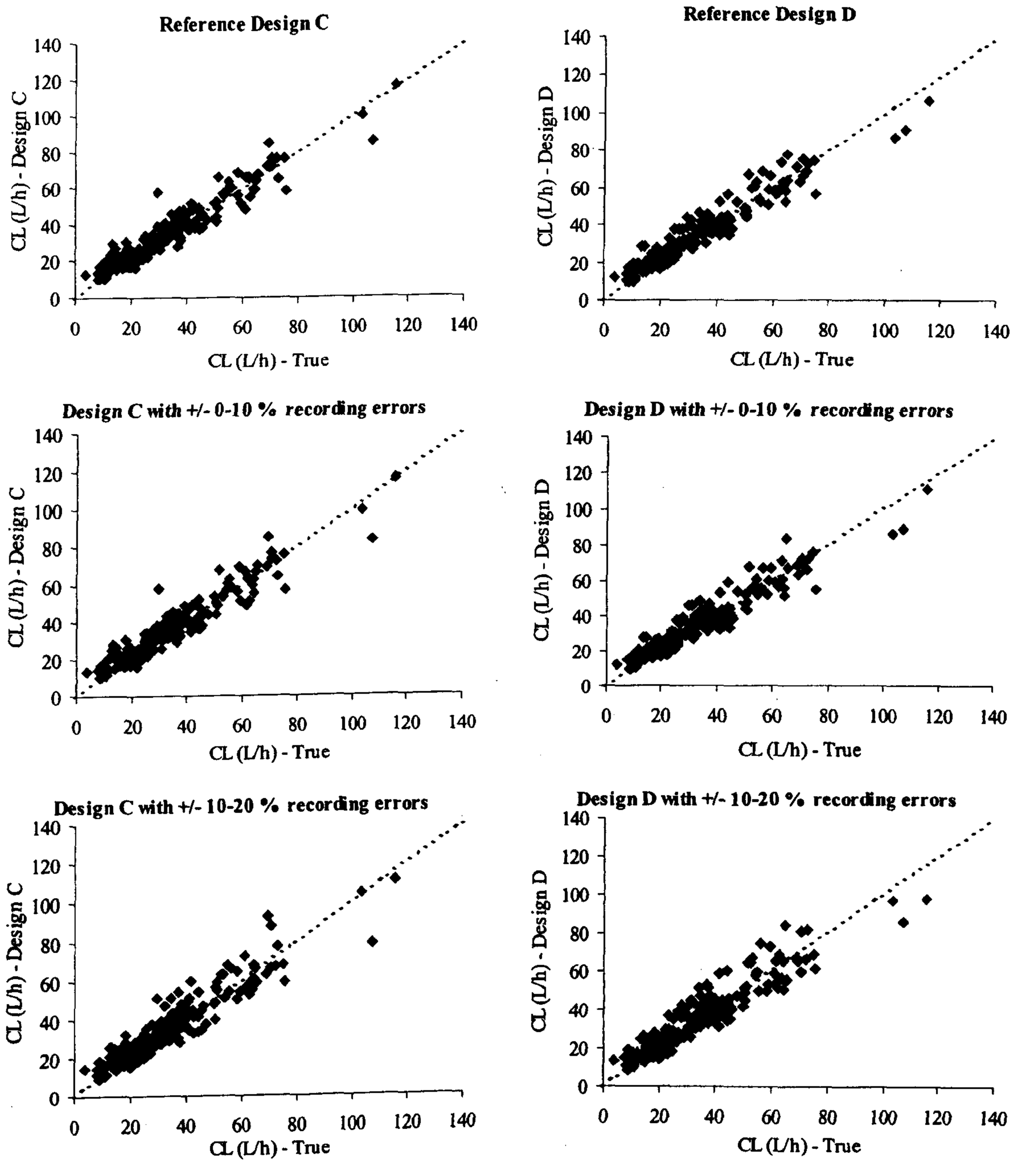
The precision and bias of CL estimates from limited sampling designs with and without recording errors

Recording Error	Design	Sampling Times			me (%)		rmse (%)	
					estimate	95 % CI	estimate	95 % CI
None	All samples				5.6	(3.0, 8.2)*	19.6	(8.5, 26.3)
	C	3 min	3 h	48 h	11.2	(7.2, 15.2)*	30.7	(16.3, 40.3)
	D	3 min	40 min	48 h	10.3	(6.7, 13.9)*	27.9	(15.7, 36.3)
	E	40 min	3 h	48 h	9.5	(6.0, 13.0)*	26.8	(13.7, 35.4)
	G	3 h	48 h		11.4	(7.4, 15.5)*	31.3	(16.6, 41.0)
0-10 % error	C	3 min	3 h	48 h	11.1	(7.1, 15.1)*	31.2	(16.4, 40.9)
	D	3 min	40 min	48 h	10.6	(6.8, 14.3)*	28.8	(26.7, 30.7)
	E	40 min	3 h	48 h	9.9	(6.3, 13.5)*	27.9	(25.9, 29.7)
	G	3 h	48 h		11.7	(7.5, 15.8)*	32.0	(29.8, 34.2)
10-20 % error	C	3 min	3 h	48 h	11.6	(7.3, 16.0)*	33.6	(31.2, 35.8)
	D	3 min	40 min	48 h	11.1	(7.1, 15.3)*	31.5	(29.3, 33.6)
	E	40 min	3 h	48 h	10.8	(6.9, 14.8)*	30.3	(28.1, 32.3)
	G	3 h	48 h		12.9	(8.4, 17.4)*	35.0	(32.5, 37.3)

* statistically significant bias, $p<0.05$

Figure 9.4 (1 of 2)

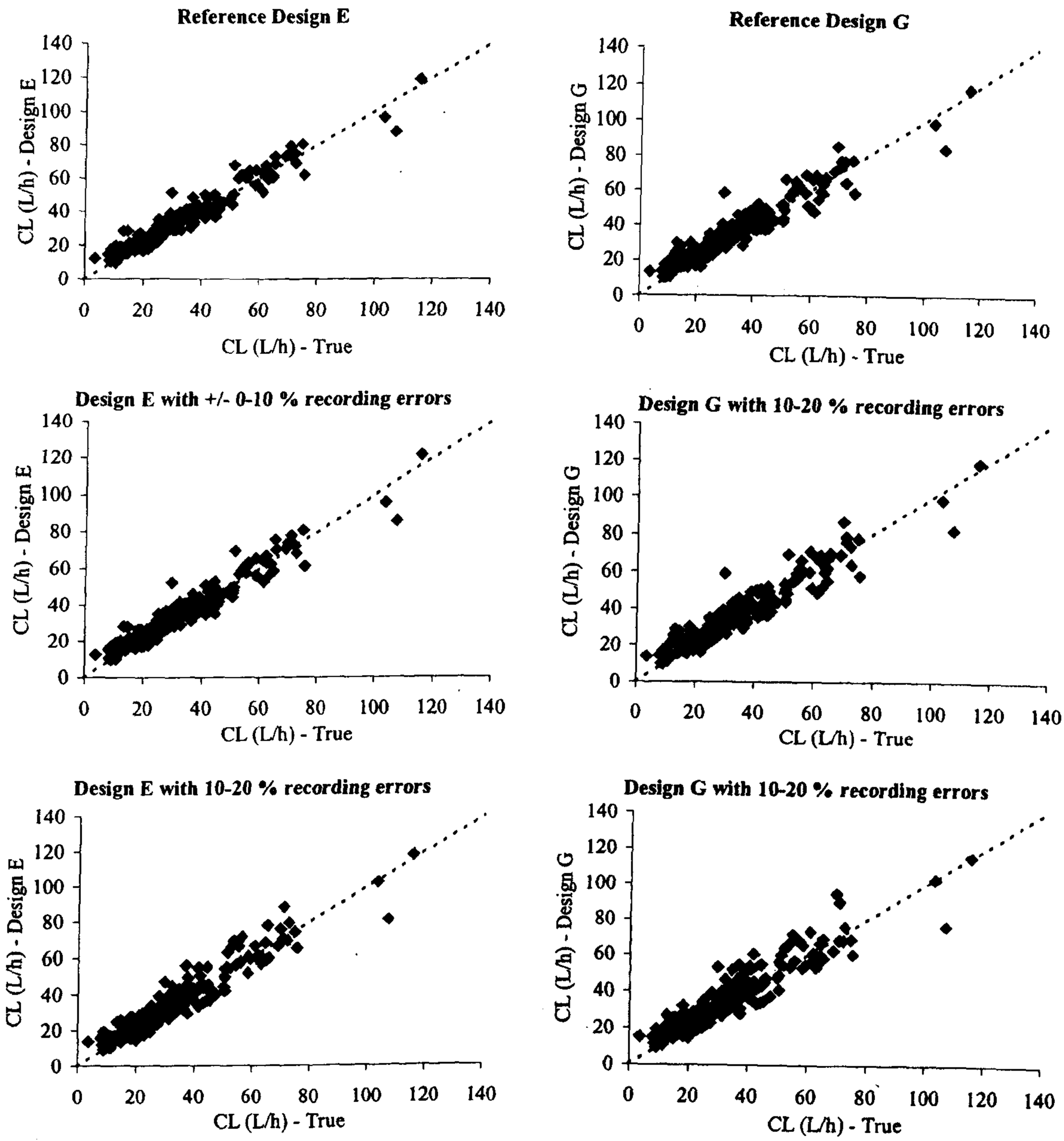
CL estimates from limited sampling designs with and without recording errors against 'true' CL values



Dotted line represents line of identity

Figure 9.4 (2 of 2)

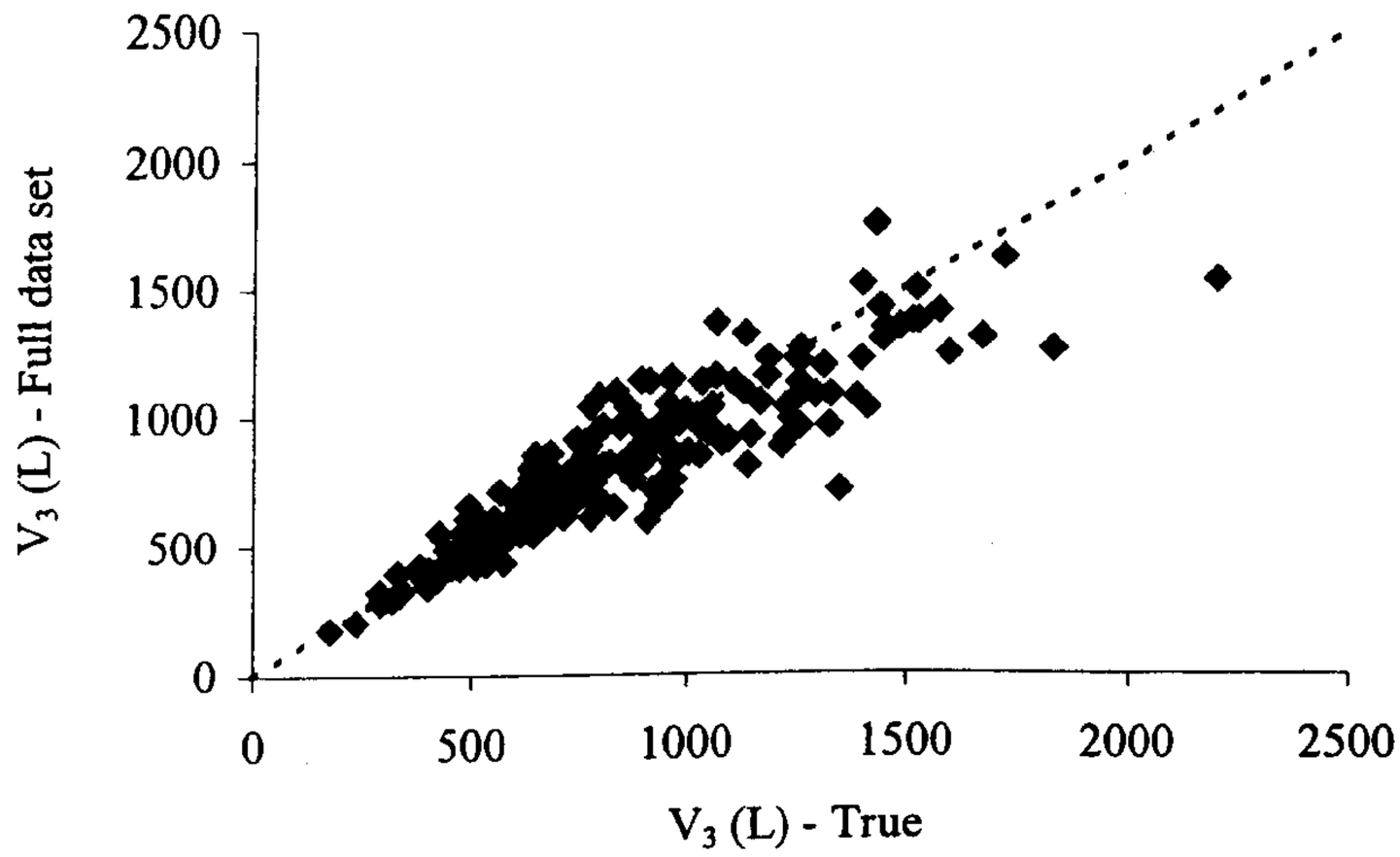
CL estimates from limited sampling designs with and without recording errors against 'true' CL values



Dotted line represents line of identity

Figure 9.5

V_3 (above) and Q_3 (below) estimates from full data set against 'true' values



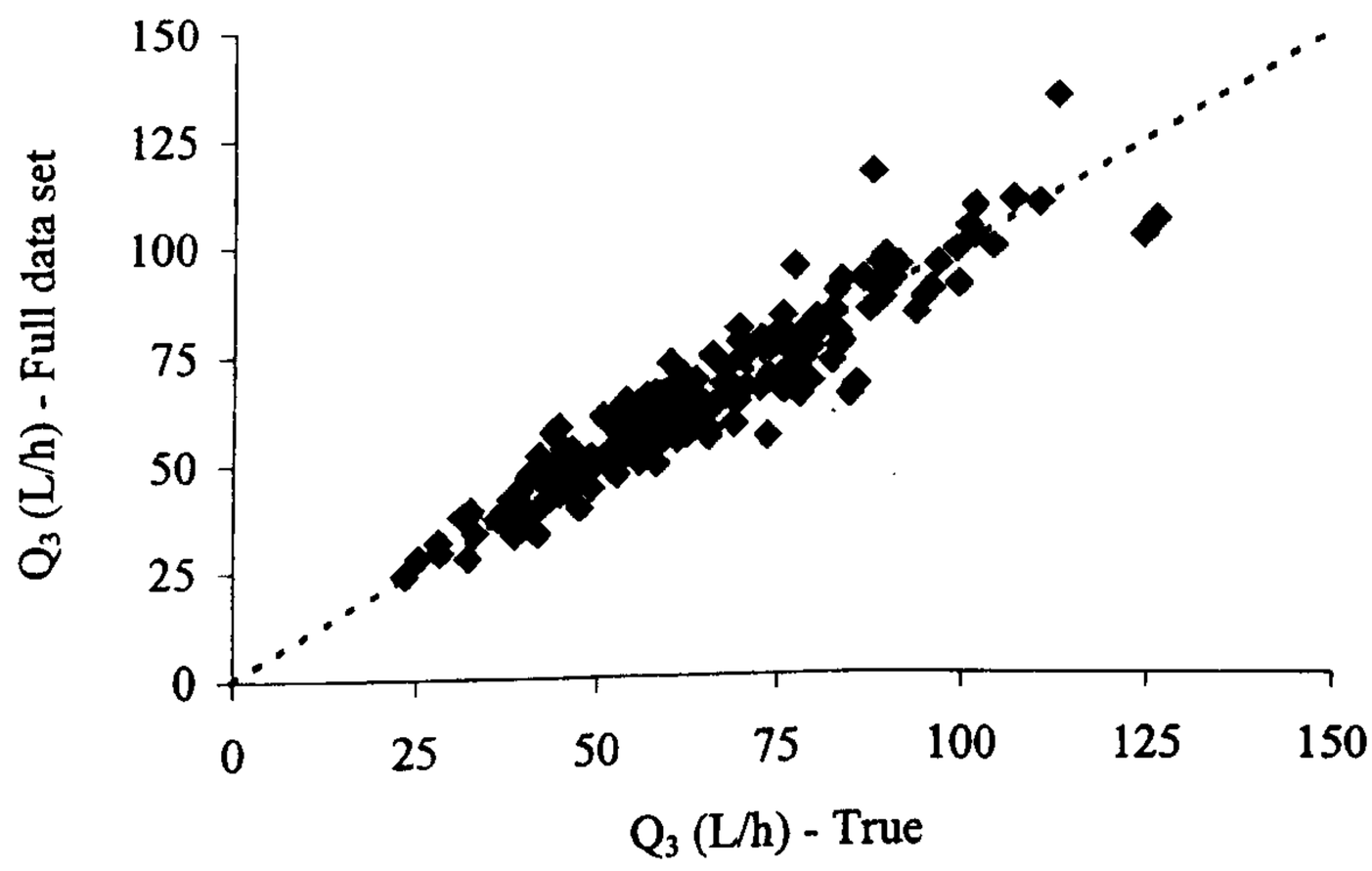


Figure 9.6

V_3 estimates from limited sampling designs against 'true' V_3 values

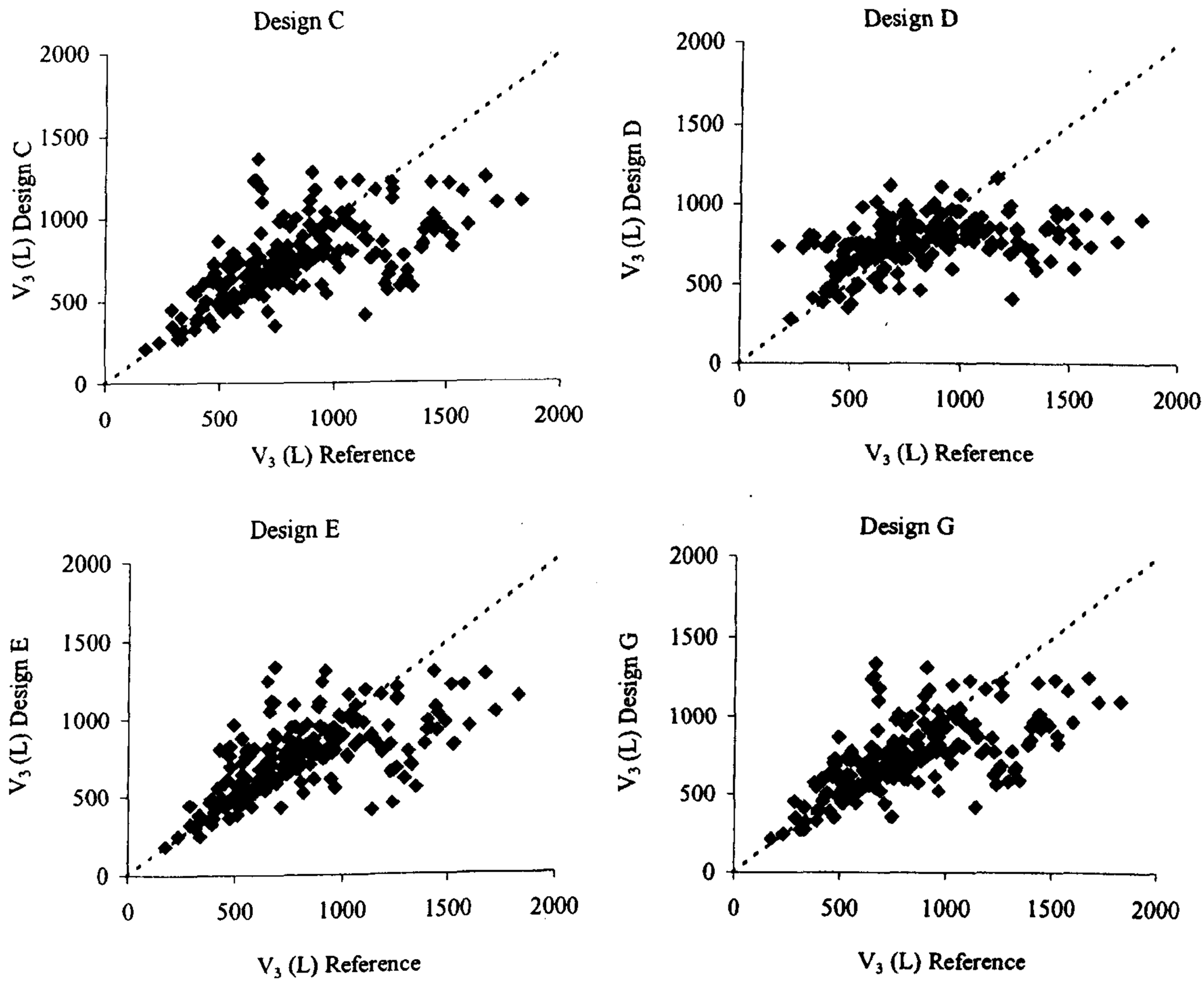


Figure 9.7

Q_3 estimates from limited sampling designs against 'true' Q_3 values

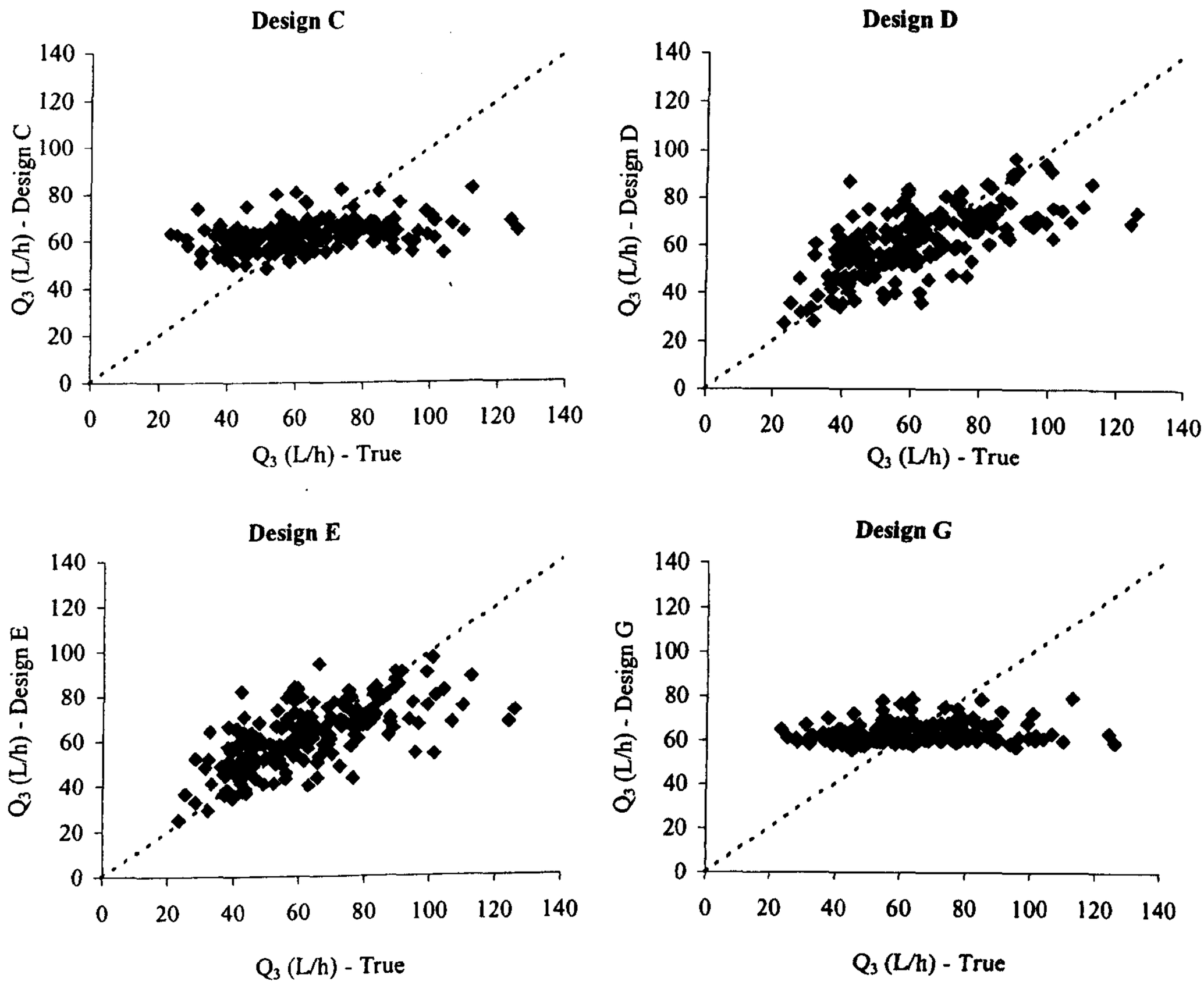


Table 9.7

The precision and bias of V_3 and Q_3 estimates from limited sampling designs

Parameter	Design	Sampling Times			me (%)		rmse (%)	
					estimate	95 % CI	estimate	95 % CI
V_3	All data				-1.0	(-3.1, 1.1)	15.1	(13.6, 16.5)
	C	3 min	3 h	48 h	-4.4	(-8.4, -0.5)*	28.6	(25.0, 31.7)
	D	3 min	40 min	48 h	3.4	(-2.7, 9.4)	43.9	(29.5, 54.6)
	E	40 min	3 h	48 h	-2.3	(-6.2, 1.7)	28.8	(24.9. 32.2)
	G	3 h	48 h		-4.5	(-8.4, -0.6)*	28.6	(25.1, 31.8)
Q_3	All data				1.5	(0.1, 2.9)*	10.3	(9.1, 11.4)
	C	3 min	3 h	48 h	10.6	(5.8, 15.5)*	36.6	(29.9, 42.3)
	D	3 min	40 min	48 h	4.5	(1.0, 8.0)*	25.6	(21.9, 28.9)
	E	40 min	3 h	48 h	6.0	(2.4, 9.5)*	26.1	(22.4, 29.4)
	G	3 h	48 h		11.4	(6.4, 16.4)*	37.7	(31.3, 43.1)

* statistically significant bias, $p<0.05$

which the imprecision was 37-38 %. The figures for Designs C and G illustrate that estimates at all values of Q_3 were shrunk towards the population value.

9B.2.2 Estimation of CL from data sets with recording errors

The CL values estimated from truncated Designs C, D, E and G with up to 10 % error in recording of blood sampling times, were plotted against the 'true' CL estimates and are shown in Figure 9.4. The figures show that even with the introduction of up to 10 % error in blood sampling times, all designs gave estimates of CL that were in close agreement with the true CL estimates. Introduction of 0-10 % recording error resulted in little change in the bias and there was only a marginal increase in imprecision (Table 9.6).

The CL values estimated from truncated Designs C, D, E and G with 10-20 % error in blood sampling times, were also plotted against the 'true' CL estimates and shown in Figure 9.4. Figure 9.4 shows that all designs still gave estimates of CL that were in close agreement with the true CL estimates even when the increase in blood sampling times errors were increased to 10 to 20 %. Introduction of 10-20 % recording error again only resulted in a marginal increase in the bias and imprecision (Table 9.6). Design E (40 min, 3 h, 48 h) was found to have the least bias and the lowest imprecision with and without recording errors and design G (3 h, 48 h) had the greatest bias and imprecision.

9.B.3 DISCUSSION

In the simulation analysis, all designs over estimated CL by approximately 10 %, which is in contrast to the results obtained with the breast cancer patients (original data set) and the hepatocellular carcinoma patients (validation data set), where none of the designs gave estimates of CL that were significantly biased. In addition, the imprecision of the designs estimated from the simulation analysis was approximately 30 % whereas in the real patient data sets it was only around 5-10 %. It should be noted that in the simulation analysis, the true individual estimates of CL are known, whereas, for the analysis using real data collected from patients, the true individual CL values were not known so were taken as those values estimated from all the available blood samples. In the simulation analysis, when CL was estimated using the all the available blood sampling times, the estimates of CL were found to be significantly biased (5 %) and rmse was 20 % relative to the true values of CL. The difference between the true CL and that estimated from the full data set was up to 12 L/h. This indicates that CL estimated from the full data set and taken as the 'true' CL for the breast cancer patients and the hepatocellular carcinoma patients are subject to error and are likely to have lead to an underestimation of the bias and imprecision of the sampling designs tested in these data sets.

In the simulation analysis, the best limited sampling design for CL was Design E as this gave the lowest bias (9.5 %) and imprecision (26.8); however, the bias and imprecision were similar for all designs. The simulation of 0-10 % or 10-20 % recording errors of the blood sampling times had negligible effect on the bias and imprecision of CL estimates and all designs appeared to be equally robust in terms of such errors.

Designs C, G and E attained estimates of V_3 with similar imprecision (29 %) to that observed for CL, although it was noted that at values above 1000 L there was a tendency for estimates to shrink towards the population value. Design D gave estimates of V_3 that were shrunken towards the population value at all values and had greater imprecision that the other designs at approximately 44 %. In contrast to the other designs, Design D does not include a 3 h concentration, suggesting that this may be an important sampling time for the estimation of V_3. Likewise, Designs D and E attained estimates of Q_3 with similar imprecision (26 %) to that observed for CL

(although values were shrunken towards the population average at values above 80 L/h), whereas Designs C and G had increased imprecision (37-38 %), and again values that were shrunken towards the population value at all values. Unlike Designs D and E, Designs C and G did not include a 40 min concentration, suggesting that this may be an important sampling time for the estimation of Q_3.

Overall, Design E gave the best estimates for all parameters and therefore this should be the limited sampling model used in any future analysis.

9C INVESTIGATION INTO REASONS FOR BIAS IN CL ESTIMATES

9C.1 METHODS

Three hypotheses were tested for the cause of the bias observed in the Bayesian estimates for CL:

1) Bias can be attributed to the high the residual error
2) Bias occurred by chance in the simulated data set
3) Bias occurred because blood samples were not taken for a long enough period post-dose. Was the bias most pronounced in patients with low CL values, and hence, longer half-lives?

9C.1.1 Assessment of the influence of residual error on bias

Using NONMEM, a data set containing 200 patients was simulated using population model parameters for epirubicin as previously described (Section 9B.1) with the exception that residual error was fixed to zero. As before, concentrations were simulated for the following sampling times: pre-dose, 3 (end of infusion), 6, 10, 20 and 40 min and 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h post-dose using a seed value of 3396450. The concentration data simulated was then used to estimate individual values of CL by Bayesian analysis and the bias and imprecision of these estimates relative to the true simulated CL values were calculated as described previously.

9C.1.2 Investigation into whether bias occurred by chance

Four further data sets each containing 200 patients were generated as described previously, using four new seeds (280874; 6258910; 4512373; 7914362). The concentration data simulated was then used to estimate individual values of CL by Bayesian analysis and the bias and imprecision of these estimates relative to the true simulated CL values were calculated as described previously.

9C.1.3 Investigation into the influence of blood sampling times on bias

Two groups of 5 new data sets, each containing 200 patients, were generated using the same seeds as above. The first group included an additional blood sample at 96 hours, the second group contained two additional blood samples, one at 96 hours and one at 168 hours post dose. The concentration data simulated for each data set were then used to estimate individual values of CL by Bayesian estimation and the bias and imprecision of these estimates relative to the true simulated CL values were calculated as described previously.

The number of patients with simulated concentrations above the limit of quantification of the assay (LOQ) at 48, 96 and 168 h post-dose was recorded and expressed as a percentage. The LOQ of the assay used to analyse epirubicin concentrations in the original data set was 1 ng/ml. However, as a lower LOQ of 0.58 ng/ml has since been achieved using an improved analytical method, the LOQ was set at 0.58 ng/ml for the current analysis (Danesi et al. 2002a).

The geometric mean of CL was estimated for each of the 5 data sets simulated up to 48, 96 and 168 h. The CL estimates within each data set were separated into 2 groups: those below the geometric mean and those above the geometric mean. The imprecision and bias was estimated for the CL values below the geometric mean and for those above the geometric mean.

9C.2 RESULTS

9C.2.1 Assessment of influence of residual error on bias

CL values estimated from the concentration data simulated without residual error had imprecision and bias 19.2 % and 9.0 %, respectively, which was consistent with that observed when residual error was included at 23 % (19.6 % and 5.0 %, respectively).

9C.2.2 Investigation into whether bias occurred by chance

The imprecision and bias of the CL values estimated by Bayesian analysis for the group of data sets containing simulated concentrations up to 48 h post-dose, are shown in Table 9.8. The bias of the estimates, as measured by me, ranged from 5.6 to 8.9 %, whereas the imprecision, as measured by rmse, ranged from 15 to 22 %. As the 95 % confidence intervals for me did not include zero, a statistically significant bias was observed in all cases.

9C.2.3 Investigation into the influence of blood sampling times on bias

The imprecision and bias of the CL values estimated by Bayesian analysis for the second group of data sets containing concentrations up to 96 h post-dose, and for the third group containing concentrations up to 168 h post-dose, are shown in Tables 9.9 and 9.10, respectively. Inclusion of a 96 h sample led to a reduction in both bias (3.0 % to 3.8 %) and imprecision (9.3 % to 11.5 %). A further improvement was seen with data sets that contained an additional measurement at 168 h post-dose. Bias ranged from 1.9 to 2.4 % and imprecision from 6.6 to 11.6 % in four of the data sets but unexpectedly high values were observed with data set No. 4 (bias 3.4 % and imprecision 18.9 %). The high bias and imprecision of data set No. 4 was attributed to one outlier patient. Removal of this patient resulted in a bias and imprecision of 2.2 and 6.7 %, respectively, which is consistent with the other data sets. Although bias was reduced when later samples were included, since the 95 % confidence intervals did not include zero a statistically significant bias remained.

The limit of quantification of the assay was assumed to be 0.58 ng/ml. The percentages of simulated concentrations that were below this value were 0.3 % in the

Table 9.8

The precision and bias of CL estimates from 5 simulated data sets in which blood samples were taken up to 48 h post-dose

	me	95 % CI	rmse	95 % CI
1	5.57	(2.97, 8.17)	19.6	(8.53, 26.3)
2	5.58	(3.63, 7.52)	15.1	(12.8, 17.0)
3	8.93	(6.10, 11.8)	22.2	(16.1, 27.0)
4	7.02	(4.97,9.06)	16.3	(12.8, 19.2)
5	5.89	(3.92, 7.86)	15.3	(11.8, 18.2)

Table 9.9

The precision and bias of CL estimates from 5 simulated data sets in which blood samples were taken up to 96 h post-dose

	me	95 % CI	rmse	95 % CI
1	3.81	(2.31, 5.31)	11.5	(8.01, 14.1)
2	3.54	(2.24, 4.83)	9.99	(7.73, 11.8)
3	2.95	(1.72, 4.28)	9.3	(7.16, 11.0)
4	3.65	(2.24, 4.85)	9.36	(7.81, 10.7)
5	3.38	(1.91, 4.86)	11.2	(6.73, 14.3)

Table 9.10

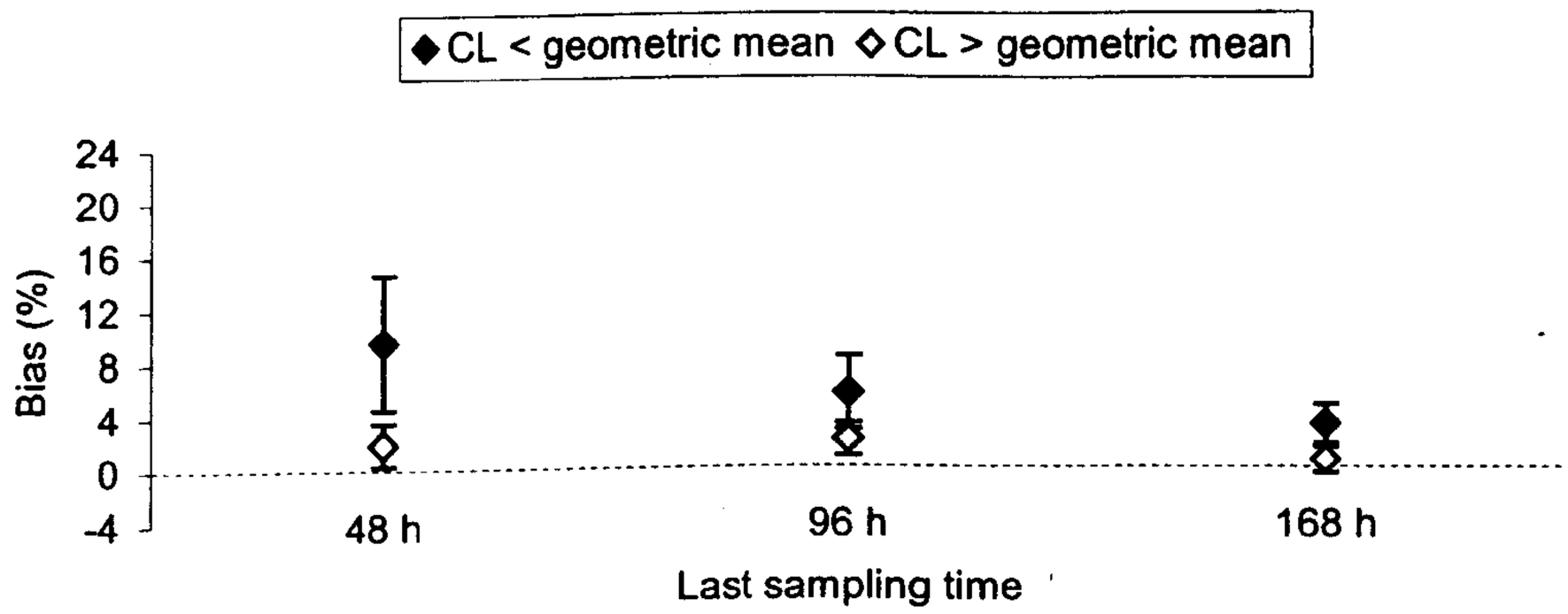
The precision and bias of CL estimates from 5 simulated data sets in which blood samples were taken up to 168 h post-dose

	me	95 % CI	rmse	95 % CI
1	1.9	(1.01, 2.80)	6.73	(5.43, 7.82)
2	2.44	(1.55, 3.33)	6.86	(5.16, 8.22)
3	2.13	(0.548, 3.71)	11.6	(7.78, 18.1)
4	3.44	(0.874, 6.01)	18.8	(15.9, 31.0)
5	1.93	(1.05, 2.81)	6.61	(5.16, 7.79)

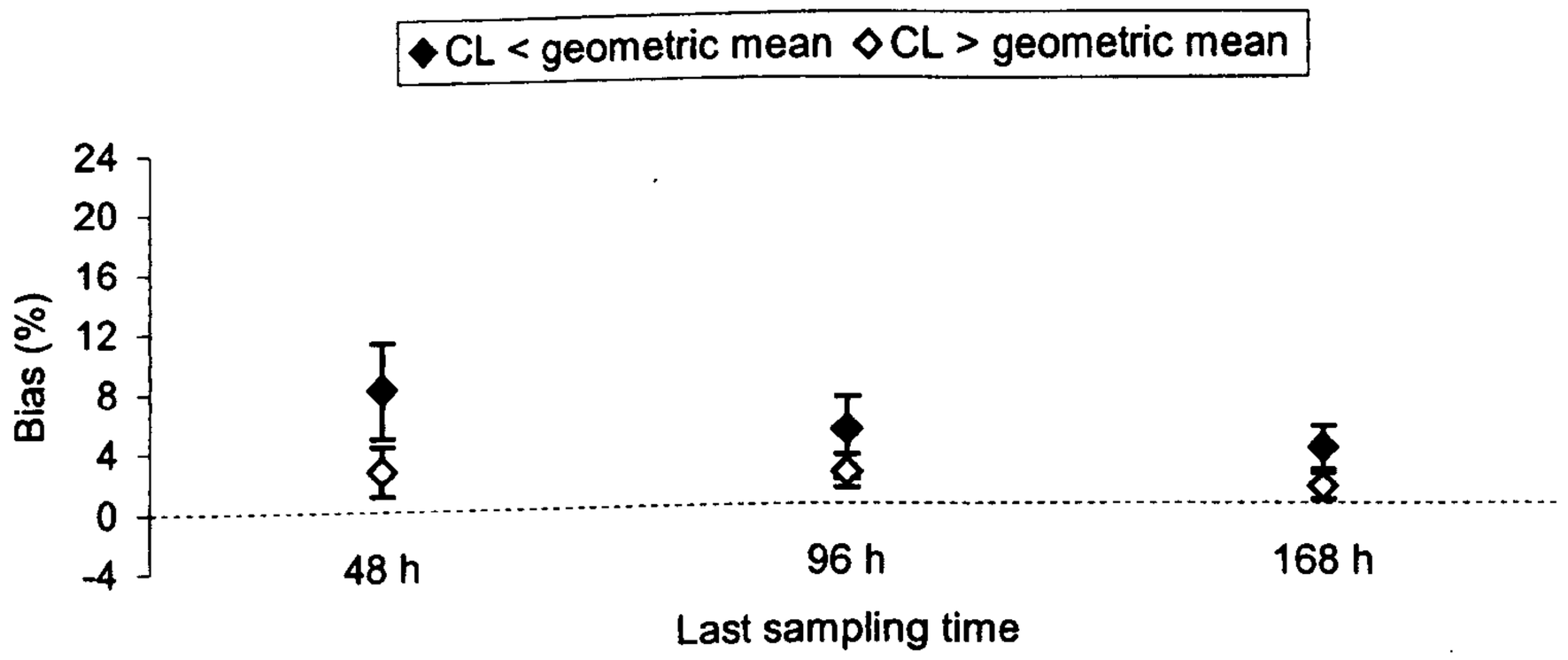
Figure 9.8 (1 of 2)

Bias calculated for CL values above geometric mean and below geometric mean in data sets with concentrations simulated up to 48, 96 and 168 h post-dose

Data set 1



Data set 2



Data set 3

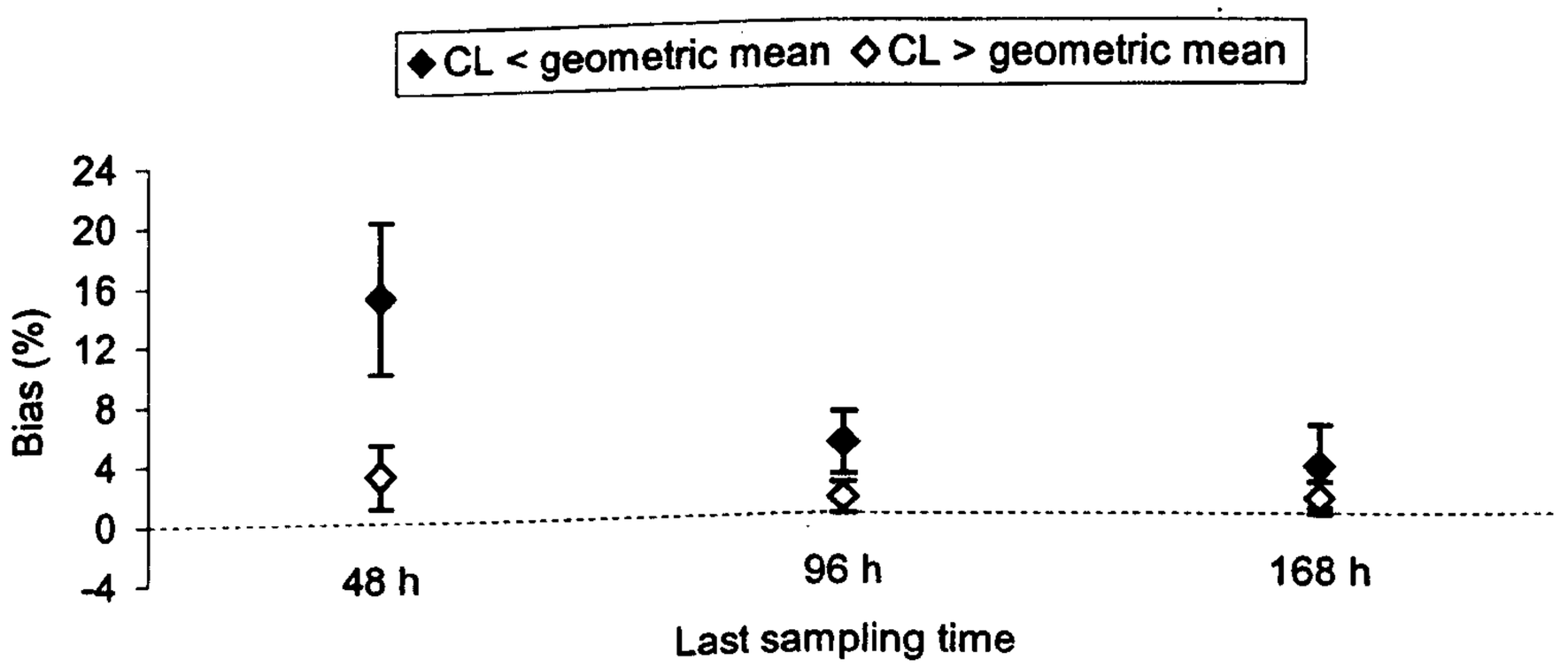
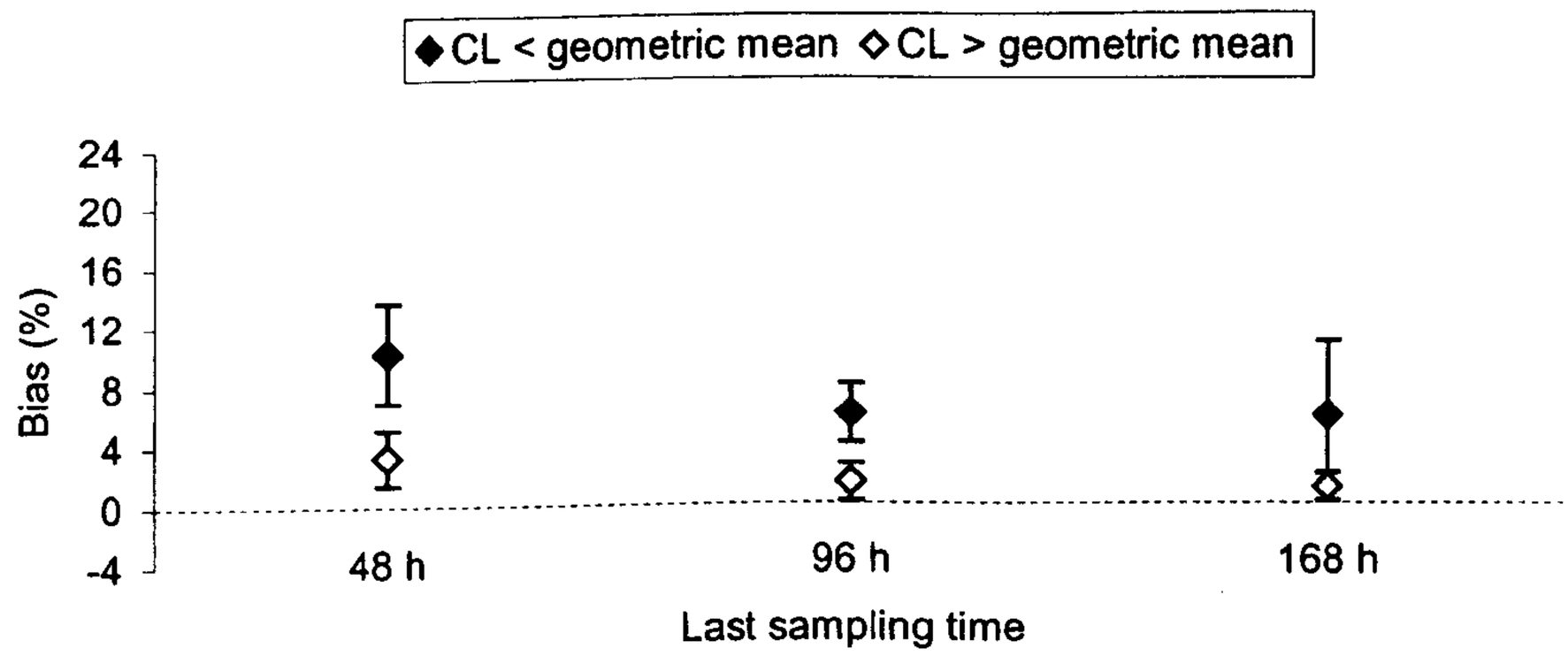


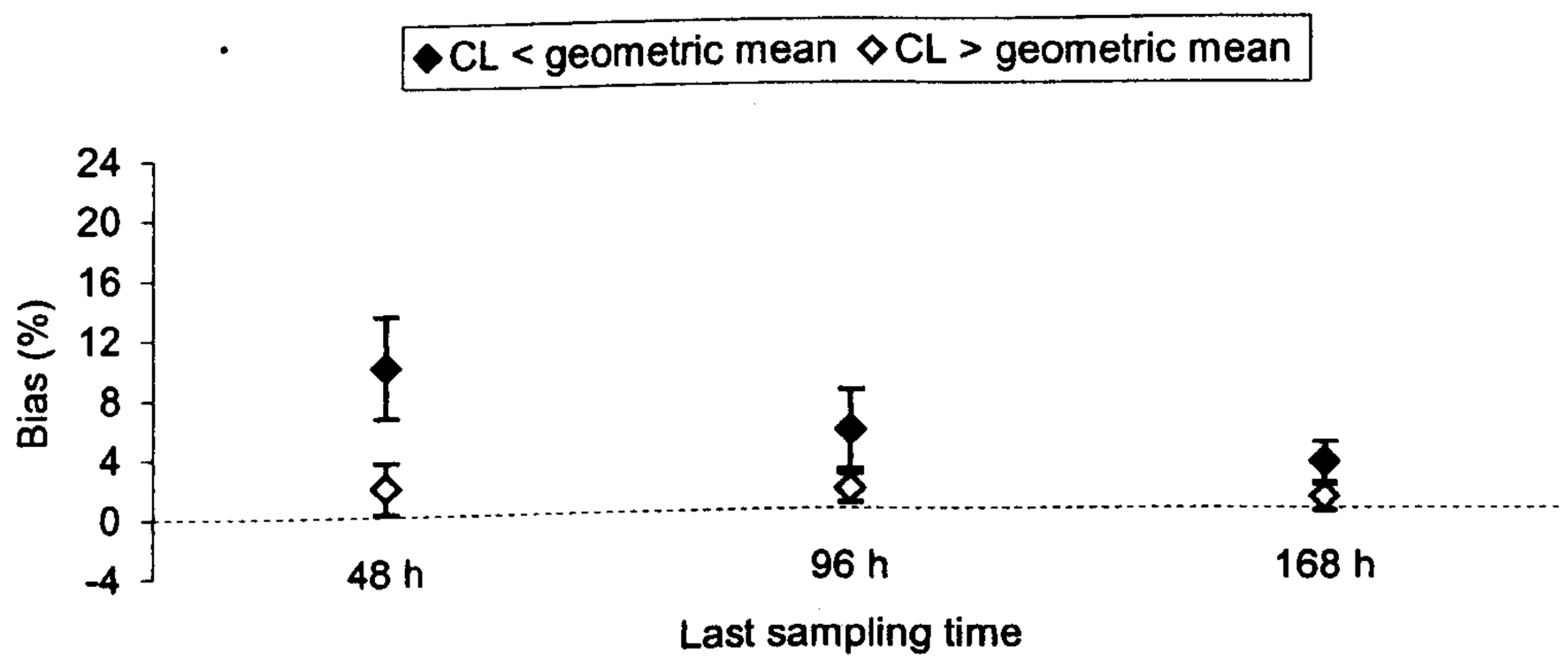
Figure 9.8 (2 of 2)

Bias calculated for CL values above geometric mean and below geometric mean in data sets with concentrations simulated up to 48, 96 and 168 h post-dose

Data set 4



Data set 5



data sets sampled up to 48 h, 10 % in data sets sampled to 96 h and 38 % in data sets sampled to 168 h.

The bias calculated in individuals whose CL was less than the geometric mean was greater than the bias calculated in individuals whose CL was greater than the geometric mean, in all data sets (Figure 9.8).

9C.3 DISCUSSION

The bias observed in the CL estimates was not influenced by the magnitude of the residual error, as no decrease in the bias was observed if the residual error was set to zero when simulating the data set. The statistically significant bias observed for the CL estimates was not a chance occurrence as repetition of the analysis with 4 new data sets gave similar bias values and in all data sets the bias was statistically significant.

When the simulated data set included concentration measurements at later time points, the extent of the bias was reduced, thus indicating that the cause of the bias was due to failure to measure blood samples for an adequate time period post-dose. It is perhaps not surprising that the estimates of CL are biased when blood samples are only measured up to 48 h, given that the population estimate of the terminal half-life for epirubicin in the original data set was approximately 30 h. It has previously been shown using a simulated data set that CL can be accurately and precisely determined if concentrations are measured for 2 half-lives (Ette 1998). Even when blood samples were simulated at 168 h (1 week) post-dose, which is more than 5 times the population elimination half-life, although the bias was reduced to approximately 2 to 3 %, it was still found to be statistically significant. It was noted that the bias was greatest in individuals with CL values below the geometric mean and hence a longer half-life. The longest half-life in the simulated data sets was calculated to be 280 h, whereas the maximum terminal half-life estimated in the patient data set was 72 h.

The limit of quantification (LOQ) of the assay used to analyse epirubicin concentrations in plasma samples in the original data set was 1 ng/ml. More recently, an improved HPLC method achieved a lower LOQ of 0.58 ng/ml (Danesi et al. 2002a). If the lower limit of quantification was set at 0.58 ng/ml, 38 % of concentrations simulated at 168 h would have been below the LOQ and therefore not available for inclusion in the analysis in a real-life clinical setting. Likewise, 10 % of the data at 96 h would not have been available. If a limited sampling approach was used, for example including two early samples and either a 96 h sample or a 168 h sample, in those patients where the concentrations were not measurable at the later time point, the individual's CL would have to be estimated using only the two early samples. Estimation of CL using only early time points was previously shown to be very poor (Section A). Thus, although bias and imprecision were slightly worse when

concentrations were only measured up to 48 h, compared to 96 h or 168 h, less than 1 % of the concentrations at 48 h would be expected to be below the LOQ of the assay. Therefore, in a clinical setting, taking a 48 h sample is likely to achieve estimates of CL that are closer to the true CL values in most patients. Thus a limited sampling design in which a 48 h sample is taken would be preferable to a design including either a 96 or 168 h sample.

9D ASSESSMENT OF JAKOBSEN'S MULTIPLE LINEAR REGRESSION LIMITED SAMPLING APPROACH

9D.1 INTRODUCTION

Using data from 78 patients who received epirubicin for treatment of advanced breast cancer, Jakobsen and coworkers (1991a) used multiple linear regression to identify blood sampling times at which measured concentrations correlated most highly with AUC. The 2 and 24 h concentrations were identified as the best combination to estimate epirubicin AUC and the 6 h concentration was identified as the best single point. In this Section, a simulation analysis was performed to compare the imprecision and bias of AUC, and hence CL, estimated using the sampling approach developed by Jakobsen to that achieved using the MAP Bayesian limited sampling approach developed in Section 9A.

9D.2 METHODS

Using NONMEM, a data set containing 200 patients was simulated using population model parameters previously estimated for epirubicin. The dose was 125 mg given as an intravenous infusion over 10 minutes to mimic the dose regimen administered to the patients studied by Jakobsen and coworkers. Individual CL values were generated by NONMEM using the 'ONLY SIM' option using a seed value of 3396450 and were designated the 'true' CL values. Concentrations were simulated at 2 and 24 h post-dose. The above methods were repeated to simulate a data set with concentrations at 6 h post-dose.

AUC values were estimated from the simulated concentrations at 2 and 24 h, using the equation previously attained by multiple linear regression by Jakobsen and coworkers (1991a):

$AUC = 9.44 \times C_2\ (ng/ml) + 62.5 \times C_{24}\ (ng/ml) + 157.7$

Likewise, AUC values were estimated from the simulated concentrations at 6 h:

$AUC = 39.6 \times C_6\ (ng/ml) + 302$

CL values were calculated from the predicted AUC values using the formula:

$CL = Dose/AUC$

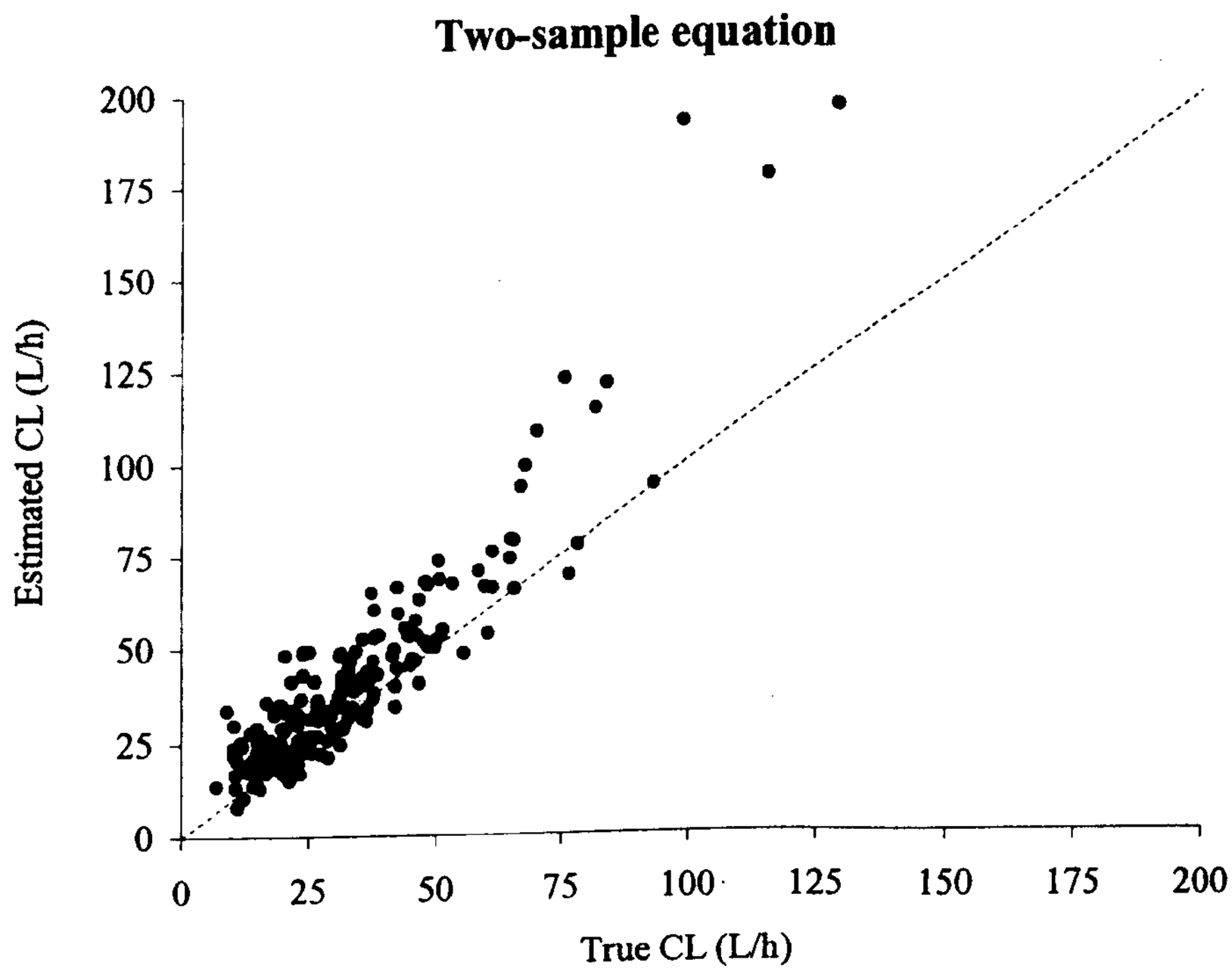
The imprecisions (rmse) and biases (me) with 95 % confidence intervals for CL estimates using the limited sampling equations relative to the 'true' CL values were calculated.

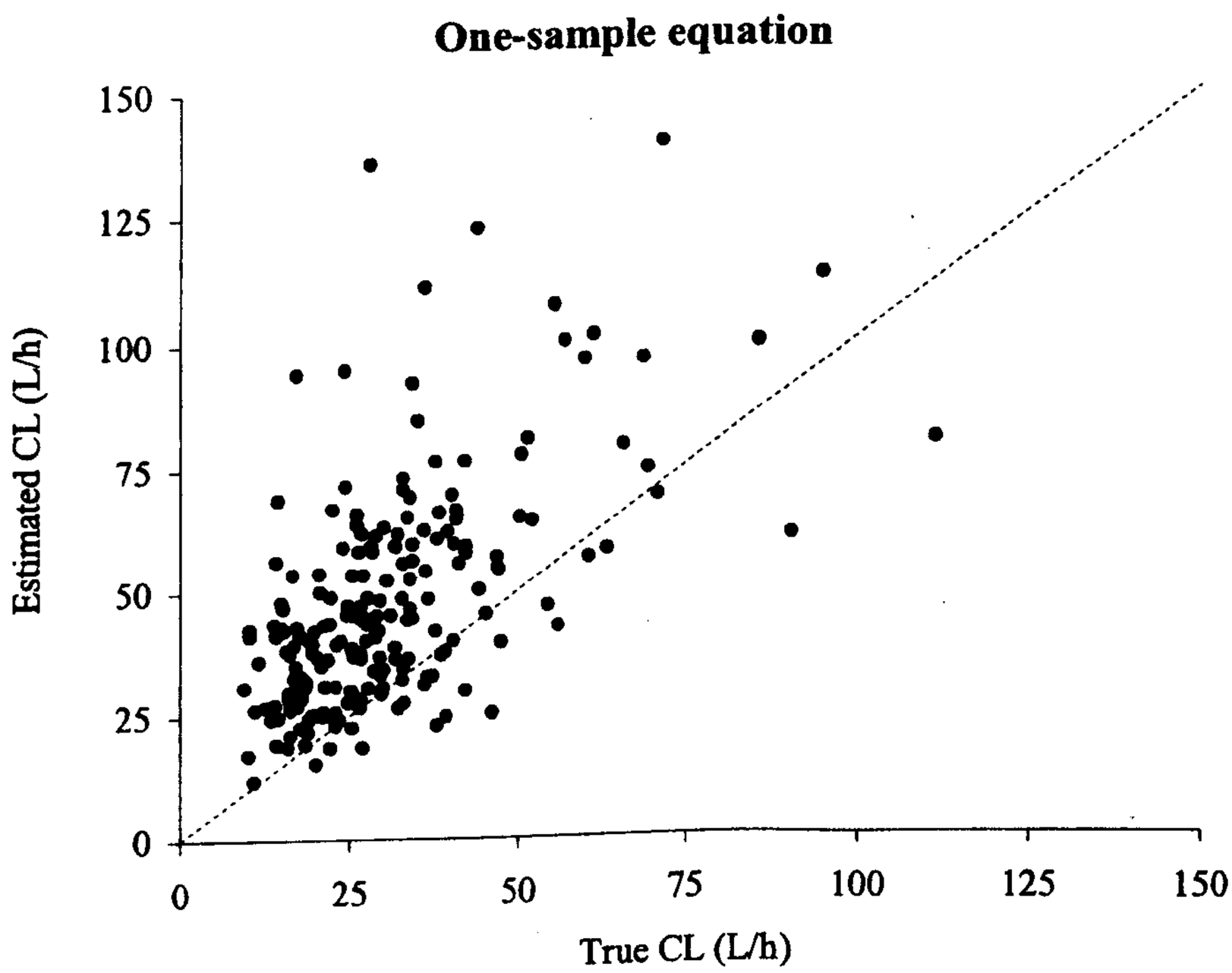
9D.3 RESULTS

CL values estimated by the two-sample and one-sample equations are plotted versus the true CL values in Figure 9.9. Both the one and two-sample equations resulted in CL values that were statistically significantly biased. The imprecision (rmse) and bias (me) of CL estimates from the two-sample equation were 47.7 (95% CI 37.1, 56.3) and 27.7 (95% CI 22.3, 33.1), respectively. The bias from the two-sample design was most noticeable at high CL values ie. above 65 L/h. For the one-sample equation the imprecision and bias of CL estimates were 107 % (95% CI 89.2, 122) and 71.8 % (95% CI 60.8, 82.8), respectively.

Figure 9.9

CL values estimated by two-sample and one-sample equations plotted versus true CL values





9D.4 DISCUSSION

The CL values estimated from the one-sample equation, previously proposed by Jakobsen were substantially biased (72 %) and were highly imprecise (rmse = 107 %). Although an improvement in bias (28 %) and imprecision (48 %) of CL estimates was observed for the two sample equation this was still very poor compared to the bias (10 %) and imprecision (27 %) observed for the best limited sampling design (Design E) developed in Section A using MAP Bayesian estimation. In addition to the improved precision and reduced bias in CL estimates obtained using MAP Bayesian estimation, this approach offers flexibility in terms of the timing of blood samples, dose level and length of the infusion, which is not possible using the multiple linear regression equation.

9.2 CONCLUSIONS TO CHAPTER

Using D-optimality to identify optimum blood sampling times, limited sampling designs were developed to facilitate estimation of epirubicin CL by MAP Bayesian estimation techniques from only 2 or 3 blood samples. These limited sampling designs combined with MAP Bayesian estimation were found to estimate epirubicin CL with greater precision than both the covariate model and a previous limited sampling model derived by multiple linear regression. Furthermore, these limited sampling designs were shown to predict CL precisely in an independent data set. A simulation analysis indicated that the proposed limited sampling designs were robust with respect to errors in the recording of sampling times, suggesting that they will work well in clinical practice.

CHAPTER 10

GENERAL CONCLUSIONS

The aims of this thesis were to use a population approach to determine typical pharmacokinetic parameters for epirubicin, and their variability, in a population of patients with normal and impaired hepatic function, to identify clinical characteristics that influence these parameters and to develop dosage guidelines to achieve a target drug exposure. The model and dosage guidelines were then evaluated using both internal and external validation methods.

Development of dosage guidelines based on clinical characteristics

The analysis of a data set obtained from UK patients identified AST as having the strongest influence on epirubicin CL. No other clinical characteristics were identified as having any additional clinically relevant influence. The population model developed was subsequently used to propose new dosage guidelines based on AST. A major advantage of these new guidelines is that, unlike the currently available guidelines, dose adjustment according to BSA is not required and is likely to reduce dosage preparation time and dosage errors. These new guidelines achieved a target AUC of 4000 ng.h/ml in the UK data set, with greater precision than UK, USA or previous guidelines proposed by Dobbs and Twelves (2003).

Despite the apparent improvement, the precision of the proposed guidelines to achieve a target AUC was still very poor. The difficulties in attaining the target AUC reflected the high inter-individual variability in CL that remained unexplained by the population model. Further work is required to identify other covariates that influence epirubicin pharmacokinetics. It may be that genetic or dietary differences account for the differences in PK between individuals. Dietary differences are likely to be difficult to measure and adjust for. Likewise, genetic differences are also difficult to identify, however, with advances in molecular biology techniques for identification of genetic polymorphisms, pharmacogenetics may offer a suitable solution in the future (Evans & Johnson 2001). Of course it is possible that there are hundreds of factors that each have a small influence on the pharmacokinetics of epirubicin and it may be that dose adjustment based on covariates may never be able to attain target PK end-points with adequate precision.

Validation of proposed dosage guidelines

Internal model validation techniques recommended by the FDA include jacknife analysis, sensitivity analysis and cross-validation. The results of this analysis found the model to be stable, not based on outliers or spurious data and suggested favourable predictive performance of CL. However, these results are inconsistent with those of the external validation, which found the population model unable to predict CL adequately in two external data sets. The value in using internal techniques, therefore, has to be questioned. The use of internal validation techniques to validate population pharmacokinetic models has become increasingly popular over the last few years (Enders Klein et al 2002; van Kesteren et al. 2002; Parke & Charles 2000). Logically, it would seem unlikely that validation of a population model, using the same data set that was used to develop it, would find the model to be anything other than valid. Of course such internal techniques are really assessing the robustness and stability of the model in terms of any spurious results. It is perhaps the terminology internal 'validation' that is responsible for many authors placing too much emphasis on the results of such analyses.

FDA guidelines recommend that the predicted performance of a population model is tested using an external data set, particularly if the results are intended to define dosage guidelines (FDA guidelines, 1999). When the model was tested in 18 patients with either breast cancer or hepatocellular carcinoma, the predictive performance of the model was found to be highly imprecise. It is possible that AST is not an appropriate marker of epirubicin CL for patients with primary liver cancer or that the model developed in patients receiving epirubicin intravenously is not suitable for patients receiving epirubicin as an intra-arterial infusion. However, the performance of model was also poor when applied to a larger data set from Sweden that included 79 patients with breast cancer. Although poor precision was predictable due to the wide variability in CL, the large bias observed in the CL estimates obtained with the Swedish data set was unexpected. The typical CL in Swedish data set was estimated to be 57 % higher than the typical CL in UK data set. The reason for this was unclear. Possible explanations for the differences in the two data sets could be the concomitant chemotherapy that was administered to the Swedish patients, or differences in prior chemotherapy, disease status or genetic factors. The results of this analysis have indicated that the 105 subjects included in the original analysis are not

representative of the true population and highlights the limitation of extrapolating results from a relatively small patient group to the population as a whole. Ideally, the population analysis should be performed on larger patient numbers and should include patients with greater heterogeneity.

Dose-adjustment based on measured drug concentrations

An alternative approach to dose adjustment based on covariate measurement (*a priori* dose adjustment) is to take blood samples after administering the dose, estimate CL from measured concentrations and adjust the next dose accordingly (*a posteriori* dose adjustment). Using D-optimality to identify optimum blood sampling times, limited sampling designs were developed. These sampling strategies enabled accurate estimation of epirubicin CL by MAP Bayesian estimation techniques from only 2 or 3 blood samples.

MAP Bayesian estimation was found to estimate epirubicin CL with better precision than the covariate model approach and a previous limited sampling model derived by multiple linear regression (Jakobsen et al. 1991a). Unlike equations derived by multiple linear regression, MAP Bayesian estimation offers flexibility in terms of the timing of blood samples and the dosage regimen. Furthermore, the proposed limited sampling designs were shown to be robust when sampling times had recording errors of up to 20%, suggesting that they will work well in clinical practice. In contrast to the dosage guidelines based on covariate analysis (AST), these limited sampling designs achieved precise estimates of CL in an independent data set that included 18 patients with breast cancer or hepatocellular carcinoma.

An advantage of measuring blood concentrations to individualise dosage from an estimate of CL (*a posteriori* methods), is that it enables identification of patients who are receiving potentially subtherapeutic doses. This is not possible when dose individualisation is performed using covariate models. An obvious disadvantage of the *a posteriori* method is that it can not be used to determine the first dose. Furthermore, taking blood samples is inconvenient to patients, samples are costly to analyse and it takes considerable training of staff to perform MAP Bayesian estimation of epirubicin CL.

Suggestions for future dosing strategies

The solution to these difficulties in epirubicin dose individualisation could be to adopt a similar approach to that used for busulphan (Sandstrom et al. 2001). Dose individualisation of busulphan according to clinical characteristics (ALT, weight and phenytoin co-administration) improved the ability to achieve the target AUC compared to non-individualised therapy. However, therapeutic drug monitoring with MAP Bayesian estimation of busulphan CL enabled the target AUC to be achieved with greater precision than using clinical characteristics. Thus, a busulphan dosing program was proposed in which the first dose of busulphan was calculated from the clinical characteristics and subsequent doses were calculated according to 3 measured concentrations. To overcome difficulties in training staff to perform MAP Bayesian estimation, the authors wrote a user-friendly program in the widely available software package Microsoft Excel using macros, in which the doses are calculated following input of basic information by the user.

Limitations of the work performed in this thesis

For dose-individualisation to be successful, inter-occasion variability needs to be less than inter-individual variability. The data sets used in this thesis included data from one dosing occasion only in each individual, and therefore, it was not possible to estimate variability in the pharmacokinetics between different dosing occasions. A previous population analysis performed on data from patients administered epirubicin estimated inter-occasion variability in CL to be 7 % and inter-individual variability to be 15 % (Sandstrom 2002). The lower inter-occasion variability relative to inter-individual variability indicates that dose individualisation has the potential to improve therapeutic outcome.

Another criterion for the successful implementation of dose individualisation is a clearly defined PK/PD relationship. If the PK target associated with either the desired response or alternatively undesirable toxicity is unknown, we cannot know how to alter the dose to achieve optimum chemotherapy. Despite the importance of this fundamental principal, there are few studies that have attempted to define the PK/PD relationship for epirubicin. PK-PD relationships in oncology are notoriously difficult to define due to the high variability in response observed and the difficulties in measuring response.

Only weak relationships were found between PK parameters and haematological toxicity in the UK data set. Although the blood cell counts were labelled as representing the nadir, they were measured in a single blood sample taken between 7 and 14 days after dosing and therefore were unlikely to represent the true nadir for all patients. If the actual day of sampling had been available, it may have been possible to model the blood cell counts over time using a transit model such as that developed by Sandstrom (2002). Unfortunately, as the data sets used in this thesis were from studies performed at least 10 years ago, it was not possible to determine the actual day of blood sampling. This highlights the sort of difficulties often experienced when using data collected from a routine clinical environment.

The most successful study, in terms of identifying a PK/PD relationship was performed in 55 patients with advanced breast cancer (Jakobsen et al. 1991b). This study identified a relationship between the AUC of epirubicin and a fall in white blood cells (WBC), expressed as the logarithmic ratio of WBC nadir to pre-treatment WBC count. In this thesis, using the relationship proposed by Jakobsen et al (1991b), an AUC of 4000 ng.h/ml was taken as a PK target in the generation of the dosage guidelines. It has not been possible to confirm if 4000 ng.h/ml is a suitable PK target, however, it is consistent with a study of patients with nasopharyngeal carcinoma treated with epirubicin in which the mean AUC associated with complete response was 4002 ng.h/ml (Hu et al. 1989). This target would therefore seem reasonable in the absence of any further information; however, the PK target may vary between individuals depending on their health and therapeutic requirements.

The work performed in this thesis has focused on CL, and hence AUC, as the parameter of primary importance in terms of dose individualisation. No investigation into other potential parameters of interest, such as C_{max}, has been performed. Studies of doxorubicin, the prototype of epirubicin, found that cardiotoxicity was reduced following administration as a prolonged infusion compared to a bolus injection, and hence, cardiotoxicity was assumed to be associated with C_{max} rather than AUC (Berrak et al. 2001). However, administration as a prolonged infusion resulted in increased severity of mucositis and bone marrow suppression (Danesi et al. 2002). An association between epirubicin C_{max} and toxicity is less evident, although one study reported a relationship between epirubicin C_{max} and alopecia (Eksborg et al. 1992). UK and USA guidelines recommend that epirubicin is

administered as a bolus injection. In contrast, some Swedish clinicians prefer to administer epirubicin as a prolonged intravenous infusion due to perceived associations between toxicity and anthracycline C_{max}, and this is reflected by the longer infusion rate (median 1 h) observed in the Swedish data set. Clearly more work is warranted to define the relationship between toxicity/response and PK markers such as C_{max}, AUC or time above a threshold concentration for epirubicin. Without more clearly defined PK-PD relationships, attempts to improve therapeutic outcome by dose individualisation are unlikely to be effective.

Concluding remarks

The importance of optimising dosage and administration procedures in cancer therapy is exemplified by the massive improvements in cure rates achieved in childhood leukaemia. In the 1960s childhood leukaemia was essentially incurable, whereas more recently the cure rate has been estimated to be 75 % (Mason & Zamboni 1997). The improvement in the cure rate is not attributed to the discovery of new drugs but rather an improvement in the methods of dose administration of the same drugs used in the 1960s. Of course the search for new methods and drugs to cure cancer must continue, but it is also essential to optimise therapy with the drugs that are currently available to maximise the chances of a favourable therapeutic response. In this thesis, investigations into the optimum dosage procedures for epirubicin to treat patients with breast cancer have been performed in an attempt to improve therapeutic outcome.

REFERENCES

Arcamone, F., Penco, S. & Vigevani, A. (1975) Synthesis and antitumour properties of new glycosides of daunomycinone and adriamycinone. *Journal of Medicinal Chemistry* **18**, 703-707

Akaike H. (1978) A Bayesian analysis of the minimum AIC procedure. *Annals of the Institute of Statistical Mathematics* **30**, 9-14

Amantea, M.A., Forrest A., Northfelt, D.W. & Mamelok, R. (1997) Population pharmacokinetics and pharmacodynamics of pegylated-liposomal doxorubicin in patients with AIDS-related Kaposi's sarcoma. *Clinical Pharmacology & Therapeutics* **61**(3), 301-311

Bachur, N.R., Yu, F., Johnson, R., Hickey, R., Wu, Y. & Malkas, L. (1992) Helicase inhibition by anthracycline agents. *Molecular Pharmacology* **41**, 993-998

Baille, P., Bruno, R., Schellens, J.H.M., Webster, L.K., Millward, M., Verweij, J. & Montay G. (1997) Optimal sampling strategies for Bayesian estimation of docetaxel (taxotere) clearance. *Clinical Cancer Research.* **3**, 1535-1538

Beal, S.L. & Sheiner, L.B. (1992) *NONMEM User Guides, parts 1-VII.* Technical Report. University of California, San Francisco

Benjamin, R.S., Wiernik, P.H. & Bachur, N.R. (1974) Adriamycin chemotherapy – efficacy, safety and pharmacologic basis of an intermittent single high-dosage schedule. *Cancer* **33**(1), 19-27

Berrak, S.G., Ewer, M.S., Jaffe, M., Pearson, P., Ried, H., Zeitz, H.A & Benjamin, R.S. (2001) Doxorubicin cardiotoxicity in children: reduced incidence of cardiac dysfunction associated with continuous-infusion schedules. *Oncology Reports* **8**, 611-614

Bodey, G.P., Buckley, M., Sathe, Y,S. & Freireich, E.J. (1966) Quantitative relationships between circulating leukocytes and infection in patients with acute leukemia. *Annals of Internal Medicine* **64**, 324-340

Bonate, P.L. (1999). The effect of collinearity on parameter estimates in nonlinear mixed effect models. *Pharmaceutical Research* **16**(5), 709-717

Box, G.E.P. & Lucas, H.L. (1959). Design of experiments in non-linear situations. *Biometrika* **46**, 77-90

Brockmoller, J. & Roots, I. (1994) Assessment of liver metabolic function. Clinical implications. *Clinical Pharmacokinetics* **27**(3), 216-248

Bruno, R., Vivier, N., Vergniol, J.C., De Phillips, S.L., Montay, G. & Sheiner, L.B. (1996) A population pharmacokinetic model for docetaxel (Taxotere®): Model building and validation. *Journal of Pharmacokinetics & Biopharmaceutics* **24**(2) 153-172

Bruno, R., Hille, D., Riva, A., Vivier, N., Huinnink, W., Oosterom, A., Kaye, S.B., Verweij, J., Fossella, F.V., Valero, V., Rigas, J.R., Seidman, A.D., Chevallier, B., Fumoleau, P., Burris, H.A., Ravdin, P.M. & Sheiner, L.B. (1998) Population pharmacokinetics/pharmacodynamics of docetaxel in phase II studies in patients with cancer. *Journal of Clinical Oncology* **16**(1),187-196

Calais da Silva, F., Denis, L., Bono, A., Bollack, C. & Bouffioux, C. (1988) Intravesical chemoresection with 4'-epi-doxorubicin in patients with superficial bladder tumors. *European Urology* **14**, 207-209

Calvert, A.H., Newell, D.R., Gumbrell, L.A. O'Reilly, S., Burnell, M., Boxall, F.E., Siddik, Z.H., Judson, I.R., Gore, M.E., & Wiltshaw, E. (1989) Carboplatin dosage: prospective evaluation of a simple formula based on renal function. *Journal of Clinical Oncology* **7**, 1748-56

Camaggi, C.M., Strocchi, E., Tamassia, V., Martoni, A., Giovannini M., Iafelice, G., Canova, N., Marraro, D., Martini, A. & Pannuti, F. (1982) Pharmacokinetic studies of 4'-epi-doxorubicin in cancer patients with normal and impaired renal function and with liver metastases. *Cancer Treatment Reports* **66**(10), 1819-1824

Camaggi, C.M., Strocchi, E., Comparsi, R., Testoni, F., Angelelli, B. & Pannuti, F. (1986) Biliary excretion and pharmacokinetics of 4'epidoxorubicin (epirubicin) in advanced cancer patients. *Cancer Chemotherapy & Pharmacology* **18**, 47-50

Camaggi, C.M., Comparsi, R., Strocchi, E., Testoni, F., Angelelli, B. & Pannuti, F. (1988) Epirubicin and doxorubicin comparative metabolism and pharmacokinetics. *Cancer Chemotherapy & Pharmacology* **21**, 221-228

Caregaro L, Menon F, Angeli P, Amodio P, Merkel C, Bortoluzzi A, Alberino F & Gatta A. (1994) Limitations of serum creatinine level and creatinine clearance as filtration markers in cirrhosis. *Archives of Internal Medicine* **154**, 201-205

Carmo-Pereira, J., Costa, F.O., Miles, D.W., Henriques, E., Richards, M.A. & Rubens, R.D. (1991) High-dose epirubicin as primary chemotherapy in advanced breast carcinoma: a phase II study. *Cancer Chemotherapy & Pharmacology* **27**, 394-396

Chiou, W.L. (1979) Potential pitfalls in the conventional pharmacokinetic studies: effects of the initial mixing of drug in blood and the pulmonary first-pass elimination. *Journal of Pharmacokinetics & Biopharmaceutics* **7**(5), 536-536

Cockcroft, D.W. & Gault, M.H. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron* **16**, 31-41

Cook, R.D. Detection of influential observation in linear regression. (1977) *Technometrics*. **19**(1), 15-18.

Coukell, A.J. & Faulds, D. (1997) Epirubicin. An updated review of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy in the management of breast cancer. *Drugs* **53**(3), 453-482

Danesi, R., Innocenti, F., Fogli, S., Gennari, A., Baldini, E., Di Paolo, A., Salvadori, B., Bocci, G., Conte, P.F. & Del Tacca, M. (2002a) Pharmacokinetics and pharmacodynamics of combination chemotherapy with paclitaxel and epirubicin in breast cancer patients. *British Journal of Clinical Pharmacology* **53**, 5080-518

Danesi, R., Conte, P.F. & Del Tacca, M. (1999) Pharmacokinetic optimisation of treatment schedules for anthracyclines and paclitaxel in patients with cancer. *Clinical Pharmacokinetics* **37**, 195-211

Danesi, R., Fogli, S., Gennari, A., Conte, P.F. & Del Tacca, M. (2002b) Pharmacokinetic-pharmacodynamic relationships of the anthracycline anticancer drugs. *Clinical Pharmacokinetics* **41**(6) 431-444

D'Argenio, D.Z. (1981). Optimal sampling times for pharmacokinetic experiments. *Journal of Pharmacokinetics & Biopharmaceutics* **9**(6), 739-756

D'Argenio, D.Z. & Schumitzky, A. (1997) *ADAPT II user's guide: pharmacokinetic/pharmacodynamic systems analysis software.* Biomedical Simulations Resource, Los Angeles.

De Jonge, M.J.A., Verweij, J., Loos, W.J., Dallaire, B.K. & Sparreboom, A. (1999) Clinical pharmacokinetics of encapsulated oral 9-aminocamptothecin in plasma and saliva. *Cancer Chemotherapy & Pharmacology* **65**(5), 491-499

Dobbs, N.A. & Twelves, C.J. (1991) Measurement of epidoxorubicin and its metabolites by high performance liquid chromatography using an advanced automated sample processor. *Journal of Chromatography* **572**, 211-217

Dobbs, N.A. & Twelves, C.J. (1998a) Anthracycline doses in patients with liver dysfunction: do UK oncologists follow current recommendations? *British Journal of Cancer* 77(7), 1145-1148

Dobbs, N.A. & Twelves, C.J. (1998b) What is the effect of adjusting epirubicin doses for body surface area? *British Journal of Cancer* **78**(5), 662-666

Dobbs, N.A., Twelves, C.J., Gregory, W., Cruickshank, C., Richards, M.A. & Rubens, R.D. (2003) Epirubicin in patients with liver dysfunction: development and evaluation of a novel dose modification scheme. *European Journal of Cancer* **39**(5), 580-586

Doroshow, J.H. (1983) Anthracycline antibiotic-stimulated superoxide, hydrogen peroxide and hydroxyl radical production by NADH dehydrogenase *Cancer Research* **43**, 4543-4551

Dubois, D. & Dubois, E.F. (1916) A formula to estimate the approximate surface area if height and weight be known. *Archives of Internal Medicine* **17**, 863-871

Effron, B. & Tibshirani, R. (1993) *An Introduction to the Bootstrap.* London: Chapman & Hall

Egorin, M.J., Van Echo, D.A., Olman, E.A. Whitacre, M.Y., Forrest, A. & Aisner, J. (1985) Prospective validation of a pharmacologically based dosing scheme for the cis-diamminedicchloroplatinum (II) analogue diamminecyyclobutanedicarboxylatoplatinum. *Cancer Research* **45**, 6502-6506

Egorin, M.J., Forrest, A. Belani, C.P., Ratain, M.J., Abrams, J.S. & Van Echo, D.A. (1989) A limited sampling strategy for cyclophosphamide pharmacokinetics. *Cancer Research* **49**(11), 3129-3133

Eksborg, S., Hardell, L., Bengtsson, N., Sjodin, M. & Elfsson, B. (1992) Epirubicin as a single agent therapy for the treatment of breast cancer – a pharmacokinetics and clinical study. *Medical Oncology & Tumor Pharmacotherapy* **9**, 75-80

Enders Klein, C., Gupta, E., Reid, J., Atherton, P.J., Sloan, J.A., Pitot, H.C., Ratain, M.J. & Kastrissios, H. (2002). Population pharmacokinetic model for irinotecan and two of its metabolites, SN-38 and SN-38 glucuronide. *Clinical Pharmacology Therapeutics* **72**(6), 638-47

Epirubicin product label, Food & Drug Administration, USA. http://www.accessdata.fda.gov/scripts/cder/onctools/labels

Ette, E.I. & Ludden, T.M. (1995) Population pharmacokinetic modelling: the importance of informative graphics. *Pharmaceutical Research*. **12**(12), 1845-1855.

Ette, E.I. Stability and performance of a population pharmacokinetic model. (1997) *Journal of Clinical Pharmacology* **37**, 486-495

Ette, E.I., Sun, H.E. & Ludden, T.M. (1998). Balanced designs in longitudinal population pharmacokinetic studies. *Journal Clinical Pharmacology* **38**, 417-423

Evans, W.E., Crom, W.R., Arbromowitch, M., Dodge, R., Look, A.T., Bowman, W.P., George, S.L. & Pui, C.H. (1986) Clinical pharmacodynamics of high-dose methotrexate in an acute lymphocytic leukemia. *New England Journal of Medicine* **314**, 471-477

Evans, W.E., Relling, M.V., Rodman, J.H., Crom, W.R., Boyett, J.M. & Pui, C.H. (1998) Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. *New England Journal of Medicine*. **338**(8), 499-505

Evans W.E & Johnson J.A. Pharmacogenomics: The inherited basis for inter-individual differences in drug response. (2001) *Annual Reviews of Genomics and Human Genetics* **2**, 9-39

Favier, M., deCazanove, F., Saint Martin, F. & Bressolle, F. (1994) Preventing medication errors in antineoplastic therapy. *American Journal of Hospital Pharmacy.* **51**, 832-833

FDA guidelines (1999). *Guidance for Industry. Population Pharmacokinetics.* US Department of Health and Human Services. Food and Drug Administration, Centre for Drug Evaluation and Research (CDER). Centre for Biologics Evaluation and Research (CBER). http://www.fda.gov/cder/guidance/index.htm

Friberg, L.E., Brindley, C.J., Karlsson, M.O. & Devlin, A.J. (2000) Models of schedule dependent haematological toxicity of 2'-deoxy-2'-methylidenecytidine (DMDC). *European Journal of Clinical Pharmacology* **56**, 567-574

Gibiansky, E., Gibiansky, L. & Bramer, S. (2001) Comparison of NONMEM, bootstrap, jacknife and profiling parameter estimates and confidence intervals for the aripiprazole population model. Poster presentation. *American Association of Pharmaceutical Scientists Annual Meeting,* Denver, USA.

Gholson, C.F. & Bacon, B.R. (1993) *Essentials of Clinical Hepatology.* pp31-40. St. Louis: Mosby-Year Book

Goodman Gilman, A., Rall, T.W., Nies, A.A. & Taylor, P. (1992). *The Pharmacological Basis of Therapeutics.* 8th Edition pp1242. Singapore: McGraw-Hill

Grochow, L.B., Baraldi, C. & Noe, D. (1990) Is dose normalisation to weight or body surface area useful in adults? *Journal of the National Cancer Institute* **82**(4), 323-325

Gurney, H.P., Ackland, S., Gebski, V. & Farrell, G. (1998) Factors affecting epirubicin pharmacokinetics and toxicity: evidence against using body-surface area for dose calculation. *Journal of Clinical Oncology* **16**(7), 2299-2304

Hayward, J.L., Rubens, R. D., Carbone, P.P., Heuson, J.C., Kumaoka, S. & Segaloff, A. (1977) Assessment of response to therapy in advanced breast cancer: project of the programme on the clinical oncology of the international union against cancer. *British Journal of Cancer* **35**, 2292-298

Hirth, J., Watkins, P.B., Strawderman, M., Schott A, Bruno, R. & Baker, L.H. (2000). The effect of individuals cytochrome CYP3A4 activity on docetaxel clearance. *Clinical Cancer Research* **6**, 1255-1258

Hu, O.Y.P., Chang, S.P., Jame, J.M. & Chen, K.Y. Pharmacokinetic and pharmacodynamic studies with 4'-epi-doxorubicin in nasophayngeal carcinoma patients. (1989) *Cancer Chemotherapy & Pharmacology* **24**, 332-337

Jakobsen, P., Steiness, E., Bastholt, L., Dalmark, M., Lorenzen, A., Petersen, D., Gjedde, S.B., Sandberg, E., Rose, C., Nielsen, O.S., Mouridsen, H.T. & Jakobsen, A. (1991a) Multiple-dose pharmacokinetics of epirubicin at four different dose levels: studies in patients with metastatic breast cancer. *Cancer Chemotherapy & Pharmacology* **28**, 63-68.

Jakobsen, P., Basholt, L., Dalmark, M., Pfeiffer, P., Petersen, D., Gjedde, S.B., Sandberg, E., Rose, C., Nielsen, O.S. & Mouridsen, H.T. (1991b) A randomised study of epirubicin at four different dose levels in advanced breast cancer. Feasibility of myelotoxicity prediction through single blood-sample measurement. *Cancer Chemotherapy & Pharmacology* **28**, 465-469

Jodrell, D.I., Egorin, M.J., Canetta, R.M., Langenberg, P., Goldbloom, E.P., Burroughs, J.N., Goodlow, J.L., Tan, S. & Wiltshaw, E. (1992). Relationships between carboplatin exposure and tumour response and toxicity in patients with ovarian cancer. *Journal of Clinical Oncology* **10**, 520-528

Jonsson, E.N. & Karlsson, M.O. (1998) Automated covariate model building within NONMEM. *Pharmaceutical Research* **15**, 1463-1468

Jonsson, E.N. & Karlsson, M.O. (1999) Xpose – an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Computer Methods & Programming in Biomedicine* **58**, 51-64

Karlsson, M.O. & Sheiner L.B. (1993) The importance of modelling interoccassion variability in population pharmacokinetic analyses. *Journal of Pharmacokinetics & Pharmacodynamics* **21**(6), 735-750

Karlsson, M.O., Port, R.E., Ratain, M.J. & Sheiner, L.B. (1995) A population model for the leukopenic effect of etoposide. *Clinical Pharmacology & Therapeutics* **57**(3), 325-334

Karlsson, M.O., Jonsson, E.N., Wiltse, C.G. & Wade, J.R. (1998). Assumption testing in population models: Illustrated with an analysis of moxonidine data from congestive heart failure patients. *Journal of Pharmacokinetics & Biopharmaceutics* **26**(2), 207-246

Kerr, D.J., Graham, J., Cummings, J., Morrison, J.G., Thompson, G.G., Bodie, M.J. & Kaye S.B. (1986). The effect of verapamil on the pharmacokinetics of adriamycin. *Cancer Chemotherapy & Pharmacology* **18**, 239-242

Kumar, P.J. and Clark M.L. (1998) *Clinical Medicine.* 4th Edition. ed. Saunders W.B. pp 289-293 Edinburgh

Lacey, L.F., Keebe, O.N., Pritchard, J.F. & Bye, A. (1997) Common noncompartmental pharmacokinetic variables: are they normally or log-normally distributed? *Journal of Biopharmaceutical Statistics* **7**(1), 171-178

Launay, M.C., Milano, G., Iliadis, A., Frenay, M. & Namer, N. (1989) A limited sampling procedure for estimating adriamycin pharmacokinetics in cancer patients. *British Journal of Cancer* **60**, 89-92

Masson, E. & Zamboni, W.C. (1997) Pharmacokinetic optimisation of cancer chemotherapy. Effect on outcomes. *Clinical Pharmacokinetics* **32**(4), 324-343

Mentre, F., Burtin, P., Merle, Y., van Bree, J., Mallet, A. & Steimer, J.L. (1995). Sparse-sampling optimal designs in pharmacokinetics and toxicokinetics. *Drug Information Journal* **29**, 997-1019

Mentre, F., Mallet, A. & Baccar, D. (1997) Optimal design in random-effects regression models. *Biometrika* **84**(2), 429-442

Mentre, F. & Ebelin, M.E. (1997). *Validation of population pharmacokinetic/pharmacodynamic analyses: Review of proposed approaches. The population approach: measuring and managing variability in response, concentration and dose.* Commission of the European Communities, pp147-160 Luxemburg: CORDIS

Miller, K.D., Munshi, N., Loesch, D., Einhorn, L.H. & Sledge, G.W. (2000) A phase II trial of high dose epirubicin in patients with advanced breast carcinoma. *Cancer* **88**(2), 375-380

Minami, H., Sasaki, Y., Saijo, N., Ohtsu, T., Fujii, H., Igarashi, T, & Itoh, K (1998). Indirect-response model for the time course of leukopenia with anticancer drugs. *Clinical Pharmacology & Therapeutics* **64**(5), 511-521

Morgan, D.J. & McLean, A.J. (1995) Clinical pharmacokinetic and pharmacodynamic considerations in patients with liver disease. *Clinical Pharmacokinetics* **29**(5), 370-391

Mouridsen, H.T., Alfthan, C., Bastholt, L., Bergh, J., Dalmark, M., Eksborg, S., Hellsten, S., Kjaer, M., Peterson, C., Skovsgard, T., Sorensen, J.B., Trope, C. & Aabo, K. (1990) Current Status of epirubicin (farmorubicin) in the treatment of solid tumours. *Acta Oncologica.* **29**(3), 257-285

Mross, K., Maessen, P., van de Vijgh, W.J.F., Gall, H., Boven, E. & Pinedo, H.M. (1988) Pharmacokinetics and metabolism of epidoxorubicin and doxorubicin in humans. *Journal of Clinical Oncology* **6**(3), 517-526

Mross, K., Hamm, K. & Hossfeld, D.K. (1993) Effects of verapamil on the pharmacokinetics and metabolism of epirubicin. *Cancer Chemotherapy & Pharmacology* **31**, 369-375

Murray, L.S., Jodrell, D.I., Morrison, J.G., Cook, A., Kerr, D.J., Whiting, B., Kaye, S.B. & Cassidy, J. (1998). The effect of cimetidine on the pharmacokinetics of epirubicin in patients with advanced breast cancer: preliminary evidence of a potentially common drug interaction. *Clinical Oncology* **10**, 35-38

Nguyen, L., Tranchand, B., Puozzo, C. & Variol, P. (2002) Population pharmacokinetics model and limited sampling strategy for intravenous vinorelbine derived from phase I clinical trials. *British Journal Clinical Pharmacology* **53**, 459-468

Panetta, J.C., Wilkinson, M, Pui, C.H. & Relling, M.V. (2002) Limited and optimal sampling strategies for etoposide and etoposide catechol in children with leukemia. *Journal of Pharmacokinetics & Pharmacodynamics* **29**(2), 171-188

Parke, J & Charles B.G. (2000) Factors affecting oral cyclosporin disposition after heart transplantation: bootstrap validation of a population pharmacokinetic model. *European Journal of Clinical Pharmacology* **56**, 481-487

Pavone-Macaluso, M., Tripi, M., Ingargiola, G.B., Corselli, G., Pavone, C. & Serretta, V. (1993) Current Views on Intravesical Treatment and Chemophrophylaxis of Superficial Bladder Cancer. The present role of epirubicin and doxorubicin. *Journal of Chemotherapy* **5**(3), 207-211

Piscitelli S.C., Rodvold, K.A., Rushing, D.A. & Tewksbury, D.A. (1993) Pharmacokinetics and pharmacodynamics of doxorubicin in patients with small cell lung cancer. *Clinical Pharmacology & Therapeutics* **53**(8), 555-561

Plosker, G.L. & Faulds, D. Epirubicin. (1993) A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cancer chemotherapy. *Drugs* **45**, 788-856

Pugh, R.N.H., Murray-Lyon, I.M., Dawson, J.L., Pietroni, M.C. & Williams, R. (1973). Transection of the oesophagus for bleeding oesophageal varices. *British Journal of Surgery*. **60**(8), 646-649.

Ratain, M.J. & Vogelzang, N.J. (1987) Limited sampling models for vinblastine pharmacokinetics. *Cancer Treatment Reports* **71**(10), 935-939

Ratain, M.J., Robert, J. & van der Vijgh W.J.F. (1991). Limited sampling models for doxorubicin pharmacokinetics. *Journal of Clinical Oncology* **9**(5), 871-876

Reich, S.D. (1978) Clinical correlations of adriamycin pharmacology. *Pharmacology & Therapeutics* **2**, 239-249

Robert, J. & Bui, N.B. (1992). Pharmacokinetics and metabolism of epirubicin administered as i.v. bolus and 48-h infusion in patients with advanced soft-tissue sarcoma. *Annals of Oncology* **3**, 651-656

Robert J (1993) Epirubicin. Clinical pharmacology and dose-effect relationship. *Drugs* **45**, 20-30

Robert, J. (1994) Clinical Pharmacokinetics of epirubicin. *Clinical Pharmacokinetics* **26**: 428-438

Robieux, I., Sorio, R., Borsatti, E., Cannizzaro, R., Vitali, V., Aita, P., Freschi, A., Galligioni, E. & Monfardini, S. (1996) . Pharmacokinetics of vinorelbine in patients with liver metastases. *Clinical Pharmacology & Therapeutics* **59**(1), 32-40

Rodman, J.H., Abromowitch, M., Sinkule, J.A., Hayes, A., Rivera, G.K. & Evans, W.E. (1987) Clinical pharmacodynamics of continuous infusion teniposide: systemic exposure as a determinant of response in a Phase I trial. *Journal of Clinical Oncology* **5**(7), 1007-1014

Rodman, J.H., Furman, W.L. & Sunderland, M. (1993) Escalating teniposide systemic exposure to increase dose intensity for pediatric cancer patients. *Journal of Clinical Oncology* **11**, 287-293

Roecker, E.B. (1991) Prediction error and its estimation for subset-selected models. *Technometrics* **33**(4), 459-468

Rousseau, A., Marquet, P., Debord, J., Sabot, C. & Lachatre, G. (2000) Adaptive control methods for the individualisation of anticancer agent. *Clinical Pharmacokinetics* **38**(4), 315-353

Rowland, M. & Tozer, T.N. (1995) *Clinical Pharmacokinetics. Concepts and Applications*. Philidelphia: Lea and Febiger

Saarto, T., Blomqvist, C., Rissanen, P., Auvinen, A. & Elomaa, I. (1997) Haematological toxicity: a marker of adjuvant chemotherapy efficacy in stage II and stage III breast cancer. *British Journal of Cancer* **75**(2), 301-305

Sandstrom, M., Freijs, A., Larsson, R., Nygren, P., Fjällskog, M.L., Bergh, J. & Karlsson, M.O. (1996). Lack of relationship between systemic exposure for the component drugs of the fluorouracil, epirubicin, and 4-hydroxycyclophosphamide regimen in breast cancer patients. *Journal of Clinical Oncology* **14**(5), 1581-1588

Sadray, S., Jonsson, E.N. & Karlsson, M.O. (1999) Likelihood-based diagnostics for influential individuals in non-linear mixed effects model selection. *Pharmaceutical Research* **16**(8), 1260-1265

Sandstrom, M., Karlsson, M.O., Ljundman, P., Hassan, Z., Jonsson, E.N., Nilsson, C., Ringden, O., Oberg, G., Bekassy, A. & Hassan, M. (2001). Population pharmacokinetic analysis resulting in a tool for dose individualization of busulphan in bone marrow transplantation recipients. *Bone Marrow Transplantation* **28**, 657-664

Sandstrom, M. (2002) *Pharmacokinetics and pharmacodynamics of anti-cancer regimens. Emphasis on busulphan and the combination therapies epirubicin-docetaxal and fluorouracil-epirubicin-cyclophoshamide.* PhD Thesis. Uppsala University.

Santini, J., Thyss, G., Renee, N., Viens, P., Ayela, P., Schneider, M. & Demard, F. (1989). 5-FU therapeutuc monitoring with dose-adjustment leads to an improved therapeutic index in head and neck cancer. *British Journal of Cancer* **59**, 287-290

Sheiner, L.B., Halkin, H., Peck, C., Rosenberg, B. & Melman, K.L. (1975) Improved computer-assisted digoxin therapy. A method using feedback of measured serum digoxin concentrations. *Annals of Internal Medicine.* **71**, 619-627

Sheiner, L.B. & Beal, S.L. (1980). Evaluation of methods for estimating population pharmacokinetic parameters. I. Michaelis-menten model: routine clinical pharmacokinetic data. *Journal of Pharmacokinetics & Biopharmaceutics* **8**, 553-571

Sheiner, L.B. & Beal, S.L. (1981a). Evaluation of methods for estimating population pharmacokinetic parameters. II. Biexponential model and experimental pharmacokinetic data. *Journal of Pharmacokinetics & Biopharmaceutics* **9(5)**, 635-651

Sheiner, L.B. & Beal, S.L. (1981b). Some suggestions for measuring predictive performance. *Journal of Pharmacokinetics & Biopharmaceutics* 9(4), 503-512

Sheiner, L.B. & Beal, S.L. (1987) A note on confidence intervals with extended least squares parameter estimates. *Journal of Pharmacokinetics & Biopharmaceutics* **15**(1), 93-98

Sinha, B.K. & Polti, P.M. (1990) *Cancer Chemotherapy and Biological Response Modifiers Annual 11*. Ed Pinedo, H.M., Chabner, B.A. & Longo D.L. pp 45-57 Elsevier Science Publishers

Sparreboom, A., De Jonge, M.J.A., Punt, C.J.A., Loos, W.J., Noote,r K., Stoter, G., Porro, M.G. & Verweij, J. (1999) Prediction of the systemic exposure to oral 9-amino-20(S)-camptothecin using single-sample analysis. *Drug Metabolism & Disposition* **27**(7), 816-820

Sorensen, B.T., Stromgren, A., Jakobsen, P. & Jakobsen, A. (1993). A limited sampling method for estimation of the carboplatin area under the curve. *Cancer Chemotherapy & Pharmacology* **31**(4), 324-327

Stewart, C.F., Arbuck, S.G., Fleming, R.A. & Evans, W.E. (1991) Relation of systemic exposure to unbound etoposide and hematologic toxicity. *Clinical Pharmacology & Therapeutics* **50**(4), 385 - 393

Summary of product characteristics, pharmorubicin® (epirubicin), Pharmacia, (2003) *Electronic Medicines Compendium* http://www.emc.medicines.org.uk

Sun, H., Fadiran, E.O., Jones, C.D., Lesko, L., Huang, S.M., Higgins, K., Hu, C., Machado, S., Maldonado, S., Williams, R., Hossain, M. & Ette, E.I. (1999) Population Pharmacokinetics. A regulatory perspective. *Clinical Pharmacokinetics* **37**(1), 41-58.

Tjuljandin, S.A., Doig, R.G., Sobol, M.M., Watson, D.G., Sheridan, W.P., Morstyn, G., Mihaly, G. & Green, D.G. (1990). Pharmacokinetics and toxicity of two schedules of high dose epirubicin. *Cancer Research* **50**, 5095-5101

Thomson, A.H. & Sie, A. (2003) Prospective evaluation of neonatal vancomycin dosage guidelines and population parameter estimates. *Paediatric & Perinatal Drug Therapy* **5**(3), 116-123

Twelves, C.J., Dobbs, N.A., Michael, Y., Summers, L.A., Gregory, W., Harper, P.G., Rubens, R.D. & Richards, M.A. (1992) Clinical pharmacokinetics of epirubicin: the importance of liver biochemistry tests. *British Journal of Cancer* **66**, 765-769

van Kesteren, C., Mathot, R.A.A., Raymond, E., Armand, J.P., Dittrich, C., Dumez, H., Roche, H., Droz, J.P., Punt, C., Ravic, M., Wanders, J., Beijnen, J.H., Fumoleau, P. & Schellens, J.H.M. (2002) Population Pharmacokinetics of the Novel Anticancer agent E7070 during Four Phase I Studies: Model Building and Validation. *Journal of Clinical Oncology* **20**(19), 4065-4073

Verbeek, R.K. & Horsmans,Y. (1998) Effect of hepatic insufficiency on pharmacokinetics and drug dosing. *Pharmacy World & Science* **20**, 183-192

Vozeh, S., Maitre, P.O. & Stanski, D.R. (1990) Evaluation of population (NONMEM) pharmacokinetic parameter estimates. *Journal of Pharmacokinetics & Biopharmaceutics* **18**(2), 161-173

Vozeh, S. (1991) *Applications of population approach to clinical pharmacokinetics and validation of the results. New Strategies in drug development and clinical evaluation: The population approach.* Commission of the European Communities, Luxemburg, 107-124

Wade, J.R., Kelman, A.W., Kerr, D.J., Robert, J. & Whiting, B. (1992) Variability in the pharmacokinetics of epirubicin: a population analysis. *Cancer Chemotherapy & Pharmacology* **29**, 391-395

Wählby, U., Jonsson, E.N. & Karlsson, M.O. (2001) Assessment of actual significance levels for covariate effects in NONMEM. *Journal of Pharmacokinetics & Biopharmaceutics* **28**(3), 231-252

Watkins, P.B., Murray, S.A., Winkelman, L.G., Heuman, D.M., Wrighton, S.A. & Guzelian, P.S. (1989) Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450. *Journal of Clinical Investigation* **83**, 688-97

Weenan, H., Lankelma, H., Penders, P.G.M., McVie, J.G., Ten Bokkel Huinink, W.W., de Planque, M.M. & Pinedo, H.M. (1983) Pharmacokinetics of 4'-epi-doxorubicin in man. *Investigational New Drugs* **1**, 59-64

Wils, J.A. Epirubicin in advanced gastrointestinal (GI) cancer. (1986) *Onkologie* **9** (1), 21-23

Wright, J.G., Boddy, A.V., Highley, M., Fenwick, J., McGill, A. & Calvert, A.H. (2001) Estimation of glomerular filtration rate in cancer patients. *British Journal of Cancer* **84**(4), 452-459

Yusa, K. & Tsuruo, T. (1989) Reversal mechanism of multidrug resistance by verapamil: direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. *Cancer Research* **49**(18), 5002-5006

APPENDIX

Control Stream for 3 compartment model with proportional error using FOCE with 'interaction' option; used in covariate model building in Chapter 3, Section 3.3.5 (Table 3.5: model 32)

```
$PROB        EPIRUBICIN
$INPUT ID AMT TIME DV RATE BILI AST ALB HGT WGT MG WHO IWGT
AGE CRCL SY BSA LMET
$DATA C:\LORRAINE\MODEL4.PRN IGNORE='C'
$SUBROUTINE ADVAN11 TRANS4
$PK    LAST=LOG(AST)
      TVCL=THETA(1)*(1-(THETA(7)*LAST))*(1+(THETA(8)*MG))
      TVCL=TVCL*(1-(THETA(9)*BSA))
      IF(LMET.EQ.1) TVCL=TVCL*THETA(10)
      TVV1=THETA(2)
      TVQ2=THETA(3)
      TVV2=THETA(4)
      TVQ3=THETA(5)*(1-(THETA(11)*ALB))
      IF(LMET.EQ.1) TVQ3=TVQ3*THETA(12)
      TVV3=THETA(6)
      CL=TVCL*EXP(ETA(1))
      V1=TVV1
      Q2=TVQ2
      V2=TVV2
      Q3=TVQ3*EXP(ETA(2))
      V3=TVV3*EXP(ETA(3))
      S1=V1/1000
$ERROR  IPRED=F
      DEL=0
      IF(F.EQ.0) DEL=1
      W=IPRED+DEL
      IRES=DV-IPRED
      IWRES=IRES/W
      Y=F+ERR(1)*W
$THETA (30) (10) (30) (40) (20) (700) (0.001) (0.0001) (0.0001) (1)
(0.0001) (1)
$OMEGA (0.1) (0.1) (0.1)
$SIGMA 0.5
$EST METHOD=1 INTERACTION MAX=9000 SIG=3 PRINT=5 NOABORT
$COV
$TABLE ID AMT TIME DV RATE BILI AST ALB HGT WGT MG WHO IWGT
AGE CRCL SY BSA LMET TVCL CL V1 Q2 V2 Q3 V3 ETA(1) ETA(2) ETA(3)
IPRED IRES IWRES FILE=RUN4.PAR NOPRINT
```

Control stream for MAP Bayesian estimation of PK parameters for Swedish data set using prior estimates from UK population model; used in Chapter 6, Section 6.2.3

```
$PROB        EPIRUBICIN
$INPUT ID TIME AMT RATE DV AGE WGT HGT BSA AST ALT ALB CREA
CRCL
$DATA C:\LORRAINE3\MARIE.PRN IGNORE='#'
$SUBROUTINE ADVAN11 TRANS4
$PK    TVCL=THETA(1)
       TVV1=THETA(2)
       TVQ2=THETA(3)
       TVV2=THETA(4)
       TVQ3=THETA(5)
       TVV3=THETA(6)
       CL=TVCL*EXP(ETA(1))
       V1=TVV1
       Q2=TVQ2
       V2=TVV2
       Q3=TVQ3*EXP(ETA(2))
       V3=TVV3*EXP(ETA(3))
       S1=V1/1000
$ERROR  IPRED=F
       DEL=0
       IF(F.EQ.0) DEL=1
       W=IPRED+DEL
       IRES=DV-IPRED
       IWRES=IRES/W
       Y=F+ERR(1)*W
$THETA (28.0) (9.88) (29.0) (35.2) (60.3) (780)
$OMEGA (0.284) (0.108) (0.194)
$SIGMA (0.0533)
$EST METHOD=1 INTERACTION MAXEVAL=0 SIG=3 PRINT=5
$COV OMIT
$TABLE ID TIME AMT RATE DV AGE WGT HGT BSA AST ALT ALB CREA
CRCL CL V1 Q2 V2 Q3 V3 ETA(1) ETA(2) ETA(3)
IPRED IRES IWRES FILE=RUN1.PAR NOPRINT
```

Control stream for simulation of a reference data set; used in Chapter 9B Section 9B.1.1

```
$PROB        SENSITIVITY
$INPUT ID AMT TIME DV RATE
$DATA C:\LORRAINE\REF.PRN IGNORE='C'
$SUBROUTINE ADVAN11 TRANS4
$PK    TVCL=THETA(1)
       TVV1=THETA(2)
       TVQ2=THETA(3)
       TVV2=THETA(4)
       TVQ3=THETA(5)
       TVV3=THETA(6)
       CL=TVCL*EXP(ETA(1))
       V1=TVV1
       Q2=TVQ2
       V2=TVV2
       Q3=TVQ3*EXP(ETA(2))
       V3=TVV3*EXP(ETA(3))
       S1=V1/1000
$ERROR  IPRED=F
       DEL=0
       IF(F.EQ.0) DEL=1
       W=IPRED+DEL
       IRES=DV-IPRED
       IWRES=IRES/W
       Y=F+ERR(1)*W
$THETA (28.0) (9.88) (29.0) (35.2) (60.3) (780)
$OMEGA (0.284) (0.108) (0.194)
$SIGMA (0.0533)
$SIMULATION (3396450) ONLYSIM
$TABLE ID AMT TIME DV RATE CL V1 Q2 V2 Q3
V3 ETA(1) ETA(2) ETA(3) IPRED IRES IWRES
FILE=RUN3.PAR NOPRINT
```