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APPLICATION OF POPULATION PHARMACOKINETIC-PHARMACODYNAMIC MODELLING TO EVALUATE AND OPTIMISE AMINOGLYCOSIDE THERAPY IN PATIENTS WITH CYSTIC FIBROSIS

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SUMMARY

Cystic fibrosis (CF) is an inherited autosomal recessive disorder that is characterised by frequent lung infections commonly caused by *P.aeruginosa*. The standard treatment for this infection is an aminoglycoside combined with a β-lactam and patients often receive multiple courses of these antibiotics over many years. Aminoglycosides are narrow therapeutic index drugs where the margin between safety and toxicity is small. Therefore, it is important to monitor patients who are on aminoglycosides to ensure safety and efficacy of therapy and advise on current and future dosage regimens. The focus of this thesis was to use population pharmacokinetic methodologies to examine how aminoglycoside pharmacokinetic parameters change over time in this group of patients and to develop and evaluate dosing regimens and data interpretation methods.

A population pharmacokinetic analysis was first conducted using the package NONMEM with the FOCE (parametric) algorithm. Aminoglycoside concentration-time profiles were available from 166 patients treated within the Glasgow Cystic Fibrosis Unit and comprised 1075 courses of therapy and 2238 concentration measurements collected over 15 years. The final, two compartment, population model identified an influence of height and creatinine clearance on clearance and height on volume of distribution of the central compartment. Inclusion of these descriptors reduced between subject variability from 23 % to 18 % for clearance and 14 % to 12 % for volume of distribution of the central compartment. Within-subject variability was low at 11 %, and there were no changes in aminoglycoside clearance over time. Internal valuation of the population model using bootstrap, prediction corrected visual predictive check and normalised prediction distribution errors indicated that this model was stable and with good predictive ability. In addition, an external model evaluation was conducted using data from The Hague that comprised tobramycin concentration measurements from 165 patients who received 415 courses of therapy. The results of this analysis indicated good performance of the model in predicting pharmacokinetic parameters and concentrations in another group of patients with cystic fibrosis.

The combined Glasgow and The Hague datasets were subsequently analysed using a non-parametric approach with the software Pmetrics (Neely MN et al., 2012). In total, data from 331 patients with 1490 courses of therapy and 3690 aminoglycoside concentration measurements were analysed. Despite the different assumptions of the two methods, the final models were the same and the final parameter estimates were very similar.

The standard dose of aminoglycoside used in patients with cystic fibrosis is 10 mg/kg administered once daily. The typical daily area under the concentration-time curve (AUC) arising from this dose was determined using pharmacokinetic parameter estimates reported in the TOPIC study (Smyth A et al., 2005) and by examining the raw data from patients within The Hague dataset who received this dosage regimen. The results of this analysis led to a target daily AUC of 106 mg.h/L (range 80-120 mg.h/L). A simulated dataset of 5000 patients was created with clinical characteristics based on patients with cystic fibrosis from Glasgow and The Hague. The final population model was then used to estimate pharmacokinetic parameters and to predict concentrations at defined time points according to the standard dose of 10 mg/kg/day and three alternative regimens (13 mg/kg/day lean body weight, 3 mg/cm/day and 326 mg/m^2/day). It was found that the dose based on height (3mg/cm/day) had the highest probability of achieving the combined targets of daily AUC range, peak concentrations of 20-30 mg/L and trough concentrations < 1 mg/L.

For standard “once daily” aminoglycoside therapy, dosage adjustment nomograms are available that help clinicians to interpret aminoglycoside concentrations and advise on dose adjustments (Nicolau DP et al., 1995). For adult patients with cystic fibrosis there is no dosage adjustment nomogram available for the 10 mg/kg aminoglycoside dose. Therefore, one of the aims of the thesis was to develop an aminoglycoside (tobramycin) dosage adjustment nomogram that could be used with doses of 10 mg/kg/day and 3 mg/kg/day. The nomogram was derived from the concentration-time profiles that were generated from the simulation approach described above and consisted of three areas representing below, within and above the target ranges. Preliminary validation work indicated that the nomogram could identify patients with low, within and above target daily area under the

concentration-time curve range from one sample point. Importantly, the nomogram was able to identify patients with poor renal function.

The goal for any antibacterial therapy is to ensure efficacy against treated organism and to achieve high probability of treatment success. In this thesis, the likelihood of treatment success for the 10 mg/kg and 3 mg/cm dosage regimens were determined against *P.aeruginosa*. This was achieved by determining the susceptibility breakpoint and cumulative fraction of response for these dosage regimens and comparing them with the breakpoints obtained from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the British Society for Antimicrobial Chemotherapy (BSAC). Breakpoints and cumulative fraction of response were also examined for doses of 12 mg/kg/day and 4 mg/cm/day. The results showed that these regimens had similar MIC breakpoints of ≤ 2 mg/L to achieve a Peak/MIC ratio ≥ 10, and MIC breakpoint of ≤ 0.5 mg/L for a daily AUC/ MIC ratio ≥ 100 mg.h/L against *P.aeruginosa*. However, they were lower than the EUCAST and BSAC susceptibility breakpoints against gram-negative pathogens (≤ 4 mg/L). Analysis of the cumulative fraction of response identified an overall treatment success of more than 90 % with all regimens for a Peak/MIC ratio ≥ 10 against *P.aeruginosa* using the EUCAST MIC distribution. At a daily AUC/MIC ratio greater than 100, the cumulative fraction of response for tobramycin indicated a success rate between 70 - 80 % for all dosage regimens, with the higher values being observed with the doses of 12 mg/kg/day and 4 mg/cm/day. However, the 12 mg/kg/day dosage regimen was associated with high peak and daily exposure and might result in more toxicity compared with the high 4 mg/cm/day dosage regimen.

The population model developed in this thesis was able to describe and predict the handling of aminoglycoside in patients with cystic fibrosis. The model was used to evaluate the current 10 mg/kg/day dosage regimen, develop a new dosage regimen and develop a dosage adjustment nomogram for clinical application. Furthermore, the model was used to predict the efficacy of the standard and new dosage regimens through determining the susceptibility breakpoints and cumulative fraction of response against gram-negative organisms. In the future, the models could be used to help estimate individual

pharmacokinetic parameters and design individualised dosage regimens using both parametric and non-parametric clinical pharmacokinetic software.

Dedication

This thesis is dedicated to my family - parents, brothers and sisters who believed that I could do it and support throughout my life.

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ومانيل المطالب بالتمني ولكن تؤخذ الدنيا غلابا

Meaning: Getting your wants is not by wishing, but by working hard for them.

Said the prince of Arabic poets Ahmed Shawqi

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LIST OF PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

- Alghanem S, Paterson I, Touw DJ, Thomson AH. Influence of Multiple Courses of Therapy on Aminoglycoside Clearance in Adult Patients With Cystic Fibrosis. Journal of Antimicrobial Chemotherapy. 2013; 68: 1338-47.

PRESENTATION

- Poster presentation- PKUK, Bristol, UK (2010). Within subject variability in aminoglycoside pharmacokinetics in patients with cystic fibrosis.
- Poster presentation- Kuwait International Pharmacy Conference, Kuwait (2011). Within subject variability in aminoglycoside pharmacokinetics in patients with cystic fibrosis.
- Oral presentation- Kuwait International Pharmacy Conference, Kuwait (2011). Within subject variability in aminoglycoside pharmacokinetics in patients with cystic fibrosis.
- Poster presentation- PAGE meeting, Athens, Greece (2011). Development of a Tobramycin Dosage Adjustment Nomogram for Patients with Cystic Fibrosis.
- Poster presentation- PKUK, Durham, UK (2011). Internal and external validation for aminoglycoside population pharmacokinetic model in patients with cystic fibrosis.
- Oral presentation - PKUK, Dorking, UK (2012). Development Of New Tobramycin Dosage Guidelines for Patient With Cystic Fibrosis.
- Poster presentation- PKUK, Dorking, UK (2012). Development Of New Tobramycin Dosage Guidelines for Patient With Cystic Fibrosis.
- Poster presentation- PAGE meeting, Glasgow, UK (2013). Comparison of NONMEM and Pmetrics Analysis for Aminoglycosides in Adult Patients with Cystic Fibrosis.

LIST OF ABBREVIATION

AUC	Area under-the-concentration time curve
B.cepacia	*Burkholderia cepacia*
BSAC	The British Society for Antimicrobial Chemotherapy
BSV	Between-subject variability
BSA	Body surface area
CF	Cystic fibrosis
CFR	The cumulative fraction of response
CL	Clearance
CV	Coefficient of variation
EUCAST	The European Committee on Antimicrobial Susceptibility Testing
FO	First order estimation method
FOCE	First order conditional estimation
GAM	Generalized additive model
H. influenzae	*Haemophilus influenzae*
IBW	Ideal body weight
IV	Intravenous
k,k_{10}	Elimination rate constant
k_{12}	Rate of transfer from central to peripheral compartment
k_{21}	Rate of transfer from peripheral to central compartment
LBW	Lean Body Weight
MIC	Minimum Inhibitory Concentration

NPEM	Nonparametric Expectation Maximization
NPAG	Nonparametric Adaptive Grid
Npde	Normalised prediction distribution error
OFV	Objective function value
pc VPC	Prediction corrected visual predictive check
PK/PD	pharmacokinetic/pharmacodynamic
P. aeruginosa	*Pseudomonas aeruginosa*
PTA	The probability of target attainment
Q	Inter-compartmental clearance
SAEM	Stochastic Approximation Expectation Maximization
S. aureus	*Staphylococcus aureus*
V	Volume of Distribution
$\mathbf{V_1}$	Volume of distribution of the central compartment
$\mathbf{V_2}$	Volume of distribution of the peripheral compartment
VPC	Visual predictive check
WT	Weight
WSV	Within-subject variability

CHAPTER 1: GENERAL INTRODUCTION

1.1 LUNG INFECTION IN PATIENTS WITH CYSTIC FIBROSIS

1.1.1 Influence of long-term aminoglycoside therapy in patients with cystic fibrosis

Cystic fibrosis is an inherited autosomal recessive disorder that is characterised by frequent lung infections. With the improved survival seen in this patient group, concerns about the development of treatment-associated toxicity has increased over the years. Since the infecting organism is often *P.aeruginosa,* aminoglycoside antibiotics are frequently used in the management of lung infections in patients with cystic fibrosis. However, these antibiotics are known to be nephrotoxic. Bertenshaw *et al* (2007) reported the incidence of acute renal failure in patients with cystic fibrosis to be between 4.6 and 10.1 cases for every 10 000 patients per year and that 80 % of the cases were associated with the administration of aminoglycosides. However, there is a lack of knowledge about the long-term effects of multiple courses of therapy of aminoglycoside in patients with cystic fibrosis. The only available study to date was conducted by Al-Aloul *et al* (2005), who investigated the influence of repeated aminoglycoside use on renal function in 80 adolescent and adult patients with cystic fibrosis. In their study, the aminoglycoside was administered with or without colistin. They used creatinine clearance as a measure of renal function and concluded that the frequent use of aminoglycosides in patients with cystic fibrosis was associated with a reduction in renal function. However, the doses used, number of courses and study follow up periods were not defined so it is difficult to assess the clinical relevance of these findings. There is evidence that gentamicin is associated with more nephrotoxicity than tobramycin (Bertenshaw C et al., 2007, Smyth A et al., 2008) and failure to separate the effects related to gentamicin or tobramycin might have influenced their results.

This thesis addresses the impact of multiple courses of aminoglycoside therapy by examining how aminoglycoside handling changes over time in patients with cystic fibrosis. A population pharmacokinetic approach using the software NONMEM (Beal SL et al., 2009) is used to determine factors that influence aminoglycoside clearance and volume of distribution, including the impact of multiple courses over long periods. In addition, random variability in pharmacokinetic parameters within a patient at different times is examined.

Few studies have previously examined this "within-subject" variability in aminoglycoside handling. Matthews *et al* (2004) examined 2567 aminoglycoside concentrations from 697 general medical patients who were treated with aminoglycoside. They defined an occasion as a dose followed by at least one measured aminoglycoside concentration and found that the inclusion of within-subject variability in both clearance and volume of distribution of the peripheral compartment improved the model fit; however, their values were small at 8 and 19 %. In patients with cystic fibrosis, two studies to date have included within-subject variability (Hennig S et al., 2007, Hennig S et al., 2013). They used the same definition as Matthews *et al* (2004), where one dosage interval with a subsequent measured concentration was considered as one occasion and also found that within-subject variability in clearance was small at 6.47% (Hennig S et al., 2007) and 12.6% (Hennig S et al., 2013). In this thesis, the nature and extent of within-subject variability in aminoglycoside pharmacokinetics in patients with cystic fibrosis is examined using a dataset with multiple courses of therapy, where an "occasion" is defined as a course of therapy, rather than a dosage interval. This is the first time that aminoglycoside handling in patients with cystic fibrosis has been examined in this way.

Model evaluation is an important step for population pharmacokinetic analysis and involves the examination of model performance and predictive ability. Typically, this is conducted using the internal validation methods that are used in this thesis. Although the best way to evaluate a model is to test its predictive ability using a new independent dataset, this "external" evaluation approach was performed in only 7 % of published pharmacokinetic studies between 2002 to 2004 (Brendel K et al., 2007), probably reflecting difficulties in obtaining new datasets. In addition to the internal evaluation, this thesis describes an external evaluation of the performance of the population model that was conducted using a new dataset.

Pmetrics (Neely MN et al., 2012) is a new version of the USC-PACK (Jelliffe RW, 1991) population modelling software, which is developed and maintained by the Laboratory of Applied Pharmacokinetics at the University of Southern California (USC) in Los Angeles,

California. It uses the nonparametric adaptive grid (NPAG) algorithm (Tatarinova T et al., 2013) to estimate pharmacokinetic parameters. To date, only one study has been published that compares results from NONMEM and Pmetrics (Tatarinova T et al., 2013). Therefore, in addition to the initial population pharmacokinetic analysis, a further analysis is performed using Pmetrics to determine whether different model and parameter estimates are obtained when a different approach is used.

1.1.2 Aminoglycosides dosage regimen in patients with cystic fibrosis

Aminoglycosides are concentration-dependent antibiotics for which either the peak concentration or the daily AUC can be used for monitoring. Although high peaks correlate with efficacy (Moore RD et al., 1987), daily AUC has been found to be a predictor of both efficacy (Nielsen EI et al., 2011, Vogelman B et al., 1988a) and nephrotoxicity (Rybak MJ et al., 1999, Croes S et al., 2012). In patients with cystic fibrosis, the target peak concentration for the standard aminoglycoside (tobramycin) dose of 10 mg/kg/day is 20 -30 mg/L and the trough concentration should be less than 1 mg/L. Although no target daily AUC has been established for this group of patients, some studies have arbitrarily defined a daily AUC target of 100 mg.h/L with an 80 to 125 % variability (Hennig S et al., 2007), while others (Coulthard KP et al., 2007, VandenBussche HL and Homnick DN, 2012) based their target of 100 mg.h/L on the established target for the dose of 7 mg/kg/day used in general medical patients (Begg EJ et al., 1995). In this thesis, an aminoglycoside (tobramycin) target daily AUC is determined using data from patients who had been administered doses of 10 mg/kg/day.

A number of studies conducted in patients with cystic fibrosis have developed aminoglycoside dosage guidelines and the common factor among them is the use of weight as the scaling factor (Hennig S et al., 2007, Hennig S et al., 2013, VandenBussche HL and Homnick DN, 2012, Lam W et al., 2007). In the TOPIC study (Smyth A et al., 2005) the recommended dose of 10 mg/kg was restricted to a maximum of 660 mg, but there was no justification provided for this restriction. Only one study used body surface area as scaling factor for a twice daily regimen (Campbell D et al., 1999), while another study

recommended lean body weight to be the scaling factor (Touw DJ et al., 1994). In this thesis, the population model is used to examine the impact of different body size measurements, including height, lean body weight and body surface area on the achievement of target concentrations and daily AUC.

The “Hartford nomogram” was the first graphical plot designed to interpret aminoglycoside concentration measurements following a dose of 7 mg/kg/day (Nicolau DP et al., 1995). Although a tobramycin dosage adjustment nomogram is currently available for patients with cystic fibrosis, it was based on a 12 mg/kg daily dose in patients aged 9 months to 20 years (Massie J and Cranswick N, 2006). There is currently no nomogram available for interpreting tobramycin concentrations in adults who receive the standard 10 mg/kg/day dose. The thesis describes how the population model was used to develop a dosage adjustment nomogram for clinical application.

The aim of antimicrobial therapy is to kill infecting organisms, and evaluation of the likelihood of treatment success is therefore required. The efficacy of antimicrobial therapy is determined by the relationship between the antibiotic’s concentration-time profile or exposure and the minimum inhibitory concentration (MIC). Since aminoglycosides are concentration-dependent antibiotics, their bactericidal effect is associated with Peak/MIC and daily AUC/MIC ratios. One of the approaches that can be used to evaluate treatment success is to use pharmacokinetic/pharmacodynamic relationships to determine the antimicrobial susceptibility breakpoint for the administered dosage regimen against the target organism. Previous PK/PD studies conducted in patients with cystic fibrosis usually defined an MIC breakpoint and examined whether the PK/PD indices of the tested aminoglycoside dosage regimen was able to achieve that breakpoint (Beringer PM et al., 2000, VandenBussche HL and Homnick DN, 2012). However, none of the available studies have determined the susceptibility breakpoint for a dose of 10 mg/kg/day. In the current thesis, the population model is used to determine the susceptibility MIC breakpoint for the standard aminoglycoside dose (10 mg/kg/day) and the new dosage guideline. Another approach that can be used to evaluate antimicrobial treatment success is to estimate the

overall treatment response against the organism. Therefore, the population model is also used to predict the efficacy of standard and new dosage regimens through determining the cumulative fraction of response against gram-negative organisms.

CHAPTER 2: BACKGROUND

2.1 CYSTIC FIBROSIS

Cystic fibrosis is an inherited autosomal recessive disease that most commonly occurs in the white population with a reported incidence of 1 in 2000 in the US (Wright SW and Morton NE, 1968) and 1 in 2500 in the UK (Dodge JA et al., 1997). The incidence is lower in other ethnic groups, for example, the incidence of cystic fibrosis has reported to be 1 in 90,000 in Asians (Wright SW and Morton NE, 1968) and 1 in 12,000 in mixed-race South Africans (Hill ID et al., 1988). In 2011, the Cystic Fibrosis Trust (Cystic Fibrosis Trust, 2013) reported that the total number of patients had been diagnosed with cystic fibrosis and registered in their database in the UK was 9749. The diagnosis is usually made early in life at around 3 months (Cystic Fibrosis Trust, 2013). The disease is caused by a defect in chromosome 7 (Knowlton RG et al., 1985, Tsui L.-C et al., 1986), which encodes for the protein "cystic fibrosis transmembrane conductance regulator". This protein normally transports electrolytes and water through chloride channels in epithelial cells and its absence may alter the volume or composition of the fluid secreted by the pancreas, hepatobiliary tree, reproductive tract, sweat glands and the airways. The majority of mutations are caused by a deletion of an amino acid on the gene position 508 (*ΔF508*) (Kerem B et al., 1989, Riordan JR et al., 1989). In the UK, the *ΔF508* mutation accounts for 90.6 % of all mutations (Cystic Fibrosis Trust, 2013). Previously the disease was associated with high mortality rate at early age; however, in more recent years the predicted survival has increased; from 35.2 in 2007 to 41.5 in 2011 (Cystic Fibrosis Trust, 2013).

The clinical manifestations of the disease are pancreatic insufficiency, abnormally high concentrations of sodium and chloride in sweat, and frequent lung infections. The consequence of pancreatic insufficiency is deficient secretion of pancreatic digestive enzymes, which leads to malabsorption of nutrients, malnutrition and possibly anaemia. In addition, because patients with cystic fibrosis are living longer, the number of patients treated for diabetes mellitus has been increasing; 18.3 % patients with cystic fibrosis were treated in the UK in 2011 (Cystic Fibrosis Trust, 2013) compared with 17.2 % in 2010 (Cystic Fibrosis Trust, 2012). However, 15 % of patients with cystic fibrosis were reported to have pancreatic sufficiency, which was reflected by normal fat absorption (Corey M et al., 1984).

This led Kerman *et al* (Kerem B et al., 1989, Kerem E et al., 1990) and Kristidis *et al* (1992) to investigate the influence of the presence and absence of pancreatic insufficiency and the severity of the disease. They found a link between mutation genotype and the severity of the disease, where patients who had a homozygote *ΔF508* genotype mutation were younger, had a higher sweat chloride concentration at the time of diagnosis, and had a more severe form of the disease, with pancreatic insufficiency and worse pulmonary disease. In contrast, patients who were heterozygotes or had another genotype mutation had a milder form of the disease. These patients were diagnosed at an older age, had a lower sweat chloride concentration, no pancreatic insufficiency and better pulmonary function. In the UK, 52.0 % of patients with cystic fibrosis have a homozygous *ΔF508* mutation, 38.6 % have heterozygous *ΔF508* mutation and 9.4 % have one of more than a thousand other genotype mutations (Cystic Fibrosis Trust, 2013). In addition, McKone *et al* (2006) developed another risk classification for patients with cystic fibrosis, according to the genotype functional defect. They defined high and low risk groups, where the *ΔF508* mutation was considered as a high risk genotype and other low risk mutations. They found a difference in mortality and median age of death between the groups, where patients in the high risk group had low survival (median 36.3 years) compared with low risk patients (median 50 years).

The clinical manifestation of the disease on sweat glands is the production of abnormally high concentrations of sodium and chloride, and the measurement of sweat chloride concentration is considered the diagnostic test for cystic fibrosis. The consequences of chloride transport defects in the pulmonary system are viscous secretions, leading to airway obstruction. Persistent airway obstruction with mucus provides a good culture medium for microorganism growth and is associated with recurrent lung infections. The most common bacterial pathogens in patients with cystic fibrosis are *Staphylococcus aureus* (*S.aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Haemophilus influenzae* (*H. influenzae*). The diversity of pathogens present in the airways of patients with cystic fibrosis that cause lung infections places a challenge to treat. In particular with the increase in survival, these patients are at risk for colonising more aggressive organisms such as *Burkholderia cepacia* complex (*B.cepacia*), non-tuberculosis *mycobacteria* species, and *Aspergillus fumigates*. In

the UK, lung infections caused by *B.cepacia* in patients with cystic fibrosis increased from 3.2 % in 2010 (Cystic Fibrosis Trust, 2012) to 3.8 % in 2011 (Cystic Fibrosis Trust, 2013). Similarly, lung infections caused by non-tuberculosis *mycobacteria* increased from 3.4 % in 2010 (Cystic Fibrosis Trust, 2012) to 3.9 % in 2011 (Cystic Fibrosis Trust, 2013).

2.2 ANTIMICROBIAL THERAPIES FOR THE MANAGEMENT OF CYSTIC FIBROSIS

2.2.1 Eradication therapy

Antimicrobial therapy plays an important role in the management of cystic fibrosis. As a result of the airway obstruction, organisms colonise the airways causing frequent lung infection with the most common organism being *P. aeruginosa* (36.5% of all infections) (Cystic Fibrosis Trust, 2013). Recurrent infections were found to be associated with a more rapid decline in lung function and increased mortality (Emerson et al., 2005, Kerem E et al., 1992). Therefore, Döring *et al* (2012) produced a recent consensus review with guidance for antibiotic treatment for lung infections in patients with cystic fibrosis. Based on the evidence they reviewed, they recommended the use of *P. aeruginosa* eradication therapy for 28 days when the *P. aeruginosa* culture is positive, without specifying a treatment protocol. They produce their recommendations based on the results of two randomised studies, the ELITE (Ratjen F et al., 2010) and EPIC (Treggiari MM et al., 2011) study. The ELITE study (Ratjen F et al., 2010) evaluated the use of inhaled tobramycin for early eradication of *P. aeruginosa* in 88 patients with cystic fibrosis. The eradication of *P. aeruginosa* was successful in 66 % of those who had 28 days treatment and 69 % in 56 days treatment and lasted for 27 months. On the other hand, the EPIC study (Treggiari MM et al., 2011) examined the influence of early eradication of four treatment protocols in 304 paediatric patients with cystic fibrosis. The examined protocols were; inhaled tobramycin combined with oral ciprofloxacin or oral placebo every three months, and inhaled tobramycin with ciprofloxacin or placebo when the quarterly culture was positive for *P. aeruginosa*. The success rate for the four examined protocols to eradicate *P. aeruginosa* was more than 80 % for 18 months with no difference between the administered protocols.

2.2.2 Management of chronic *P. aeruginosa* infection

At early stages of the disease, *P. aeruginosa* can be eradicated and failure to do so could lead to chronic *P. aeruginosa* infection (Mayer-Hamblett N et al., 2012). In this case, long term inhaled antibiotics could be used (Ryan G et al., 2011, Flume PA et al., 2007). A recently published Cochrane review examined the current evidence for the use of long term inhaled antibiotics in this group of patients (Ryan G et al., 2011). The authors concluded after reviewing 19 trials with 1724 patients that the use of inhaled antibiotics improved lung function and reduced exacerbation rates. Similarly, the Cystic Fibrosis Foundation (Flume PA et al., 2007) concluded from the evidence reviewed that chronic use of inhaled antibiotics reduced exacerbations and improved lung function, and hence they recommended the use of inhaled antibiotic in patients with chronic *P. aeruginosa*. However, the Cochrane review also noted that there was an increased rate of resistance when long term inhaled antibiotics were used.

2.2.3 Management of acute pulmonary exacerbation

Patients with cystic fibrosis can also suffer from an acute pulmonary exacerbation, where systemic antimicrobial therapy is recommended (Döring et al., 2012, The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). Pulmonary exacerbation is usually characterised by an increased productive cough, breathlessness, decreased exercise tolerance, loss of appetite, change in appearance or volume of sputum, fever, and/or fall in respiratory function (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). Intravenous antibiotics are preferred over oral or inhaled antibiotics to ensure achievement of high concentration of the antibiotic in the lung. The most common organism causing pulmonary exacerbations is *P. aeruginosa* and hence antibiotic therapy should be effective against it. Usually, a combination of antibiotics with different mechanisms of action is prescribed and a combination of β-lactam and aminoglycoside is commonly used (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). Aminoglycoside therapy is the main focus of this thesis.

In addition to *P. aeruginosa*, other organisms colonise the airways in patients with cystic fibrosis including *B.cepacia*, non-tuberculosis *mycobacteria* species, and *Aspergillus fumigates*, which increase the virulence of *P. aeruginosa*. If these organisms are suspected to cause the exacerbation, then appropriate antimicrobial therapy should be considered (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009).

2.2.4 Prophylaxis therapy

Although the most common pathogen causing lung infection in patients with cystic fibrosis is *P. aeruginosa*, *S.aureus* is considered the second most common pathogen (15.7 %) (Cystic Fibrosis Trust, 2013). Stutman *et al* (2002) examined the use of anti-staphylococcal antibiotics as prophylactic therapy for 7 years in infants and children with cystic fibrosis. They found that the growth of *S.aureus* was suppressed, but with an increased incidence of positive *P. aeruginosa* culture in the treated arm compared with placebo, leading to more incidences of pulmonary exacerbations. Therefore, routine anti-staphylococcal prophylaxis was not recommended in children with cystic fibrosis.

2.2.5 Anti-inflammatory therapy

Macrolides were recommended for the use as a long term therapy in patients with cystic fibrosis because of their anti-inflammatory effect and influence on *P. aeruginosa* biofilm formation (Flume PA et al., 2007, The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). Long term treatment with macrolides (azithromycin) for 3 months and up to 24 weeks was associated with 3.7 – 6.2 % improvement in lung function (Equi A et al., 2002, Wolter J et al., 2002, Saiman L et al., 2003) and was associated with a reduction in the incidence of pulmonary exacerbations (Saiman L et al., 2003). However, improvement in lung function was only seen in patients who were infected with *P. aeruginosa* and not in those uninfected with *P. aeruginosa* (Saiman L et al., 2010).

2.3 PHARMACOKINETICS IN PATIENTS WITH CYSTIC FIBROSIS

The consequence of cystic fibrosis on drug handling was expected to alter as a result of the alteration in pathophysiology of gastrointestinal tract, liver and kidney. Rey *et al* (1998) reviewed publications focused on examining pharmacokinetics in patients with cystic fibrosis. The authors hypothesized that drug absorption in patients with cystic fibrosis might be decreased as a result of gastric acid hypersecretion and bile acid malabsorption. However, the results from the reviewed studies indicated that the drug absorption was slower for some drugs and not changed for most of the drugs. Therefore, the authors recommended that these recommendations should not be generalised to all drugs used in this patient group.

As a result of pancreatic insufficiency, these patients are usually malnourished, which might affect the distribution of drugs. Volume of distribution of drugs in patients with cystic fibrosis was found to be highly influenced by the body size measurement use for normalisation (Rey E et al., 1998). This issue will be discussed further in the next section with a focus on aminoglycoside pharmacokinetics in patients with cystic fibrosis.

Cystic fibrosis can also cause hepatic dysfunction, which affects hepatic enzyme production. Therefore elimination of hepatically metabolised drugs was expected to change in this group of patients in favour of increased elimination for some drugs (Rey E et al., 1998). Moreover, the influence of cystic fibrosis on renally cleared drugs has been extensively studied, showing conflicting results. Aminoglycosides are renally cleared drugs and their clearance in patients with cystic fibrosis has been shown to increase (Kearns GL et al., 1982, Mann HJ et al., 1985, Levy J et al., 1984) or not change (Mann HJ et al., 1985, Hennig S et al., 2013). The current thesis will examine aminoglycoside handling in patients with cystic fibrosis in detail.

2.4 AMINOGLYCOSIDES

Aminoglycosides are one of the most frequently prescribed types of antibacterial agents for the treatment of pulmonary exacerbations in patients with cystic fibrosis. Aminoglycosides are hydrophilic antibiotics with poor oral absorption. They are mainly administered intravenously or intramuscularly for systemic indications (Schentag et al., 2006). When aminoglycosides are transported across bacterial cell membranes, they bind to 30S and 50S ribosomal subunits to cause misreading of genetic codes, which impairs bacterial protein synthesis. This induces membrane damage, and an increase in intracellular osmotic pressure and bacterial cell death. They are active against aerobic gram-negative organisms including *Escherichia coli*, *Proteus*, *Enterobacter*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Serratia*, and *Providencia* species (Schentag et al., 2006).

2.4.1 Aminoglycoside pharmacokinetics, pharmacodynamics and toxicity

2.4.1.1 Pharmacokinetics

Aminoglycosides are polar drugs with low albumin binding ranging from zero for gentamicin and tobramycin to 35 % for streptomycin (Gordon RC et al., 1972, Myers DR et al., 1977), whose distribution is essentially limited to the extracellular fluid compartment, although they can diffuse into synovial, peritoneal and ascitic fluids (Dee TH and Kozin F, 1977, Gill MA and Kern JW, 1979, Marsh DCJr et al., 1974, Rodriguez V et al., 1970, Chow A et al., 1971) and cross the placenta (Bernard B et al., 1977, Yoshioka H et al., 1972). Animal studies have demonstrated active transport into the inner ear and renal proximal tubule and accumulation in these tissues has been associated with the development of ototoxicity and nephrotoxicity. Aminoglycosides have poor penetration into lung tissue and bronchial secretions (Levy J, 1986).

Aminoglycosides are eliminated unchanged, mainly through renal elimination by glomerular filtration (Gyselynck AM et al., 1971, Plantier J et al., 1976, Kirby WM et al., 1976). They are also actively reabsorbed by the proximal tubule, which may lead to renal toxic effects (Luft FC and Kleit SA, 1974, Schentag JJ et al., 1977, Schentag JJ et al., 1982). Small amounts of

aminoglycoside have also been found in bile (Mendelson J et al., 1973, Pitt HA et al., 1973, Smithivas T et al., 1971). Table 2.1 shows summary of aminoglycosides (gentamicin, tobramycin and amikacin) pharmacokinetic parameters obtained from the literature for general medical patients.

Table 2.1 Summary of aminoglycoside (gentamicin, tobramycin and amikacin) pharmacokinetic parameters for general medical patients obtained from the literature.

Parameter	Gentamicin	Tobramycin	Amikacin
Volume of distribution (L/kg)	0.31 ± 0.1	0.33 ± 0.04	0.27 ± 0.06
Protein binding (%)	< 10	< 10	4
Clearance (mL/min/kg)	0.82 CrCL + 0.11	0.98 CrCL ± 32 %	0.6 CrCL ± 0.14
Elimination half-life (hour)	2.0 – 3.0	2.2 ± 0.1	2.3 ± 0.4

Reference: (Hardman JG et al., 2001)
Key: CrCL is creatinine clearance.

2.4.1.2 Pharmacodynamic and resistance patterns

Aminoglycosides are bactericidal antibiotics that exhibit concentration dependent-killing, which is defined as a progressive increase in the killing rate with increasing antibacterial concentration (Sanchez-Navarro A and Sanchez Recio MM, 1999). Achievement of a higher aminoglycoside peak level to MIC ratio is associated with a positive therapeutic outcome including decrease in high temperature, decrease in leukocyte counts and resolve of signs of infection at the site of infection (Moore RD et al., 1987), although, daily AUC to MIC ratio has also been suggested to be related to the antibacterial effect of aminoglycosides (Nielsen EI et al., 2011). Furthermore, aminoglycosides have a post-antibiotic effect, which represents the time that inhibition of bacterial growth continues after exposure of the bacterium to an antibacterial, even though the antibacterial concentration has fallen below the bacterial MIC (Sanchez-Navarro A and Sanchez Recio MM, 1999). The duration of the aminoglycoside post-antibiotic effect has been reported as two to four hours *in vitro* and up to ten hours *in vivo* against gram negative bacilli (Craig WA et al., 1991). However, in an *in vitro* pharmacokinetic model simulating human pharmacokinetics, the tobramycin post-

antibiotic effect was observed to decrease as the concentration decreased during the 12 hour dosing interval and it completely disappeared after the drug concentration reached the MIC for *P. aeruginosa* (Den Hollander JG et al., 1996). The authors therefore questioned the use of a long post-antibiotic effect as supportive evidence for prescribing aminoglycoside with an extended interval dosage regimen such as once daily. Aminoglycosides also exhibit a post-antibiotic leukocyte enhancement phenomenon, whereby the antibiotic enhances bacterial susceptibility to leukocyte phagocytosis and killing in the presence of white blood cells (neutrophils) (Fantin B et al., 1991, Kapusnik JE et al., 1988, Vogelman B et al., 1988b).

Organisms that are initially sensitive to an aminoglycoside can develop resistance to the drug. Poole (2005) reviewed the current publications on *P. aeruginosa* resistance to aminoglycosides and reported that several possible mechanisms contributed to the resistance, including enzymatic and non-enzymatic mechanisms. An enzymatic mechanism through modifying enzymes such as aminoglycoside phosphoryl-transferase, aminoglycoside acetyl-transferase, and aminoglycoside adenyl-transferase, which inactivate the drug, is well-established. A non-enzymatic induced resistance is also another possible mechanism by affecting aminoglycoside permeability and reduction of its uptake by the bacterial cell.

Another type of bacterial resistance to aminoglycosides that is influenced by the duration of antibiotic exposure and not to genetic mutation, is called adaptive resistance (Barclay ML and Begg EJ, 2001). During the first several hours of aminoglycoside exposure, bacterial killing will be in a concentration dependant fashion. After a prolonged exposure to the aminoglycoside, the bacterial pathogen down-regulates aminoglycoside transport (Daikos GL et al., 1990, Jackson GG et al., 1990). However, this type of resistance is unstable and can be reversible within few hours, yielding pathogens that are fully susceptible to the aminoglycoside killing effect if they have a drug-free period. More recent *in vitro* work identified a multidrug efflux pump system, known as the MexXY efflux pump, as a possible explanation for the reduction of aminoglycoside accumulation within the bacterial cell and the occurrence of adaptive resistance (Sobel ML et al., 2003). The authors observed a fast activation of MeXY production (within 2 hours) after *P. aeruginosa* exposure to an

aminoglycoside and a reduction in the efflux pump expression when the organism was no longer exposed to the antibiotic.

2.4.1.3 Toxicity

The main aminoglycoside side effects are nephrotoxicity and ototoxicity, and also neuromuscular blockade to a small degree. The reported incidence of nephrotoxicity in general medical patients was up to 20 % (Kahlmeter G and Dahlager JI, 1984, Barza M et al., 1996, De Jager P and Van Altena R, 2002). The predisposing factors for aminoglycoside induced nephrotoxicty are elevated serum trough levels (>2 mg/L), abnormal baseline renal function (Selby NM et al., 2009) and the use of concomitant nephrotoxic drugs, such as vancomycin (Rybak MJ et al., 1999) and furosemide (Prins JM et al., 1996). However, aminoglycoside dose and duration of course were not associated with the development of the nephrotoxic effect. Although aminoglycosides are eliminated mainly by glomerular filtration, a fraction of the dose is reabsorbed into the proximal tubule and this is thought to be the primary site for nephrotoxicity (Schentag JJ et al., 1979, Schentag JJ and Plaut ME, 1980). Aminoglycosides are reabsorbed and transported into the renal proximal tubule by pinocytosis and sequestered in lysosomes, mitochondria and the body of the Golgi apparatus (Sundin DP et al., 2001). They induce cellular death by inhibiting protein synthesis and alteration of lysosomal membrane permeability, causing disruption of the membrane and release of lysosomal enzymes into the cytoplasm. Moreover, molecular studies have shown other possible mechanisms. Proximal tubule calcium receptors might be activated by the antibiotic leading to renal damage (Ward DT et al., 2002) but this requires further investigation. Another suggested mechanism is by reducing glucose reabsorption in kidney by reducing its transporters (Takamoto K et al., 2003).

The incidence of aminoglycoside induced ototoxicity was small, less than 20 % (Kahlmeter G and Dahlager JI, 1984, De Jager P and Van Altena R, 2002). Aminoglycoside induced ototoxicity includes cochlear and vestibular dysfunction (Schentag et al., 2006). Cochlear toxicity may manifest as tinnitus, hearing loss, pressure, and sometimes pain in the ear,

while vestibular toxicity symptoms include dizziness, vertigo, ataxia, and nystagmus. Symptoms of aminoglycoside induced ototoxicity can occur as early as three to five days after starting therapy or even up to four to six weeks after stopping therapy. Several factors have been reported to predispose a patient to the drug's ototoxic effect, including prior renal insufficiency, prior abnormal audiogram, older age, septicaemia, dehydration, high temperature, total cumulative dose, prolonged duration of therapy (2-3 weeks), prior aminoglycoside exposure, peak serum concentration, trough serum concentration, and concomitant administration of ototoxic drugs, such as loop diuretics. Current thinking regarding a possible predisposing factor for the antibiotic's ototoxic effect is through genetic mutation. The predisposing mutation is the *A1555G* in the 12S ribosomal RNA gene (Prezant TR et al., 1993), and was associated with 30 % of hearing loss cases (Fischel-Ghodsian N et al., 1997). The genetic mutation does not stop at the individual level, but can be inherited (Gardner JC et al., 1997, Hu DN et al., 1991). Gardner *et al* (1997) reported a Southern African family in which nine members went deaf following streptomycin treatment, while Hu *et al* (Hu DN et al., 1991) reported 36 Chinese families that had a family history of aminoglycoside-induced hearing loss. This suggests the need to take a family history before aminoglycoside administration. Guthrie (2008) reviewed *in vitro* and *in vivo* work to explain possible mechanisms for aminoglycoside ototoxicity. He reported that the possible molecular mechanism to induce vestibulocochlear damage was by formation of an aminoglycoside-iron complex that produced oxidative stress in the inner ear. Recently, the production of oxidative stress was found to be associated with genetic mutations at the oxidative stress-related gene (p*NOS3*, *GSTZ1*, and *GSTP1* gene) to induce aminoglycoside-vestibulotoxicity (Roth SM et al., 2008). Current research is moving towards finding possible protection strategies, for example, the use of iron chelators and free radical scavengers.

Aminoglycoside induced neuromuscular blockade is a rare but potentially fatal adverse effect (Schentag et al., 2006). The effect can be clinically relevant in patients with pre-existing neuromuscular disease, such as myasthenia gravis, patients who are hypocalcaemic or hypomagnesaemic, concomitant administration with other neuromuscular blocking drugs or anaesthetic agents, and patients receiving calcium channel blockers. The drug interferes with presynaptic uptake of calcium, thus reducing the release of acetylcholine, and binds to

the postsynaptic acetylcholine receptor-channel complex. Usually the toxicity can be reversible in mild cases by stopping the drug, whereas in more severe cases pharmacological intervention is needed, including administration of calcium gluconate or neostigmine.

2.4.2 Aminoglycoside pharmacokinetics in patients with cystic fibrosis

2.4.2.1 Volume of distribution in patients with cystic fibrosis

A number of studies compared estimates of aminoglycoside volume of distribution in paediatric and young adult patients with and without cystic fibrosis and found that patients with cystic fibrosis had higher estimates of volume of distribution when normalised to body weight (Kearns GL et al., 1982, Levy J et al., 1984). On the other hand, other studies failed to find any difference between volume of distribution in patients with cystic fibrosis compared to those without the disease (Mann HJ et al., 1985, Hennig S et al., 2013). The reason may be related to participant characteristics, including nutritional state. Levy *et al* (1984) suggested that malnourished patients had higher extracellular volume on the basis of weight, and because aminoglycoside is a hydrophilic drug then it would distribute to extracellular fluid. Another possible explanation could be the influence of body size measurement used to normalised volume of distribution. For example, when Levy *et al* (1984) normalised volume of distribution to weight, a difference was observed. However, when volume of distribution was normalised to body surface area, no difference in volume of distribution was observed between patients with and without cystic fibrosis. Hennig *et al* (2013) used lean body weight (Janmahasatian S et al., 2005) to estimate volume of distribution and no difference was observed between patients with and without the disease. Patients with cystic fibrosis are usually malnourished and lack adipose tissue; this difference in body composition is taken into account in addition to body size when lean body weight was used compared with weight. Therefore, the use of lean body weight eliminated the difference between patients with cystic fibrosis and those without the disease.

2.4.2.2 Clearance in patients with cystic fibrosis

Findings from several studies in paediatric and adult patients with cystic fibrosis supported higher aminoglycoside clearance values, both when compared to control and to historical estimates (Kearns GL et al., 1982, Mann HJ et al., 1985, Levy J et al., 1984). A possible explanation for these differences might be an additional, non-renal elimination pathway. Sputum has been suggested as an alternative route of aminoglycoside elimination, particularly for tobramycin (Levy J et al., 1984). Because mucus plugs in patients with cystic fibrosis are composed of negatively charged glycoproteins, positively charged compounds, such as tobramycin, could bind and be eliminated (Levy J, 1986, Hunt BE et al., 1995, Ramphal R et al., 1988). In contrast, MacDonald *et al* (1983) suggested that the severity of the disease might influence aminoglycoside pharmacokinetics, where patients with mild disease had higher drug clearance. In contrast, a recent study conducted in eight centres did not find any difference in clearance in patients with cystic fibrosis compared to the general population (Hennig S et al., 2013). The difference in results could be related to the sample size; studies that observed an increased aminoglycoside clearance in patients with cystic fibrosis included less than 30 patients and the age group was limited to paediatric and young adults (less than 18 years old) (Kearns GL et al., 1982, Mann HJ et al., 1985, Levy J et al., 1984). However, in the study of Hennig *et al* (2013), a total of 732 patients were included with a wide range of age (0.01 to 66.4 years old). Usually, paediatric patients have higher clearance compared with adults and because the mean age of Kearns *et al* (1982), Mann *et al* (1985) and Levy *et al* (1984) was between 11 and 16 years old, that might explain the increased aminoglycoside clearance that was observed. In addition, the method used to estimate renal function might influence the results. Levy *et al* (1984) used two methods to estimate renal function; clearance of iothalamate and creatinine clearance by measuring creatinine concentration in the urine. Mann *et al* (1985) estimated creatinine clearance by the Cockcroft and Gault equation with no information on how to handle low serum creatinine. There is some evidence showed that the use of low serum creatinine values (less than 60 µmol/L) to estimate creatinine clearance by the Cockcroft and Gault equation might result in overestimation of creatinine clearance (Touw DJ et al., 1996). On the other hand, Kearns *et al* (1982) used 12 or 24 hour urine collection to estimate creatinine clearance. However, Hennig *et al* (2013) used the serum creatinine, age and lean

body weight, which take into account body size and composition, to estimate aminoglycoside clearance.

2.5 AMINOGLYCOSIDE USE IN PATIENTS WITH CYSTIC FIBROSIS

Patients with cystic fibrosis suffer from frequent lung infections caused mainly by *P. aeruginosa* (Cystic FibrosisTrust, 2013) and the UK Cystic Fibrosis Trust recommend the use of intravenous antibiotics to treat pulmonary exacerbations or low grade symptoms which are not responding to oral antibiotics (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). The Trust recommends a combination of antibiotics with different mechanisms of action to be prescribed and a combination of ceftazidime and tobramycin is commonly used.

2.5.1 Aminoglycoside dosing

2.5.1.1 Extended interval dosing

Traditionally, aminoglycosides were administered every six or eight hours, but further understanding of their pharmacodynamics has moved dosing regimens towards extended interval, twelve hourly and particularly once daily administration. The rationale behind this change is that aminoglycosides have concentration-dependent activity so high peaks correlate better with efficacy. They also have a long post-antibiotic effect, from three to ten hours against *P.aeruginosa* (Fantin B et al., 1991, Vogelman B et al., 1988b, Craig WA, 1993), which is even more prolonged in the presence of neutrophils (Kapusnik JE et al., 1988). Once daily administration reduces the development of adaptive resistance, although the exact duration is still unknown (Daikos GL et al., 1991, Gilleland LB et al., 1989, Daikos GL et al., 1990). Moreover, once daily aminoglycoside dosing has been associated with less drug being accumulated in the kidney and hence a reduced risk of nephrotoxicity (De Broe ME et al., 1991, Verpooten GA et al., 1989).

The efficacy and safety of once daily aminoglycoside dosing was compared with three times daily dosing in a meta-analysis conducted by Munckhof *et al* (1996), who evaluated 20 studies including 2881 adult patients. The authors found that once daily dosing resulted in a small improvement in clinical efficacy with a difference of 3.5 % (95% confidence interval, 0.5 to 6.5 %; p = 0.027) compared with three time daily dosing, but with a similar rate of nephrotoxicity (difference: 1.3 %; 95 % confidence interval, -3.1 to 5 %; p = 0.19) and ototoxicity assessed by audiometry (difference 0.7 %; 95 % confidence interval, – 3.1 to 4.5 %; p = 0.84). In addition, Hatala *et al* (1996) evaluated thirteen randomised controlled trials that compared the efficacy and safety of aminoglycoside once and three times daily dosage regimens in adult general medical patients. The results from the meta-analysis showed that both regimens had equivalent efficacy with risk ratio of 1.02 (95 % confidence interval, 0.99 to 1.05). However, although the differences were non-significant, the once daily dosage regimen was associated with a relative risk reduction of nephrotoxicity of 13 %, a relative risk reduction of ototoxicity of 33 %, and a relative risk reduction of 9 % for mortality compared with three times daily. Furthermore, Contopoulos-Ioannidis *et al* (2004) examined 24 studies that compared the efficacy and safety of once and three times daily dosage regimens in paediatric patients treated with aminoglycosides. The results indicated a trend for better efficacy associated with once daily compared with three times daily dosing with a risk ratio of 0.71 (95 % confidence interval, 0.45 to 1.11; p = 0.13) and with comparable rate of nephrotoxicity, risk ratio 0.97 (95% confidence interval, 0.55 to 1.69; p =0.90) and ototoxicity, risk ratio 1.06 (95% confidence interval, 0.51 to 2.19; p = 0.92). Moreover, aminoglycoside associated nephrotoxicity was compared for twice and once daily dosage regimen in general medical patients (Rybak MJ et al., 1999). The authors reported six cases of nephrotoxicity in the twelve hourly dosing and no cases in the once daily dosage regimen group. These results confirmed that less frequent aminoglycoside dosing was associated with reduced risk of nephrotoxicity. The available evidence encouraged the use of extended interval aminoglycoside dosing in different patient groups treated for susceptible infections.

2.5.1.2 Aminoglycoside dosing in patients with cystic fibrosis

The dose recommended by the cystic fibrosis Trust for both children and adults is tobramycin 10 mg/kg/day every 24 hours by infusion over 30 minutes up to a maximum of 660 mg. This dose should be administered for two weeks with "trough" levels being monitored before the second and eighth doses. The target concentration for samples taken 18 hours post dose is <1 mg/L (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). In addition, plasma creatinine should be monitored before the first dose of tobramycin and again before the eighth dose. It is recommended that baseline audiometry is performed at the beginning of each treatment with intravenous tobramycin, i.e. within the first two days after the first dose (Scheenstra RJ et al., 2010). However, not all cystic fibrosis units follow these guidelines. The current practice in Glasgow is to prescribe a tobramycin dose of 120 mg/m^2 twice daily, according to the work of Campbell *et al* (1999), aiming for a peak of 8-12 mg/L and trough less than 1 mg/L (*Personal Communication, cystic fibrosis pharmacist at Gartnavel Hospital, Glasgow, May 11, 2010*).

The Cystic Fibrosis Trust recommendations resulted from cumulative evidence supporting the effectiveness and safety of once daily tobramycin. One piece of evidence came from the TOPIC study (Smyth A et al., 2005), which contained 219 patients given a dose of 10 mg/kg tobramycin once daily or eight hourly for 14 days. This is considered as one of the largest clinical trials comparing once to three times daily dosing of aminoglycosides in patients with cystic fibrosis. There was no difference in efficacy in patients with acute pulmonary exacerbations but a tendency for a greater increase in serum creatinine concentrations was observed in adult patients with cystic fibrosis in the once daily group. In addition, patients from the TOPIC study were further investigated for the occurrence of ototoxicity assessed by audiometry (Mulheran M et al., 2006). The analysis showed no difference in the incidence of ototoxicity between once and three times daily dosing. In addition, Scheenstra *et al* (2010) examined the incidence of ototoxicity for tobramycin dose of 10 mg/kg/day administered in two divided doses, and found no hearing loss in patients with cystic fibrosis.

The results from the TOPIC study are in agreement with other publications that showed an equivalent improvement in lung function with once daily and three times daily dosing of tobramycin with no difference in nephro- or ototoxicity in patients with cystic fibrosis (Bates RD et al., 1997, Vic P et al., 1998, Master V et al., 2001, Burkhardt O et al., 2006). However, Vic *et al* (1998) favoured once daily dosing as it reached higher tobramycin sputum concentrations compared with three times daily dosing. In addition, although the difference was small, patients treated with three times daily dosing had higher microglobulinuria on day 14 compared with the once daily group, suggesting better renal tolerance for once daily dosing. Bates *et al* (1997) reported also an increase in blood urea nitrogen level but without nephrotoxicity following once daily dosing. However, blood urea nitrogen is not a specific indicator for nephrotoxicity and the authors suggested a high protein diet effect or corticosteroid administration might have caused this increased level. On the other hand, Master *et al* (2001) reported ototoxicity in the form of tinnitus as a result of rapid administration of tobramycin with both three times daily and once daily dosing.

Despite the evidence supporting once daily administration of tobramycin in patients with cystic fibrosis, Beringer *et al* (2000) and Burkhardt *et al* (2006) raised some concerns about administering once daily aminoglycosides in these patients. Although the pharmacokinetic /pharmacodynamic index of peak to MIC ratio was greater with once daily compared to twelve or eight hourly, and it had greater bactericidal activity, the time below the MIC for *P. aeruginosa* with once daily dosing exceeded the post-antibiotic effect in both *in vitro* and *in vivo* studies in adult patients with cystic fibrosis (Beringer PM et al., 2000). The authors were concerned about a possible emergence of *P. aeruginosa* resistance following once daily dosing as a result of the prolonged time below the MIC. This may allow greater bacterial re-growth during the dosing interval and the development of resistance. This observation was supported by the study of Burkhardt *et al* (2006) in patients with cystic fibrosis. They found an increase in the MIC of *P. aeruginosa* after 24 hourly dosing, whereas the MIC after 8 hourly dosing did not change. In addition, Master *et al* (2001) reported that both once and three times daily tobramycin dosage regimens led to an increase in MIC against *P. aeruginosa* where the once daily tobramycin dosage regimen resulted in a statistical significant increase with multiple courses of the antibiotic. This raises another concern

about developing resistance because patients with cystic fibrosis have recurrent lung infections and would require more frequent treatment with aminoglycoside. Further evidence comes from a UK evaluation susceptibility test study in a range of patients, which showed that *P. aeruginosa* resistance rates to the β-lactam, aminoglycoside and quinolone agents was low, less than twelve per cent (Henwood et al., 2001). However, a patient sub-group analysis showed that high resistance rates were reported for isolates from patients with cystic fibrosis.

CHAPTER 3: WITHIN SUBJECT VARIABILITY IN AMINOGLYCOSIDE PHARMACOKINETICS IN PATIENTS WITH CYSTIC FIBROSIS

3.1 INTRODUCTION

Cystic fibrosis is an inherited autosomal recessive disease characterised by viscous secretions within the respiratory tract leading to persistent airway obstruction with mucus, which provides a good culture medium for microorganism growth. Chronic and intermittent infections with *P. aeruginosa* commonly occur in this group of patients (Cystic FibrosisTrust, 2013) and are typically treated with a combination of an aminoglycoside (usually tobramycin) and a β-lactam, such as ceftazidime (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). Since patients with cystic fibrosis now live longer, with a median survival of 41 years reported in 2010 and 2011 compared with 34 years in 2009 (Cystic FibrosisTrust, 2013), they are potentially at increased risk of developing aminoglycoside induced renal toxicity through exposure to multiple courses of therapy over a prolonged period of time.

The incidence of acute renal failure has been reported as 4.6-10.5 per 10 000 patients with cystic fibrosis per year in the UK, and 88 % of these patients were prescribed an aminoglycoside (Bertenshaw C et al., 2007). Previous studies have mainly focused on nephrotoxicity arising from single courses of aminoglycoside therapy and have studied patients within a range of clinical specialties, including cystic fibrosis (Prestidge C et al., 2011, Smyth A et al., 2005), general medicine (Drusano GL and Louie A, 2011, Sweileh WM, 2009), critical illness (Galvez R et al., 2011), and tuberculosis (De Jager P and Van Altena R, 2002). However, there is currently little information on the impact of multiple courses of aminoglycoside therapy on renal function in patients with cystic fibrosis.

3.2 AIMS OF STUDY

The aims of the present study were as follows:

- to investigate the influence of covariates on aminoglycoside pharmacokinetic parameters in patients with cystic fibrosis.
- to determine the nature and extent of within-subject variability in aminoglycoside pharmacokinetics in patients with cystic fibrosis.

- to determine how pharmacokinetic parameters change over time in patients with cystic fibrosis who have received multiple courses of therapy and their impact on future dosage recommendations.

3.3 METHODS

3.3.1 Patient data and setting

The study was a retrospective analysis of data contained within a database of aminoglycoside therapeutic drug monitoring for adult patients with cystic fibrosis data covering the period 1993 to 2009. A total of 219 courses of therapy from 51 of these patients, collected between 1993 and 1997, have been analysed and reported previously (Campbell D et al., 1999). In addition, data from a maximum of 2 courses from 163 patients were included in a recent population meta-analysis that compared tobramycin pharmacokinetics in children and adults with and without cystic fibrosis (Hennig S et al., 2013). The project was conducted using the data that were routinely collected by the therapeutic drug monitoring service provided by clinical pharmacists to the Adult Cystic Fibrosis Unit, Gartnavel General Hospital, Glasgow. The number of active patients within the unit in 2011 was 217 (Cystic FibrosisTrust, 2013). All files containing therapeutic drug monitoring data from patients who were treated in the cystic fibrosis unit with an aminoglycoside antibiotic and were stored within the database in the pharmacy department were eligible for inclusion in the study. Aminoglycoside dose and concentration data are routinely analysed using a MAP Bayesian pharmacokinetic package, OPT (Kelman et al., 1982). Hard copies of OPT data collection forms and output are stored within the pharmacy department and electronic copies of OPT files are stored on the hospital computer network.

The database was constructed using the spreadsheet package Excel. The following clinical data were entered into the database and summarised: anonymised patient identification number, date of start of therapy, time of drug administration, duration of therapy, age, sex, actual weight, height, and serum creatinine concentrations. If clinical characteristics were missing, the median of the patient group was imputed. No patient identification data were stored in the final database. The following pharmacokinetic data were also entered into the

database: drug name, administered dose amounts, dates, times and rates of infusion, dates and times of concentration measurements. Ethical approval was obtained from the West of Scotland Research Ethics Service committee (Reference number 09/S0709/50). A copy of the ethics approval letter is shown in APPENDIX I. Patient consent was not obtained since the data were retrospective and had been anonymised by clinical pharmacists before entry into the database.

3.3.2 Serum creatinine and drug assay

Serum creatinine was measured by the Jaffé method and there was no change over time in the analytical method. Aminoglycoside concentrations were measured by the clinical microbiology laboratory of the hospital using Fluorescence Polarization Immunoassay (TDx, Abbott Laboratories) with no change in methodology over the data collection period. The limit of quantification was 0.1 mg/L and 2.5 % of the concentrations were reported as 0.1 mg/L. Five concentration measurements were below this limit were excluded from the analysis. The inter-assay coefficients of variation were 6.3% at 1 mg/L, 3.7% at 4 mg/L and 4.3% at 8 mg/L.

3.3.3 Population data analysis

The data were analysed using the population pharmacokinetic software NONMEM (Beal SL et al., 2009). The modelling approach within NONMEM is divided into three sub-models: structural, statistical and covariate model. The structural sub-model describes the time course of the drug in the body in a specific individual (the drug behaviour in the body), which is decided based on the available drug concentration data to follow one or multi-compartment model, using fixed effects parameters. The fixed effect parameters include the typical population estimates of pharmacokinetic parameters such as clearance and volume of distribution, that can be a function of various covariates (Sheiner LB and Beal SL, 1981a). A covariate is any variable that is specific to an individual and may influence the pharmacokinetics or pharmacodynamic of a drug including age, weight, dose, and presence of concomitant medication. The statistical sub-model accounts for the random effect parameter, which are the amount of pharmacokinetic variability including between-subject

variability (BSV), within-subject variability (WSV) and residual variability, e.g. assay error, doses times, and incorrect pharmacokinetic model.

3.3.3.1 Structural model

In the current study, one compartment and two compartment linear models were compared. They were tested using NONMEM version 7.1 (Beal SL et al., 2009) using the first order conditional estimation with interaction algorithm (FOCE I). The FOCE is an estimation algorithm that conditions the linearization of the model around each individual parameter estimate for the between-subject variability random effects. In addition, the Stochastic Approximation Expectation Maximization (SAEM) algorithm was tried. The SAEM algorithm is a stochastic approximation version of the expectation maximization algorithm linked to a Monte Carlo procedure to estimate the maximum likelihood (Kuhn E and Lavielle M, 2005). To define a compartment model in NONMEM, an "ADVAN" subroutine should be selected, and to re-parameterise these parameters to the pharmacokinetic parameters requested by the modeller the "TRANS" subroutines could be selected also. There are several re-parameterisation options available within NONMEM, and TRANS 2 was chosen for the one compartment model. This converts the basic parameters k (the elimination rate constant) and V (the volume of distribution) into clearance (CL) and volume of distribution (V) according to the following relationship

$$k = CL/V$$

For the two compartment model, ADVAN 3 was used with TRANS 4, which re-parameterised the basic pharmacokinetic parameters for ADVAN 3 to:

CL clearance
V_1 central volume
Q intercompartmental clearance
V_2 peripheral volume

according to the following relationships

$$k_{10} = CL/V_1$$

$$k_{12} = Q/V_1$$

$$k_{21} = Q/V_2$$

Initial values for the pharmacokinetic parameters were obtained from the paper by Campbell *et al* (1999). The initial estimate of clearance used for the one-compartment model was 4.3 L/h and the volume of distribution estimate was 14 L. For the two compartment parameters, both clearance and volume of distribution of the central compartment values were similar to those used in the one compartment model. Different initial estimates for volume of distribution of the peripheral compartment and inter-compartmental clearance values were tried.

Between-subject variability was modelled using an exponential model because pharmacokinetic data are usually right-skewed (Lacey LF et al., 1997) and log- normal distribution is assumed, and to force the parameters to be greater than zero (to get positive parameters) as follows;

$$CLi = TVCL \exp(\eta i)$$

Where CLi is clearance for i^{th} subject, TVCL is the typical population clearance estimate; ηi is the deviation from the typical for the i^{th} subject with zero mean and variance ω^2. The initial values for all BSV variance were set at 0.05. The block matrix was tried and was retained in the model if it improved the fit of the data. Because log-normal distribution was assumed the estimated variance is in log scale, it should be converted to the original scale by estimating a coefficient of variation. The coefficient of variation can be estimated then using the following formula:

$$CV\ (\%) = \sqrt{\omega^2} \times 100$$

Within-subject variability (WSV) was then added to the structural model applying the Karlsson and Sheiner model (Karlsson MO and Sheiner LB, 1993) as follow:

$$CLi = TVCL\ exp\ (\eta i + \eta j_1\ OCC_1 + \eta j_2\ OCC_2 + ...\eta_{jn}\ OCCj)$$

Where j is the occasion number e.g. j=1,2...O.; ηj_n is the deviation from the population typical estimate due to variability from occasion j. It is assumed that each ηj_n has zero mean and variance $\omega^2 j$. A coefficient of variation can be estimated using the CV formula previously stated. The value of the WSV variance was constant across all occasions; APPENDIX II shows an example of a control file that contains the coding for WSV. The definition of one occasion was one aminoglycoside treatment course. Residual error was also modelled where additive, proportional and combined error models were tried and compared using the base model.

The criteria set for choosing the structural model were: first, a significant reduction in the difference in objective function value (ΔOFV) = 13.82 for two degree of freedom, $P < 0.001$. Secondly, an improvement in the goodness of fit plots, such as observed versus population and individual predicted concentrations, and conditional weighted residual errors (CWRES) versus time after dose and population predicted concentrations. CWRES is the weighted difference between the model prediction and data calculated using the FOCE method (Hooker AC et al., 2007). Scatter plots for the measured versus predicted concentrations were examined for agreement.

In order to decide on which algorithm to be taken forward and used for covariate modelling, run times for the FOCE I and SAEM algorithms were compared. In addition bias and imprecision in pharmacokinetics and individual concentration predictions were examined using the Sheiner *et al* approach (1981b). The algorithm which was fast and produced unbiased and precise predictions was used for covariate modelling.

Bias and imprecision were estimated for the pharmacokinetic parameters of interest, clearance and V_1, and the measured versus population predicted concentrations. Bias was defined as mean difference in prediction error if the results were normally distributed or

median if they were not (Sheiner LB and Beal SL, 1981b). Lower bias indicates higher accuracy in model predictions. Bias was then assessed by comparing mean prediction errors with zero using the Student's t test if the data were normally distributed and the median prediction using the Wilcoxon signed rank test otherwise with statistical significance set at $p < 0.05$. The 95% confidence interval of the difference was also examined using Minitab Version 15 (Minitab Ltd.). The following formulas were used to estimate the prediction errors for pharmacokinetic estimates and concentrations;

$$PK\ Estimate\ Prediction\ Error = Individual\ PK\ Estimate - Population\ PK\ Estimate$$

$$Concentration\ Prediction\ Error = Measured\ Concentration - Predicted\ Concentration$$

Imprecision was based on the root mean squared prediction error if the data were normally distributed, or the median absolute (unsigned) error if the data were non-normally distributed. A lower value indicates higher precision in model predictions.

3.3.3.2 Covariate model

The covariates tested were age, weight, height and serum creatinine concentration. In addition, other derived covariates were tested, including lean body weight (LBW) (Janmahasatian S et al., 2005), and body surface area (BSA) (Mosteller RD, 1987). Patient's nutrition status was determined using the body mass index (BMI) (World Health Organisation, 2011) grouped according to the World Health Organisation categorisation into four groups; underweight (BMI < 18.5 kg/m^2), normal weight (BMI = 18.5 to 24.99 kg/m^2), overweight (BMI = 25 to 29.99 kg/m^2), and obese (BMI ≥ 30 kg/m^2) (World Health Organisation, 2011). The formulas used to determine the derived body size measurements and body mass index are as follow;

$$LBW\ for\ males\ (kg) = \frac{9270 \times Weight\ (kg)}{6680 + 216 \times BMI\ (^{kg}/_{m^2})}$$

$$LBW\ for\ females\ (kg) = \frac{9270 \times Weight\ (kg)}{8780 + 244 \times BMI(^{kg}/_{m^2})}$$

$$BSA\ (m^2) = \sqrt{\frac{Height\ (cm) \times Weight\ (kg)}{3600}}$$

$$BMI = \frac{Weight\ (kg)}{(Height\ (m)^2)}$$

In addition, renal function was another tested covariate and it was estimated by the Cockcroft and Gault equation (1976) using three approaches. The first approach was to use the measured concentration and the second was to fix serum creatinine concentration to 60 µmol/L if the measured concentration was less than 60 µmol/L, as recommended by Duffull *et al* (1997) and Rosario *et al* (1998).

$$CLcr\ if\ Female = \frac{1.04\ \times (140 - Age) \times Weight\ (kg)}{Serum\ creatinine\ (\mu mol/L)}$$

$$CLcr\ if\ Male = \frac{1.23\ \times (140 - Age) \times Weight\ (kg)}{Serum\ creatinine\ (\mu mol/L)}$$

A further approach was to multiply the Cockcroft and Gault estimate of creatinine clearance by 0.69 for individuals who were underweight by 15% or more (Khuu T et al., 2010). Weight was used in the Cockcroft and Gault formula. The methodology of Khuu *et al* (2010) was followed whereby ideal body weight was determined by the Devine method (Devine BJ, 1974), then percent underweight was calculated as follows:

$$IBW\ for\ Female = 45.5\ kg + 2.3\ kg\ (Height\ in\ inches - 60)$$

$$IBW\ for\ Male = 50\ kg + 2.3\ kg\ (Height\ in\ inches - 60)$$

$$Percentage\ underweight = \left(1 - \left(\frac{ABW}{IBW}\right)\right) * 100$$

The mechanistic model proposed by Matthews *et al* (2004) and Anderson and Holford (2009), which separates drug clearance into renal and non-renal components, was also investigated. This model assumes different creatinine production rates for patients whose creatinine concentration is above or below 60 µmol/L. A full mechanistic model, in which all model parameters were estimated from the dataset, and a model that included the fixed parameters for creatinine clearance reported by Matthews *et al* (2004) were compared. An example of the control file used for this model is shown in APPENDIX III. In addition, other potential covariates including aminoglycoside type (gentamicin or tobramycin), the number of courses of therapy and the time since the first course were evaluated.

The relationships between individual pharmacokinetic estimates and covariates were visually examined by scatter plots using the NONMEM post-processing package Xpose 4 (version 4.3.5) (Jonsson and Karlsson, 1999) implemented in R (version 2.15.1) (R Core Team, 2012). Covariates that were identified as having a potentially strong relationship with a pharmacokinetic parameter were included in the model first. In addition, a generalised additive model (GAM) (Mandema JW et al., 1992) analysis of covariates and parameters was performed using Xpose 4. Covariates suggested by the GAM analysis with the lowest Akaike number were added to the model.

There is no consensus of how best to do covariate modelling when WSV is to be used, before or after the addition of WSV. In the current study, the addition of covariates was modelled first without WSV. Within-subject variability was then added to the potential final covariate models. The reason behind this decision was to avoid the long computation time associated with the very complex model. Covariates were retained in the model when a statistically significant improvement in the fit of the model to the data was observed. This significant improvement was defined as a reduction in the OFV of 6.63 ($P < 0.01$) during the

stepwise addition of covariates and an increase in OFV of ≥10.3 ($P < 0.001$) during the stepwise removal of covariates. Furthermore, an increase in goodness-of-fit caused by the introduction was examined by the measured and predicted concentrations, and conditional weighted residuals against time after dose and population predicted concentrations. The addition of more covariates into the model was decided after examining parameter variability (etas) plots against covariates to assess whether anything was missing from the model.

Linear and allometric relationships with weight and power relationships for time and course number were tested. In addition, clinical factors were centred or scaled to their median value as appropriate. The following different structures for modelling covariates were used singly or in combination:

Linear modelling:

$CLi = (TVCL (1+\Theta_{n+1} (Covariate - Median)) EXP (\eta i)$

$CLi = (TVCL \times Body\ size\ measurement (1+\Theta_{n+1}(Covariate - Median)) EXP (\eta i)$

$CLi = (\Theta_{n+1} \times Body\ size\ measurements + \Theta_{n+1} (Covariate - Median)) EXP (\eta i)$

Non-linear model:

$CLi = (TVCL ((Covariate/Median)^{\Theta n+1})) EXP (\eta i)$

Allometric scale model:

$CLi = (TVCL (Weight/70)^{0.75}) EXP (\eta i)$

Mechanistic model:

$CLi=(\Theta_{n+1}(LBW/70)^{0.75}+\Theta_{n+1}(CrCL(L/h)/7.26\ L/h/70\ kg \times (LBW+0.211(Weight–LBW)\ /70)^{0.75}))$ $EXP(\eta i)$

Where CLi is the clearance value for the i^{th} subject, TVCL is the typical clearance estimate, Θ_{n+1} is the proportional change of typical value per unit of covariate, ηi is between-subject

variability and represents the difference between the individual parameter and the typical population value for the i^{th} subject with zero mean and variance ω^2. The term "7.26 L/h/70kg" in the mechanistic model refers to the standard GFR for an adult patient weighing 70 kg, and the 0.211 is a correction term for the contribution of fat mass to GFR.

Models investigated for V_1 included direct linear relationships with body size measurements, linear relationships with an intercept, non-linear relationships and allometric scaling as presented below:

Linear model:

$V_{1i} = (TVV_1 \times \text{Body Size}) \text{ EXP } (\eta i)$

$V_{1i} = (TVV_1 (1+ \Theta_{n+1}(\text{body size - median}))) \text{ EXP } (\eta i)$

Non-linear model:

$V_{1i} = (TVV_1 (\text{body size / median})^{\Theta n+1}) \text{ EXP } (\eta i)$

Allometric scale model:

$V_{1i} = (TVV_1 (\text{Weight / 70}) \text{ EXP } (\eta i)$

Mechanistic model:

$V = (TVV_1 \times (LBW+ (WT-LBW)/70))) \text{ EXP } (\eta i)$

$V = (TVV_1 (1+ \Theta_{n+1} (LBW+ (WT-LBW)/70))) \text{ EXP } (\eta i)$

Where V_{1i} is the volume of distribution of the central compartment of the i^{th} subject, TVV_1 is the typical population value for V_1, Θ_{n+1} is the proportional change of typical value per unit of covariate, ηi is between-subject variability and represents the difference between the individual parameter and the typical population value for the i^{th} subject with zero mean and variance ω^2. An example of a covariate modelling control file is shown in APPENDIX II and III.

In addition, time since the first course was modelled as covariates. A non-linear (power) relationship was used to test relationships between CL and time as follow;

IF(OBST.EQ.0)THEN
CLi= TVCL x EXP (ηi)
ELSE
CLi= TVCL x ((OBST/Median)$^{\Theta n+1}$) EXP (ηi)
ENDIF

Where CL_i is the clearance of the i^{th} subject, TVCL is the typical population value for CL, Θ_{n+1} is the proportional change of typical value per unit of covariate, ηi is between-subject variability and represents the difference between the individual parameter and the typical population value for the i^{th} subject with zero mean and variance ω^2, OBST refers to the observation time in years.

3.3.3.3 Distribution and elimination half-lives estimates

The individual parameter estimates obtained with the final model were used to calculate individual estimates of distribution and elimination half-life. For the one compartment model, the elimination half-life was estimated as follows:

$$t_{1/2} = \frac{0.693}{k}$$

Where k is the elimination rate constant, as discussed in the structural model section.

For the two-compartment model, the distribution half-life was estimated as follows:

$$t_{1/2}^{\lambda 1} = \frac{0.693}{\lambda_1}$$

Where λ_1 was calculated with the following formula:

$$\lambda_1 = \frac{1}{2}\left[(k_{12} + k_{21} + k_{10}) + \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4 \times k_{21} \times k_{10}}\right]$$

The elimination half-life was estimated with the following formula:

$$t_{1/2}^{\lambda 2} = \frac{0.693}{\lambda_2}$$

Where λ_2 was calculated with the following formula:

$$\lambda_2 = \frac{1}{2}\left[(k_{12} + k_{21} + k_{10}) - \sqrt{(k_{12} + k_{21} + k_{10})^2 - 4 \times k_{21} \times k_{10}}\right]$$

where k_{12} and k_{21} are the transfer rate constants from central to peripheral compartment and vice versa and k_{10} is the elimination rate constant from V_1. These parameters were estimated from:

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

$$k_{21} = \frac{Q}{V_2}$$

$$k_{10} = \frac{CL}{V_1}$$

3.3.3.4 Pharmacokinetic parameter changes over time

The influence of multiple courses of therapy on aminoglycoside clearance was examined visually by plotting individual clearance estimates against course number for all patients who received more than one and more than 10 courses of therapy and by comparing first and final clearance estimates in patients who received at least 10 courses of therapy (paired Student's t-test, $p<0.05$ significant). Data were plotted using Minitab® version 15, (Minitab Ltd, Coventry, UK).

3.4 RESULTS

3.4.1 Patient characteristics

Aminoglycoside concentration-time data were available from 166 patients of whom 81 were male and 85 female. The demographic and clinical characteristics of all 166 patients and grouped according to gender are summarized in Table 3.1. The majority of patients were young, with a median age of 23 years. Although the eldest patient was 66 years of age, patients were typically less than 40 years of age for most of their courses of therapy, as shown in the histogram plot of age in Figure 3.1. Information from 22 patients (58 courses) was available when they were more than 40 years old. One female patient had a missing height, so the median female height was used. Since the highest estimate of BMI was 29 kg/m^2, none of the patients was obese, but patients were defined as overweight (BMI = 25

to 29.99 kg/m^2) in 4% of courses and underweight (BMI < 18.5 kg/m^2) in 40% of courses. Tobramycin was used in 96% of courses and gentamicin in 4%. Females were significantly smaller in body size compared to males with median weights and heights of 48 kg and 158 cm versus 55 kg and 171 cm respectively (P <0.001). Figure 3.2 shows patients' characteristics as a matrix plot. The difference in body size measurements because of gender is clearly seen in lean body weight as expected from Janmahasatian *et al* (2005) equation against weight and body surface area. These matrix plots indicated also a poor correlation between weight and height in this group of patients.

Twenty nine of the measured serum creatinine concentrations had already been fixed to 60 when the data were collected and the actual values were not available. Of 1075 creatinine concentration measurements, 136 (13%) were < 60 μmol/L and were fixed to 60 μmol/L for the purpose of calculating creatinine clearance. Patients typically had normal renal function; however, one patient had moderate renal impairment with an estimated creatinine clearance that ranged between 35 and 48 mL/min during different courses of therapy. No trend was observed in this patient's renal function over time. A frequency distribution of the measured serum creatinine concentrations and creatinine clearance estimated by the Cockcroft and Gault equation for all courses is shown in Figure 3.3.

Table 3.1 Summary of the demographic and clinical characteristics for Glasgow patients as overall and group based on gender.

Patient Characteristic	Glasgow data Median (Range)	Male Median (Range)	Female Median (Range)
Number*	166	81	85
Age (years)**	23 (14 - 66)	24 (14 – 66)	22 (14 – 56)
Weight (kg)**	50 (30- 86)	55 (32 – 86)	48 (30 – 80)
Height (cm)**	163 (139 - 191)	171 (150 – 191)	159 (139 – 174)
Serum creatinine (µmol/L)**	71 (29 - 203)	76 (38 – 203)	67 (29 – 112)
BSA (m^2)**	1.5 (1.07 – 2.08)	1.6 (1.2 – 2.1)	1.4 (1.1 – 1.9)
LBW (kg)**	37.4 (22.1 - 65.8)	48 (32 – 66)	33 (22 – 46)
BMI (kg/m^2)**	19.1 (11.5 - 29.3)	19 (12 – 28)	19 (13 – 29)
CGCL (ml/min)**	92 (35 - 181)	104 (35 – 181)	85 (50 – 128)
EGCL (mL/min)**	93 (35 - 228)	105 (35 – 181)	86 (50 – 228)
FGCL (mL/min)**	82.4 (24.3 – 227)	82 (24 – 181)	82 (40 – 228)

Key: BSA= Body Surface Area, LBW= Lean Body Weight, BMI= Body Mass Index, CGCL= Corrected estimated creatinine clearance using the Cockcroft and Gault equation (C&G) (Cockcroft DW and Gault MH, 1976) with minimum serum creatinine concentration fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998), ECGCL= estimated creatinine clearance using C&G (Cockcroft DW and Gault MH, 1976) and the reported serum creatinine, FGCL= estimated creatinine CL using C&G and a factor for individuals who were 15 % and more underweight (Khuu T et al., 2010).
***based on number of patients = 166**
****based on total number of courses = 1075**

Figure 3.1 Frequency histogram of patient age in the Glasgow dataset.

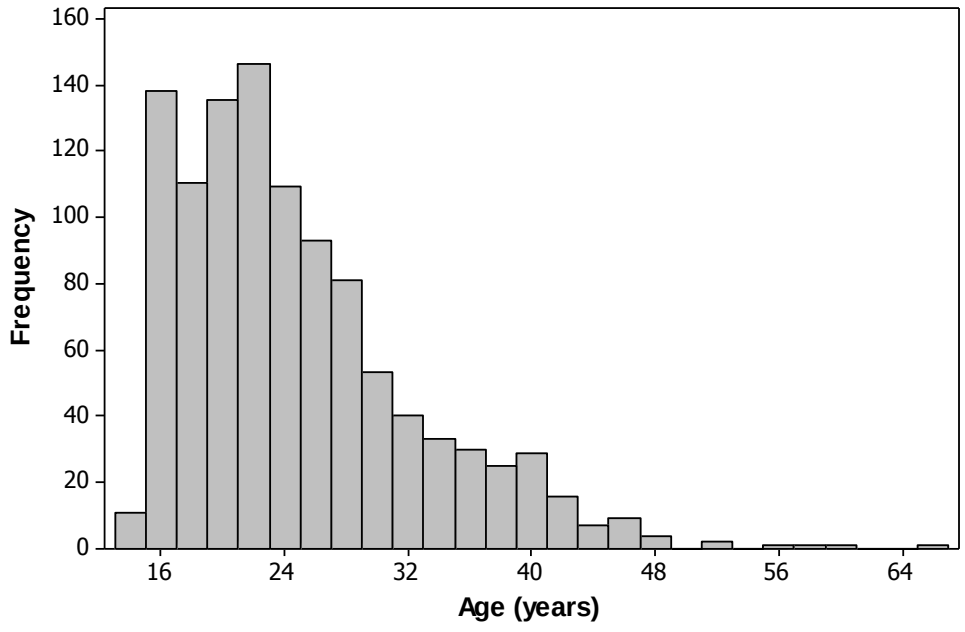
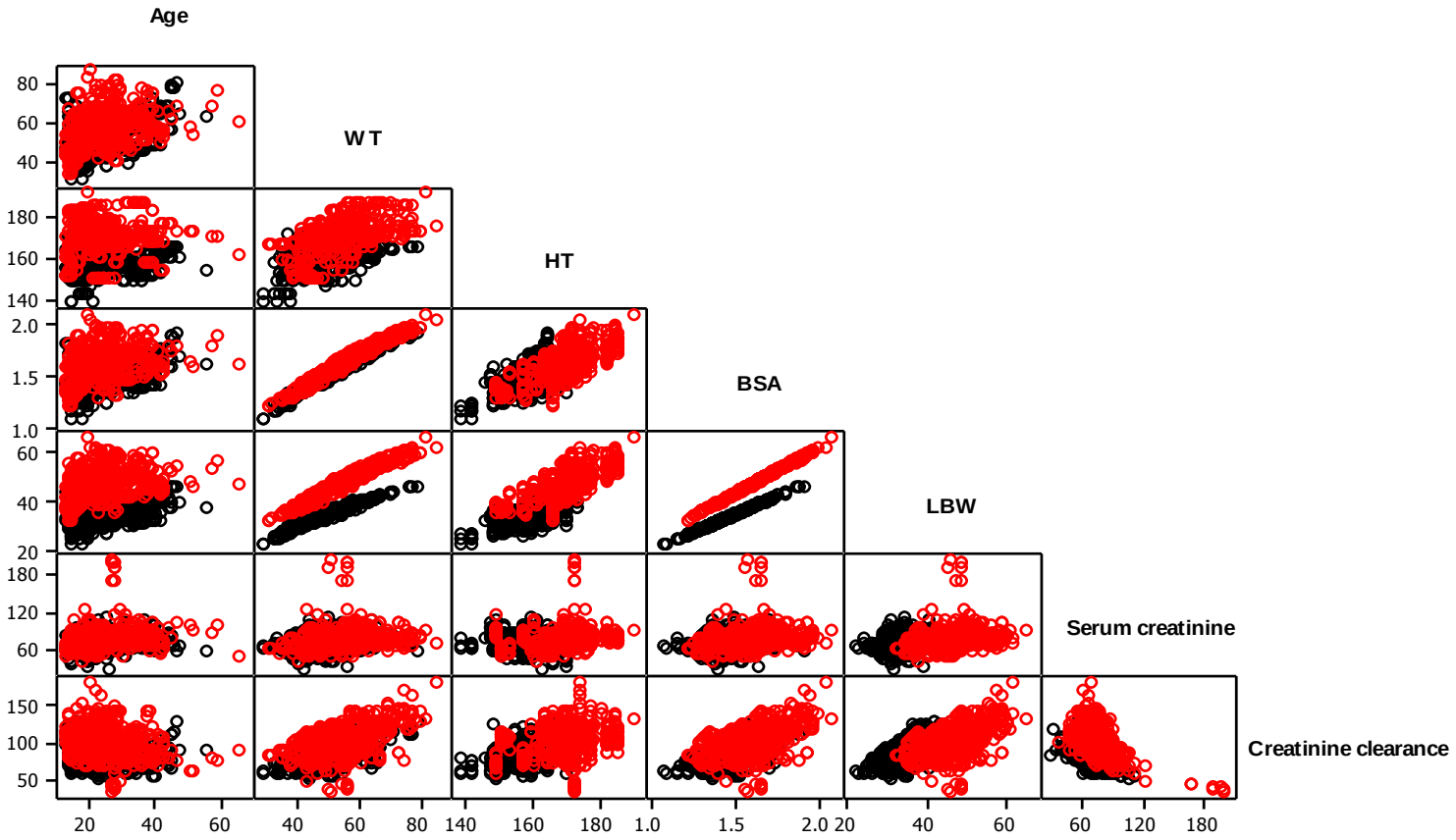
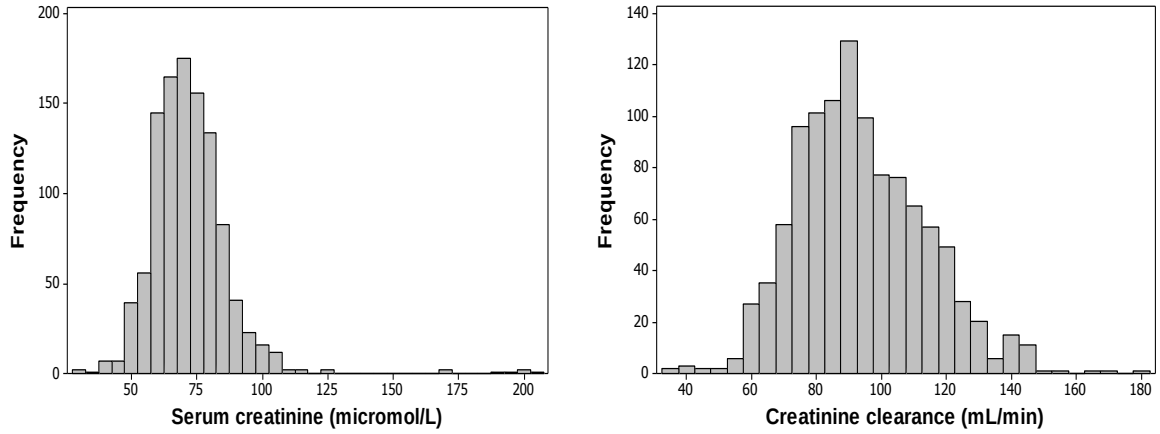


Figure 3.2 Matrix plots of patient characteristics grouped according to gender.



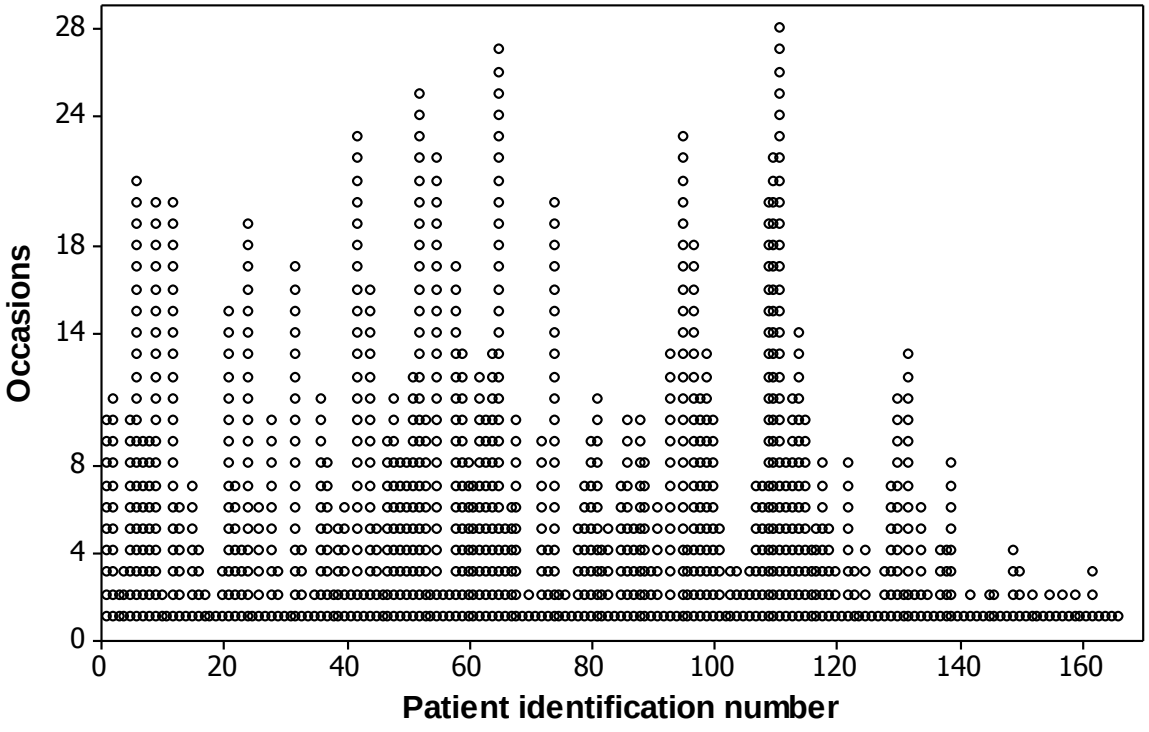
Key: The black open circles indicate females and the red open circle indicated males.
WT= Weight (kg), HT= Height (cm), BSA= Body Surface Area (m^2) (Mosteller RD, 1987), LBW= Lean Body Weight (kg) (Janmahasatian S et al., 2005), serum creatinine (µmol/L), Creatinine clearance estimated by Cockcroft and Gault equation(1976) with serum creatinine concentrations below to 60 µmol/L fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998).

Figure 3.3 Frequency distribution of serum creatinine and creatinine clearance estimates from 1075 courses in 166 patients.



In total, there were 1075 courses of therapy, ranging from 1 to 28 per patient with a median of 5 occasions, as illustrated in Figure 3.4. Of the 166 patients, 38 (23%) had only one course of aminoglycoside therapy, 57 (34%) had up to 5 courses, 38 (23%) had up to 10 courses, 25 (15%) had up to 20 courses and 8 (5 %) more than 20 courses.

Figure 3.4 Number of occasions versus patient identification number.



3.4.2 Aminoglycoside doses and concentrations

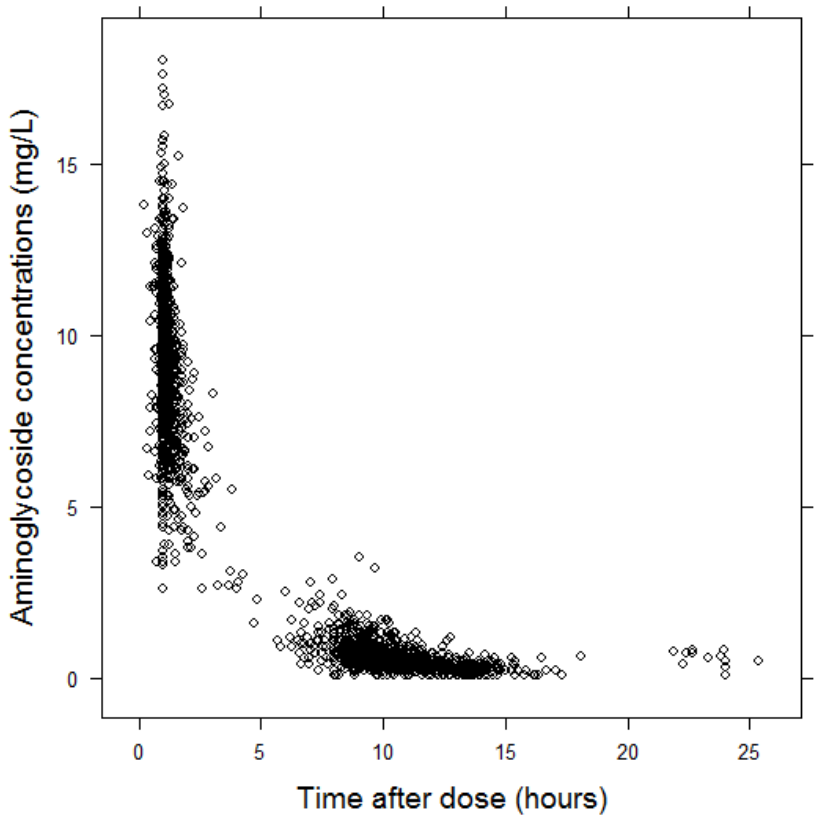
Aminoglycoside doses were administered by bolus injection or 5 minute infusion. The median dose was 360 mg/day and ranged from 120 to 660 mg/day. Overall, 44 (4 %) of the courses were administered 8 hourly, 1022 (95 %) 12 hourly and 9 courses (1%) 24 hourly. A total of 2238 aminoglycoside serum concentrations were available and the number of samples per occasion per patient ranging from 1 to 11, with a median of 2. Table 3.2 shows a summary of the peak, mid-dose and trough measured concentrations. The majority of samples were peak concentrations (49%, typically 1 hour post dose, median 9.3 mg/L, range 2.6 – 18 mg/L) or trough concentrations (37%, median 0.4 mg/L, range 0.1 – 3.2 mg/L) and most (83%) were withdrawn within 72 hours of starting drug therapy. The measured concentration versus time after dose plot is presented in Figure 3.5.

Table 3.2 Summary of the measured aminoglycoside concentrations (n = 2238).

Aminoglycoside Concentrations	Median	Range
Peak Concentration (mg/L)* (n = 1086)	9.3	2.6 - 18
Mid-dose Concentration (mg/L) (n = 316)	0.8	0.1 – 8.9
Trough Concentration (mg/L) (n = 836)	0.4	0.1 – 3.2

Key: * "Peak" concentrations were measured within the first 2 hours after the infusion

Figure 3.5 Scatter plot of the Glasgow observed aminoglycoside concentrations versus time after dose.



3.4.3 Population pharmacokinetic analysis

3.4.3.1 Structural model

A two-compartment model (OFV 33 for FOCE I and 30 for the SAEM algorithm) provided a better fit of the data than a one compartment model (OFV 177 for FOCE I and 168 for the SAEM algorithm). Although the OFV indicated an advantage in using a two compartment model, this was not clearly observed in scatter plots of the measured concentrations plotted against predicted concentrations with either the FOCE I (Figure 3.6 above) or SAEM algorithms (Figure 3.6 below). With both models, there was more variability in the higher concentrations. There was no difference between the FOCE I and SAEM algorithms in regard to model preference or parameter estimates, as shown in Table 3.3. However, run times were longer using the SAEM algorithm (1715 for the one and 9922 seconds for the two

compartment model) compared with the FOCE I algorithm (12.9 for the one and 79.6 seconds for the two compartment model) regardless of the compartment model used.

The FOCE I algorithm produced unbiased clearance (-0.02 L/h (95 % confidence interval; -0.07, 0.03)) and inter-compartmental clearance (-0.001 L/h (95 % confidence interval; -0.003, 0.002)) estimates with high precision (0.6 and 0.02 L/h), whereas the SAEM algorithm produced biased clearance (0.06 L/h (95 % confidence interval; 0.006, 0.11)) estimates but with high precision (0.6 L/h). On the other hand, the FOCE I algorithm produced biased V_1 estimates (-0.165 L (95 % confidence interval; -0.28, -0.06) compared with the SAEM algorithm (-0.10 L (95 % confidence interval; -0.22, 0.006)) and slightly lower imprecision (1.15 L and 1.19 L). Both algorithms produced biased V_2 estimates (0.15 L (95 % confidence interval; 0.08, 0.21) and 1.44 L (95 % confidence interval; 1.15, 1.75)) but the SAEM algorithm produced less precise estimates compared with the FOCE I algorithm (2.78 vs 0.79 L). The FOCE I algorithm produced unbiased population concentration predictions (0.015 mg/L (95 % confidence interval; -0.01, 0.046), whereas the SAEM algorithm produced biased population concentration predictions (-0.042 mg/L (95 % confidence interval; -0.071, 0.013)) but both algorithms were highly precise (0.38 mg/L).

The conditional weighted residual versus time after dose plots shown in Figure 3.7 (a and b) did not indicate any improvement in fit with the more complex model since both plots had a random distribution around zero and an acceptable range of error (-4 to 4). Both models tended to under-predict concentrations 15 hours after administration; however, this trend was less pronounced for the two-compartment model. The conditional weighted residuals against population model predictions plot indicated that both models predicted concentrations with a few outliers whose conditional weighted residuals were greater than ±4. Since there was no difference between the FOCE I and the SAEM algorithms and the run time was much longer using the SAEM algorithm, the FOCE I algorithm was used for covariate modelling. Eta shrinkage using FOCE I algorithm was low for drug clearance (7.4%) and volume of distribution of the central compartment (V_1) (18.5%) but high for volume of

distribution of the peripheral compartment (V_2) (75%) and inter-compartmental clearance (Q) (69%) in comparison to a typical accepted shrinkage value of less than 30 %.

Table 3.3 One and two compartment structural model comparison using the FOCE I and the SAEM algorithms.

Parameter	One compartment		Two compartments	
	FOCE I	**SAEM**	**FOCE I**	**SAEM**
OFV	177	168	33.1	30.1
CL (SE) (L/h)	4.56 (0.08)	4.51 (0.09)	4.63 (0.10)	4.60 (0.09)
V_1 (SE) (L)	14.7 (0.19)	14.6 (0.21)	14 (0.22)	13.7 (0.26)
V_2 (SE) (L)	-	-	6.81 (1.79)	5.64 (1.52)
Q (SE) (L/h)	-	-	0.37 (0.04)	0.48 (0.08)
Additive error (SE) (mg/L)	0.17 (0.01)	0.17 (0.01)	0.16 (0.01)	0.15 (0.01)
Proportional error (SE) (%)	16.9 (0.007)	16.9 (0.007)	16.9 (0.007)	16.9 (0.007)

Key: FOCE I= First order conditional estimation with Interaction, SAEM= Stochastic Approximation Expectation Maximization, OFV= Objective function value, CL= Clearance, V_1= Volume of distribution of the central compartment, V_2= Volume of distribution of the peripheral compartment, Q= Inter-compartmental clearance, SE= Standard error.

Figure 3.6 Observed versus predicted aminoglycoside concentrations panel a) one compartment model using FOCE I (above) and SAEM (below algorithms, and panel b) two compartment structural models using FOCE I (above) and SAEM (below) algorithms. The black line is the line of unity and the black dashed line is a smooth.

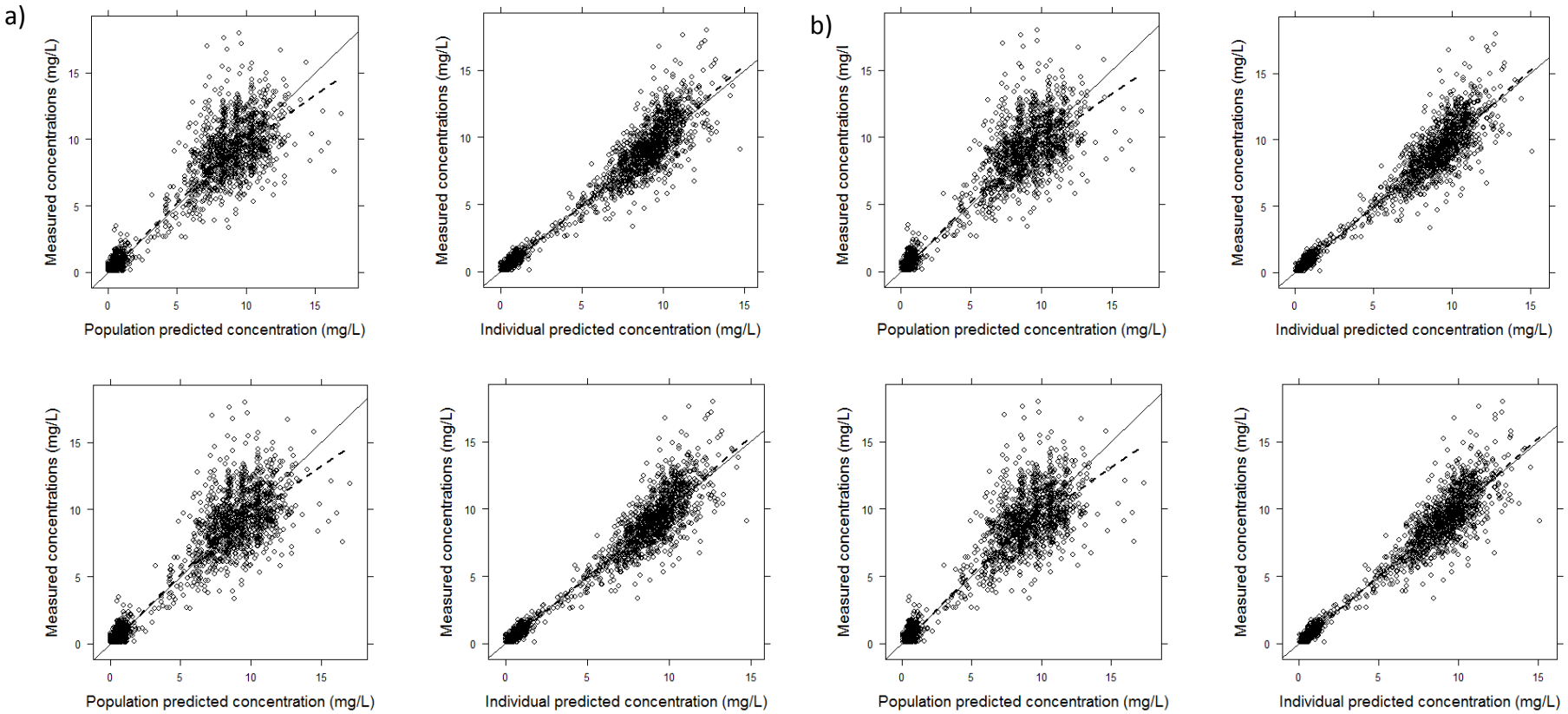
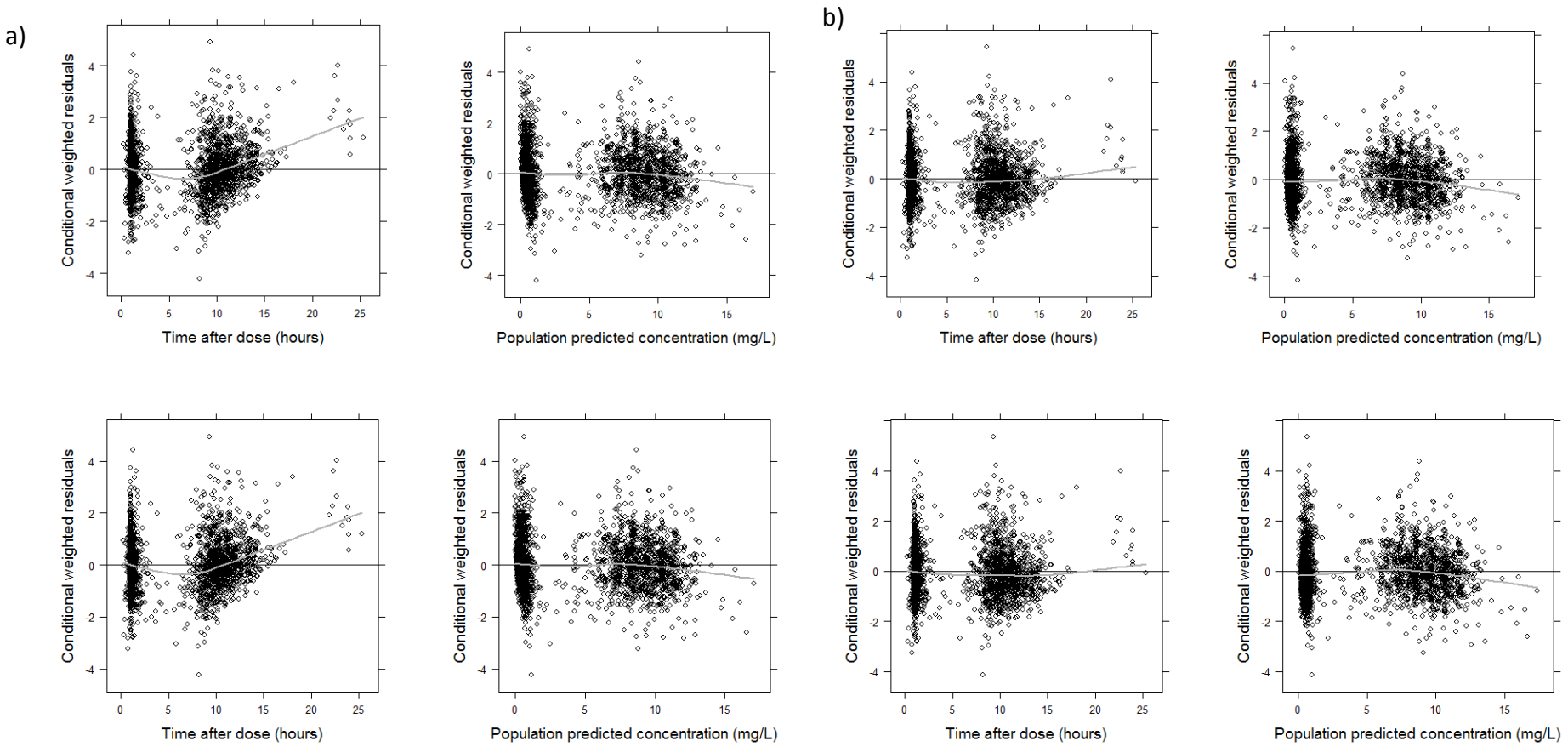


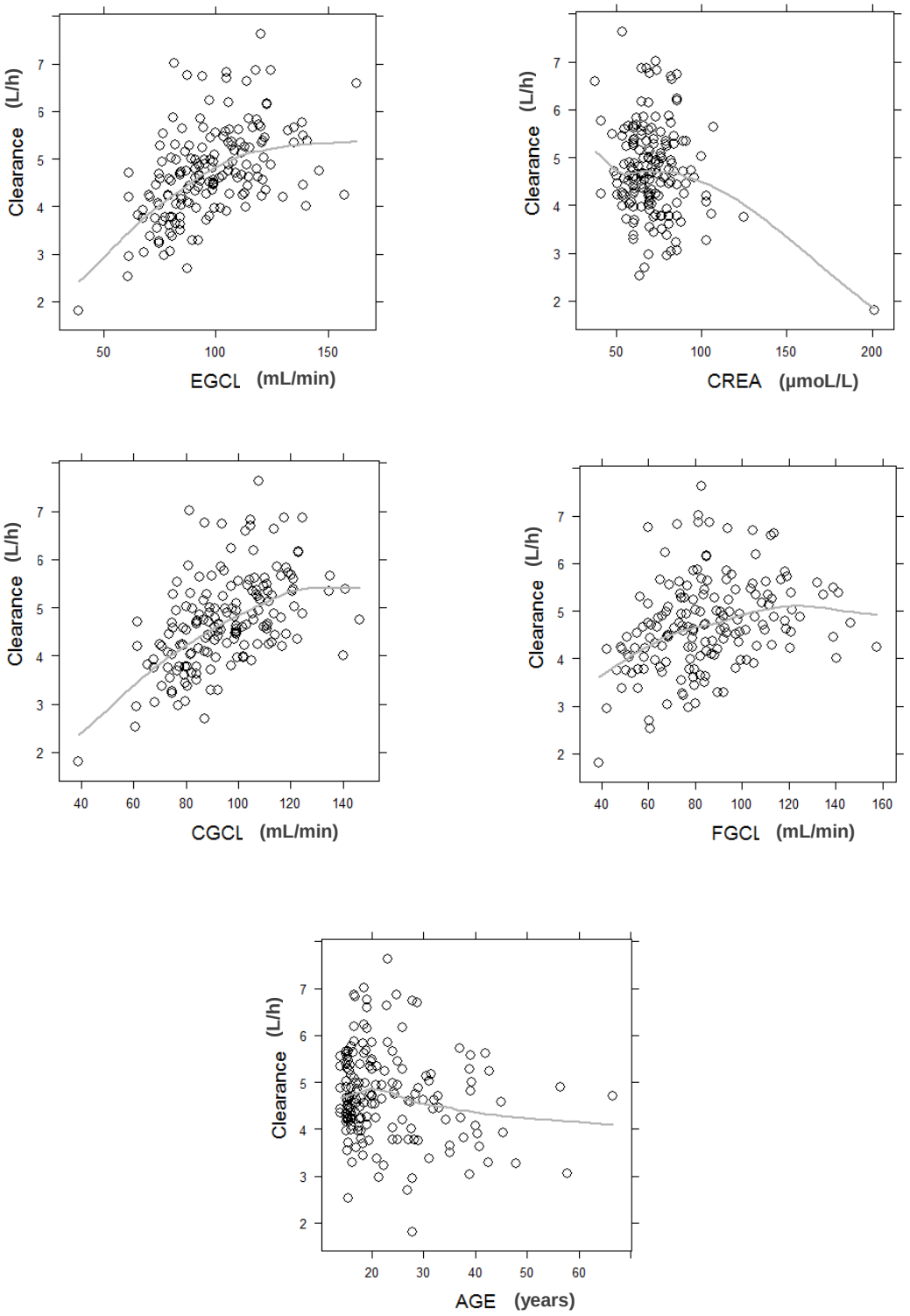
Figure 3.7 Scatter plot of conditional weighted residual versus time after dose and population predicted concentrations for the one comaprtmnet model (panel a) and two compartment model (panel b) using FOCE I (above) and SAEM (below) algorithms.



3.4.3.2 Covariate model

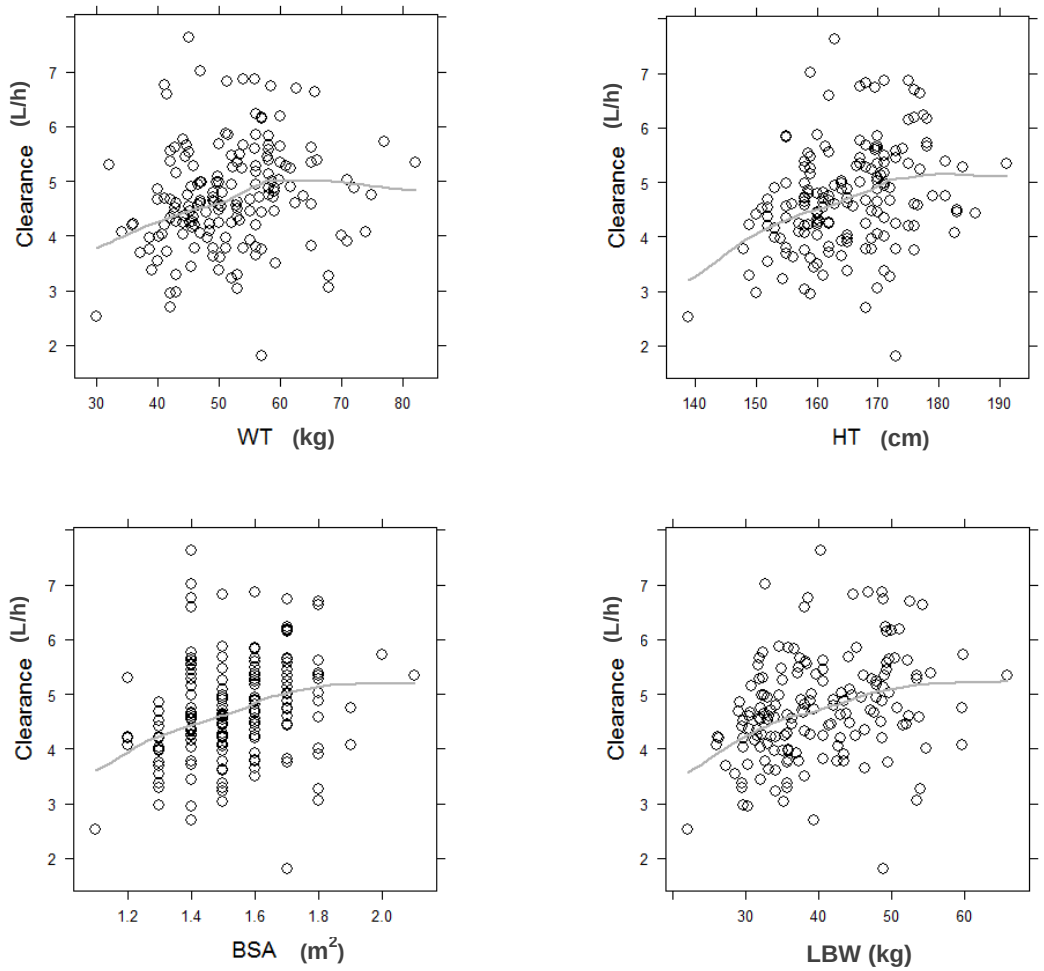
Figures 3.8 and 3.9 shows scatter plots of individual estimates of clearance against the various covariates. The strongest relationships with clearance were seen with estimated creatinine clearance using actual serum creatinine values (EGCL) and when creatinine concentrations below 60 μmol/L were fixed to 60 μmol/L (CGCL). Negative trends were seen with serum creatinine. Body size measurements, including weight, height, body surface area, and lean body weight showed weaker correlations with aminoglycoside clearance. Figure 3.10 (a and b) shows the GAM analysis results for potential covariates for clearance that resulted in the lowest Akaiki values and residuals versus potential covariates. The suggested covariates on clearance were height and a non-linear relationship with estimated creatinine clearance (CGCL). In Figure 3.11 the individual influence on the GAM fit showed that two patients (40 and 89) had a high influence on the fit and the certainty in the fit would depend on these data points.

Figure 3.8 Scatter plots of individual estimates of clearance versus serum creatinine, creatinine clearance measurements, and age. The grey line represents a smooth through the data points.



Key: CREA= serum creatinine in µmol/L, CGCL= estimated creatinine clearancein mL/min using the Cockcroft and Gault equation (1976) with the lowest serum creatinine set to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998), EGCL= estimated creatinine clearance in mL/min using the Cockcroft and Gault equation (1976) using the actual serum creatinine values, FGCL= estimated creatinine clearance in mL/min using the Cockcroft and Gault equation using a factor for patients with serum creatinine less than 60 µmol/L (Khuu T et al., 2010),Clearance is in L/h, and Age is in years.

Figure 3.9 Scatter plots of individual estimates of clearance versus the different body size measurements. The grey line represents a smooth through the data points.



Key: WT= weight in kg, HT= height in cm, BSA= body surface area (Mosteller RD, 1987) in m^2, LBW= lean body weight (Janmahasatian S et al., 2005) in kg.

Figure 3.10 Clearance GAM analysis showing a) Akaike value for different covariate models and b) residuals versus potential covariates.

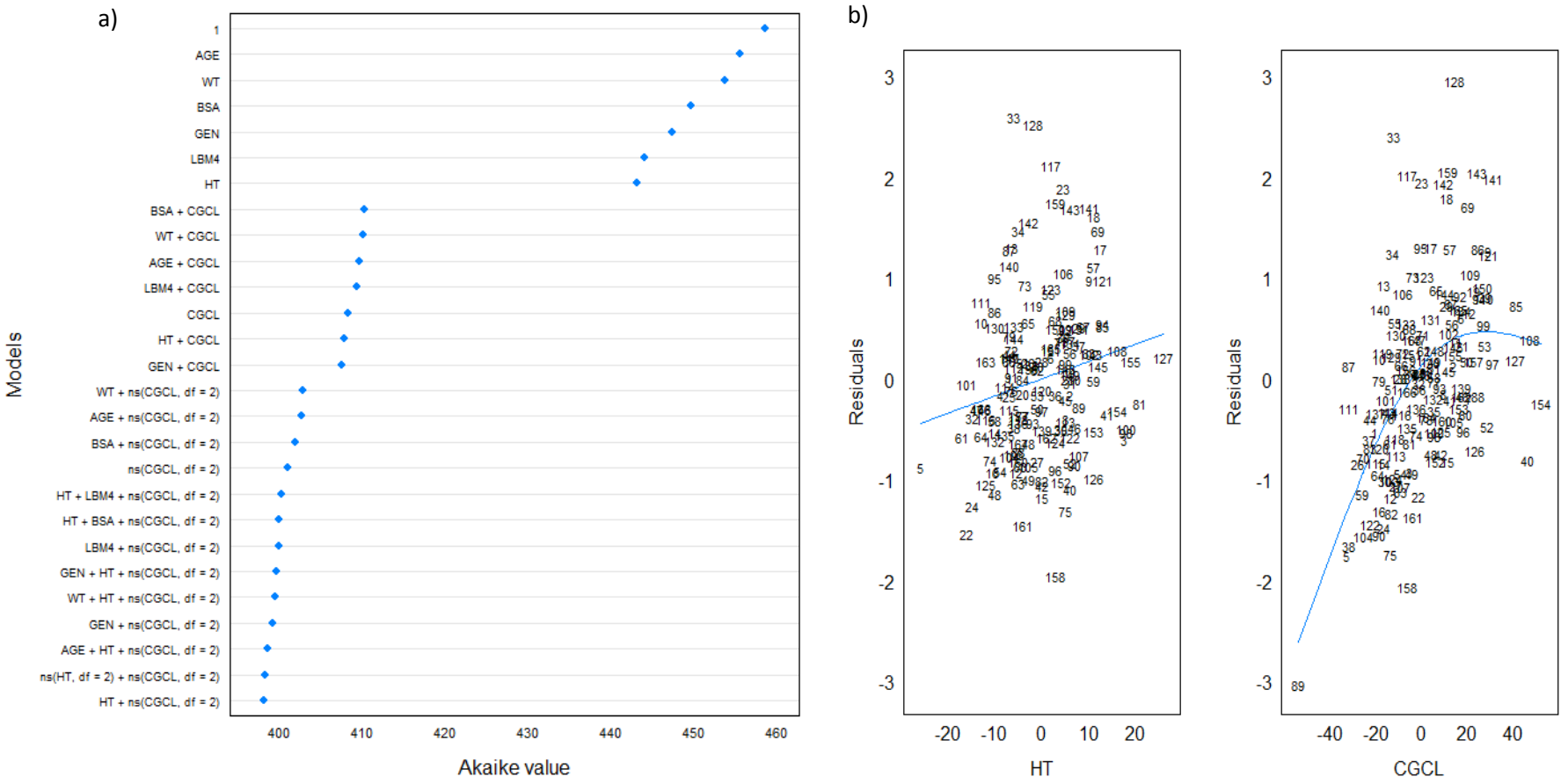
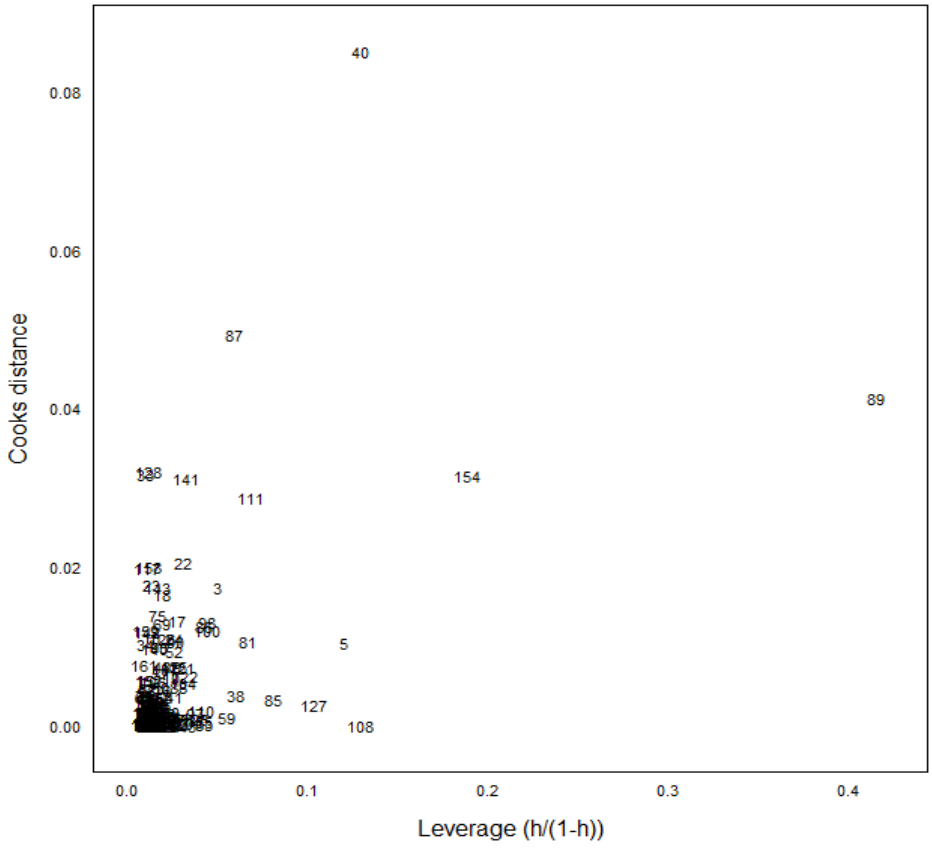
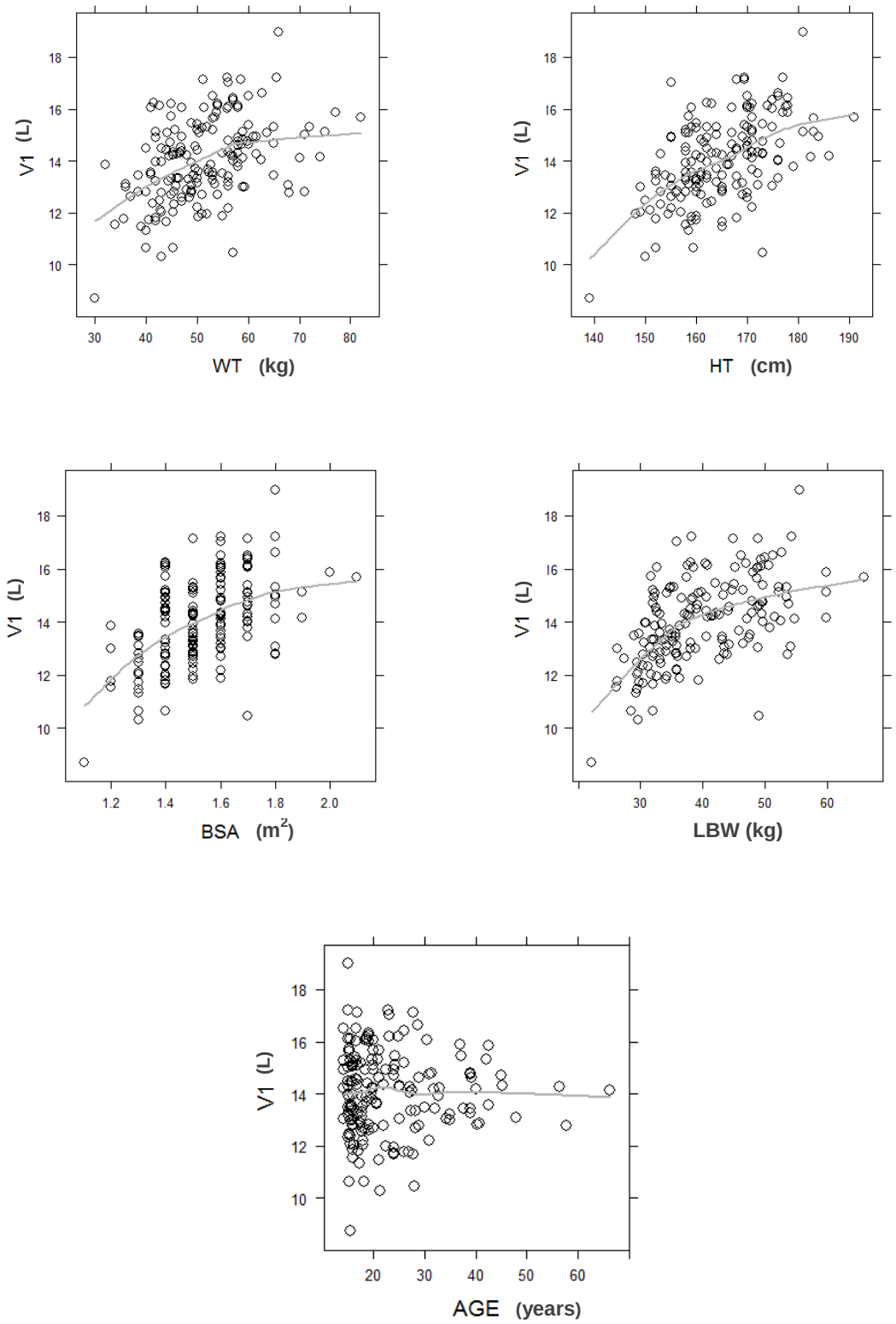


Figure 3.11 Individual influence on GAM fit for clearance.



Relationships between covariates and aminoglycoside V_1 are illustrated in Figure 3.12. Although there was a trend with weight, the plots suggested that height, body surface area and lean body weight were better descriptors. The GAM analysis suggested height, a non-linear relationship with lean body weight and estimated creatinine clearance (CGCL) as potential covariates for V_1 (Figure 3.13). In Figure 3.14 the individual influence on GAM fit showed that two patients (89 and 127) had high influence on the fit and the certainty in the fit would depend on these data points.

Figure 3.12 Scatter plots for V_1 versus covariates. The grey line is a smooth.



Key: WT= weight in kg, HT= height in cm, BSA= body surface area (Mosteller RD, 1987) in m^2, LBW= lean body weight (Janmahasatian S et al., 2005) in kg.

Figure 3.13 V_1 GAM analysis showing a) Akaki values for different covariate models and b) residuals versus potential covariates.

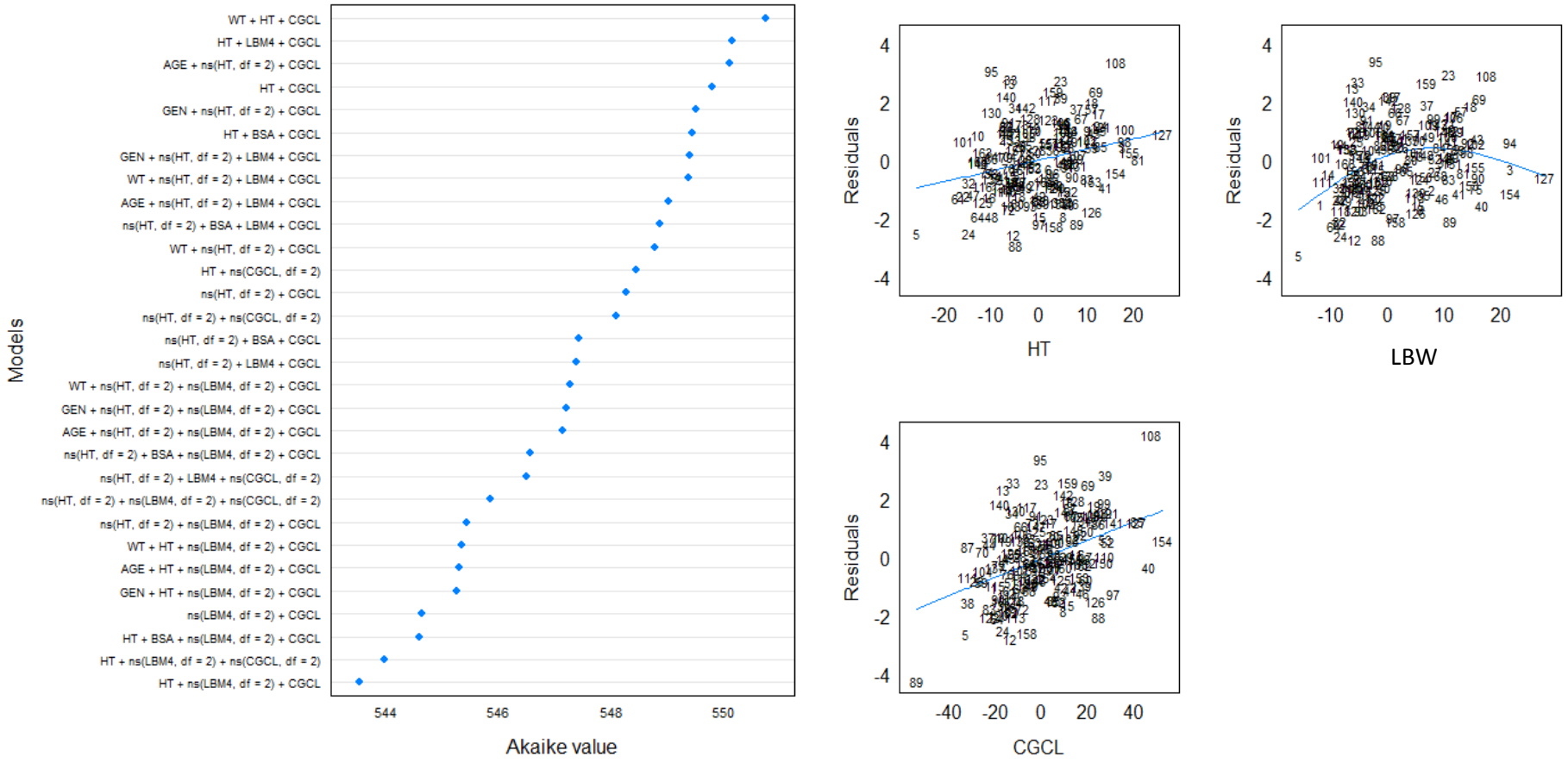
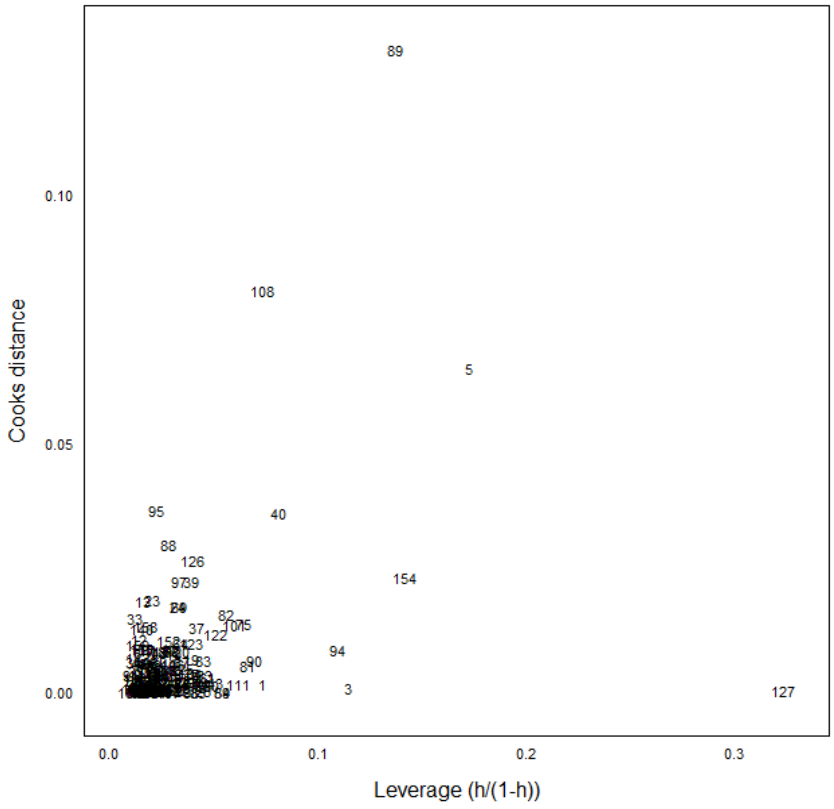


Figure 3.14 Individual influence on GAM fit for V_1.



For V_2 the GAM analysis showed that height and a non-linear relationship with creatinine clearance (CGCL) could be potential covariates. The potential covariates for Q were a non-linear relationship with height and serum creatinine. The scatter plots for V_2 and Q against the different covariates failed to identify any trends.

Table 3.4 shows the results obtained when covariates were added singly and in combination into the population model. A reduction in the OFV was obtained when the different renal function estimates (FGCL, CGCL and EGCL) were added to clearance model. Although estimated creatinine clearance using actual serum creatinine measurements (EGCL) produced the lowest OFV, there was no difference in BSV and residual errors obtained from models using the actual serum creatinine (EGCL) or setting a minimum serum creatinine level (CGCL). Weight was also included into clearance model using allometric scaling, the OFV increased to 193 with no change in BSV when the power was fixed to 0.75. However, when the power was estimated the OFV reduced to 22. The addition of body size

measurement to the V_1 model resulted in further reduction in OFV and BSV. When clinical factors were combined in the population model, statistically significant improvements in fit were identified with CGCL and height on clearance and height in the V_1 model. These reduced BSV from 23 to 20 % for clearance and 14 to 10 % for V_1. This model is highlighted in red in Table 3.4. When height in clearance model was centred on median height, an additional parameter was required that was close to 0 and did not improve the fit (change in objective function value = 0). The simpler model was therefore chosen. Using actual weight or lean body weight rather than height to describe clearance again produced a poor fit with an increase in OFV from -77 to 184 with actual weight and to 57 with lean body weight. BSV also increased, from 23% to 24.5% with actual weight and to 25% with lean body weight. Using body surface area, a slight improvement was seen in the model fit (OFV 33 to 10). An additional model in which age was modelled as categorical variable with a cut off at 18 years old was also tested. However, OFV (33) did not change and both group of patients had similar clearance estimates of 4.6 L/h.

With the mechanistic model, a better fit was obtained when CrCL parameters were estimated rather than taken from the model of Matthews *et al* (2004) (OFV -75 vs -63) and reduced BSV from 23 to 20 %, and height was a better descriptor than allometrically scaled size for “non-renal” clearance (OFV -75 compared to -39). For both the empirical and the mechanistic models, CrCL only had a small influence on clearance. Estimated CrCL using both the mechanistic approach and the Cockcroft and Gault equation were highly correlated, as shown in Figure 3.15. However, patients whose serum creatinine was 60 µmol/L or less tended to have higher estimates of CrCL using the mechanistic approach (maximum 264 mL/min) compared with the Cockcroft and Gault equation (maximum 181 mL/min).

Although the effect was less pronounced, with both the empirical and the mechanistic models, V_1 was also better described using height rather than actual weight or lean body weight. BSV estimates for V_2 and Q were poorly characterised and removal of these parameters had no effect on OFV or other model parameter estimates. A series of analyses

in which V_2 and Q were assumed to be related to size consistently found that such assumptions produced poorer fits of the data, rounding errors, unsuccessful minimisations and/or unrealistic parameter estimates.

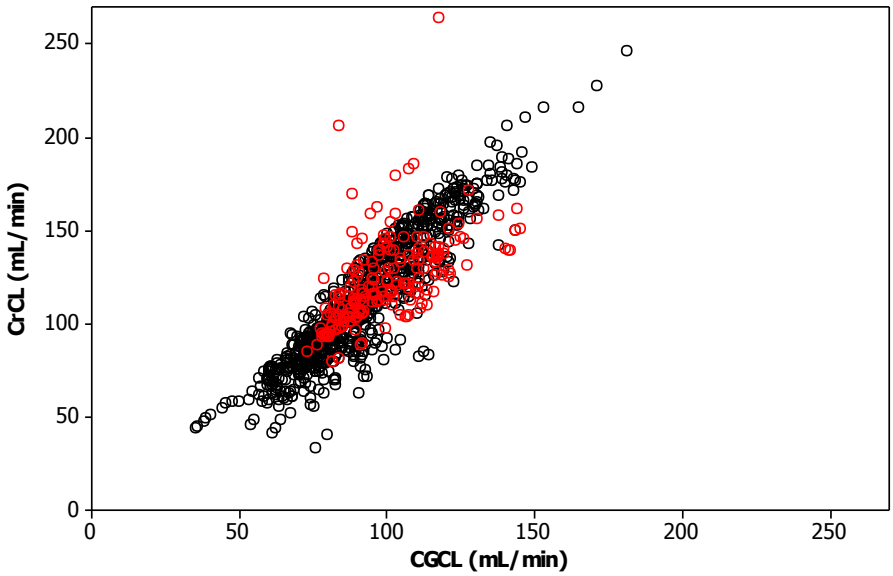
Neither the number of courses of therapy nor time since the first course had any influence on aminoglycoside clearance. Aminoglycoside type (gentamicin or tobramycin) had no influence on the model. The main population pharmacokinetic model parameter estimates for the empirical and mechanistic models are listed in Table 3.4.

Table 3.4 Summary of covariate sub-model building results.

OFV	CL model (L/hr)	BSV_{CL} %	V_1 model (L)	BSV_{V1} %
	Empirical approach (no WSV)			
33.1	4.63	23	14.0	14
22.8	$4.84 \times (WT/70)^{0.14}$	22	13.9	14
-13.3	4.60 + 0.007(FGCL-82)	21	14.0	14
-16.2	4.60 + 0.011 (CGCL-92)	20	14.0	14
-18.7	4.58 + 0.009 (EGCL-93)	20	14.0	14
184	0.088 x WT+ 0.00003 (CGCL-92)	24.5	13.6 x (1 + 0.0158 (WT-50))	11
58.1	0.116 x LBW + 0.0003 (CGCL-92)	25	12.0 x (1 + 0.214 (LBW-37))	11
10.5	2.98 x BSA + 0.001 (CGCL-92)	21	9.07 x BSA	10
-6.14	$5.04 \times (WT/70)^{0.28}$	22	$15.5 \times (WT/70)^{0.35}$	11
-57.6	4.58 x (1+ 0.00264 x (CGCL-92))	20	13.8 x(1+0.00820x(HT -163))	10
-77.1	0.0278 x HT + 0.0112 (CGCL-92)	20	13.7 x (1 + 0.0109 (HT-163))	10
	Mechanistic approach (no WSV)			
-38.6	$5.24 \times (LBW/70)^{0.75}$ + 0.704 x (CrCL (L/h)/ 7.26 L/h/70 kg x (LBW+0.211(Weight–LBW) /70)$^{0.75}$))	19	8.39 x (1+0.879 x (LBW+(WT-LBW)/70))	11
-62.6	0.022 x HT + 0.866 x (CrCL (L/h)/ 7.26 L/h/70 kg x (LBW+0.211(Weight–LBW) /70)$^{0.75}$))	20	13.8 x (1+0.00939(HT-163))	11
-75.4	0.0225 x HT + 0.654 x (CrCL (L/h)/ 7.26 L/h/70 kg x (LBW+0.211(Weight–LBW) /70)$^{0.75}$))	20	13.7 x (1+ 0.00949 (HT-163))	11

Key: OFV= Objective function value, CL= clearance, BSV_{CL}= Between-subject variability in clearance, V_1= Volume of distribution of the central compartment, BSV_{V1}= Between-subject variability in V_1, WSV= Within-subject variability, WT= weight in kg, CGCL= estimated creatinine clearance in mL/min using the Cockcroft and Gault equation (1976) with the lowest serum creatinine is 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998), HT= height in cm, BSA= body surface area (Mosteller RD, 1987) in m^2, LBW= Lean body weight (Janmahasatian S et al., 2005) in kg, CrCL= creatinine clearance estimated using the mechanistic approach (Matthews et al., 2004, Anderson BJ and Holford NHG, 2009).

Figure 3.15 Scatter plot of creatinine clearance estimated by the mechanistic approach against the estimates from the Cockcroft and Gault equation.



Key: The black open circle is patients whose serum creatinine was greater than 60 µmol/L. The red open circles are patients whose serum creatinine was 60 µmol/L or less. CrCL= creatinine clearance estimated by mechanistic approach (Anderson BJ and Holford NHG, 2009, Matthews et al., 2004). CGCL= estimated creatinine clearance using the Cockcroft and Gault equation (Cockcroft DW and Gault MH, 1976) with the lowest serum creatinine is 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998).

3.4.3.3 Within -subject variability

The inclusion of WSV on clearance achieved a further improvement in fit (OFV -304), before the addition of covariates, with a reduction in the BSV from 23 to 21 % and the residual error from 0.16 mg/L and 17 % to 0.08 mg/L and 15 %, but adding WSV to V_1 had no effect. The creatinine clearance (CGCL) alone reduced BSV from 21 to 18.5% and the addition of height reduced it further to 18 %. A similar improvement in fit was obtained when WSV was added to the mechanistic model (OFV -310) but CrCL parameters could not be estimated and instead were fixed to the values obtained using the model without WSV.

The final population pharmacokinetic model parameter estimates for the empirical and mechanistic models are listed in Table 3.5. The empirical model identified a typical clearance estimate of 4.65 L/h at the median height of 163 cm and median CrCL of 92 mL/min. The typical V_1 was 13.3 L and changed by 11% for every 10 cm difference from 163 cm. Similar results were obtained with the mechanistic model. Using the final empirical model, the aminoglycoside distribution and elimination half-lives were estimated. The first estimated

population half-life ($t^{\lambda 1}_{1/2}$) was 1.74 hours (range 1.19-3.26) and the second population half-life ($t^{\lambda 2}_{1/2}$) was 12.5 hours (10.8-15.2).

Table 3.5 Summary of model with within subject variability included.

OFV	CL model (L/hr)	BSV_{CL} %	WSV_{CL} %	V_1 model (L)	BSV_{V1} %
Empirical approach (full model with WSV)					
-210	4.72 *(0.0942)*	21	11	13.6 *(0.225)*	16
-304	0.0285 (*0.000504*)xHT+ 0.0114 (*0.00221*) (CGCL-92)	18	11	13.3(*0.189*)(1+0.0113 (*0.00127*) (HT-163))	12
Mechanistic approach (full model with WSV)					
-256	5.07 (*0.289*) x $(LBW/70)^{0.75}$+ 0.83 (*0.100*)x (CrCL/ 7.26 L/h/70 kg x $(LBW+0.211(Weight–LBW)/70)^{0.75}$))	17	11	8.84 (*0.953*) (1+0.718 (*0.223*)x (LBW+(WT-LBW)/70))	13
-303	0.0216 (*0.00143*) x HT+ 1.01 (*0.192*)x (CrCL/7.26 L/h/70 kg x $(LBW+0.211(Weight–LBW)/70)^{0.75}$))	18	11	13.4 (*0.199*)(1+ 0.00958 (0.000123) (HT-163))	12
-310	0.0226 (*0.00117*) x HT+0.709 (*0.126*)x (CrCL/7.26 L/h/70 kg x $(LBW+0.211(Weight–LBW)/70)^{0.75}$))	18	11	13.4 (*0.192*) (1+0.00974 (*0.00105*) (HT-163))	12

Key: OFV= Objective function value, CL= clearance, BSV_{CL}= Between-subject variability in clearance, V_1= Volume of distribution of the central compartment, BSV_{V1}= Between-subject variability in V_1, WSV= Within-subject variability, WT= weight in kg, CGCL= estimated creatinine clearance in mL/min using the Cockcroft and Gault equation (1976) with the lowest serum creatinine is 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998), HT= height in cm, BSA= body surface area (Mosteller RD, 1987) in m^2, LBW= Lean body weight (Janmahasatian S et al., 2005) in kg, CrCL= creatinine clearance estimated using the mechanistic approach (Matthews et al., 2004, Anderson BJ and Holford NHG, 2009). Standard errors of each parameter estimate are shown in italics.

Figures 3.16 and 3.17 show the measured concentrations versus final model population and individual predictions and the conditional weighted residuals against time after dose and population predicted concentrations. There is a close correlation between measured and predicted concentrations although the model tended to over predict concentrations greater than 10 mg/L. It had also the tendency for higher prediction errors with samples obtained after 15 hours of aminoglycoside administration, as shown in the CWRES plot versus time after dose. Both the conditional weighted residual plots contained a few outliers whose conditional weighted residuals were outside ±4. In addition, the absolute individual weighted residual plot shown in Figure 3.18 does not show any trend and confirmed that the residual error model is adequate.

Figure 3.16 Measured versus predicted aminoglycoside concentration plots using the final model. The solid black line is the line of unity and the dotted black line is a smooth.

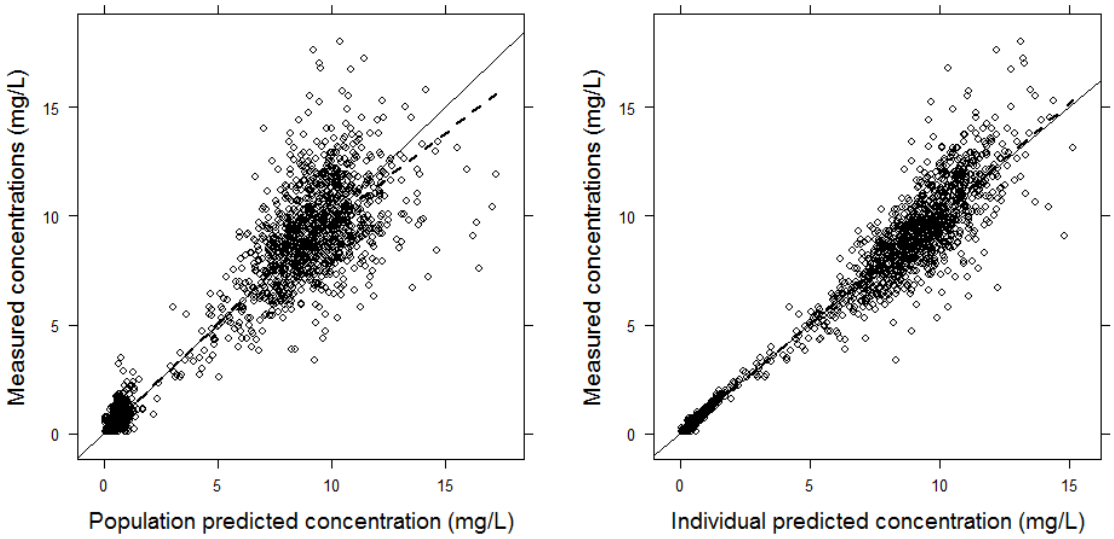


Figure 3.17 Plot of conditional weighted residual versus time after dose (left) and population predicted concentrations (right) using the final model.

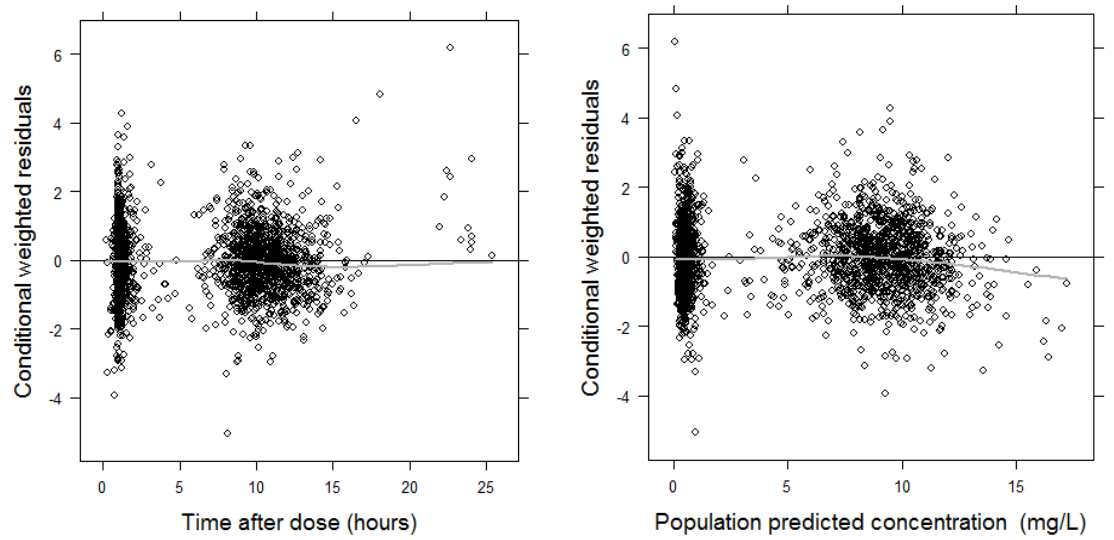
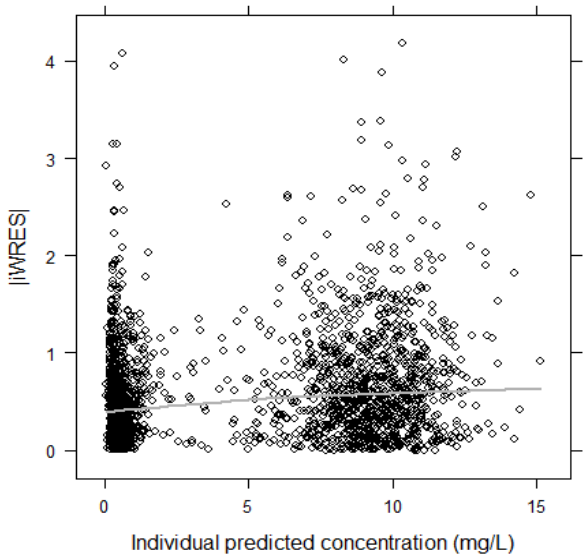


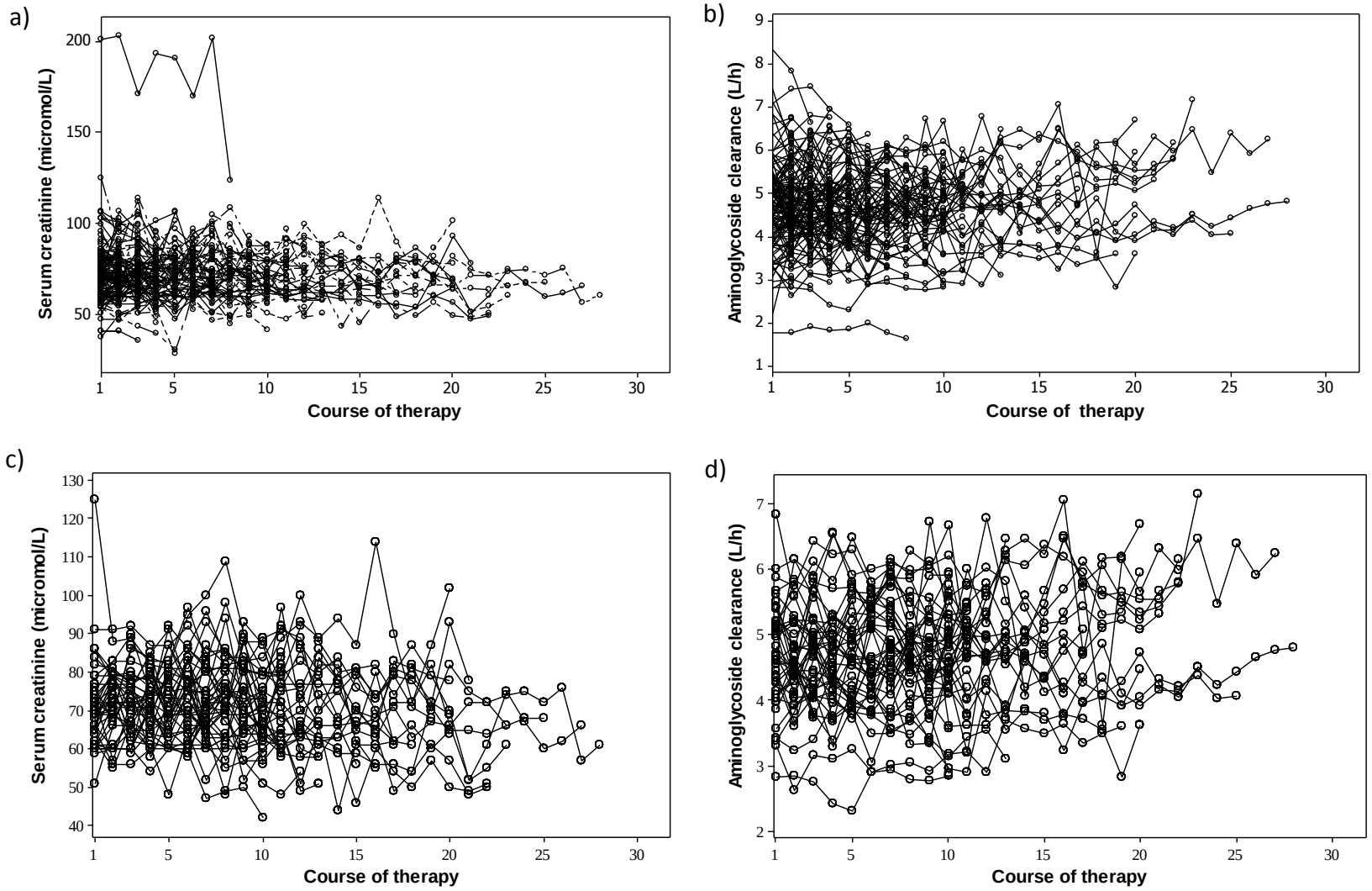
Figure 3.18 The absolute individual weighted residuals versus individual concentration predictions using the final model. The grey line is a smooth.



3.4.4 Clearance change over time

Figure 3.19 shows serum creatinine concentration and aminoglycoside clearance plotted against the number of courses of therapy for the 128 patients who had more than one course and the 43 patients who had at least 10 courses of therapy. No trend can be seen with either group. Furthermore, there was no significant change in clearance in those patients who had at least 10 courses of therapy (mean (SD) 4.71 (0.80) L/h first course vs 4.88 (0.99) L/h last course, p = 0.139, 95 % confidence interval; -0.39 to 0.057).

Figure 3.19 Scatter plots of (a and c) serum creatinine and (b and d) aminoglycoside clearance versus the number of courses of therapy for (a and b) the 128 patients who had more than one course of therapy and (c and d) the 43 patients who had 10 or more courses of therapy.



3.5 DISCUSSION

3.5.1 Introduction

The aim of the current study was to investigate factors that influence aminoglycoside pharmacokinetics and examine how these parameters vary over time in a group of patients with cystic fibrosis who received multiple courses of therapy. Height and creatinine clearance were the best descriptors for clearance and height for V_1. These descriptors reduced between-subject variability from 23% to 18 % for clearance and 14% to 12 % for V_1. The model included between occasion variability in clearance; however, the value was only 11 %. The results also indicated that aminoglycoside clearance did not change over time, despite multiple courses of therapy.

3.5.2 Patients

This study is one of the largest population pharmacokinetic analyses in patients with cystic fibrosis, as it included 1075 different courses of aminoglycoside therapy from 166 patients. The age range included in the current analysis was wide compared with previously published studies with 13 % of patients older than 40 years old. The patients had similar weight range with other published studies in this group of patients (Campbell D et al., 1999, Touw DJ et al., 2007, Burkhardt O et al., 2006). However, 40% of the patients were underweight at one or multiple courses. In the current study, patients with high serum creatinine were included, whereas previously published population pharmacokinetic studies excluded such patients (Burkhardt O et al., 2006, Touw DJ et al., 2007). Although a wider range for patients' estimated creatinine clearance were included in the current study compared to previously published population pharmacokinetic studies in patients with cystic fibrosis (Campbell D et al., 1999, Touw DJ et al., 2007, Aminimanizani A et al., 2002), only one patient had a creatinine clearance estimate below 50 mL/min.

3.5.3 Structural model

Aminoglycoside pharmacokinetics are known to be best described by a multi-compartment model as reported by Schentag *et al* (1977), who identified a long terminal elimination half-life of 100 hours. In the present study, the two-compartment model produced a lower OFV

than a one-compartment model (difference in OFV = -144). Although the plots of observed versus predicted concentration were very similar, the conditional weighted residual versus time after dose plot indicated a small trend for the two-compartment model to produce less biased predictions at later times. This was confirmed using both FOCE I and SAEM algorithms, which performed similarly in the present study with the FOCE I algorithm running faster. Gibiansky *et al* (2012) compared the performance of a number of algorithms available in NONMEM 7 and found that SAEM algorithm provided similar parameter estimates and standard error to the FOCE I algorithm. In addition, Plan *et al* (2012) found that the FOCE I algorithm in NONMEM was fast, robust, accurate and precise. Therefore, the FOCE I algorithm was used to develop the covariate model in the present work. A two-compartment model provided a better fit in the present population analysis and this is consistent with findings from other studies in patients with cystic fibrosis (Aminimanizani A et al., 2002, Burkhardt O et al., 2006), cancer (Rosario MC et al., 1998) and general medical conditions (Matthews et al., 2004). However, a one-compartment model is often assumed for aminoglycoside therapeutic drug monitoring data (Campbell D et al., 1999, Touw DJ et al., 2007, Touw DJ et al., 1997).

Using an extensive sampling strategy, Aminimanizani *et al* (2002) characterised a distribution half-life of 0.4 hours following a single daily dosing regimen of tobramycin in 6 patients with cystic fibrosis. The apparent distribution half-life reported in the present study was 1.75 hours, which is closer to the typical elimination half-life of 3 hours reported elsewhere (Aminimanizani A et al., 2002), and is therefore likely to reflect a mixture of distribution and elimination. Moreover, the apparent elimination half-life of 12.5 hours reported in the present study probably represents a mixture of the principal elimination phase and the slow terminal elimination that occurs with aminoglycoside antibiotics (Schentag JJ et al., 1977). Similar issues have been reported in previous population studies of aminoglycoside pharmacokinetics that used sparse data (Matthews et al., 2004, Rosario MC et al., 1998). This could be related to limitation in study design, which prevented the identification of a longer elimination half-life. The last sample in the study of Aminimanizani *et al* (2002) was withdrawn within 8 hours after administration. On the other hand, concentration measurements in the current study were withdrawn within 24 hour post-dose

with the majority within the first 2 hours and at 10-12 hour post-dose and no concentration measurements were available after 24 hours. In aminoglycoside treated patients a third compartment has been demonstrated with half-lives of 100 hours (Schentag JJ et al., 1977). This phase results from re-distribution of the drug from deeper tissues including within the kidney and might be associated with the risk of toxicity (Schentag and Jusko, 1977, Schentag JJ et al., 1977, Fabre J et al., 1976). The reported elimination half-life in the current study was shorter (12.5 vs 100 hours), because most of the concentration measurements were withdrawn within the first three days of therapy and drug tissue accumulation would not be complete.

3.5.4 Identification of the covariate model

Patient height and creatinine clearance provided the best description of aminoglycoside pharmacokinetic parameters. Creatinine clearance was an expected covariate because aminoglycosides are hydrophilic drugs and eliminated mainly by glomerular filtration, which makes renal function a good predictor for drug clearance and was consistent with previous publications (Matthews et al., 2004, Rosario MC et al., 1998, Levy J et al., 1984, Touw DJ et al., 1996). However, creatinine clearance was estimated using the Cockcroft and Gault equation (1976) with serum creatinine concentrations below 60 µmol/L fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998). Using serum creatinine values less than 60 µmol/L can result in an overestimate of creatinine clearance (Touw DJ et al., 1996), and an improvement in the population predictions of gentamicin clearance was reported when serum creatinine concentrations below 60 µmol/L were fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998). A small reduction in OFV (-3.2) was observed in the current study when the actual serum creatinine measurements were used to estimate creatinine clearance. However, since only 136 of 1075 creatinine concentration measurements were less than 60 µmol/L, a fixed minimum serum creatinine value had little impact.

An alternative approach was also investigated to estimate creatinine clearance in patients whose serum creatinine was less than 60 µmol/L. This involved including a factor for patients who were 15% and more underweight in the Cockcroft and Gault equation (1976),

as recommended by Khuu *et al* (2010). Although the author suggested that using an adjustment factor for estimating renal function in underweight patients was more precise and less biased than using a fixed serum creatinine concentration (Khuu T et al., 2010), the difference was not statistically significant. Both methods were found to be superior to estimating renal function using the measured serum creatinine concentration. However, in the current population pharmacokinetic analysis, both estimating creatinine clearance using the actual creatinine concentration and fixing serum creatinine measurements less than 60 μmol/L to 60 μmol/L produced a lower OFV compared with using an adjustment factor. The little improvement observed when the Khuu *et al* (2010) approach was applied might be related to body weight not being closely related to serum creatinine in the studied group. Underweight patients had a wide range of serum creatinine concentrations and did not always have low levels.

The typical aminoglycoside clearance estimate of 4.65 L/h reported from the analysis is in agreement with values ranging from 4.28 to 5.4 L/h that were found in other studies in this group of patients (Campbell D et al., 1999, Beringer PM et al., 2000, Touw DJ et al., 1994, Touw DJ et al., 1996), but lower than the estimates of 5.5 L/h reported elsewhere (Burkhardt O et al., 2006, Aminimanizani A et al., 2002). Although between subject variability in clearance was lower than has been reported in a general population of hospital patients who receive aminoglycosides (Matthews et al., 2004), it was consistent with values of 12-14% reported in other cystic fibrosis studies (Campbell D et al., 1999, Hennig S et al., 2007). This difference probably reflects the narrow range of clinical characteristics compared to a general patient population in which age, weight, renal function, co-morbidities and co-medications are more variable.

The final model indicated that creatinine clearance had low contribution to aminoglycoside clearance as it only reduced BSV from 23% to 18.5 %. This probably reflects the narrow range of serum creatinine concentrations (96 % were below 100 μmol/L) available and the observation that only one patient had poor renal function in the current database. Difficulties in characterising relationships between aminoglycoside clearance and renal

function in this patient group have been reported previously (Touw DJ et al., 1997, Soulsby N et al., 2010, Campbell D et al., 1999, Hennig S et al., 2007, Touw DJ et al., 1996, Hennig S et al., 2013). Hennig *et al* (2007) were unable to identify any factor other than size as influencing clearance and Soulsby *et al* (2010) found no relationship between tobramycin clearance and measured glomerular filtration rate. Consequently, the population model cannot be used to predict aminoglycoside clearance in patients with cystic fibrosis who also have renal insufficiency.

The present study provided no evidence that adult patients with cystic fibrosis have higher estimates of aminoglycoside clearance than other patient groups. Kirkpatrick *et al* (1999) reported a typical clearance of 4.0 L/h (range 0.68 to 12.5 L/h) in 957 general medical patients with a wide range of renal function. Recently, Hennig *et al* (2013) compared 465 paediatric and adult patients with cystic fibrosis with 267 paediatric and adult patients without cystic fibrosis, and found no difference in tobramycin pharmacokinetics in patients with and without cystic fibrosis. In addition, Hendeles *et al* (1987) found no difference in pharmacokinetic parameters in patients with cystic fibrosis and without cystic fibrosis. In contrast to other studies that reported higher aminoglycoside clearances in patients with cystic fibrosis, which were typically conducted in small groups of paediatric and young adult patients (Kearns GL et al., 1982, Levy J et al., 1984, Mann HJ et al., 1985) and were not adequately corrected for size.

In the current study, height produced a better OFV than weight when used to describe aminoglycoside clearance. Although height only provided a small improvement in fit, reducing BSV from 18.5% with creatinine clearance alone to 18% with the combination, when weight was included in the clearance model, the OFV increased substantially to 184. Although a wide range of weights was available in the raw data, they were not mirrored by a wide range of clearance estimates, which more closely matched the narrow range of heights. In addition, there was a poor correlation between weight and height in this patient group; some patients with low heights had relatively high weights, whereas some tall patients had low weights. Additionally, there was no relationship between low weight and

low creatinine concentration, i.e. low serum creatinine concentrations were seen at a wide range of weight values. Substituting lean body weight had similar issues; when lean body weight was included into clearance model the OFV value increased. Similar results were obtained when the mechanistic approach was applied; height proved a better descriptor of clearance than allometric scaling with weight. A better relationship was observed with height because it had a narrower range and was constant within an individual patient over time.

Height was also the best V_1 descriptor, which is the first time this finding has been documented in this group of patients, but was documented recently in critically ill patients (Conil J-M et al., 2010). Usually weight (Massie J and Cranswick N, 2006, Touw DJ et al., 2007) is the covariate explaining between subject variability in aminoglycoside or other derived body size measures such as body surface area (Campbell D et al., 1999) or lean body weight (Touw DJ et al., 1994). The previous papers used a one-compartment model, which might account for the difference in findings, as Wade *et al* (1994) pointed out. They concluded from their work with quinidine and netilmicin that the choice for a "correct" covariate and statistical sub-models is highly depending on the structural model. For example, weight and gestation age were found to influence V when a one-compartment model was used to describe netilmicin data in neonates. However, the influence of these covariates was lost when a two-compartment model was used. In the present study, height provided a better fit than total body weight and lean body weight (Janmahasatian S et al., 2005) on V_1. This finding might be related to the observation that a high proportion of the patients were underweight (40% at one or multiple courses). The lean body weight formula used in the current study was developed from a total of 373 subjects who were 70 % overweight or obese and no underweight subjects were included (Janmahasatian S et al., 2005).

Since the data used in the present study were collected over 15 years and included patients who grew up during this time period age might have been expected to influence aminoglycoside pharmacokinetics. However, there was no relationship between clearance

(or V_1) and age. In addition, there was no difference in clearance estimates when age was modelled as categorical variable. No effect of age was seen in the current analysis, which is not surprising since renal function matures by one year of age, and the majority of the patients were mainly young adults. In the present study, 69 patients (207 courses) were under 18 years old with the youngest patient 14 years old and the median age was 23 years. A similar finding was documented by VandenBussche *et al* (2012), who found no difference in pharmacokinetic parameters between paediatric patients and young adults (less than 18 years old) or adults (greater than 18 years old). On the other hand, Hennig *et al* (2013) found that patient age influenced tobramycin clearance. They were able to find an effect because their study more paediatric patients with cystic fibrosis (351 patients) and over a wider range of age (0.01 to 17.9 years old) were included compared to the present study. Therefore, the main focus of the present work was on adult patients with cystic fibrosis. Using height as a covariate is an advantage of the model, because it is easy to measure and practical to use in clinical setting compared with using derived body size measurements. In addition, derived body size measurements usually have more than one method for estimation and there is a higher chance of calculation error.

The results with the mechanistic model were similar to those obtained with the empirical approach. Renal function explained much of the variability in clearance and height was again superior to allometric scaled weight for both clearance and V_1. The apparent "non-renal" component of clearance was very similar for the mechanistic and empirical models.

The typical value of V_1, 13.3 L, was consistent with other studies (Burkhardt O et al., 2006, Aminimanizani A et al., 2002, Campbell D et al., 1999, Touw DJ et al., 1994, Touw DJ et al., 1996), but slightly lower than estimates of 15.7 L obtained by Beringer *et al* (2000). This could be explained by the patients in the Beringer study being slightly bigger in size with median weight of 54 kg. Touw *et al* (2007) found that volume of distribution decreased with increasing age, which they suggested was related to aminoglycosides being water soluble drugs that distribute into the extracellular fluid, which decreases with increasing age. This observation was not found in the current analysis because the patient group included were

mainly young adults. However, in the Touw *et al* (2007) analysis, both paediatric and young adult patients were included and hence there was more scope to identify a reduction in volume of distribution. There was no evidence in the current study that patients with cystic fibrosis had higher estimates of aminoglycoside volume of distribution than other patients, which contrasts with some (paediatric and young adults) studies (Levy J et al., 1984, Kearns GL et al., 1982) but is consistent with others (MacDonald NE et al., 1983, Mann HJ et al., 1985, Hennig S et al., 2013).

The V_2 and Q estimates, 6.62 L and 0.45 L/h, were in agreement with the values reported by Aminimanizani *et al* (2002). However, it was difficult to characterise between subject variability for V_2 and Q, which indicates that the sparse data used in this analysis did not contain enough information to quantify this parameter. Therefore, between-subject variability in V_2 and Q were removed from the final model. In the current study, no difference in pharmacokinetics between gentamicin and tobramycin was found, which is in agreement with Bauer *et al* (1983).

3.5.5 Within subject variability

A unique aspect of the present study was the availability of data from patients who were administered several courses of aminoglycoside over a prolonged period of time, which facilitated the estimation of within-subject variability. Karlsson *et al* (1993) recommended including within-subject variability into the population pharmacokinetic model, because they found that including within-subject variability in a previously published covariate model led to explaining some of the variabilities. In another example, the effect of a covariate was highly dependent on the inclusion of within-subject variability (Karlsson MO and Sheiner LB, 1993). The authors concluded that failure to include within-subject variability might bias the parameter estimates. The magnitude of within-subject variability can be valuable for decisions regarding drug therapy. Two studies to date included within-subject variability in patients with cystic fibrosis examined data from 35 patients with an average of 4 to 5 occasions (Hennig S et al., 2007) and 465 patients with an average of 4 to 7 occasions (Hennig S et al., 2013), but defined an “occasion” as one dosing interval within a single

course of therapy rather than one course of therapy. The reported within-subject variability in clearance was small at 6.47% (Hennig S et al., 2007) and 12.6% (Hennig S et al., 2013), which indicated that patients' pharmacokinetic parameters are stable from one dosage interval to another. Therefore in the present study, an "occasion" was defined as a course of therapy rather than a dosage interval to examine how the pharmacokinetics of aminoglycosides vary between different courses of therapy in patients with cystic fibrosis. Although the addition of within-subject variability in the present study improved the fit, its magnitude was small at 11 %, indicating little variability within a patient between courses of aminoglycoside therapy. This indicated a little variation in dose requirements within a patient over time and reflects the stable nature of this particular patient group.

3.5.6 Clearance change over time

The long duration of this study (up to 15 years) with up to 28 courses of therapy allowed the long term risks of nephrotoxicity to be evaluated. No changes in aminoglycoside clearance were identified with multiple courses of therapy. These findings contrast with the results of the TOPIC study (Smyth A et al., 2005), in which a trend towards an increase in serum creatinine was identified during a two week course of treatment with once daily tobramycin. However, renal damage has previously been found to resolve after stopping treatment (Steinkamp G et al., 1986, Bertenshaw C et al., 2007) and the present study is consistent with these findings. The lower aminoglycoside doses used (median 7.2 mg/kg/day compared to 10 mg/kg/day in the TOPIC study) may also have contributed. Smyth *et al* (2008) investigated the risk factors for developing acute renal failure in patients with cystic fibrosis. They found no influence of cumulative exposure to aminoglycosides during the previous year on the development of acute renal failure. In contrast, Al-Aloul *et al* (2005) did find an association between repeated aminoglycoside use and long-term renal damage. However, it is difficult to compare their results with the findings of the present study since they did not document the aminoglycoside doses used or the study time scale. In addition, Andrieux *et al* (2010) followed 112 paediatric patients with cystic fibrosis over 8 years and found no correlation between the cumulative aminoglycosides dose and glomerular filtration rate. They found also a low renal impairment regardless of aminoglycoside used. However, they raised the question of what is the best method to

estimate or measure glomerular filtration rate in cystic fibrosis patients. Halacova *et al* (2008) evaluated the use of serum cystatin C and creatinine and their clearances to estimate glomerular filtration rate in patients with cystic fibrosis on amikacin. They found that serum cystatin C and cystatin C clearance were better predictors of glomerular filtration rate in patients with cystic fibrosis than serum creatinine and creatinine clearance.

3.5.7 Conclusions

A two-compartment model that included within subject variability in clearance provided the best fit of the data

- The covariate model for clearance included estimated creatinine clearance using the Cockcroft and Gault equation with serum creatinine concentrations below 60 μmol/L fixed to 60 μmol/L and height.
- The V_1 covariate model included height.
- The impact of within subject variability was small from a clinical perspective.
- Clearance did not change over multiple administrations of aminoglycoside in patients with cystic fibrosis.

Although creatinine clearance was a statistically significant clinical characteristic in explaining variability in clearance, it was not a powerful factor due to its narrow range. Because of the few data points collected, usually peak and trough measurements, NONMEM faced some difficulties in estimating V_2 and Q values and their BSV. Modelling within subject variability yielded a more stable model; however, its magnitude was not of clinical significance. Multiple dosing did not influence aminoglycoside clearance over time.

CHAPTER 4: MODEL VALIDATION

4.1 INTRODUCTION

Basic model performance can be investigated by examining the standard diagnostic plots as discussed and illustrated in Chapter 3. However, more sophisticated model diagnostic methods, for example simulation-based numerical and graphical diagnostics can provide additional information.

Bootstrap analysis is an example of a numerical diagnostic and was first introduced by Efron for general model evaluation (Efron B, 1979). It provides information on model robustness and assesses statistical accuracy and precision. Bootstrapping involves creating new datasets by random sampling with replacement from the original data then applying the same analysis steps to each of the new datasets as was performed on the original data. The purpose of applying the bootstrap with re-sampling is to assess the stability of the final parameter estimates. The resulting distributions from a large number of bootstrap samples are then used to provide confidence intervals for the parameter estimates. The main drawback of bootstrap is the extensive computation time to evaluate hundreds of datasets.

Another simulated-based method, the "Visual Predictive Check" (VPC) suggested by Holford *et al* (Holford, 2005, Karlsson M and Holford N, 2008), is to generate a set of simulated datasets using the model to be evaluated. The purpose of a VPC is to assess whether the model can reproduce the median and variability in the observed data graphically when plotted against an independent variable (Bergstrand M et al., 2011). A number of simulations with the model of interest are performed, typically using at least 1000 simulated datasets. Then, the real data observations are compared with the distribution of the simulated observations graphically by plotting them against an independent variable, usually time or time after dose. The percentiles of the simulated data are compared to the corresponding percentiles of the observed data graphically.

VPC plots show the level of agreement between observations and simulated predictions. However, there are some drawbacks with the traditional VPC methods if doses, dosing

times, observation times, and/or covariate values vary between subjects (Bergstrand M et al., 2011). Under these circumstances, stratification by important variables might be necessary (Karlsson M and Savic RM, 2007). However, a number of strata might be required and diagnosis for each stratum might become less informative as the number of graphs increases and the amount of data available per graph decreases. Bergstrand *et al* (2011) developed another VPC method that tries to account for these limitations and known as the prediction corrected VPC (pcVPC). In this approach, concentrations are subjected to prediction correction before the statistics are calculated (Bergstrand M et al., 2011). The purpose of a pcVPC is to correct for the differences within a bin due to independent variables, such as time and dose, and due to covariates that are included in the model. The approach facilitates diagnosis of model misspecifications in both fixed and random effects. However, this advantage is dependent on how large the differences are in expected variability between observations in a specific bin.

Normalised prediction distribution error (npde) is another simulation-based model evaluation technique, developed by Comets *et al* (2008). It is a normalised version of the prediction discrepancies developed by Mentré and Escolano (2006), which is the percentile of an observation and its prediction distribution with the assumption that the evaluated model adequately describes the data. Using npde is preferred over prediction discrepancies (Mentré F and Escolano S, 2006), because prediction discrepancies were found to be affected by within-subject correlation, while npde had the advantage of decorrelating multiple observations per subject (Comets E et al., 2008, Brendel K et al., 2010). This is an important issue to be considered in the present study, where multiple observations per subject and multiple courses of therapy were included.

The previous model diagnostic methods evaluated model performance internally. However, a more powerful method involves testing model performance with a new, independent dataset. This is done by estimating model predictions then determining the bias and precision of these predictions. In addition, the previous simulation based methods, pc VPC

and npde, can be used to evaluate the adequacy of the population model to describe a new dataset.

4.2 AIMS

To evaluate the adequacy of the developed empirical and mechanistic models listed in Table 4.1 to describe the data, using internal and external validation methods. The internal validation methods were bootstrapping, prediction corrected visual predictive check and normalised prediction distribution error. The external validation was conducted using a dataset that was generated in the Netherlands.

Table 4.1 Different covariate models tested using the validation datasets.

OFV	CL model (L/hr)	BSV_{CL} %	WSV_{CL} %	V_1 model (L)	BSV_{V1} %
		Empirical approach			
-304	0.0285 x HT+ 0.0114 x (CGCL-92)	18	11	13.3 x (1+0.0113 x (HT-163))	12
		Mechanistic approach			
-310	0.0226 x HT+0.709 x (CrCL (L/h)/ 7.26 L/h/70 kg x (LBW+0.211(Weight–LBW) /70)$^{0.75}$))	18	11	13.4 x (1+0.00974 x (HT-163))	12
-256	5.07 x (LBW/70)$^{0.75}$+ 0.83 x (CrCL (L/h)/ 7.26 L/h/70 kg x (LBW+0.211(Weight–LBW) /70)$^{0.75}$))	17	11	8.84 (1+0.718 x (LBW+(WT-LBW)/70))	13

Key: CGCL= estimated creatinine clearance in mL/min using the Cockcroft and Gault equation (1976) with the lowest serum creatinine set at 60 μmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998), HT= height in cm, LBW= Lean body weight (Janmahasatian S et al., 2005) in kg, CrCL= creatinine clearance estimated using the mechanistic approach (Anderson BJ and Holford NHG, 2009, Matthews et al., 2004).

4.3 METHODS

4.3.1 Internal model validation

4.3.1.1 Bootstrap

One thousand bootstrap data sets were generated using Perls-Speaks NONMEM (PsN version 3.2.12) (Lindbom L et al., 2004, Lindbom et al., 2005) then the parameter estimates for each of the 1000 samples were re-estimated using NONMEM version 7.1 (Beal SL et al.,

2009) for the empirical model listed in Table 4.1. The median and 95th and 5th percentiles of the median obtained for each of the parameters with the bootstrap samples were then compared with the NONMEM estimates from the empirical population model. The following is an example of the command used to perform the bootstrap:

bootstrap -samples=1000 -seed=4361 -dir=Boot-dir2 Run436b.mod -threads=5

The meaning of the command is as follow:

bootstrap	To call PsN to run bootstrap
-samples=N	To set the number of bootstrap datasets to generate
-seed=N	A seed to generate a random number
-dir=	The directory name where the output run would be stored
Run436b.mod	Name of the model file
-threads=N	The number of parallel processes

4.3.1.2 Prediction-corrected visual predictive checks

The pc VPC was performed using NONMEM 7.1 (Beal SL et al., 2009) and PsN 3.2.12 (Lindbom et al., 2005, Lindbom L et al., 2004) with 1000 simulated replicates of the original dataset. In the current study, prediction-corrected VPC was performed using the *–predcorr* option. Binning can help to visualise the results better when observation times are heterogeneous between subjects and a range of binning approaches were tried. One method involved binning the independent variable such as time after dose with equal intervals, an alternative is to have equal number of measured concentrations in each bin. The data had variability in sampling times between subjects and hence binning based on a defined list of values of the independent variable (sampling schedule binning) was also tested. Time after dose was used as the independent variable in the current study. An example of pc VPC command is shown below:

VPC Run436bVPC.mod -samples=1000 -idv=TAD -predcorr -bin_by_count=0
-bin_array=1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24

The meaning of the command is as follow:

VPC	*To* call PsN to run VPC
Run436bVPC.mod	Name of the model file
-samples=N	The number of simulated datasets to generate
-idv	The independent variable to bin on
-predcorr	Perform prediction correction of dependent variable values
-bin_by_count=0 -bin_array=1,…	Bins based on a defined list of values of the independent variable

The output from PsN was graphically presented using the package Xpose 4 in R version 2.15.1 (R Core Team, 2012). In the pc VPC plots, the observed and simulated data were presented as median (50^{th} percentile) and 2.5^{th} and 97.5^{th} percentiles, which corresponded to a 95% prediction interval. Bins correspond to the 95% confidence interval of simulated datasets. To plot pc VPC the following command was used in R following loading the xpose 4 package:

Library(xpose4)

then

xpose.VPC()

to generate more customised VPC plot, the following options were added in the command line:

xpose.VPC(main=NULL,PI.real="lines",PI.real.up.col=1,PI.real.down.col=1,PI.real.med.col=1,type="n",xlb="Time after dose (hours)", ylb=" Concentrations (mg/L)", PI.ci="area", PI.ci.up.arcol="gray70", PI.ci.down.arcol="gray70",PI.ci.med.arcol="gray22",ylim=c(-2,22))

main	Plot title
PI.real	Plot the percentiles of the real data in the various bins
PI.real.up.col	The colour of the upper PI.real
PI.real.down.col	The colour of the lower PI.real
PI.real.med.col	The colour of the median PI.real

PI.ci	Plot the prediction interval of the simulated data's percentiles for each bin. They can be plotted as lines or area or both
PI.ci.up.arcol	The colour of the upper PI.ci
PI.ci.down.arcol	The colour of the lower PI.ci
PI.ci.med.arcol	The colour of the median PI.ci
Type	Character used to show the observation on the plot. To not show the observation on the plot, then use the character "n"
xlb, ylb	Label for x- and y-axis
ylim	to set up y-axis limits

4.3.1.3 Normalised prediction distribution error (npde)

The normalised prediction distribution error was performed using the add on package for R version 1.2 (Comets E et al., 2008). Two files were required to compute npde, the dataset to be evaluated and called the "observed data", and a simulated data file. Three important columns should be included in the npde data file, patient identification, the independent variable (xobs or xsim) such as time after dose which was used in the present study, and the dependent variable (yobs or ysim) such as concentration. In addition, a missing data column might be required, where a code of 1 indicated missing data and 0 indicated observed data. Other columns might be present in the file but would not be used by the program. The files should be saved as a text file (.tab file). The simulations were performed using NONMEM 7.1 (Beal SL et al., 2009), where 1000 simulated datasets were generated using the $SIMULATION option. The evaluated model parameter estimates listed in Table 4.1 were used. An example of the control file used to generate the npde simulations is attached in APPENDIX IV. The function used to compute npde within R is as follow:

Library(npde)

x<-autonpde ("Obsdata.tab","Simdata12.tab",iid=1,ix=2,iy=3,imdv=4, boolsave=TRUE, namsav = "npde12", output = TRUE)

autonpde	The function used to call npde

Obsdata.tab	Name of data file to be evaluated
Simdata12.tab	Name of the simulated data
iid	Number of column in which ID is located in the observed data file
ix	Number of column in which the independent variable is located in the observed data file
iy	Number of column in which the dependent variable is located in the observed data file
imdv	Number of column in which the missing data variable is located in the observed data file
boolsave	Whether to save the graphs and results in to a file
namsav	Name of the file in which results should be saved
output	Whether the function returns the results

The assumption used in npde is that the model concentration predictions follow a normal distribution with a mean of zero and variance of 1 (Comets E et al., 2008). The program is setup to do the following default statistical tests; the Wilcoxon signed rank test to test whether the mean is different from zero, the Fisher test of variance to test whether the variance is different from 1, and the Shapiro-Wilks test to assess whether the distribution is different from a normal distribution. The authors of the program used another test called a global test, which combines the p values from the previous three tests with a Bonferroni correction and reported a p value multiplied by 3. To say that the model describes the data adequately, the results from the statistical test should be non-significant. In addition to the numerical results, four graphs are plotted following computing npde; the QQ-plot of the npde, the histogram of the npde, a scatter plot of npde versus the independent variable (x), and scatter plot of the npde versus the predicted dependent variable (Y). If the model is adequate then no trend should be seen in the scatter plots.

4.3.2 External model validation

A new set of data from adult patients with cystic fibrosis was received from the Apotheek Haagse Ziekenhuizen and Haga Teaching Hospital in The Netherlands. In the Netherlands, ethical approval is needed when the patient undergoes a procedure or an intervention. Since these data were routinely monitored data, anonymised and no intervention or procedure was performed, ethical approval was not required *(Personal Communication, Dr Touw, the Apotheek Haagse Ziekenhuizen and Haga Teaching Hospital, the Netherlands, October 05, 2013)*. The database included patients with cystic fibrosis who had received tobramycin. The data were supplied in an Excel spreadsheet format exported from the software MWPharm (Mediware, Groningen, The Netherlands) (Proost JH and Meijer DKF, 1992). The file initially contained 500 different courses of therapy. The first task was to identify which courses came from the same patient (but at different times). Patients who had the same date of birth were assumed to be the same individual. Courses of therapy that contained insufficient data or suspected errors were removed from the dataset. For example, a course of therapy was excluded if date of birth was not documented, if the patient had the same date of birth but different sex or if the patient was less than 14 years old. Further exclusions included cases for which the course of therapy was not clear or was repeated with different concentration measurements at the same sampling times.

The dataset comprised clinical and demographic data, the dosage regimen(s) and a list of measured concentrations. This format was not suitable for a NONMEM analysis since dosage information is required before each measured concentration. Lack of detailed dosage information required some assumptions to be made. These were as follows:

- "Peak" concentrations were assumed to be measured one hour after starting the infusion.
- If a measured concentration was recorded without information about the previous dose, a dose was assumed to be administered similar to a previous recorded dose and dosage interval.
- Steady state coding was added to the data file. Samples taken at least 60 hours following the start of therapy were assumed to be at steady state.

These assumptions were confirmed as valid by Dr Touw, who had supplied the data from the Apotheek Haagse Ziekenhuizen and Haga Teaching Hospital, the Netherlands. External evaluation was performed for the models listed in Table 4.1. The control file contained the population model and parameter estimates for fixed and random parameters. The estimation step was omitted by using the code MAXEVAL = 0. Population pharmacokinetic parameters and concentrations were estimated for each course of therapy and individual parameters and concentrations were also obtained (using the POSTHOC option in the estimation step). An example of the control file used is shown in APPENDIX V. In addition, pc VPCs and npdes were performed for the evaluated models using the new dataset. The Hague dataset was then combined with the Glasgow dataset and the parameters of the final model were re-estimated.

4.3.2.1 Serum creatinine and drug assay

The Jaffé method was used to measure serum creatinine concentration over the data period. Concentrations measured in The Hague (external validation data set) were also measured using FPIA but on an AxSym platform (Abbott Laboratories). On this system, the assay error was described by the following equation: SD = 0.011596 + 0.042146*C + 0.002791*C_2. The reported limit of quantification was 0.3 mg/L. The data set contained 10 concentrations below this value; the lowest was 0.1 mg/L.

4.3.3 Statistical analysis of the external validation

The Mann-Whitney test was applied to compare the characteristics of the patients in the model development and validation datasets. Statistical significance was set at a p value <0.05.

Bias and imprecision were estimated for the pharmacokinetic parameters of interest, clearance and V_1, and the measured versus population predicted concentrations. The analysis was conducted according to the Sheiner method for measuring predictive performance (Sheiner LB and Beal SL, 1981b). Bias was defined as mean difference in prediction error if the results were normally distributed or median if they were not (Sheiner

LB and Beal SL, 1981b). Lower bias indicates higher accuracy in model predictions. Bias was then assessed by comparing mean prediction errors with zero using the Student's t test if the data were normally distributed and the median using the Wilcoxon signed rank test otherwise, with statistical significance set at $p < 0.05$. The 95% confidence interval of the difference was also examined using Minitab Version 15 (Minitab Ltd.). The following formulas were used to estimate the prediction errors for pharmacokinetic estimates and concentrations;

$$PK\ Estimate\ Prediction\ Error = Individual\ PK\ Estimate - Population\ PK\ Estimate$$

$$Concentration\ Prediction\ Error = Measured\ Concentration - Predicted\ Concentration$$

Imprecision was based on the root mean squared prediction error if the data were normally distributed, or the median absolute (unsigned) error if the data were non-normally distributed. A lower value indicates higher precision in model predictions.

4.4 RESULTS

4.4.1 Internal model validation

4.4.1.1 Bootstrap

In total, 166 runs out of the 1000 runs terminated with rounding errors. The median, 95th and 5th percentiles for each parameter were estimated by the bootstrap samples using all replicates and only those replicates which minimised successfully, and are shown in Table 4.2 . The results obtained with the bootstrap and the empirical model coincided well with a narrow confidence interval range. This indicates good precision in the empirical model parameter estimates. There was no difference in the median and percentile confidence interval of the parameter estimates obtained from all replicates or replicates which minimised successfully.

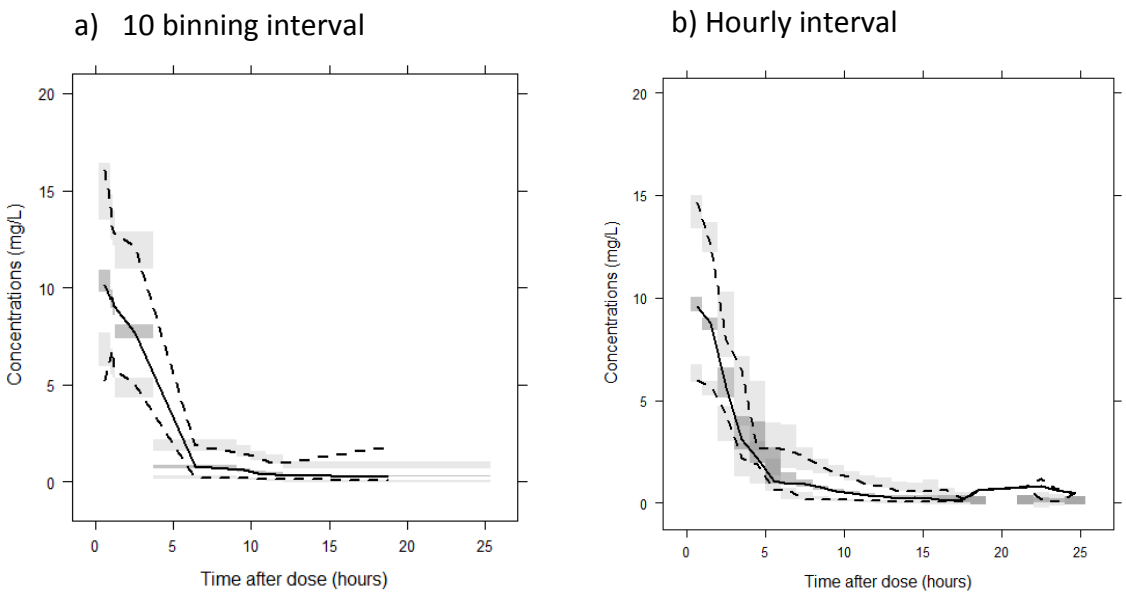
Table 4.2 The empirical model parameter estimates and bootstrap results using all and minimised successfully replicates.

Parameter	Final population model	All		Minimised successfully	
		Bootstrap median estimate	Bootstrap 5th and 95th percentiles	Bootstrap median estimate	Bootstrap 5th and 95th percentiles
OFV	-304	-322	-567, -81.8	-319	-559, -80.3
θ_{HT} CL (L/h)	0.0285	0.0284	0.0276, 0.0293	0.0284	0.0276, 0.0293
θ_{renal} CL (L/h)	0.0114	0.0112	0.0077, 0.0149	0.0112	0.0077, 0.0149
BOV (CL)	11.4%	11.0%	9.68%, 12.3%	11.0%	9.48%, 12.2%
BSV (CL)	18.0%	17.9%	14.8%, 21.4%	17.8%	14.8%, 21.3%
θ_{V1} (L)	13.3	13.4	13.1, 13.7	13.3	13.0, 13.6
θ_{HT} V_1	0.0113	0.0114	0.0091, 0.0134	0.0114	0.0089, 0.0133
BSV (V_1)	11.6%	11.4%	9.49%, 13.2%	11.5%	9.48%, 13.2%
V_2 (L)	6.62	6.69	5.19, 8.62	6.54	5.16, 8.39
Q (L/h)	0.452	0.447	0.379, 0.522	0.449	0.381, 0.522
Additive error (mg/L)	0.086	0.0846	0.0665, 0.118	0.0852	0.0662, 0.118
Proportional error (CV %)	14.8	14.8	13.8, 15.8	14.7	13.8, 15.7

4.4.1.2 Prediction-corrected visual predictive checks

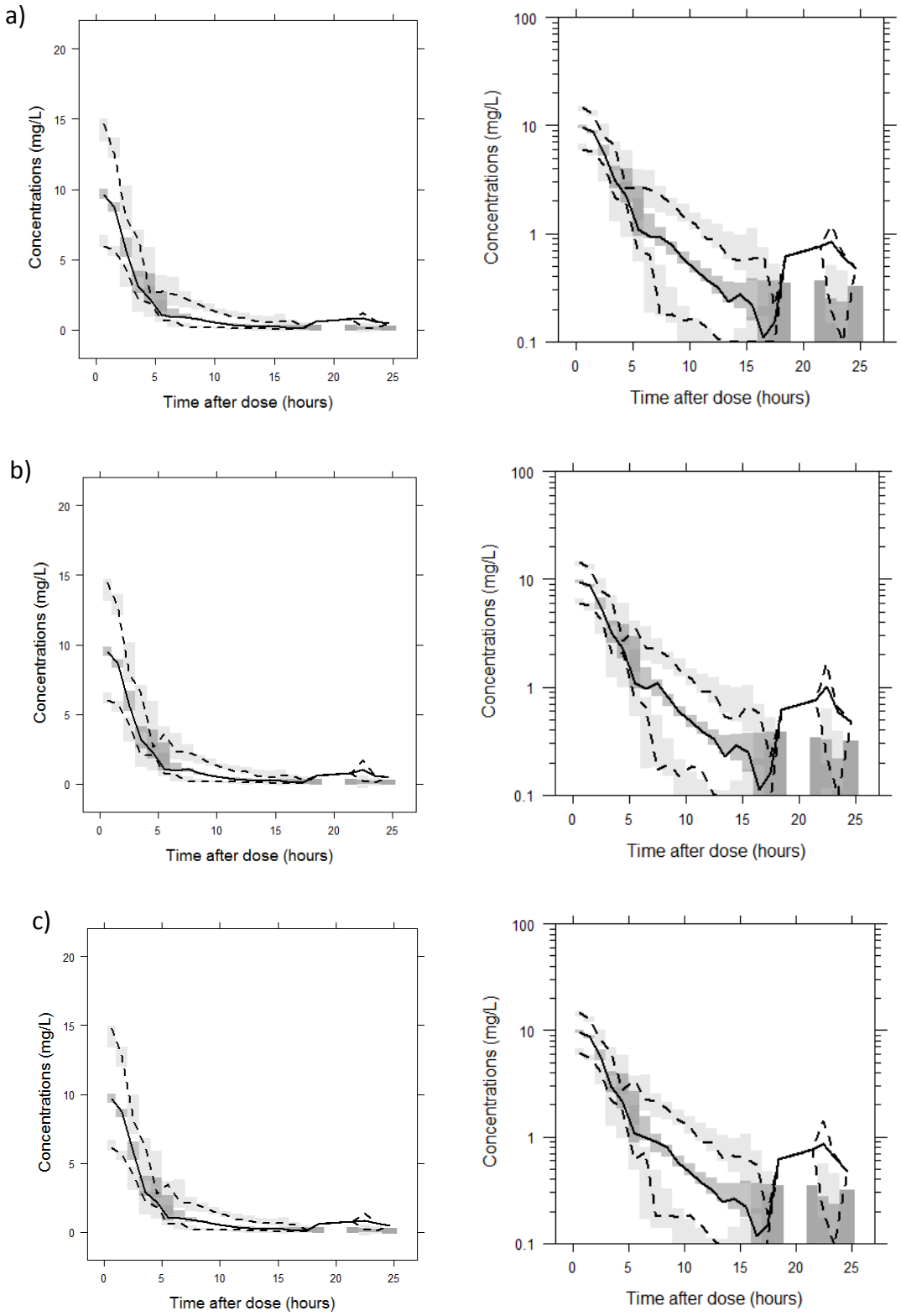
Figure 4.1 shows the pc VPC for the empirical model using different binning approaches. Binning by width and dividing the intervals by hourly intervals resulted in more informative pc VPC plots. There was no difference between the empirical and mechanistic model pc VPCs as shown in Figure 4.2, which indicated that the evaluated models provided a satisfactory description of the data.

Figure 4.1 Prediction corrected Visual Predictive Check using the empirical model after binning the data by count (a) and by width (b).



Key: The black dotted lines are the 97.5th and 2.5th percentiles for the observed concentrations and correspond to 95% prediction interval. The black solid line represents the median of the observed concentrations. The shaded areas represent the binning and correspond to the 95% confidence intervals for the 97.5th, 50th and 2.5th percentiles of the simulated dataset.

Figure 4.2 Prediction corrected Visual Predictive Check using (a) the empirical model, the mechanistic model (b) with height and with (c) allometric scale of weight after binning the data by width in normal (right) and logarithmic (left) scale.



Key: The black dotted lines are the 97.5th and 2.5th percentiles for the observed concentrations and correspond to 95% prediction interval. The black solid line represents the median of the observed concentrations. The shaded areas represent the binning and correspond to the 95% confidence intervals for the 97.5th, 50th and 2.5th percentiles of the simulated dataset.

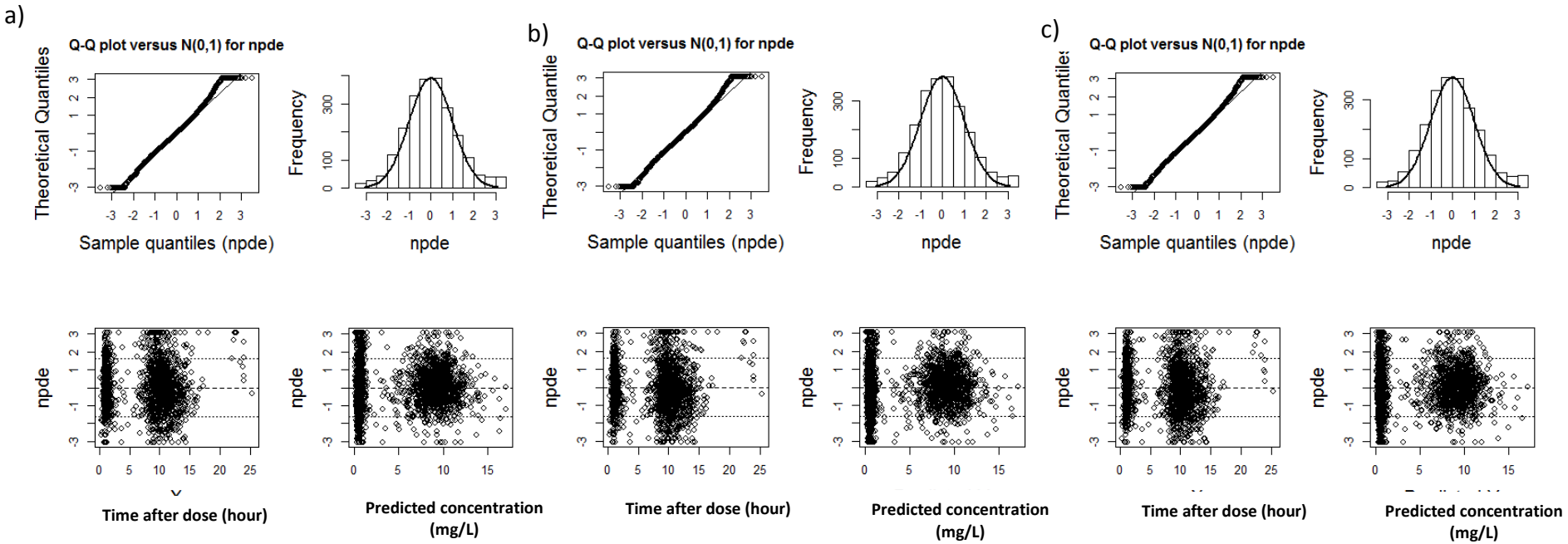
4.4.1.4 Normalised prediction distribution error

There was no difference in the distribution of npde and the statistical tests when the three models were evaluated, as illustrated in Table 4.3. The values of mean, skewness and kurtosis were close to zero and slightly higher than 1 for the variance of the examined models. Although the Fisher variance test, Shapiro- Wilks test, and the global tests were significant, the quantile-quantile and histogram of npde plots in Figure 4.3 showed that the normality assumption was not rejected. The npde scatter plots against the independent variable (time after dose) and the predicted concentrations plots were satisfactory in general and did not show any trend. However, npde scatter plots against the independent variable shows more positive npdes for samples obtained after 15 hours following the start of infusion for the evaluated models.

Table 4.3 Results for the distribution of npde and statistical tests used to evaluate the assumption of normality.

Npde test	**Independent variable (Time after dose)**		
	Empirical model	**Mechanistic approach**	
		With height	**With allometric scale of weight**
Distribution of npde			
Mean	0.016	-0.012	0.008
Variance	1.45	1.47	1.47
Skewness	0.191	0.165	0.177
Kurtosis	0.157	0.129	0.092
Statistical tests			
Wilcoxon signed rank test	0.700	0.183	0.556
Fisher variance test	0.001	0.001	0.001
Shapiro- Wilks test	< 0.001	< 0.001	< 0.001
Global test	0.001	0.001	0.001

Figure 4.3 The npde graphical output using (a) the empirical model and (b) the mechanistic approach with height (c) the mechanistic approach with an allometric scale of weight. Quantile-quantile plot of npde versus expected standard normal distribution (upper left); Histogram of npde with density of overlaid standard normal distribution (upper right); Scatter plot of npde versus independent variable X (time after dose) (lower left); scatter plot of npde versus predicted Y (concentration) (lower right).



4.4.2 External validation

4.4.2.1 Demographics, dosage history and concentration measurement results

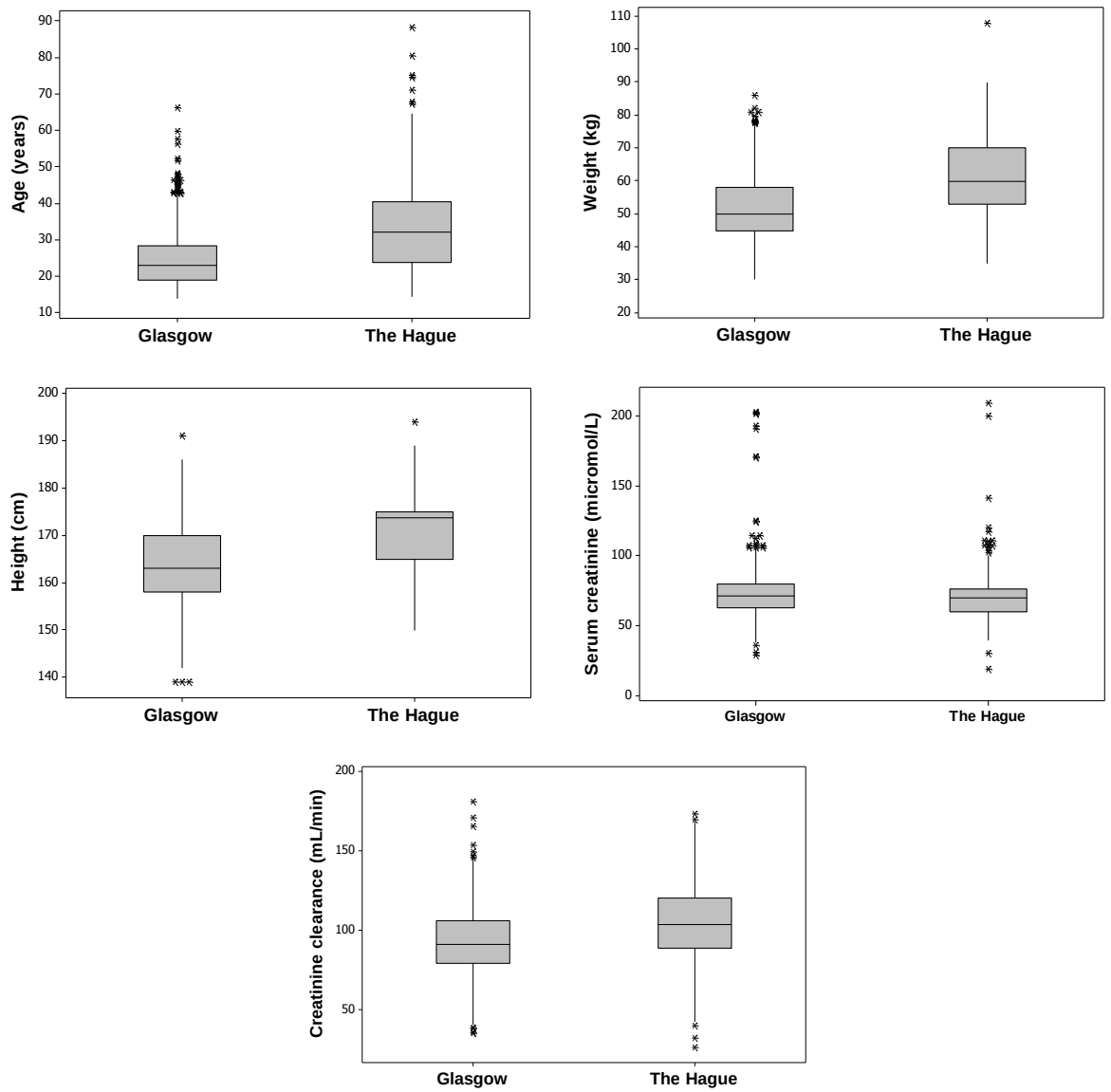
The final validation dataset comprised 165 patients and 415 courses of therapy, ranging from 1 to 13 with a median of 2 courses per patient. Table 4.4 shows a summary of patient characteristics for both the Glasgow and The Hague datasets. Patients in the validation dataset were significantly older, taller and heavier than patients in the model development dataset but there was no difference in the distributions of serum creatinine. Figure 4.4 shows box plots summarising the clinical characteristics of the patients in each dataset and shows clearly the difference between the datasets in age, weight, height and creatinine clearance, in addition to a few outliers.

Table 4.4 Summary of patient characteristics in the Glasgow and The Hague datasets.

Patient characteristics	Glasgow (n = 166 patients)		The Hague (n = 165 patients)		P value
	Median	Range	Median	Range	
Age (years)	23	14 - 66	32	14 – 88	< 0.001
Weight (kg)	50	30 - 86	60	35 – 108	< 0.001
Height (cm)	163	139 – 191	174	150 – 194	< 0.001
Serum creatinine (µmol/L)	71	29 – 203	70	19 – 209	0.06
Creatinine clearance (mL/min)	92	35 – 181	104	26 – 174	< 0.001

Key: Creatinine clearance estimated by the Cockcroft and Gault equation (1976) with the lowest serum creatinine value fixed to 60 µmol/L (Duffull SB et al., 1997).

Figure 4.4 Box plots illustrating the distributions of clinical characteristics of patients in the Glasgow and The Hague datasets.



Key: The box lines represent the first, median and third quartile from bottom to top. The black solid line represents the inter-quartile range and the starts represent the outliers.

There was only one 8 hourly aminoglycoside course in The Hague dataset, and had a 210 mg daily dose (70 mg 8 hourly). Twelve hourly dosing accounted for 33 % of courses with a median daily dose of 560 mg (240 – 880), and 24 hourly dosing for 65 % with a median daily dose of 500 mg (120 – 800). Three courses were 36 hourly and two patients had 48 hourly dosing and were administered doses of 240 to 300 mg daily. Figure 4.5 shows the distribution of the daily doses divided according to the dosage interval. In total, 1452 concentration measurements were available. Peak concentrations accounted for 37 %,

troughs accounted for 8 % and the majority (54%) of samples were mid-dose. The concentration-time profiles are presented in Figure 4.6. Table 4.5 shows a summary of the measured concentrations categorised according to the dosage intervals.

Figure 4.5 The distribution of daily doses divided by dosage interval.

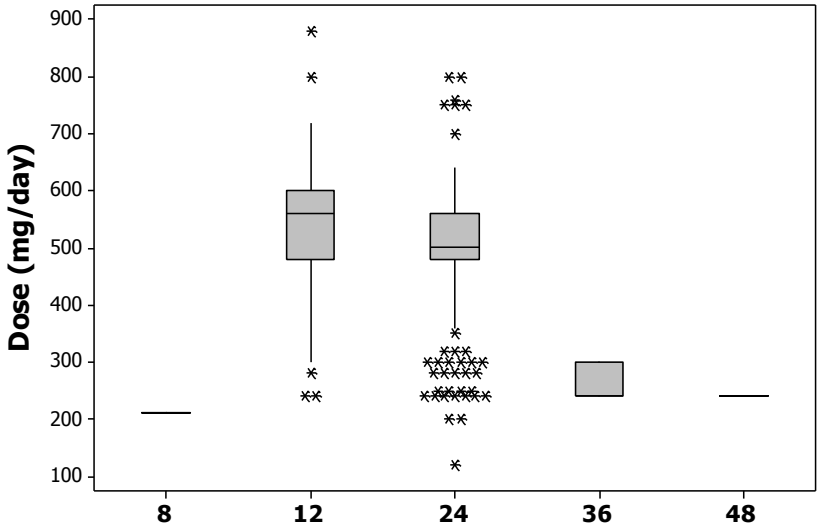


Figure 4.6 Scatter plot for The Hague concentration-time profile.

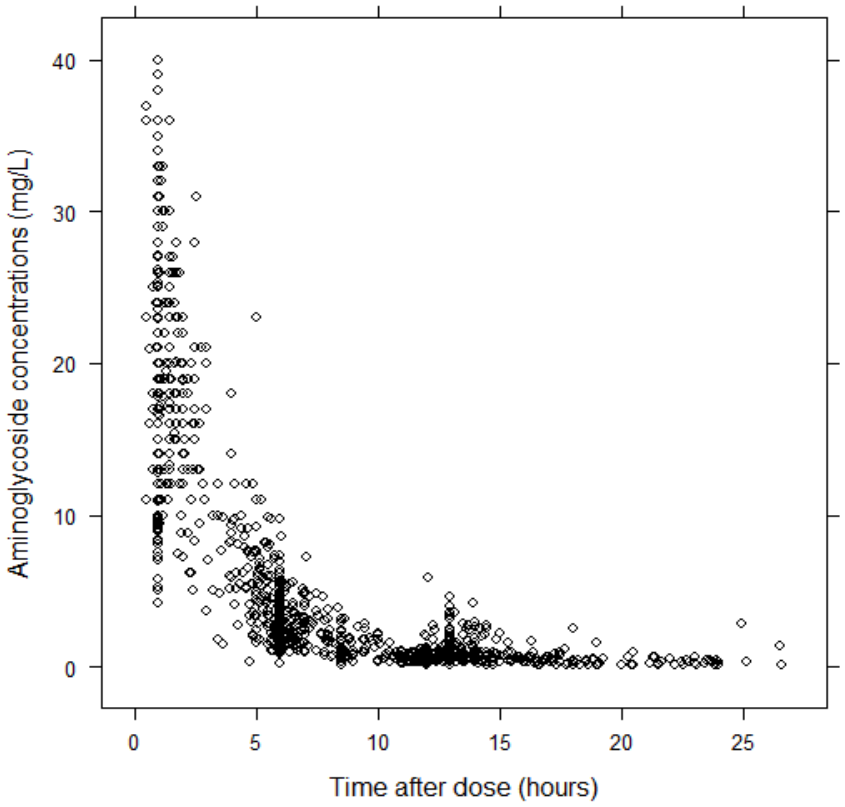


Table 4.5 Summary of The Hague measured concentrations in general and according to dosage interval.

Variable	Number of samples	Median	Range
Peak concentration (mg/L)	544	17.0	4.20- 40.0
Mid-dose concentration (mg/L)	790	1.80	0.10 – 31.0
Trough concentration (mg/L)	118	0.40	0.10 – 4.60
8 hourly (n = 1 courses)			
Peak concentration (mg/L)	-	-	-
Mid-dose concentration (mg/L)	1	1.60	-
Trough concentration (mg/L)	1	4.60	-
12 hourly (n = 137 courses)			
Peak concentration (mg/L)	306	15.0	4.20 -28.0
Mid-dose concentration (mg/L)	251	1.90	0.10 – 18.0
Trough concentration (mg/L)	67	0.52	0.10 – 4.60
24 hourly (n=272 courses)			
Peak concentration (mg/L)	229	21.0	8.80 – 40.0
Mid-dose concentration (mg/L)	526	1.60	0.15 – 31.0
Trough concentration (mg/L)	49	0.21	0.10 – 1.20
36 hourly (n= 3 courses)			
Peak concentration (mg/L)	7	13.0	11.0 – 19.0
Mid-dose concentration (mg/L)	9	2.50	1.40 – 4.20
Trough concentration (mg/L)	1	1.40	-
48 hourly (n =2 courses)			
Peak concentration (mg/L)	2	14.5	9.00 – 20.0
Mid-dose concentration (mg/L)	3	3.20	2.80 – 14.0
Trough concentration (mg/L)	-	-	-

Key: Peak concentration was defined as samples obtained within the first 2 hours after starting the infusion. Mid- dose concentration was defined as samples obtained between 2 to 9.9 hours for 12 hour dosing, 2 to 17.9 hour for 24 hour dosing, and after 2 to 24 hour for 36 and 48 hour dosing. Trough concentration was defined as samples obtained 10 hours post dose for 12 hourly dosing and 18 hours for 24 hourly dosing.

4.4.2.2 Model performance

Figure 4.7 shows the measured concentrations against population and individual predicted concentrations for the different models tested. The three evaluated models showed good agreement between the measured concentrations and predicted concentrations with no favour for one model over another. Figure 4.8 shows the population and individual predictions for clearance and V_1 using the different covariate models. All models provided a narrow range for clearance and V_1 population predictions that ranged from 2.39 to 6.49 L/h for clearance and 11.3 to 18.6 L for V_1. However a problem was identified with the mechanistic model for creatinine clearance estimate where patients who were older than 70 years had negative estimates as shown in Figure 4.9.

Figure 4.7 Measured concentrations versus population and individual predictions for (a) empirical model, (b) mechanistic model with height and (c) mechanistic model with allometrically scaled weight.

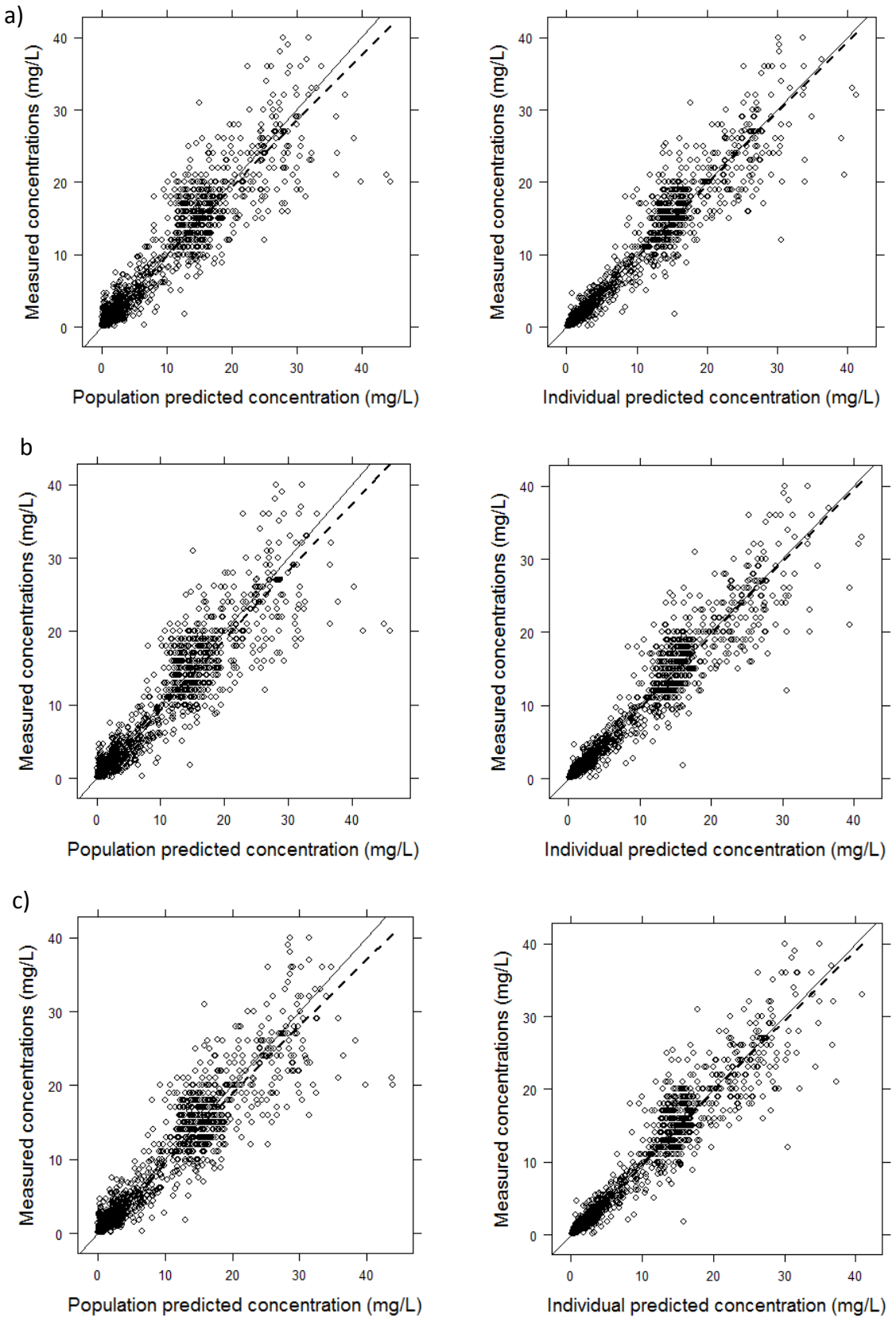


Figure 4.8 Population versus individual estimates of clearance and volume of the central compartment for (a) empirical model, (b) mechanistic model with height and (c) mechanistic model with allometric scale weight.

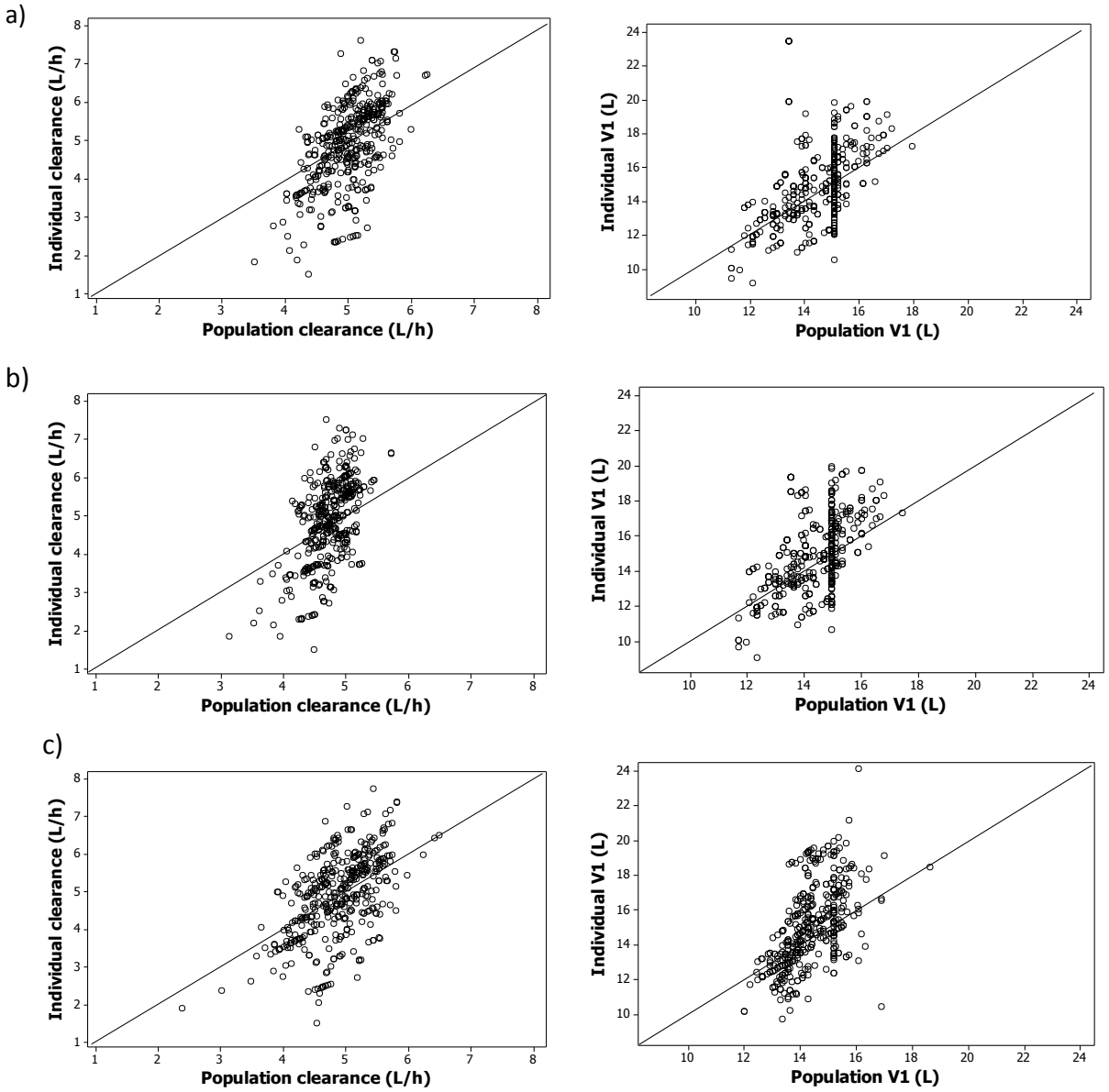
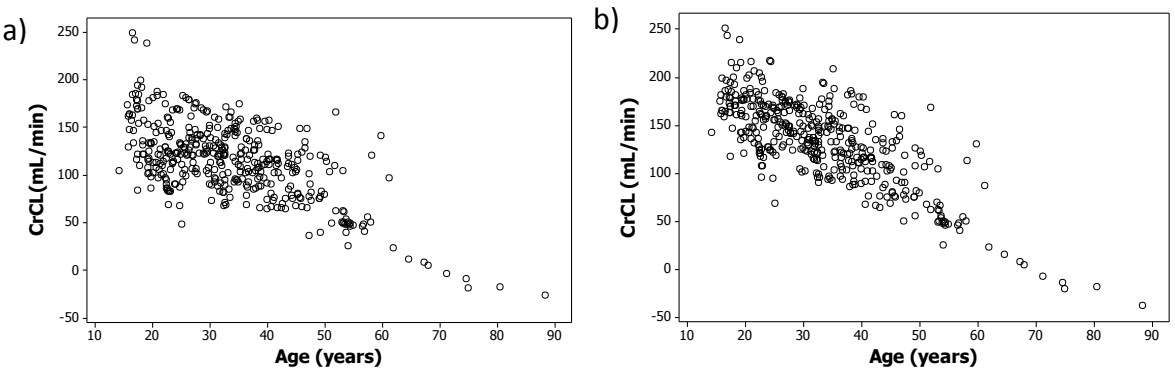


Figure 4.9 Scatter plot for creatinine clearance estimated using the mechanistic approach by parameter estimates generated from the model using (a) height and (b) allometric scale versus age.



Tables 4.6 and 4.7 show the results for bias calculations using the evaluated models. The empirical model and the mechanistic model with the allometric scale produced unbiased predictions for clearance with similar imprecision of 0.5 L/h. However, the mechanistic model with height produced biased clearance predictions, $P < 0.05$, but with low imprecision of 0.6 L/h. All three models produced biased V_1 predictions, with the empirical model being the lowest, and comparable imprecision of 1 L. Predicted population concentrations from the mechanistic approach with either height or an allometric scale of size were biased for peak and mid-dose concentrations, while accurate predictions were produced from the empirical model with comparable imprecision values around 2.7 and 0.6 mg/L. Unbiased trough concentrations were predicted using the mechanistic approach models compared with biased predictions from the empirical model with low imprecision value around 0.18 mg/L. Figures 4.10 and 4.11 shows the distribution of imprecision (absolute prediction error) arising from the evaluated models.

Table 4.6 Median prediction error calculation results for clearance and V_1 predictions when the different covariate models were used.

Model	Median Prediction error	95% Confidence interval
Clearance (n = 415 courses)		
Empirical model	-0.08 L/h	-0.16, 0.00
Mechanistic model with HT	0.15 L/h	0.06, 0.26*
Mechanistic model with allometric scale	0.04 L/h	-0.04, 0.13
V_1 (n = 415 courses)		
Empirical model	0.36 L	0.19, 0.52*
Mechanistic model with HT	0.44 L	0.28, 0.61*
Mechanistic model with allometric scale	0.53 L	0.33, 0.69*

***indicates p < 0.05.**

Table 4.7 Median prediction error calculation results for the population predicted concentration when the different covariate models were used divided by sampling time into peak, mid-dose and trough concentrations.

Model	Median Prediction error	95% Confidence interval
Peak conc = 544		
Empirical model	-0.06 mg/L	-0.42, 0.30
Mechanistic model with HT	-0.40 mg/L	-0.78, -0.04*
Mechanistic model with allometric scale	-0.50 mg/L	-0.86, -0.14*
Mid conc = 790		
Empirical model	-0.03 mg/L	-0.09, 0.04
Mechanistic model with HT	-0.25 mg/L	-0.33, -0.16*
Mechanistic model with allometric scale	-0.10 mg/L	-0.18, -0.03*
Trough conc =118		
Empirical model	0.06 mg/L	0.00, 0.11*
Mechanistic model with HT	-0.00 mg/L	-0.06, 0.06
Mechanistic model with allometric scale	0.04 mg/L	-0.01, 0.09

***indicates p < 0.05.**

Figure 4.10 Imprecision for clearance and V_1 predictions using the tested models presented as box plots for (1) empirical model, (2) mechanistic model with height and (3) mechanistic model with allometric scale size.

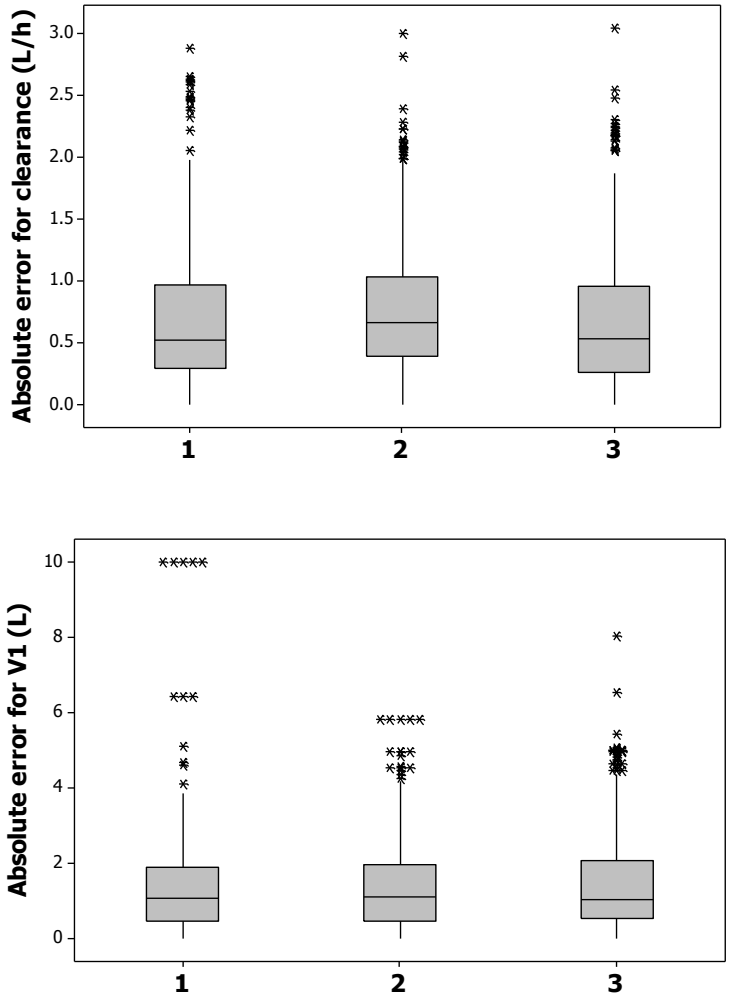


Figure 4.11 Imprecision for population concentration divided according to sampling time for (a) peak, (b) mid-dose and (c) trough concentrations, using the tested models presented as box plots for (1) empirical model, (2) mechanistic model with height and (3) mechanistic model with allometric scale size.

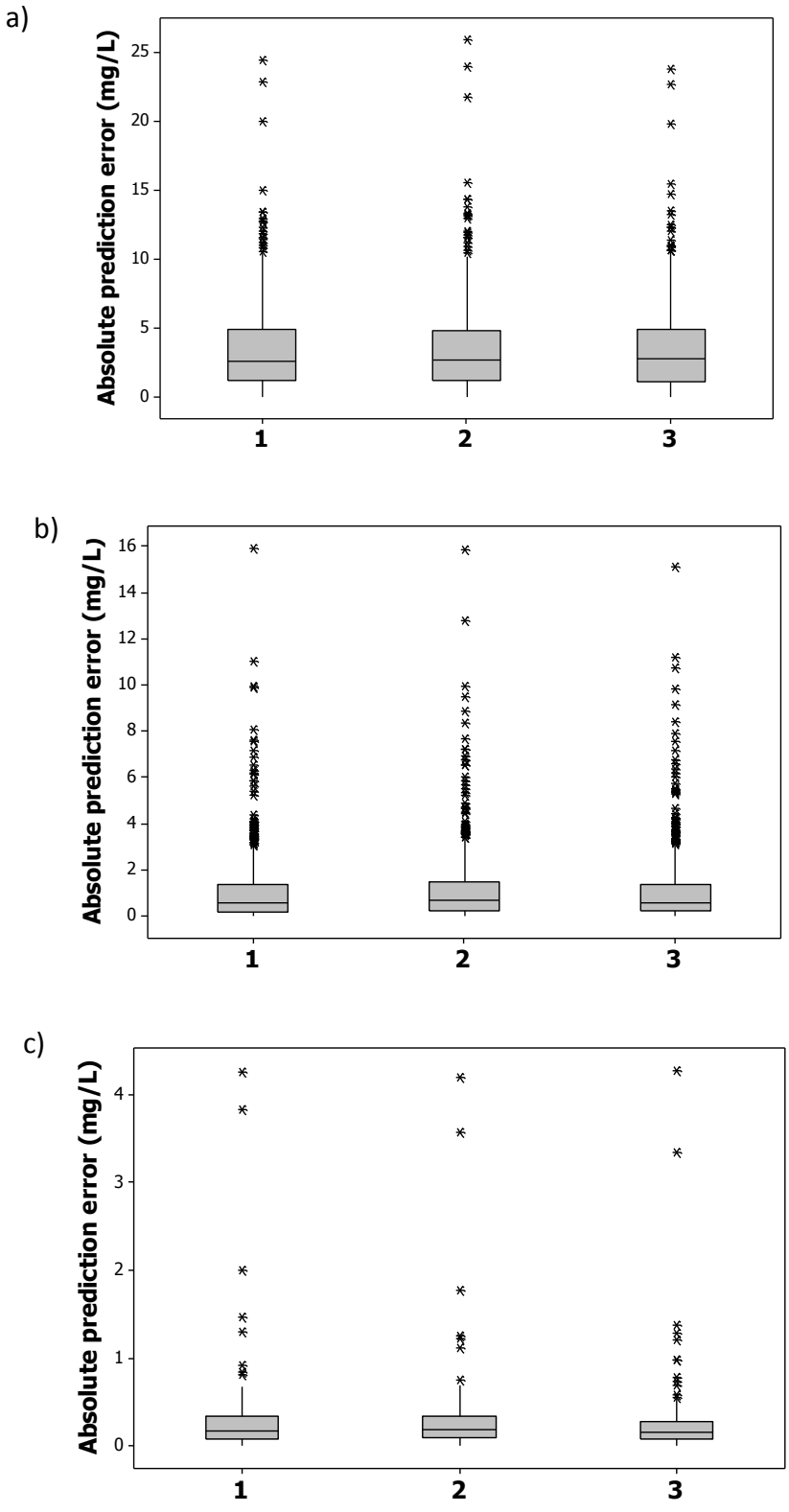
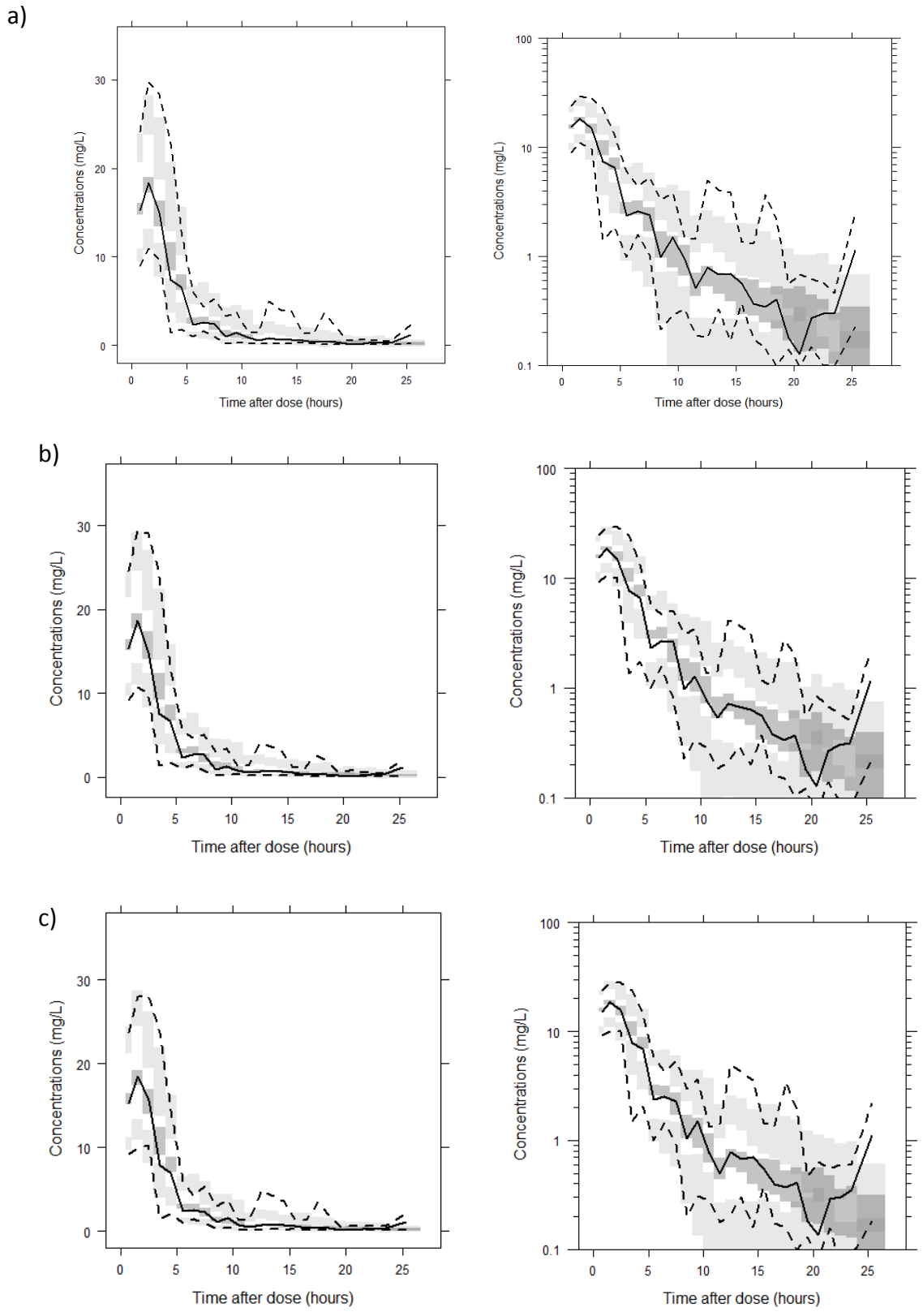


Figure 4.12 shows the results from pc VPC using The Hague dataset with the different evaluated models and indicated no model preference. The pc VPC indicated a tendency of the model to under-predict concentrations, particularly at 2 to 5 hours following the start of infusion. Similarly, there was no difference in the distribution of npde and the statistical tests were observed when the three models were evaluated, as shown in Table 4.8. The values of mean, skewness and kurtosis were close to zero and higher than 1 for the variances of the examined models. Although the Fisher variance test, Shapiro- Wilks test, and the global tests were significant, the quantile-quantile and histogram of npde plots in Figure 4.13 showed that the normality assumption was not rejected. The npde scatter plots against the independent variable (time after dose) was satisfactory and did not show any trend, whereas the npde versus predicted concentration plots showed a negative npde for concentrations higher than 30 mg/L. However, the histogram of npde with the density of the standard normal distribution overlaid indicated that more observations had value of 3 for the empirical model and the mechanistic approach using allometric scaling compared with the model using mechanistic approach with height.

Figure 4.12 Prediction corrected Visual Predictive Check using (a) empirical model, (b) mechanistic model with height and (c) mechanistic model with allometric scale weight in normal (left and log (right) scale.



Key: The black dotted lines are the 97.5th and 2.5th percentiles for the observed concentrations and correspond to 95% prediction interval. The black solid line represents the median of the observed concentrations. The shaded areas represent the binning and correspond to the 95% confidence intervals for the 97.5th, 50th and 2.5th percentiles of the simulated datasets.

Table 4.8 Results for the distribution of npde and statistical tests used to evaluate the normality assumption using The Hague dataset.

Npde test	Independent variable (Time after dose)		
	Empirical model	Mechanistic approach	
		With height	With allometric scale of weight
Distribution of npde			
Mean	0.007	-0.052	-0.034
Variance	2.30	2.16	2.16
Skewness	0.074	0.112	0.108
Kurtosis	-0.518	-0.440	-0.432
Statistical tests			
Wilcoxon signed rank test	0.914	0.082	0.211
Fisher variance test	0.001	0.001	0.001
Shapiro- Wilks test	< 0.001	< 0.001	< 0.001
Global test	0.001	0.001	0.001

Figure 4.13 The npde graphical output using (a) the empirical model and (b) the mechanistic approach with height (c) the mechanistic approach with allometric scale weight using The Hague dataset. Quantile-quantile plot of npde versus expected standard normal distribution ((upper left); Histogram of npde with density of overlaid standard normal distribution (upper right); Scatter plot of npde versus independent variable X (time after dose) (lower left); scatter plot of npde versus predicted Y (concentration) (lower right).

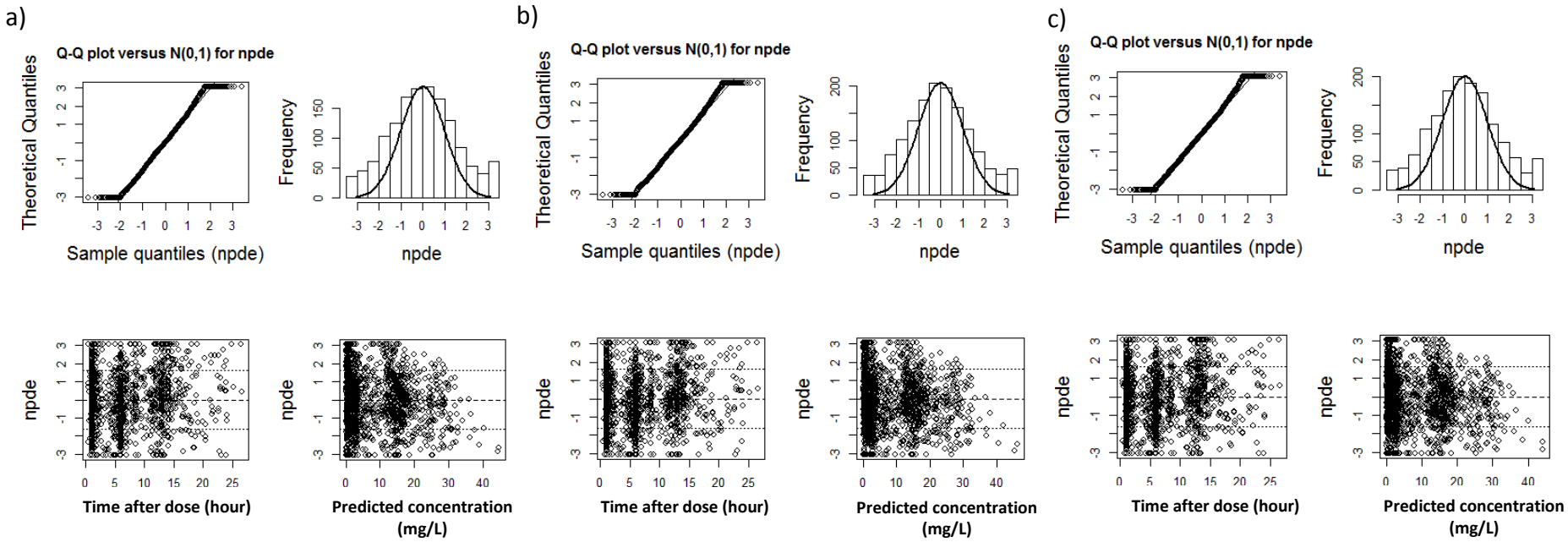


Table 4.9 shows the parameter estimates from the empirical model based on the Glasgow dataset and the estimates from the combined Glasgow and The Hague datasets. There was no difference between the parameter estimates obtained from the Glasgow dataset and when The Hague dataset was added. The residual error slightly increased when The Hague dataset was added.

Table 4.9 Summary the Glasgow compared with combing both Glasgow and The Hague datasets parameter estimates using the final empirical model.

Parameter	Glasgow dataset	Combined datasets
Θ_{HT} CL (L/h)	0.0285	0.0286
Θ_{renal} CL (L/h)	0.0114	0.0135
BOV (CL)	11.4	12.0
BSV (CL)	18.0	19.1
Θ_{V1} (L)	13.3	13.2
Θ_{HT} V_1	0.0113	0.0118
BSV (V_1)	11.6	12.4
V_2 (L)	6.62	6.68
Q (L/h)	0.452	0.583
Additive error (mg/L)	0.086	0.0872
Proportional error (%)	14.8	20.2

4.5 DISCUSSION

The results from various model evaluation methods confirmed the adequacy of the empirical model in describing the data and are encouraging to support further work with the model for clinical applications.

4.5.1 Bootstrap

Bootstrap was used to test the performance of the population model and its predictive accuracy. Bootstrap parameter estimates were found to be sensitive to the number of replicates and in order to obtain stable estimates the minimal recommended number of replicates is 1000 (Gastonguay MR and El-Tahtawy A, 2005, Efron and Tibshirani, 1993). Therefore, 1000 bootstrap samples were generated and the 95^{th} and 5^{th} percentiles were estimated. The results from bootstrapping confirmed that the parameter estimates values coincided well and were stable. In the current bootstrap, the results obtained using all replicates, regardless of their termination status, and only those replicates which terminated successfully, were examined. There was no difference in parameter estimates and confidence intervals obtained from both approaches. Holford *et al* (2006) recommended using all successful bootstrap samples and found that it provided accurate and precise description of NONMEM estimates, while Gastonguay *et al* (2005) found that minimisation status had minimal impact on bootstrap results. They recommended the use of all replicates regardless of their minimisation for the 95% confidence interval estimation. Bootstrapping has the advantage of evaluating the entire tested model dataset and is a useful technique for evaluating the stability of parameter estimates in a population pharmacokinetic model (Ette EI, 1997). However, it does not indicate whether the model adequately describes the data. Prediction-corrected visual predictive checks and normalised prediction distribution errors are more powerful because they provided a visual representation of the data and simulations.

4.5.2 Prediction-corrected visual predictive check

Bergstrand *et al* (2011) illustrated the usefulness for pc VPC methodology in a number of examples over the traditional VPC. In pc VPC, the dependent variable was subject to prediction correction before calculating the statistics. The aim of these corrections was to correct for differences arising from the independent variable, which would facilitate picking up model misspecifications in both parameter estimates and variabilities. In the present study, the pc VPC plots for the evaluated models did not show any obvious differences with the tendency of poor ability to predict concentrations at late time points, which might indicate a poor ability of the model to predict patients with renal impairment. In the Glasgow dataset, only one patient with renal impairment was included and hence little information was available on the performance of the model in patients with renal impairment. Therefore the developed model should not be used in patients with renal impairment. There was a gap in the pc VPC between 18 and 22 hours, which reflected a lack of concentrations at these time points, as shown in Figure 3.5.

4.5.3 Normalised prediction distribution errors

Another method used to evaluate the models was npde (Comets E et al., 2008). The recommended number of simulated datasets is 1000 (Comets E et al., 2008) and hence 1000 simulated datasets were generated in the present study. Although the normality assumption was rejected and the variance was significantly different from 1, the mean was not different from zero. These assumptions appeared to be rejected because of the large sample size in the present study. However, the npde plots for all examined models described the data well and did not show deviation from normality or model misspecification. The problem with failing the statistical tests despite having plots that look satisfactory is recognised as an issue when very large datasets are analysed (personal communication – from Emmanuelle Comets).

4.5.4 External validation

External evaluation is the most powerful method for testing the predictive ability of a model because it allows testing the developed model using an independent dataset with different patient characteristics. However, external evaluation was performed in only 7 % of published pharmacokinetic studies between 2002 to 2004 (Brendel K et al., 2007). Fortunately, a new dataset from patients with cystic fibrosis from the Netherlands was available for analysis.

Patients from The Hague were significantly bigger than the Glasgow patients who comprised the model development dataset. In addition, in The Hague dataset, one patient managed to live up to 88 years with cystic fibrosis. In general, patients with cystic fibrosis tended to have short life spans; the current median survival rate is 41.5 years old (95 confidence interval; 35.7, 46.0) in the UK (Cystic FibrosisTrust, 2013). However, this patient's long survival could be explained by having a "mild" *CFTR* genotype, as suggested by Mckone *et al* (2006). They classified the five cystic fibrosis mutations according to the mechanism by which they disrupt chloride channel function into two broader genetic risk groups, high-risk and low-risk groups, based on the effect of the mutation functional class on phenotype and mortality (McKone EF et al., 2003, McKone EF et al., 2006). They found that there was a difference in survival and median age at death. However, these differences in survival were not fully explained by clinical measures of lung function, nutrition, and pancreatic insufficiency, suggesting that the *CFTR* genotype was an independent predictor of survival. The authors concluded that patients having a milder *CFTR* genotype were more likely to have a less severe clinical course. In the Netherlands, *A455E* is the most common cystic fibrosis mutation (Kazazian HH, 1994). This mutation was found to be associated with less frequent *P.aeruginosa* colonisation and mild lung disease (Gan K-H et al., 1995, McKone EF et al., 2003), and could explain having a patient who managed to live with cystic fibrosis for 88 years. In contrast, 52.0 % of mutations in the UK are homozygous with the *ΔF508* mutation (Cystic FibrosisTrust, 2013), which is classified as a high-risk *CFTR* genotype group (McKone EF et al., 2006) and characterised by more severe clinical manifestations (Kerem E et al., 1990).

Bias and imprecision calculations indicated the accuracy and precision of the empirical model in predicting clearance, peak and mid-dose concentrations. However, the mechanistic model with either height or allometric scale of weight resulted in biased clearance and peak and mid-dose concentrations. Problems were encountered with the mechanistic model when applied to The Hague dataset. Since one of the age parameters for creatinine clearance was 70, negative estimates were obtained with older patients. This problem resulted in limited use of the mechanistic model for the Glasgow data only.

Results from the diagnostic plots, pc VPC and npde, failed to show model preference. The examined models had the tendency to under-predict concentrations obtained 2 to 5 hours after the start of infusion, which might be related to the absence of concentrations at these time points in the Glasgow dataset as shown in the concentration-versus-time profile. Checking the data showed clear outliers between the documented measured and predicted concentrations, which raised the issue for sampling or documentation errors. When the Glasgow and The Hague datasets were combined and the empirical model was used, no difference in the parameter estimates was identified with the exception of the residual errors. There was slight increase in the proportional error component, which could be related to the quality of data collected or difference in assay measurements.

The strength behind the current validation is the use of an independent dataset with comparable sample size with the model development datasets (165 vs 166 patients) and with multiple aminoglycoside courses of therapy. Few population pharmacokinetic studies have performed external validation using independent datasets. The majority of researchers perform bootstrapping and VPC and a minority use data splitting. All these model diagnostics involve using the model development dataset. However, there were some limitations for the current analysis that need to be considered when interpreting the results. Firstly, the data were from an external source and, unlike the development dataset, it was impossible to return to the raw data and check individual patient records. A number of assumptions had to be made about the dose and sample times, which might have introduced data errors and increased variability.

4.5.5 Conclusion

In conclusion, both internal and external validation methods aided in choosing the final model and showed good predictions of pharmacokinetic parameters and concentration measurements. However, despite the fact that the populations were very different, the empirical model fitted the data well. The mechanistic model was limited by the age parameter used to estimate renal function. The empirical model including height and creatinine clearance in CL and height in V_1 was chosen as the final model. The validation results encourage the use of the developed model for clinical applications.

CHAPTER 5: NONPARAMETRIC MODELLING OF AMINOGLYCOSIDE DATA FROM PATIENTS WITH CYSTIC FIBROSIS USING PMETRICS

5.1 INTRODUCTION

Pmetrics (Neely MN et al., 2012) is the new developed software by the Laboratory of Applied Pharmacokinetics at the University of Southern California (USC) in Los Angeles, California, and uses the nonparametric adaptive grid (NPAG) algorithm (Tatarinova T et al., 2013), which is a nonparametric methods to estimate pharmacokinetic parameters. The lab developed the first program in the early 1990s and it was known as the USC-PACK (Jelliffe RW, 1991), which used the nonparametric expectation maximization (NPEM) algorithm (Schumitzky A, 1991). Then further development was performed to include in addition to the NPEM algorithm the NPAG algorithm and renamed the MM-USCPACK. The NPEM algorithm was used to develop population pharmacokinetic model in patients with epilepsy treated with carbamazepine (Bondareva IB et al., 2006) and valporic acid (Bondareva IB et al., 2004), and tramadol in post-operative orthopaedics patients (Gan SH et al., 2004). However, the NPEM algorithm required a powerful computer and long time to obtain the results. Therefore, a new algorithm, NPAG, was developed by Leary *et al* (Leary RH et al., 2002, Tatarinova T et al., 2013). The NPAG algorithm required less computational time (Bustand A et al., 2006).

In this approach, no assumptions are made about the shape of the parameter distributions while in the parametric approach a normal or log-normal distribution of parameters is assumed. The advantages of using nonparametric methods was illustrated in a paper by Neely *et al* (2012). They showed a simulation example of a group of patients who included slow and rapid metabolisers and outlier. Although the nonparametric and parametric approaches were able to identify the true central tendency of the data, the nonparametric approach was able to identify the subpopulations and fitted the outlier in a small group of patients (50 subjects). In addition, the nonparametric approach produces discrete support points (one for each subject) where each point has a set of the model parameter estimates and its associated probability (Tatarinova T et al., 2013, Neely MN et al., 2012). The sum of all support points probabilities is one. The obtained support points would allow the use of multiple-model design of dosage regimen (Tatarinova T et al., 2013, Jelliffe R et al., 2000,

Bayard DS et al., 1994). However, a major disadvantage of nonparametric approach is the inability to estimate confidence limits (Neely MN et al., 2012).

5.2 AIMS

The aims of the present study were to develop a population pharmacokinetic model using nonparametric method with aminoglycoside data from Glasgow and The Hague and compare the results with those obtained from the parametric FOCE I method implemented in NONMEM.

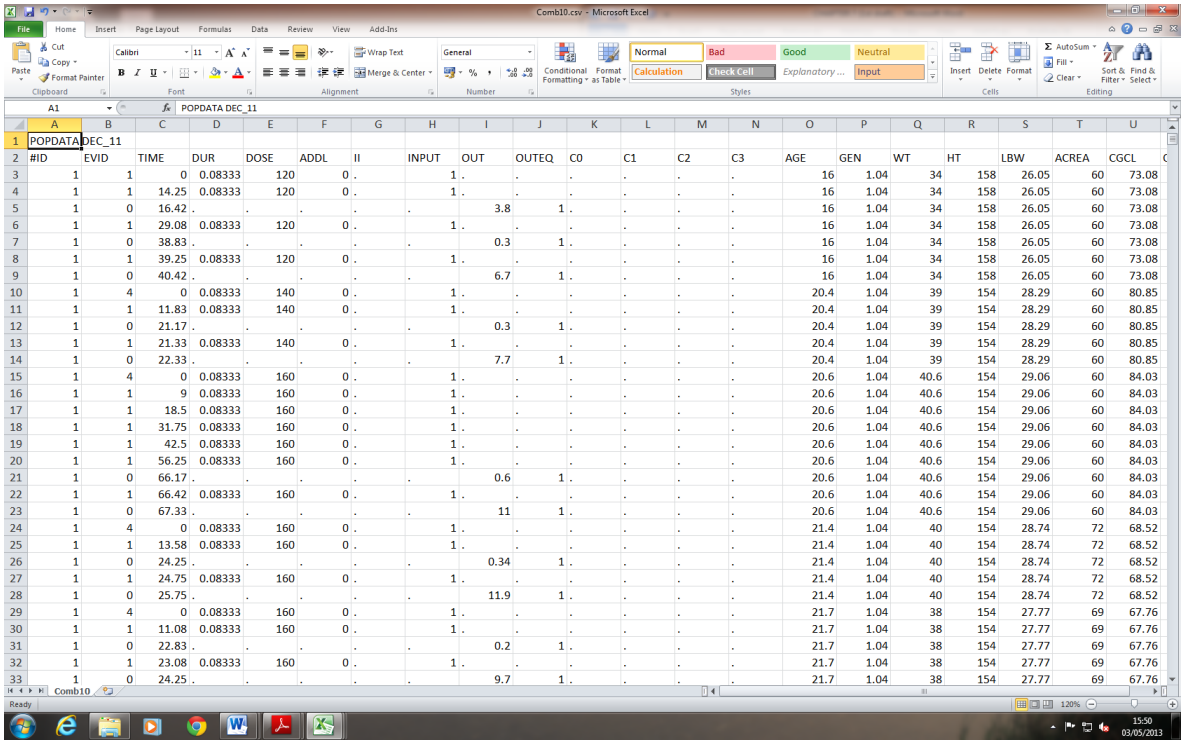
5.3 METHODS

The present analysis focused on population pharmacokinetic modelling using a nonparametric approach with the software Pmetrics (Neely MN et al., 2012). Pmetrics can be downloaded from the website of the Laboratory of Applied Pharmacokinetics, University of Southern California (http://www.lapk.org/) as a zip file then installed into R (R Core Team, 2012). In the current study, Pmetrics version 0.3 was used within R version 2.15.1 (R Core Team, 2012).

There were two algorithms used with Pmetrics to estimate pharmacokinetic parameters, one was the parametric iterative 2-stage Bayesian (IT2B) algorithm, and the other was the nonparametric algorithm, Nonparametric Adaptive Grid (NPAG) (Neely MN et al., 2012). The aim of the IT2B is to estimate initial ranges for the parameters, which can then be used in the NPAG run.

The data file was reformatted within an Excel spread-sheet to be suitable for Pmetrics analysis. The data file included the patients' dosage histories, measured concentrations (column name was "OUT"), and the covariates. An example of the Pmetrics data file used is shown in Figure 5.1.

Figure 5.1 Pmetrics data file.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	POPDATA DEC_11																				
2	#ID	EVID	TIME	DUR	DOSE	ADDL	II	INPUT	OUT	OUTEQ	C0	C1	C2	C3	AGE	GEN	WT	HT	LBW	ACREA	CGCL
3	1	1	0	0.08333	120	0	.	1	.	.	.	.	.	.	16	1.04	34	158	26.05	60	73.08
4	1	1	14.25	0.08333	120	0	.	1	.	.	.	.	.	.	16	1.04	34	158	26.05	60	73.08
5	1	0	16.42	.	.	.	.	.	3.8	1	.	.	.	.	16	1.04	34	158	26.05	60	73.08
6	1	1	29.08	0.08333	120	0	.	1	.	.	.	.	.	.	16	1.04	34	158	26.05	60	73.08
7	1	0	38.83	.	.	.	.	.	0.3	1	.	.	.	.	16	1.04	34	158	26.05	60	73.08
8	1	1	39.25	0.08333	120	0	.	1	.	.	.	.	.	.	16	1.04	34	158	26.05	60	73.08
9	1	0	40.42	.	.	.	.	.	6.7	1	.	.	.	.	16	1.04	34	158	26.05	60	73.08
10	1	4	0	0.08333	140	0	.	1	.	.	.	.	.	.	20.4	1.04	39	154	28.29	60	80.85
11	1	1	11.83	0.08333	140	0	.	1	.	.	.	.	.	.	20.4	1.04	39	154	28.29	60	80.85
12	1	0	21.17	.	.	.	.	.	0.3	1	.	.	.	.	20.4	1.04	39	154	28.29	60	80.85
13	1	1	21.33	0.08333	140	0	.	1	.	.	.	.	.	.	20.4	1.04	39	154	28.29	60	80.85
14	1	0	22.33	.	.	.	.	.	7.7	1	.	.	.	.	20.4	1.04	39	154	28.29	60	80.85
15	1	4	0	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
16	1	1	9	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
17	1	1	18.5	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
18	1	1	31.75	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
19	1	1	42.5	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
20	1	1	56.25	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
21	1	0	66.17	.	.	.	.	.	0.6	1	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
22	1	1	66.42	0.08333	160	0	.	1	.	.	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
23	1	0	67.33	.	.	.	.	.	11	1	.	.	.	.	20.6	1.04	40.6	154	29.06	60	84.03
24	1	4	0	0.08333	160	0	.	1	.	.	.	.	.	.	21.4	1.04	40	154	28.74	72	68.52
25	1	1	13.58	0.08333	160	0	.	1	.	.	.	.	.	.	21.4	1.04	40	154	28.74	72	68.52
26	1	0	24.25	.	.	.	.	.	0.34	1	.	.	.	.	21.4	1.04	40	154	28.74	72	68.52
27	1	1	24.75	0.08333	160	0	.	1	.	.	.	.	.	.	21.4	1.04	40	154	28.74	72	68.52
28	1	0	25.75	.	.	.	.	.	11.9	1	.	.	.	.	21.4	1.04	40	154	28.74	72	68.52
29	1	4	0	0.08333	160	0	.	1	.	.	.	.	.	.	21.7	1.04	38	154	27.77	69	67.76
30	1	1	11.08	0.08333	160	0	.	1	.	.	.	.	.	.	21.7	1.04	38	154	27.77	69	67.76
31	1	0	22.83	.	.	.	.	.	0.2	1	.	.	.	.	21.7	1.04	38	154	27.77	69	67.76
32	1	1	23.08	0.08333	160	0	.	1	.	.	.	.	.	.	21.7	1.04	38	154	27.77	69	67.76
33	1	0	24.25	.	.	.	.	.	9.7	1	.	.	.	.	21.7	1.04	38	154	27.77	69	67.76

Variabilities within Pmetrics can be separated into assay error and other environmental sources of variability. The program is set up to estimate the assay standard deviation using a polynomial model and hence the coefficients of the assay error polynomial for each concentration was required to be included in the data file (Neely MN et al., 2012). The program assay standard deviation polynomial model was as follows;

$$SD = C_0 + C_1 x\,(obs) + C_2(obs)^2 + C_3(obs)^3$$

Where SD is the assay standard deviation and C_0 to C_3 represent the coefficients of the polynomial for concentration. Obs represents the measured serum concentration, obs^2 is the concentration squared and obs^3 is the concentration cubed. If there is no available information for these coefficients, then the user can estimate them using the "ERRrun" script within R and before running NPAG. This approach was applied in the present study. In addition, other environmental sources of error, such as errors in preparation and administration of the drug and documentation errors of samples and doses were taken into account. The environmental error can be modelled using proportional or additive models by multiplying (gamma) or adding (lambda) the error to the assay error polynomial. In the

present study, both gamma and lambda terms were tested. The initial estimate was set at 5 for gamma or 0.4 (5 times C_0) for lambda. The script used to estimate the coefficients of the assay error polynomial within Pmetrics was as follows;

Library(Pmetrics)

then

ERRrun(model="1comp.txt", data="Comb10.csv", instr = "PMCom2instr1")

ERRrun	To run assay error module
model	Name of the control file
data	Name of the data file
instr	Name of the instruction file

A model file was also required to run Pmetrics, which consists with a series of "blocks". The default setting in Pmetrics has the primary parameters as rate constants and volume of distribution. For the current analysis, the primary variables were parameterised as CL and V for the one compartment model and CL, V_1, V_2 and Q for the two compartment model. To achieve this, the rate constants were re-parameterised within the "secondary variables" block as follows: $K_e=CL/V_1$; $K_{12}=Q/V_1$; $K_{21}=Q/V_2$. Examples of the default control file and the modified model control file used in the current study are shown in Figure 5.2 (a, b) to illustrate the difference.

Figure 5.2 An example of Pmetrics model file using (a) the default setting and (b) when the elimination rate constant for a one compartment model was re-parameterised to CL and V.

a)

```
#Pri
Ke
V

#Cov
AGE
GEN
WT
HT
LBW
ACREA
CGCL

#Sec


#Out
Y(1)=X(1)/V
```

b)

```
#Pri
CL
V

#Cov
AGE
GEN
WT
HT
LBW
ACREA
CGCL

#Sec
Ke=CL/V

#Out
Y(1)=X(1)/V
```

In addition, to run iterative 2-stage Bayesian (IT2B) or NPAG an instruction file is required. In case a file was not available, before running IT2B or NPAG the user need to answer questions and supply some necessary information. These instructions were saved as text file and were used to run other models. The instruction file was modified as required. Before running NPAG, the IT2B method, which is a parametric algorithm, was used to estimate initial ranges of parameter values to be used in NPAG run. The scripts used to run IT2B and NPAG after loading Pmetrics were as follows:

Library(Pmetrics)

then

ITrun(model="1comp.txt", data="Comb10.csv", instr=-99)

ITrun	To run IT2B
model	Name of the control file
data	Name of the data file

instr	Name of the instruction file, if available

NPrun(model="1comp.txt", data="Comb10.csv", instr ="PMCom2instr1")

NPrun	To run NPAG
model	Name of the control file
data	Name of the data file
instr	Name of the instruction file, if available

5.3.1 Identification of structural model

The datasets from Glasgow and The Hague had previously been analysed using a traditional parametric population modelling approach using NONMEM with the FOCE algorithm (version 7) (Beal SL et al., 2009), as described in Chapters 3 and 4. These combined datasets were used to develop the model using Pmetrics with the NPAG algorithm. Both one and two compartment models were examined.

5.3.2 Identification of covariate model

The influence of covariates was investigated using the NPAG algorithm. Several covariates were examined for their influence on the model fit, including demographic and biomedical data, such as weight, height and serum creatinine. In addition, derived covariates were examined, including lean body weight (Janmahasatian S et al., 2005), body surface area (Mosteller RD, 1987) and creatinine clearance (Cockcroft DW and Gault MH, 1976). Serum creatinine concentrations less than 60 µmol/L was set to 60 µmol/L for the estimation of creatinine clearance (Rosario MC et al., 1998, Duffull SB et al., 1997). The median covariate values from the combined data (Glasgow and The Hague) were used to normalise the models. These covariates were modelled using both the empirical approach and the mechanistic approach described by Matthews *et al* (2004) and Anderson *et al* (2009). In addition, clearance estimates using age with a cut off at 18 years old, and the influence of

gender and different populations (Glasgow and The Hague) were examined as categorical variables.

The program was set up to perform a stepwise linear regression analysis to examine relationships between covariates and Bayesian posterior parameters using the "step ()" function in the statistics package R (R Core Team, 2012). The relationships between each covariate and parameter in the model were tested in a step-wise multivariate linear regression with forwards inclusion and backwards elimination. This analysis would generate a P value for the relationship of covariates to Bayesian posterior parameter value. A value of "NA" would indicate that the variable was not retained in the final model.

An improvement in model fit was based on an increase in the likelihood (a reduction in the -2 log likelihood value) with improvement in model bias and precision values for the individual predicted concentrations. Bias was determined as the mean prediction error of observation subtracted from individual predicted concentration. Bias was then assessed by comparing mean prediction errors with zero using the Student's t test with statistical significance set at $p < 0.05$. The 95% confidence interval of the difference was also examined using Minitab Version 15 (Minitab Ltd.). Imprecision in the observed compared to the predicted concentration was evaluated using the root mean squared error of the prediction error. In addition, plots of the measured against the predicted concentrations and the residual error were examined for any improvement in model fit. The model with the highest likelihood and lowest bias and imprecision was chosen to be the final model.

5.3.3 Comparison of Pmetrics with NONMEM results

Equivalent models were compared between Pmetrics and NONMEM. The parameter estimates and the measured versus predicted concentrations were compared for both the base two compartment model without within-subject variability and the final covariate model without within-subject variability. Bias and imprecision of Pmetrics and NONMEM individual predicted concentrations were also compared.

5.4 RESULTS

5.4.1 Description of the data file

Table 5.1 shows a summary of the Glasgow and The Hague patients' characteristics. The full dataset included 331 patients with 1490 courses of aminoglycoside therapy (for more details refer to Chapters 3 and 4). In brief, Glasgow patients were younger and smaller in size compared to patients from The Hague, and both groups had good renal function. In total, the full dataset included 3690 aminoglycoside concentration measurements, where 44% of the measured concentrations were peak, 23 % were mid samples and 33 % were trough concentrations.

Table 5.1 Summary of patient characteristics in the Glasgow and The Hague datasets.

Patient characteristics	Glasgow data (n = 166 patients)		The Hague data (n = 165 patients)	
	Median	Range	Median	Range
Age (years)	23	14 - 66	32	14 – 88
Weight (kg)	50	30 - 86	60	35 – 108
Height (cm)	163	139 – 191	174	150 – 194
Serum creatinine (µmol/L)	71	29 – 203	70	19 – 209
Creatinine clearance* (mL/min)	92	35 – 181	104	26 – 174

Key: *Creatinine clearance estimated by the Cockcroft and Gault equation (Cockcroft DW and Gault MH, 1976) with the lowest serum creatinine value fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998).

5.4.2 Identification of the structural model

The IT2B run converged successfully for both one and two compartment base model with a wide range of parameters initial ranges as shown in Table 5.2. However, NPAG would not run with these initial ranges. Therefore, the initial ranges of the parameters were obtained from the results of the NONMEM analysis performed in Chapter 3. The two compartment model was superior to the one compartment model with an improvement in the negative log likelihood value from 9618 to 9004. In addition, although both models produced

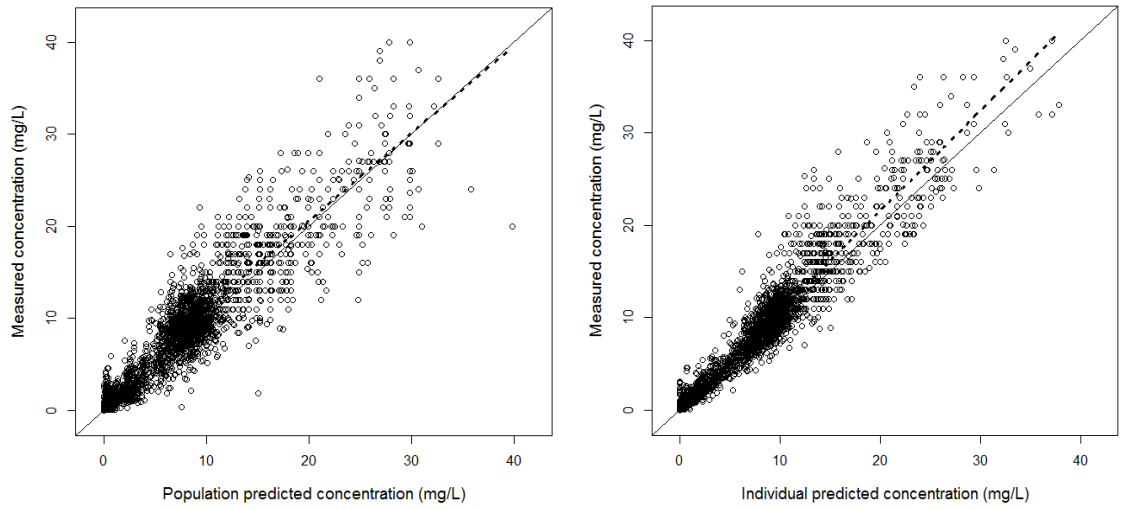
significantly biased predictions, bias and imprecision in predictions were lower for the two compartment model (0.29 and 1.48 mg/L vs 0.35 and 1.54 mg/L). The inclusion of proportional error gave a better the model fit compared with additive error (9004 vs 9049) and with value of 1.11. There was also a slight improvement in the measured versus predicted concentration where the smooth line of the data was closer to the line of unity for the two compartment compared with one compartment model as shown in Figure 5.3. Although the residual error plots shown in Figure 5.4 for one and two compartment model were similar, the mean weighted prediction error for the two compartment model was comparable to the one compartment model (0.16 vs 0.20 mg/L). The residual error plot did not indicate any trend with the errors all were within the range -4 to 4 and there were few outliers with errors above -4. There were 42 and 46 support points for the population joint parameters generated from the one and two compartment base models, respectively. Each support point had a value for each parameter and its probability. The highest probably was 0.122 with clearance and volume of distribution values of 4.56 L/h and 13.7 L using the one compartment model. For the two compartment model, the highest probability was 0.105 and was for clearance, V_1, $V_2$and Q values of 4.59 L/h, 13.2 L, 7.99 L and 0.3 L/h.

Table 5.2 The initial parameters ranges for one and two compartment base model following IT2B.

Parameter	One compartment	Two compartment
Clearance (L/h)	1×10^{-8} - 11.1	1×10^{-8} – 10.9
V_1 (L)	1.32 - 29.5	1×10^{-8} – 28.9
Q (L/h)	-	1×10^{-8} – 2.32
V_2 (L)	-	1×10^{-8} – 33.5

Figure 5.3 Scatter plots of measured versus population and individual predicted concentrations from the one (a) and two (b) compartment base models using Pmetrics. The black solid line is the line of unity and the black dashed line is a smooth line through the data.

a)



b)

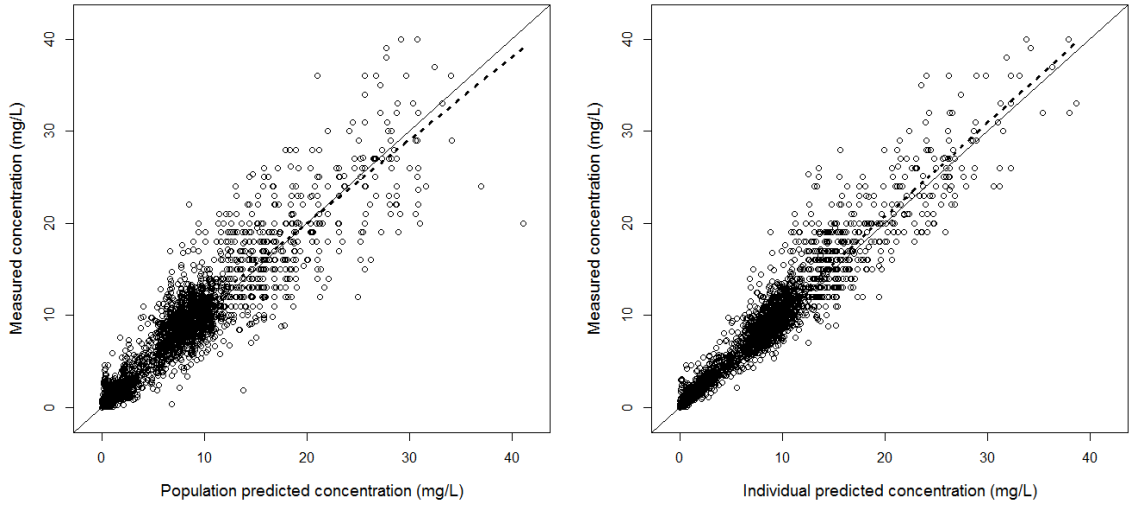
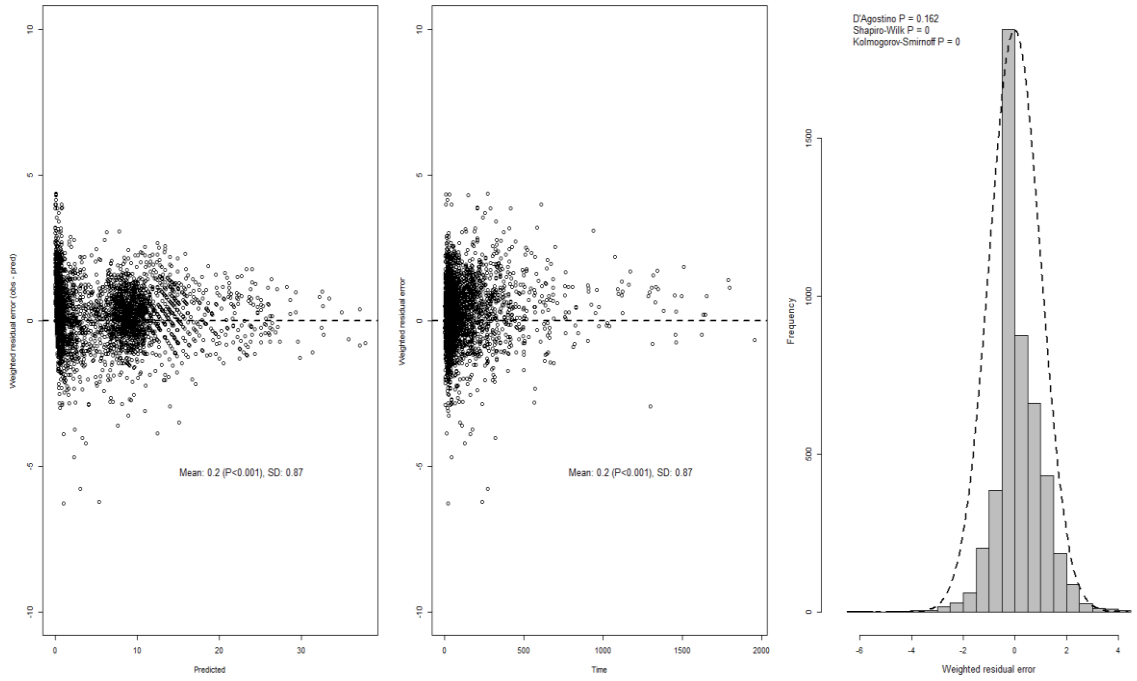


Figure 5.4 The residual error plot for the (a) one and (b) two compartment base models. Panels 1 and 2 show the weighted residual error versus the individual predicted concentrations and time. Panel 3 shows the distribution of the weighted residual errors.

a)



b)

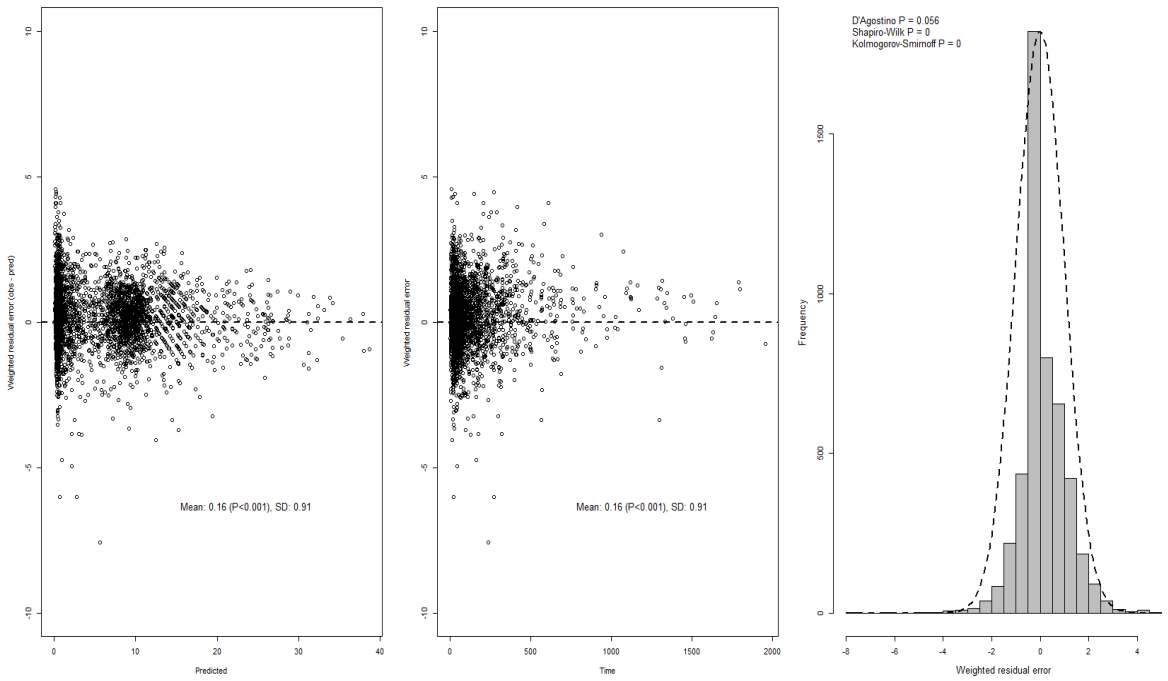


Figure 5.5 shows the distribution of the two compartment model parameter estimates following the NPAG run in Pmetrics. There was a wide range of clearance estimates with high probability for a value of 4.5 L/h, but few patients had clearance less than 3 L/h. On the other hand, V_1 ranges were narrower with the highest probability value being around 13.5 L. There were a few outliers with low (less than 10 L) and high (greater than 20 L) V_1 estimates. There were clearly two groups of Q estimates, one with low (0.3 L/h) and another with high (0.5 L/h) estimates. Similarly, V_2 estimates were grouped with low (6 L) and high (8 L) values.

Figure 5.5 The probability distribution of the two compartment model parameters following NPAG run in Pmetrics.

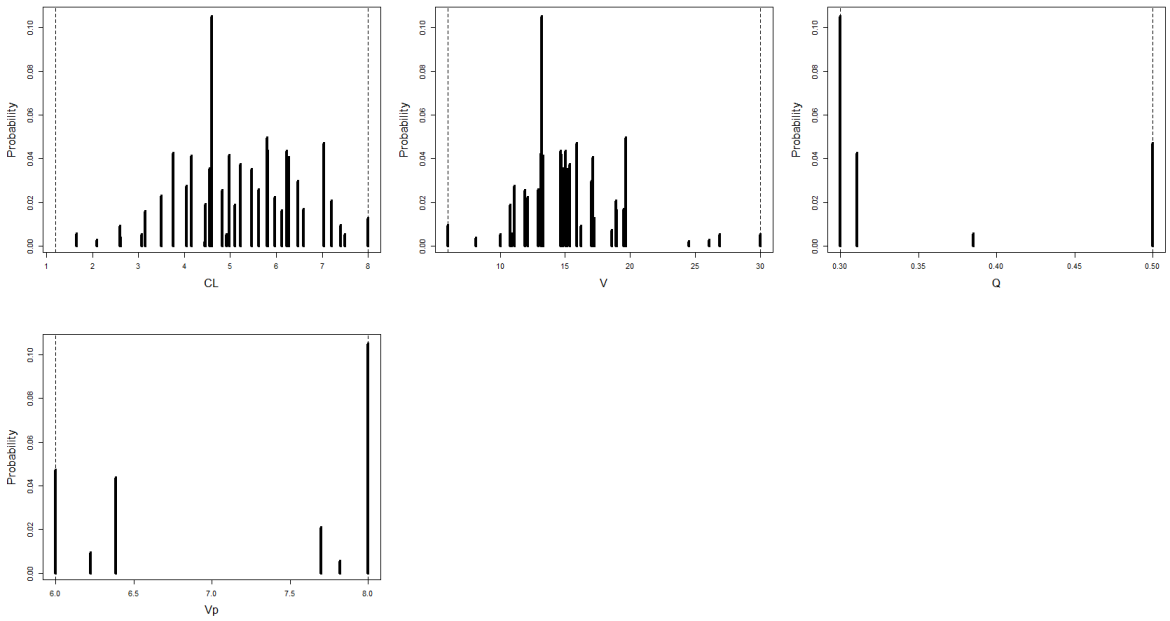


Table 5.3 shows the parameter estimates following the Pmetrics run compared with the NONMEM run results for the base model. Parameter estimates following NPAG are shown as mean and median, and they had similar values. Parameter estimates and variabilities from the Pmetrics analysis were close to the NONMEM values. However, the mean clearance estimate obtained from Pmetrics was slightly higher (5.24 L/h) compared with the NONMEM estimate (4.85 L/h). Between-subject variability in clearance was close for both approaches (23 % for Pmetrics and 25 % for NONMEM), but slightly higher for variability in V_1 estimated from Pmetrics (21 % for Pmetrics and 15 % for NONMEM). On the other hand, variabilities in Q (79 % and 21 %) and V_2 (59 % and 13 %) were higher for NONMEM

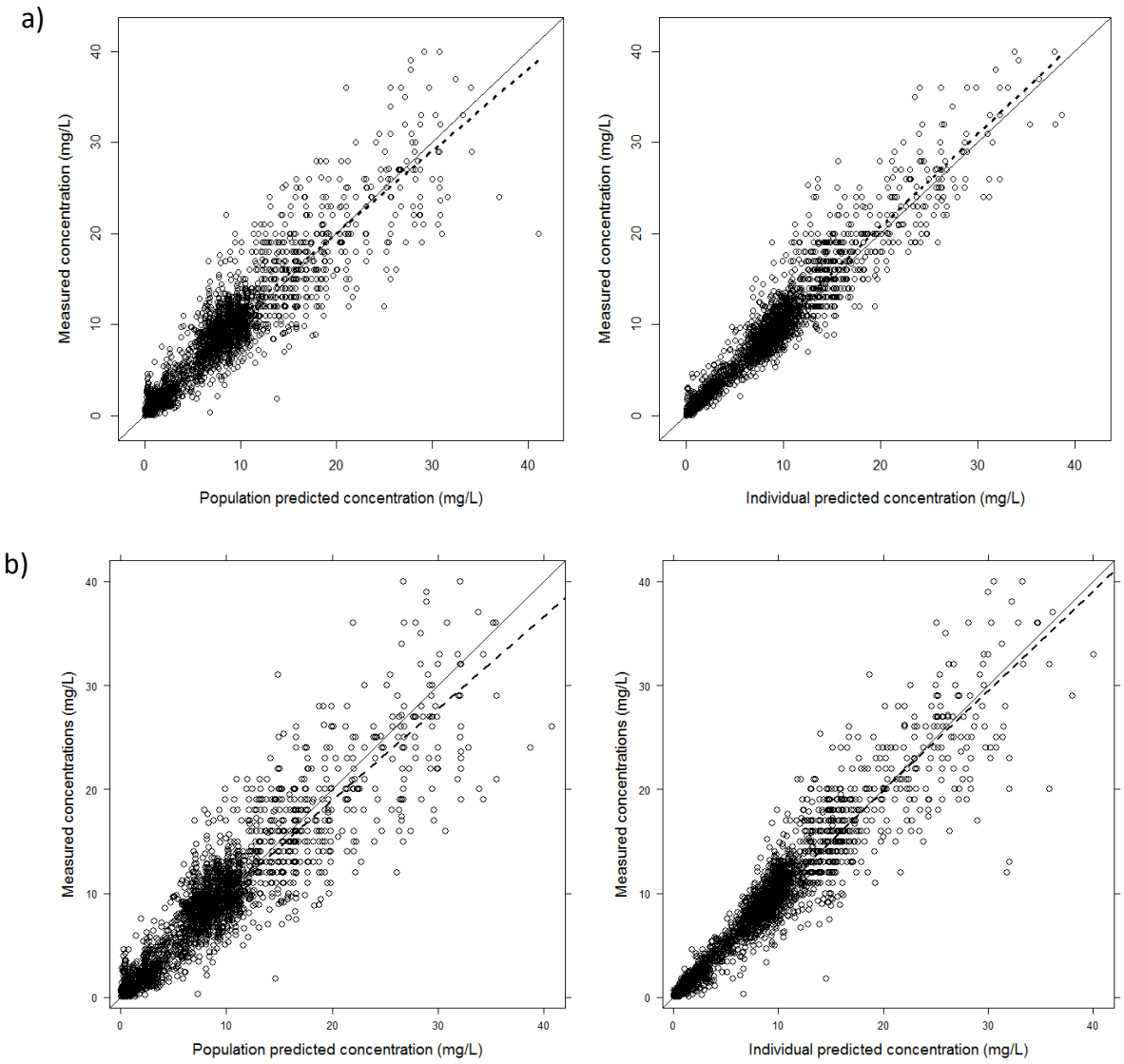
compared with Pmetrics. The measured versus predicted concentrations from Pmetrics and NONMEM were also very close. However, predictions generated by Pmetrics were closer to the line of unity, as shown in Figure 5.6.

Table 5.3 Parameter estimates obtained using Pmetrics and NONMEM for the two compartment base model.

Parameter	Pmetrics			NONMEM	
	Mean	Median	BSV (CV %)	Typical value	BSV (CV %)
CL (L/h)	5.24	5.22	23 %	4.85	25 %
V_1 (L)	14.8	14.7	21 %	14.1	15 %
Q (L/h)	0.434	0.499	21 %	0.596	79 %
V_2 (L)	7.08	7.99	13.8 %	7.18	59 %

Key: BSV= between subject-variability

Figure 5.6 Scatter plot of measured versus population and individual predicted concentration from base two compartment model using (a) Pmetrics and (b) NONMEM. The black solid line is the line of unity and the black dashed line is a smooth line.



5.4.3 Covariate model

The stepwise covariate parameter regression analysis was performed for the two compartment base model and the results are shown in Table 5.4. This analysis suggested weight, height and creatinine clearance to be potential covariates for clearance. For V_1, gender and creatinine clearance were suggested as potential covariates. For Q, the only potential covariate was creatinine clearance whereas for V_2 gender was the potential covariate.

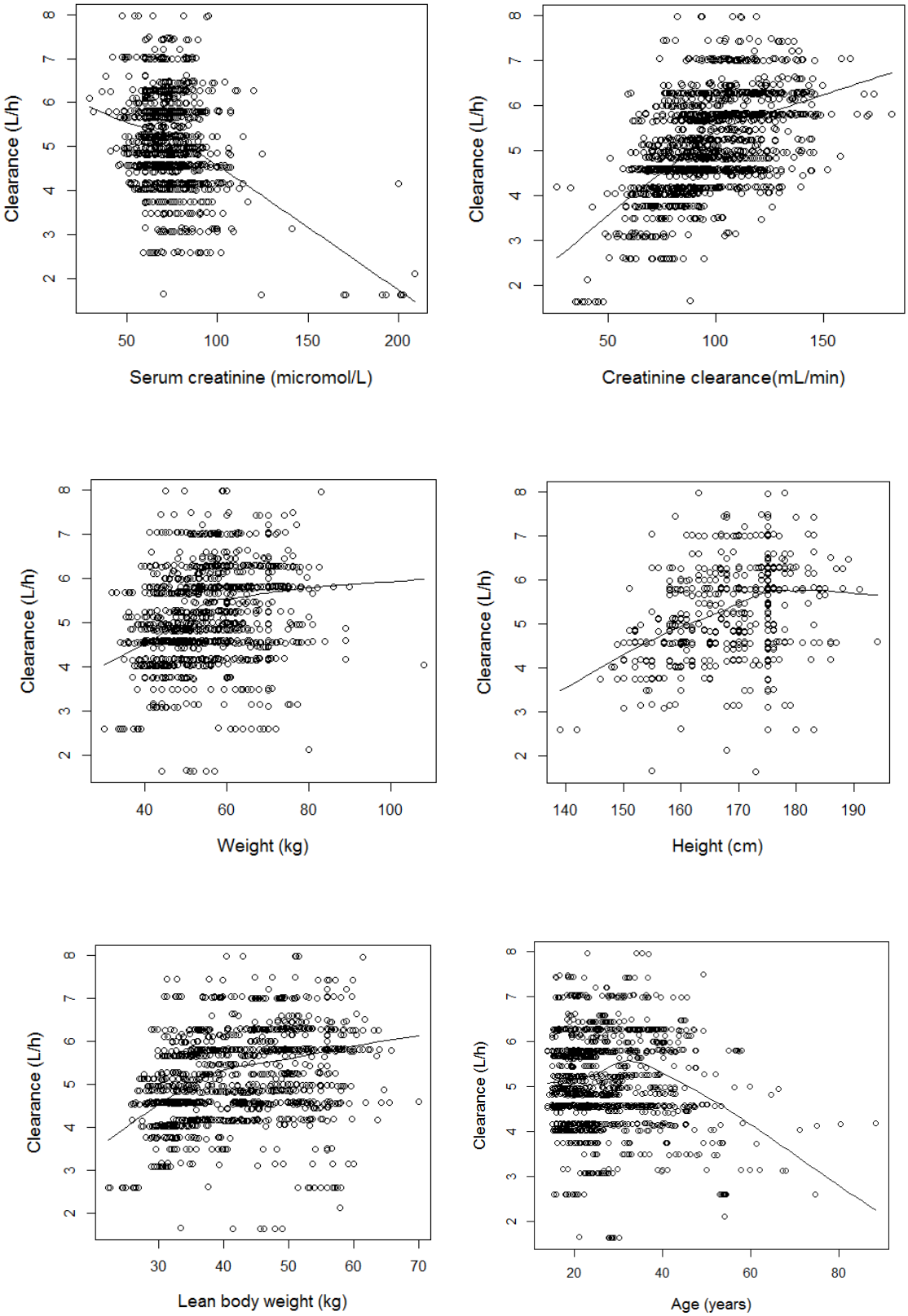
Table 5.4 The multi-variate P values for the relationship between pharmacokinetic parameter and covariates using the stepwise linear regression analysis for two compartment base model.

Covariate	CL	V_1	Q	V_2
Age (years)	NA	NA	NA	NA
Gender	NA	0.0005	NA	0.028
Weight (Kg)	0.016	NA	NA	NA
Height (cm)	0.036	NA	NA	NA
Lean body weight (kg)	NA	NA	NA	NA
Serum creatinine (µmol/L)	NA	0.005	NA	NA
Creatinine clearance (mL/min)	< 0.001	< 0.001	< 0.001	0.06

Key: NA = not applicable and indicates that the variable was not retained in the final model.

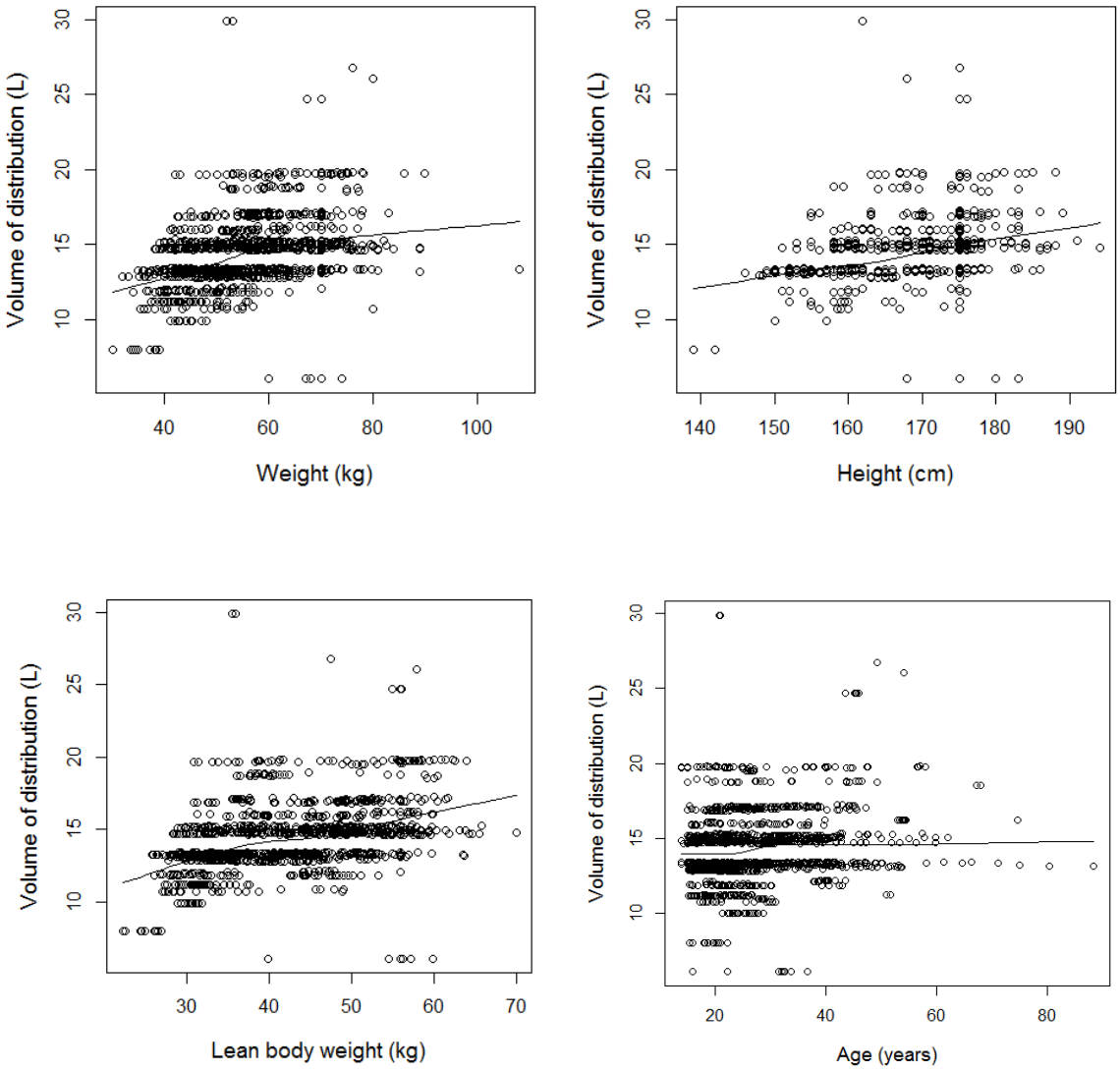
Scatter plots of parameters against covariates are shown in Figures 5.7 and 5.8. A clear relationship was observed between clearance versus creatinine clearance, and clearance versus height compared with clearance versus weight and lean body weight. The scatter plot of clearance and age showed that there was no relationship between clearance and patients younger than 40 years old, whereas a negative relationship started to appear after 40 years of age. On the other hand, plots of V_1 versus covariates showed a slight trend with weight, height and lean body weight and no trend with age. These plots showed clearly a separation of V_1 estimate into two groups. In contrast, scatter plots of Q and V_2 did not show any trend with covariates.

Figure 5.7 Scatter plots of clearance versus covariates. The black solid line is a smooth line.



Key: Creatinine clearance estimated by the Cockcroft and Gault equation (Cockcroft DW and Gault MH, 1976) with the lowest serum creatinine value fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998).

Figure 5.8 Scatter plots of volume of distribution of the central compartment versus covariates. The black solid line is a smooth line.



The inclusion of age as categorical variable with a cut off at 18 years old resulted in an improved log likelihood, but there was no difference in clearance estimates between the groups (median clearance: 5.2 and 5.1 L/h). These findings were consistent with the NONMEM results reported in Chapter 3. Similarly, clearance estimates for patients from Glasgow and The Hague were similar (median clearance: 5.14 and 5.07 L/h). Gender was modelled as categorical variable where males and females had different V_1 estimates; however, the model did not improve the fit; there was no change in log likelihood value (9004) or model prediction bias and imprecision (0.28 and 1.47 mg/L). On the other hand, when the estimate of V_1 was divided into two groups based on population (Glasgow or The

Hague patients), the log likelihood improved (from 9004 to 8730) but the parameter estimate distributions and mean values were similar (14.5 L for Glasgow and 14.9 L for The Hague patients).

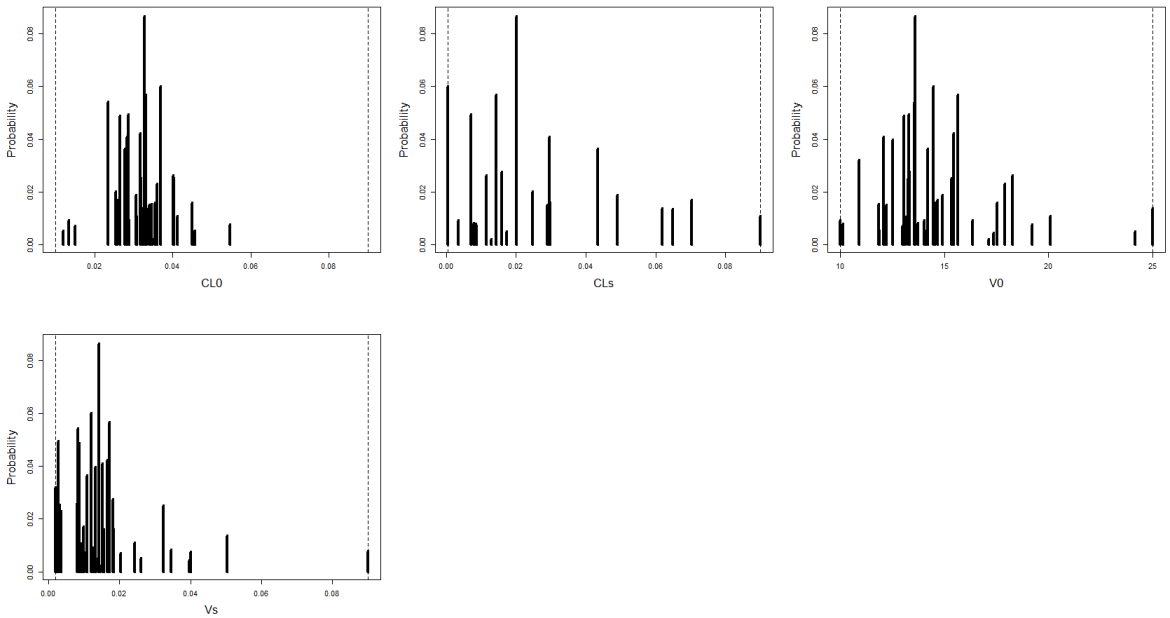
Table 5.5 shows the covariate model building. Including weight as covariate in both clearance and V_1 improved the log likelihood value (from 9004 to 8989), but it increased concentration prediction bias from 0.29 to 0.32 and imprecision from 1.48 to 1.56. In contrast, including lean body weight resulted in a worse log likelihood function (from 9004 to 9061) and the imprecision increased from 1.48 to 1.51. Including body surface area improved the model fit from log likelihood 9004 to 8675. Although the mechanistic model using the creatinine clearance parameter estimates reported in Matthews *et al* (2004) paper improved the log likelihood with a value of 8789, between-subject variability in clearance and V_1 increased to 33 and 34 %. Therefore, creatinine clearance parameters were estimated, but the run did not converged despite reaching the maximum number of cycles allowed in Pmetrics or ended with a hessian error. The best model fit with the lowest bias and imprecision was achieved when both height and creatinine clearance were included in the model for clearance and height in the model for V_1 (8568). Including creatinine clearance as covariate on Q improved the model fit, but with no improvement in model predictions based on bias and imprecision. However, there was a difference between the mean and median Q estimates (0.009 and 0.006 L/h) and the estimate of variability increased. Therefore, the values of Q and V_2 were fixed in the final model. There were 49 supports points generated from the final covariate model. The final model gamma value was small at 1.06. The distribution of the final model parameters is shown in Figure 5.9. The final model identified a mean clearance estimate of 4.98 L/h at the median height of 166 cm and median CrCL of 94 mL/min. The mean V_1 was 14.2 L and changed by 11% for every 10 cm difference from 166 cm.

Table 5.5 Summary of covariate models, likelihood values, parameter estimates and between-subject variabilities obtained using Pmetrics.

Likelihood	Clearance (L/h) Mean	BSV_{CL} %	V_1 (L) Mean	BSV_{V1} %
9004	5.24 (*0.06*)	23	14.8 (*0.17*)	21
9061	0.12 (*0.002*)x LBW + 0.01 (*0.001*) (CGCL- 94)	25	14.2 (*0.11*) (1+0.01 (*0.0002*) (LBW-40))	15
8989	0.09 (*0.001*)x WT + 0.01 (*0.0004*)(CGCL- 94)	24	13.8 (*0.13*)(1 + 0.02 (*0.001*) (WT - 53))	17
8789	5.35 (*0.09*)(LBW/70)$^{0.75}$ +1.35 (*0.04*)x (CrCL/7.26 L/h/70 kg x (LBW+0.211(Weight–LBW) /70)$^{0.75}$))	33	6.28 (*0.12*) (1+0.03 (*0.001*) (LBW+ (WT-LBW)/70))	34
8675	3.20 (*0.04*) x BSA + 0.01 (*0.001*) (CGCL- 94)	22	14.5 (*0.14*) (1+0.75 (*0.014*)(BSA - 1.6))	17
8568	0.03 (*0.0003*)x HT + 0.02(*0.001*) (CGCL- 94)	20	14.2 (*0.13*) (1 + 0.01 (*0.001*)(HT - 166))	17

Key: LBW= Lean body weight in kg (Janmahasatian S et al., 2005), CGCL= Creatinine clearance in mL/min estimated by the Cockcroft and Gault equation (1976) with the lowest serum creatinine value fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998), WT= Weight in kg, CrCL= creatinine clearance in L/h estimated using the mechanistic approach (Matthews et al., 2004, Anderson BJ and Holford NHG, 2009), BSA= Body surface area m^2 (Mosteller RD, 1987), HT= Height in cm. Standard errors of each parameter estimate are shown in italics.

Figure 5.9 The probability distribution of the final model parameters following an NPAG run in Pmetrics.



Key: CL_0= represents the intercept of clearance, CL_s= represent the slop of clearance, V_0= represents intercept of V_1, V_s= represents of V_1.

5.4.4 Comparison of results from Pmetrics and NONMEM

The covariates chosen for the final model were the same for Pmetrics and NONMEM. In addition, the parameter estimates obtained with Pmetrics and NONMEM were similar, as shown in Table 5.6. Although NONMEM produced lower and non-significant bias in predictions of individual concentrations (0.05mg/L; 95% confidence interval: -0.01, 0.10) compared with Pmetrics predictions (0.28 mg/L; 95% confidence interval: 0.24, 0.32), imprecision was higher for NONMEM at 1.89 mg/L compared to 1.48 mg/L with Pmetrics. Figure 5.10 shows scatter plots of the measured versus the predicted concentrations obtained from the Pmetrics final model and those obtained from NONMEM. Predictions obtained from Pmetrics were closer to the line of identity and with fewer outliers compared with NONMEM predictions. Figure 5.11 shows concentration predictions obtained from Pmetrics and NONMEM. The plots show good agreement in predictions obtained from Pmetrics and NONMEM and with few outliers where NONMEM had the tendency to over-predict concentration that was more pronounced at concentrations greater than 20 mg/L.

Table 5.6 Comparison of the final model parameter estimates obtained from Pmetrics and NONMEM.

Parameter	Pmetrics (mean values)	NONMEM
CL (L/h)	0.0311 x HT + 0.0184 x (CGCL - 94)	0.0287 x HT+ 0.0135 x (CGCL - 94)
V_1 (L)	14.2 x(1 + 0.0127 x (HT - 166))	13.9 x(1 + 0.0108 x(HT - 166))
Q (L/h)	0.600	0.602
V_2 (L)	8.00	5.79

Key: HT= Height in cm, CGCL= Creatinine clearance in mL/min estimated by the Cockcroft and Gault equation (1976) with the lowest serum creatinine value fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998).

Figure 5.10 Scatter plots of the measured versus population and individual predicted aminoglycoside concentrations using the final model from (a) Pmetrics (b) NONMEM. The dashed line is a smooth line of the data.

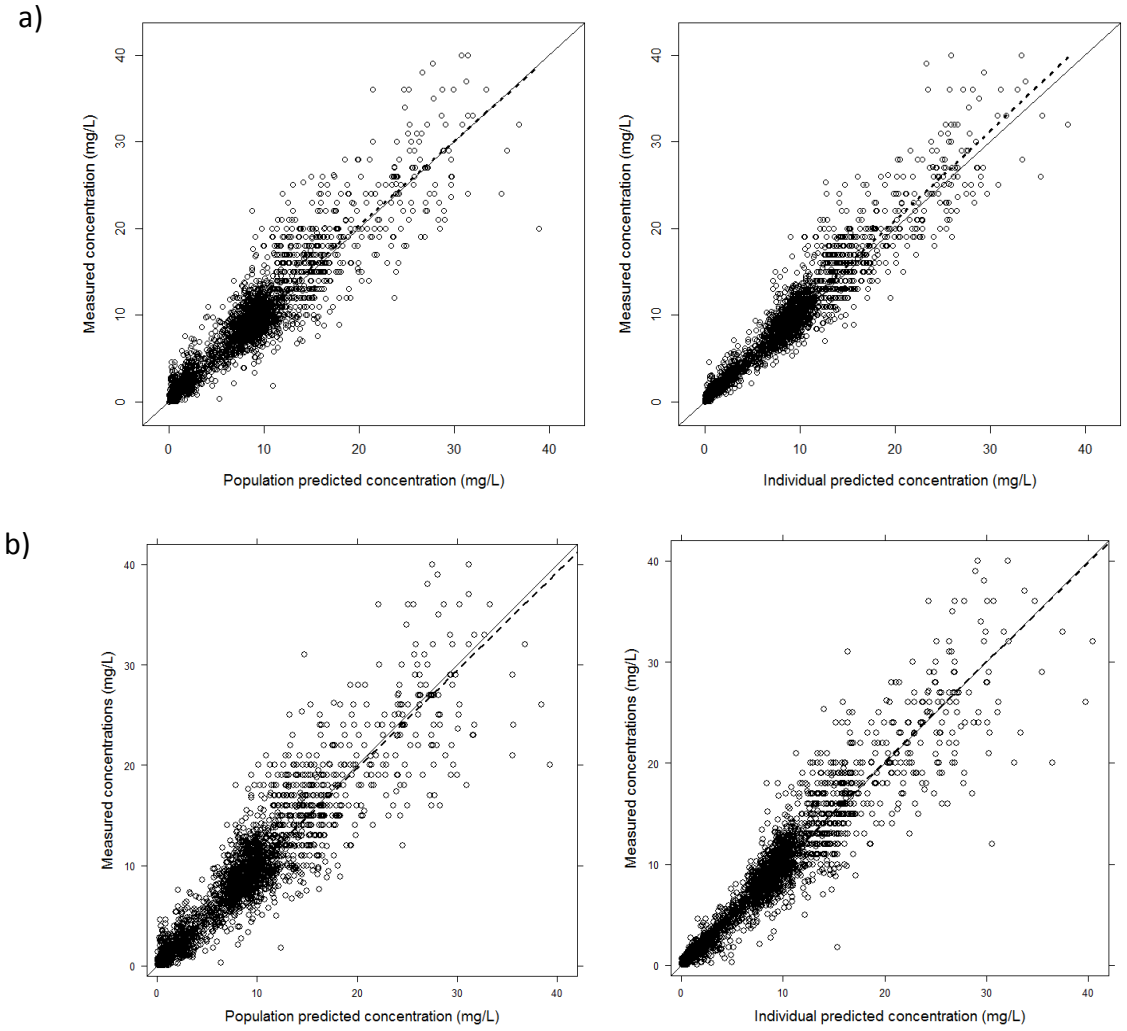
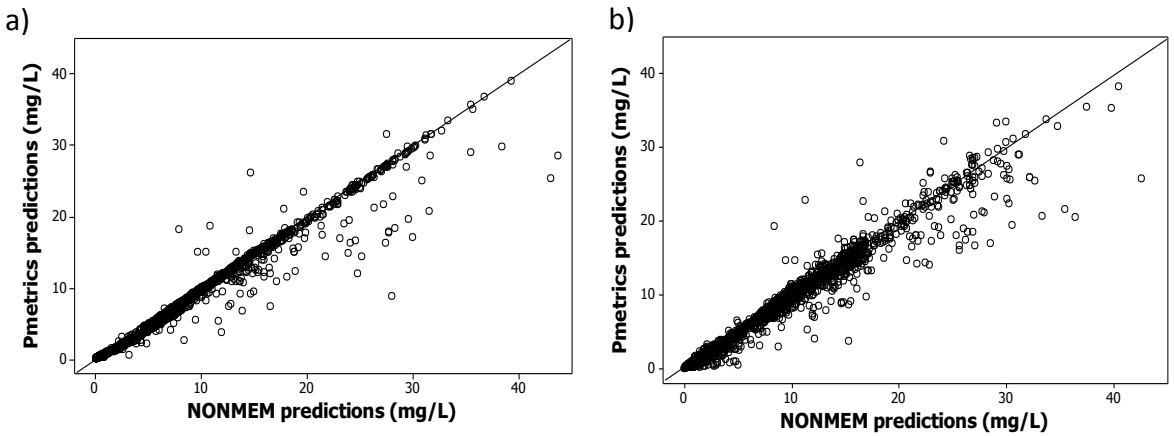


Figure 5.11 Scatter plots of Pmetrics versus NONMEM predicted (a) population and (b) individual predicted concetrations.



5.5 DISCUSSION

In the current study, population pharmacokinetic modelling was performed using the nonparametric approach implemented in Pmetrics (Neely MN et al., 2012) and compared with the results from parametric approach performed using NONMEM (Beal SL et al., 2009). Both approaches yielded similar final models, where height and creatinine clearance in clearance and height in V_1 model were the best descriptors. Parameter estimates were very close; however, clearance parameter estimates were slightly higher for Pmetrics. In addition, individual concentration predictions were more precise using the nonparametric approach.

In order to obtain the initial parameter estimates for the NPAG run, an IT2B was first used. However, NPAG would not run with these initial ranges, whereas the use of parameter ranges obtained by from previous NONMEM analysis resulted in successful run. This could be related to the wide range of parameter estimates obtained following the IT2B run compared with constraining a narrower range of the parameter when obtained from previous knowledge with NONMEM results. In addition, this reflected how sensitive NPAG is for the initial range estimates.

A two compartment model fitted the aminoglycoside data better than one compartment model, which is in agreement with NONMEM analysis performed previously in Chapter 3 and with other publications in this patient group (Aminimanizani A et al., 2002, Burkhardt O et al., 2006). Clearance estimates obtained from NPAG were higher compared with NONMEM but with similar between-subjects variability (23 and 25 %). V_1 estimates were similar using both approaches; however, the nonparametric approach produced higher variability in V_1 compared with the parametric approach (21 and 15 %). In addition, Q and V_2 estimates obtained from Pmetrics and NONMEM were similar, but variabilities were higher for NONMEM Q (79 % from NONMEM and 21 % from Pmetrics) and V_2 (59 % from NONMEM and 13 % from Pmetrics) compared with Pmetrics. The results showed that Pmetrics (NPAG) faced difficulty to estimate Q and V_2, which was reflected in the distribution of estimates and being divided into two district groups. This reflected the sparse

nature of data where majority of samples were peak (44 %) and trough (33 %) concentrations, which did not contain enough information to quantify this parameter.

The difference seen in clearance estimates and variabilities obtained from Pmetrics versus NONMEM could be related to the method of estimation, where the nonparametric approach does not assume a shape for parameter distribution and thus allowing the detection of subpopulations and outliers within the examined group of patients (Neely MN et al., 2012, Bustand A et al., 2006). Prémaud *et al* (2011) documented a similar finding when they compared the parametric and nonparametric approach using mycophenolic acid data from 34 paediatric renal transplant patients. They found that parameter estimates obtained from NPAG were higher than those obtained from the FOCE algorithm. In addition, Tatarinova *et al* (2013) illustrated the advantage of NPAG algorithm in detecting outliers in a simulation study of 35 infants who were administered zidovudine. They found that both NPAG and FOCE algorithms were able to find the same pharmacokinetic parameter typical values, but NPAG was able to estimate the true pharmacokinetic parameter distribution and detected two group of patients with slow and rapid drug clearance. The FOCE algorithm that is used in NONMEM was not able to find this true distribution and subsequently was not able to identify these two groups of patients.

Moreover, Carlsson and his colleagues (2009) developed a gabapentin population pharmacokinetic model in 16 adult patients with chronic neuropathic pain using parametric and nonparametric approaches, and found no difference in gabapentin parameter estimates obtained from the FOCE algorithm and the NPAG algorithm. In addition, Bustad *et al* (2006) found following their study in 16 adult patients treated with amikacin for urinary tract infection, that between-subject variability obtained from the parametric approach (IT2B) was narrower compared to the nonparametric approaches (NPEM and NPAG) suggesting that the narrow range of parameter estimates arose as a result of the normality assumption. They also performed another simulation study to evaluate the ability of the nonparametric approach to detect subpopulations. Twenty subjects were simulated with two groups, slow and rapid metabolises. They showed that the NPEM algorithm was able to

detect the two groups even without including covariates in the model. In addition, Bustad *et al* (2006) performed another simulation study for amikacin with between 25 to 800 simulated subjects to assess the consistency of parametric and nonparametric algorithms in estimating the true parameter values. They found that as the number of subjects increased, the estimated mean by NPAG algorithm was closer to the true parameter value. However, the mean value estimated by the IT2B and FOCE algorithms deviated from the true value as the number of simulated subjects increased. These results indicated that the NPAG algorithm had had consistent behaviour. In addition, their results showed that less bias and more precise concentration predictions was obtained by using the nonparametric method compared with the parametric method.

Measured versus predicted concentration plots from both Pmetrics and NONMEM were similar. Although bias was low for both algorithms, NONMEM produced non-significant predictions and hence was more accurate compared with Pmetrics. On the other hand, Pmetrics produced more precise individual concentration predictions. The current study finding was in agreement with Prémaud *et al* (2011) who found that more precise concentration predictions were obtained from the nonparametric (NPAG) approach compared with parametric (FOCE) approach.

The data included patients with a wide range of age (14 – 88 years old) and hence age was examined for its influence on aminoglycoside pharmacokinetics. However, the results showed no difference in clearance estimates for adolescents (less than 18 years old) and adults (greater than 18 years old). This was shown previously in NONMEM analysis performed in Chapter 3 and is consistent with the finding of VandenBussche *et al* (VandenBussche HL and Homnick DN, 2012). Although, scatter plots for V_1 against different body size measurements showed a possible two groups that could be related to gender effect, modelling gender did not improve the model fit. Although data from Glasgow and The Hague were combined, there was no difference in clearance and V_1 estimates. Including weight improved the model fit; however, bias and imprecision in concentration predictions increased. A similar observation was documented in NONMEM analysis in Chapter 3, where

weight worsened the model fit with an increase in OFV from 33 to 184 and an increase in between-subject variability from 23 % to 25 %. The best model fit was obtained when height and creatinine clearance were included into clearance and height into the V_1 model. Although weight slightly improved the model fit, height had a better model fit and produced lower bias and imprecision. On the other hand, including lean body weight worsened the model fit. A similar problem was observed in the previous NONMEM analysis conducted in Chapter 3. Including lean body weight worsened the model fit, OFV increased from 33 to 58, and between-subject variability increased from 23 % to 25 %. The second best model was when body surface area and creatinine clearance were included into clearance and body surface area in V_1 models with comparable bias and imprecision to the height model. However, similar model in NONMEM resulted in a worse model fit.

An important advantage of the parametric method is the ability to separate variabilities into between-subject, within-subject and residual variability whereas the nonparametric approach separated variabilities into assay error and environmental variability (Neely MN et al., 2012, Bustand A et al., 2006). A unique aspect of the available dataset was the availability of multiple courses of aminoglycoside therapy over 15 years and the influence on within-subject variability could be examined. This was examined by NONMEM in Chapter 3. However, modelling within-subject variability is not feasible in the current version of Pmetrics. This issue was discussed with the Pmetrics developer, who stated that future versions of Pmetrics may include this option. In the present study, the overall source of variability without the assay error was estimated in the form of gamma. A value of 1 suggests no other source of variability (or noise) than the assay error (Bustand A et al., 2006). In the current study gamma value for the final model was 1.06 and this suggested a small environmental noise.

The mechanistic model described by Matthews *et al* (2004) and Anderson *et al* (2009) improved the model fit but resulted in increase in between subject variability. In Pmetrics, creatinine clearance parameters estimates from Matthews *et al* (2004) study were used, which might contribute to the increase in between subject variabilities in clearance and V_1.

Attempts to estimate these parameters resulted in maximum cycle been reached but the run did not converged or the run ended with a hessian error.

The strength with the parameteric software NONMEM is the ability to evaluate the model internally using a variety of methods. However, in the nonparametric software Pmetrics, these methods are under development namely bootstrap and visual predictive check.

By using the nonparametric approach to estimate the parameters, discrete support points were obtained where each point had a set of the model parameter estimates and its associated probability and represented possible different models for the patients (Neely MN et al., 2012, Tatarinova T et al., 2013). One application of this type of population model is to including it within software that aids dose optimisation of individual patients (Tatarinova T et al., 2013, Jelliffe R et al., 2000, Bayard DS et al., 1994). For example, population models were used within the multiple-model design of dosage regimen (Bayesian adaptive dosage) algorithm (Bayard DS et al., 1994) that was previously implemented within the USC-PACK package (Jelliffe RW, 1991) developed and maintained by the Laboratory of Applied Pharmacokinetics, University of Southern California. With the new development and improvements in their nonparametric population pharmacokinetic program, Pmetrics, the clinical software is now available separately as a Windows® program and renamed "BestDose", although it still uses the same approach.

In the present study, the model included 49 support points. The small number of support points obtained from the current analysis despite the relatively large number of patients (331 patients) reflected the low variability within the studied group of patients. The final model could be used in the clinical program "BestDose" to individualise dosage regimens for adult patients with cystic fibrosis treated with tobramycin. In order to illustrate the usefulness of the multiple-model design of dosage regimen for therapeutic drug monitoring and to advise on the initial and future dosage regimens to achieve a defined target goal, Neely *et al* (2008) presented four cases of patients who were treated with antiretroviral

drugs (efavirenz, nelfinavir, amprenavir, and atazanavir). The method had proven its usefulness through the identification of patient with unexpected pharmacokinetics such as low drug clearance or high volume of distribution and to develop an individualised dosage schedule to fit patient's need to ensure adherence.

A potential future study would be to evaluate the performance of the model within the "BestDose" software and compare the results with current practice, which involves using a different software package, a MAP Bayesian method call OPT (Kelman et al., 1982). Data have been collected from 40 new patients with 63 courses of therapy. However, at present, this study is not feasible because the current version of BestDose only accepts a model that contains with rate constants and volume of distribution, whereas the final model of the current study re-parameterised the rate constants to clearance and volume of distribution. In addition, the current version of BestDose only allows creatinine clearance to be used as covariate for clearance and weight as a covariate for volume of distribution. This was not the case in the present final model where height and creatinine clearance were the covariates for clearance and height for volume of distribution. The BestDose developers are planning to change the current program format and made future versions more flexible to accept different models with different parameterisations and covariates but this is still in the development stage and the timescale for completion of this work is unknown.

5.5.1 Conclusion

In conclusion, the current analysis indicated that both parametric and nonparametric approaches performed similar when were used to analyse aminoglycoside data from patients with cystic fibrosis. The results obtained following nonparametric analysis confirmed the results from parametric analysis where the final model that best fitted that data was a two compartment model and included height and creatinine clearance in clearance and height in V_1. Although, parameter estimates were very close, clearance parameter estimates were slightly higher for the nonparametric approach, which indicated that the nonparametric approach was able to detect subpopulations and outliers within the

examined group of patients. In addition, individual concentration predictions were more precise using the nonparametric approach.

CHAPTER 6: DEVELOPMENT AND VALIDATION OF A TOBRAMYCIN DOSAGE ADJUSTMENT NOMOGRAM FOR PATIENTS WITH CYSTIC FIBROSIS

6.1 INTRODUCTION

Nicolau *et al* (1995) were the first group to propose "once daily" or "extended interval" dosing of aminoglycoside antibiotics. Their guidelines of a fixed 7 mg/kg dose and a dosage interval based on the patient's calculated creatinine clearance were intended for use in general medical patients. The regimen was designed to produce a peak concentration at one hour of 20 mg/L, approximately ten times an MIC of 2 mg/L. To allow easy interpretation of drug concentration measurements, they also proposed a dose adjustment nomogram with a single random blood sample obtained between 6 and 14 hours after the start of infusion.

In patients with cystic fibrosis, however, a higher aminoglycoside dose of 10 mg/kg/day has been recommended to ensure that high concentrations, that are likely to be active against *P.aeruginosa*, are achieved at the site of infection, which is the lung in this case (Smyth A et al., 2005, The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009). Current monitoring of these high doses is typically based on a peak concentration between 20 and 30 mg/L, obtained 30 minutes after a 30-minute infusion and a trough (pre-dose) concentration ≤1 mg/L. There is currently no dosage adjustment nomogram available to help clinicians interpret aminoglycoside concentration measurements associated with this high dose. In Australia, daily exposure is the recommended monitoring approach to monitor once daily tobramycin (Begg EJ et al., 1995). They have a defined target daily AUC for general medical patients but there is no daily AUC target for CF patients yet. Aminoglycosides dose are usually scaled based on weight in patients with cystic fibrosis (Aminimanizani A et al., 2002, Beringer PM et al., 2000, Kearns GL et al., 1982, Massie J and Cranswick N, 2006). However, other body size measurements such as body surface area (Campbell D et al., 1999) and lean body weight (Touw DJ et al., 1994) were used to scale the dose in this patient group. In this chapter, different dosage scaling factors including weight would be evaluated for the best that achieve target concentrations and exposure of tobramycin.

6.2 AIMS

- To determine a daily area under the concentration-time curve (daily AUC) target.
- To determine which dosage scaling factor provides the best opportunity to achieve target concentrations and exposure of tobramycin.
- To develop tobramycin dosage adjustment nomograms for patients with cystic fibrosis given a dose of 10 mg/kg every 24 hours and given a dose based on the best scaling factor.
- To validate the weight scale based dose nomogram using routine tobramycin concentration-time measurements obtained from a new set of patients with cystic fibrosis.

6.3 METHODS

6.3.1 Identification of daily exposure target and range using real data

Since a target range for tobramycin daily exposure in patients with cystic fibrosis was not available from the literature, the range was derived from routine clinical data. Daily doses and individual CL estimates from the Glasgow and The Hague datasets were used to predict daily AUC ranges by estimating daily AUCs for each patient. The daily AUC was calculated from daily dose/individual CL estimate. The TOPIC study (Smyth A et al., 2005) is the largest randomised controlled trail conducted in patients with cystic fibrosis to compare the safety and efficacy of a 10 mg/kg dose administered once daily or in three divided doses. In that study, 219 patients from 21 cystic fibrosis centres in the UK, 15 paediatric and six adult centres, were included. A pharmacokinetic analysis was performed using 136 patients who had complete data on tobramycin doses, administration dates and times, and tobramycin concentrations with recorded sampling times (Touw DJ et al., 2007). Therefore, the typical daily AUC value obtained with the recommended daily dose of 10 mg/kg/day tobramycin was estimated from the TOPIC (Touw DJ et al., 2007) study using their mean estimates of elimination rate constant (0.318 h^{-1}) and V (0.294 L/kg). The target daily AUC and ranges were then used as the monitoring parameter for once daily tobramycin to determine the dose scaling factors and develop the nomogram.

The Glasgow dataset included 166 adult patients with cystic fibrosis and 1075 courses of aminoglycoside therapy. The median dose was 360 mg/day and ranged from 120 to 660 mg/day. Overall, 44 (4 %) of the courses were administered 8 hourly, 1022 (95 %) 12 hourly and 9 courses (1%) 24 hourly. The Hague dataset comprised 165 patients with 415 courses of tobramycin. These patients received a median dose of 500 mg/day (range 120 to 880 mg/day). One course was administered 8 hourly, 137 (33 %) of the courses were 12 hourly, 272 (65 %) were 24 hourly, 3 were 36 hourly and 2 were 48 hourly.

To determine a possible daily AUC range for the 10 mg/kg/day dose, data from patients in The Hague dataset who received 24, 36 or 48 hourly doses were selected for further analysis. The daily AUC range was determined after first identifying the doses that achieved the target peak concentrations of 20-30 mg/L and troughs ≤ 1 mg/L (Smyth A et al., 2005). For the purpose of this analysis, "peak" concentrations were defined as measurements obtained between 0.95 to 1.5 hours and "trough" concentrations between 17.5 to 20 hours after the start of the infusion. The daily AUC distributions associated with peak and trough concentrations below, within and above these targets were identified and used to determine the lower and upper daily AUC range.

6.3.2 Creation of simulated patient dataset

Five thousand simulated patients with age, weight, height, serum creatinine, and CrCL distributions that mirrored the combined datasets were created using NONMEM (Beal SL et al., 2009). A log normal distribution for the variance of these clinical characteristics from combining the Glasgow and The Hague datasets was used to ensure similar distributions to the raw data characteristics. The categorical variable, gender, was simulated and the NONMEM code was constrained to simulate 50% of patients to be males and 50% to be females. The typical relationship between weight, height and gender was identified by regression analysis and included in the simulation to ensure that the combinations of weight and height within a patient made physiological sense. Creatinine clearance was estimated by the Cockcroft and Gault (Cockcroft DW and Gault MH, 1976) equation using the simulated values of gender, age, weight and serum creatinine. The minimum serum

creatinine value was fixed at 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998). The simulation data file included the following information for one patient: the median age, weight, height, serum creatinine and CrCL values derived from the combined dataset. Figure 6.1 shows the data file used to simulate the weight scaled dose (10 mg/kg). In order to avoid simulated clinical characteristics that were outside the range of characteristics in the combined datasets, the ranges were limited using the "IF" code. This indicated that if the simulated value was lower than the minimum or greater than the maximum value in the combined dataset then it should be replaced with the median value that was included in the simulation data file. The NONMEM coding that was used to generate the simulated patients' characteristics is presented in Figure 6.2.

A dosage adjustment factor (F1) was included in the control file to correct the dose included in the data file. F1 is the fraction of the patient's simulated scaling factor such as simulated weight, height, lean body weight or body surface area and the median scaling factor (e.g. weight, height, lean body weight or body surface area) used in the data file (F1=SIMWT/WT).

Then the simulated amount would be the amount used in the data file multiplied by the dosage adjustment factor (F1) value (AMT2=AMT*F1).The final two compartment model and pharmacokinetic estimates derived from the analysis of the model development datasets were used to generate 5000 simulated patients using the "$SIMULATION" code. Figure 6.2 shows the control file used to generate weight scaled dose (10 mg/kg) simulations.

Different dosing scaling factor options were examined including the current weight scaled dose (10 mg/kg with and without restricting the daily dose to 660 mg/day) (Smyth A et al., 2005) and the height, LBW (Janmahasatian S et al., 2005) and BSA (Mosteller RD, 1987) scaled doses. For each examined dosage regimen, a new data file was created with only change in the dose, which is based on the scaling factor to be used such as LBW, height or

BSA. For the derived body size measurements, LBW and BSA, their formulas were coded within the NONMEM control file to estimate their values from the simulated weight and height values generated. Tobramycin concentrations were predicted for each simulated patient at 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hours after the start of the infusion.

Figure 6.1 Example of the data file used to generate the simulated patients and concentrations**.**

#ID	TIME	AMT	RATE	EVID	AGE	WT	HT	GEN	CREA	CGCL	DV
1	0	530	1060	1	24.6	53	166	0	70	94	0
1	1	0	0	0	24.6	53	166	0	70	94	0
1	2	0	0	0	24.6	53	166	0	70	94	0
1	4	0	0	0	24.6	53	166	0	70	94	0
1	6	0	0	0	24.6	53	166	0	70	94	0
1	8	0	0	0	24.6	53	166	0	70	94	0
1	10	0	0	0	24.6	53	166	0	70	94	0
1	12	0	0	0	24.6	53	166	0	70	94	0
1	14	0	0	0	24.6	53	166	0	70	94	0
1	16	0	0	0	24.6	53	166	0	70	94	0
1	18	0	0	0	24.6	53	166	0	70	94	0
1	20	0	0	0	24.6	53	166	0	70	94	0
1	22	0	0	0	24.6	53	166	0	70	94	0
1	24	0	0	0	24.6	53	166	0	70	94	0

Figure 6.2 An example of the control file used to generate weight scaled dose (10 mg/kg) simulations.

```
$PROB NOMOGRAM DEVELOPMENT BY SIMULATION
$INPUT ID TIME AMT RATE EVID AGE WT HT GEN CREA CGCL DV
$DATA C:\Nomogramsimulations\COMBINEDNOMOGRAM.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$PK

SIMHT=HT*EXP(ETA(4))
IF(SIMHT.LT.139)SIMHT=HT
IF(SIMHT.GT.194)SIMHT=HT

IF(ICALL.EQ.4.AND.NEWIND.NE.2) THEN
GEN1=0 ;MALE
CALL RANDOM(2,R)
GENDER=R
IF(GENDER.LT.0.50) THEN
GEN1=1 ;FEMALE
ENDIF
ENDIF

SIMAGE=AGE*EXP(ETA(5))
IF(SIMAGE.LT.14)SIMAGE=AGE
IF(SIMAGE.GT.88)SIMAGE=AGE

SIMCREA=CREA*EXP(ETA(6))
IF(SIMCREA.LT.60)SIMCREA=60
IF(SIMCREA.GT.209)SIMCREA=CREA

SIMWT=(-67.1+1.09 *GEN1+0.731*SIMHT)*EXP(ETA(7))
IF(SIMWT.LT.30)SIMWT=WT
IF(SIMWT.GT.108)SIMWT=WT
F1=SIMWT/WT ; DOSING ADJUSTMENT FACTOR
AMT2=AMT*F1
IF(GEN1.EQ.1) THEN
SIMCGCL=((1.04*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ; LOGCRCL VARAINACE
ELSE
SIMCGCL=((1.23*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ;LOGCRCL VARIANCE
ENDIF

IF(SIMCGCL.LT.26.3)SIMCGCL=CGCL
IF(SIMCGCL.GT.181.4)SIMCGCL=CGCL

         TVCL=THETA(1)*SIMHT*+THETA(2)*(SIMCGCL-92)
         TVV1=THETA(3)*(1+THETA(4)*(SIMHT-163))
         TVV2=THETA(5)
         TVQ=THETA(6)
         CL=TVCL*EXP(ETA(1)+ETA(2))
         V1=TVV1*EXP(ETA(3))
         V2=TVV2
         Q=TVQ
         S1=V1
         AUC=AMT2/CL

$ERROR   IPRED=F
$ERROR   W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
         IRES=DV-IPRED
         IWRES=IRES/W
         Y=IPRED+W*ERR(1)

$THETA   0.0285 0.0114 13.3 0.0113 6.62 0.452 0.086 0.148
$OMEGA   0.0129  ;IOV CL
$OMEGA BLOCK(2) 0.0325 0.0140 0.0134;IIV CL BLOCK MATRIX IIV V1
$OMEGA   0.003 0.112 0.042 0.035 0.05 ;LOG NORMAL DISTRIBUTION VARIANE VALUE FOR ETA 4 5 6 7 8
$SIGMA 1 FIX
$SIMULATION (22032012) (812 UNIFORM)ONLYSIM SUBPROBLEMS=5000
$TABLE ID TIME AMT2 EVID DV GEN1 SIMAGE SIMWT SIMHT SIMCREA SIMCGCL TVCL TVV1 TVV2 TVQ CL AUC V1 V2 Q
NOPRINT ONEHEADER FILE=Simcombine12B2.TAB
```

Key: SIMHT = simulated height, HT= median height value in the data file, ETA = log normal distribution variance obtained for the combined datasets, SIMWT = simulated weight, GEN1 = simulated gender, WT, median weight, F1 = dosage adjustment factor, AMT2 = doses adjusted for the simulated scaling factor value (e.g. weight)

6.3.3 Creation of simulated datasets with a range of dosing scalers

The median dose required to achieve the typical daily AUC was calculated using the median CL estimate obtained with the population pharmacokinetic model (4.92 L/h) and using the following formula;

Median dose (mg) = Typical daily AUC (mg.h/L) x Clearance (L/h)

This median dose was then scaled by dividing the median values of lean body weight (LBW) (Janmahasatian S et al., 2005), height and body surface area (BSA) (Mosteller RD, 1987) obtained from the combined datasets, to create three dosage regimens, i.e.

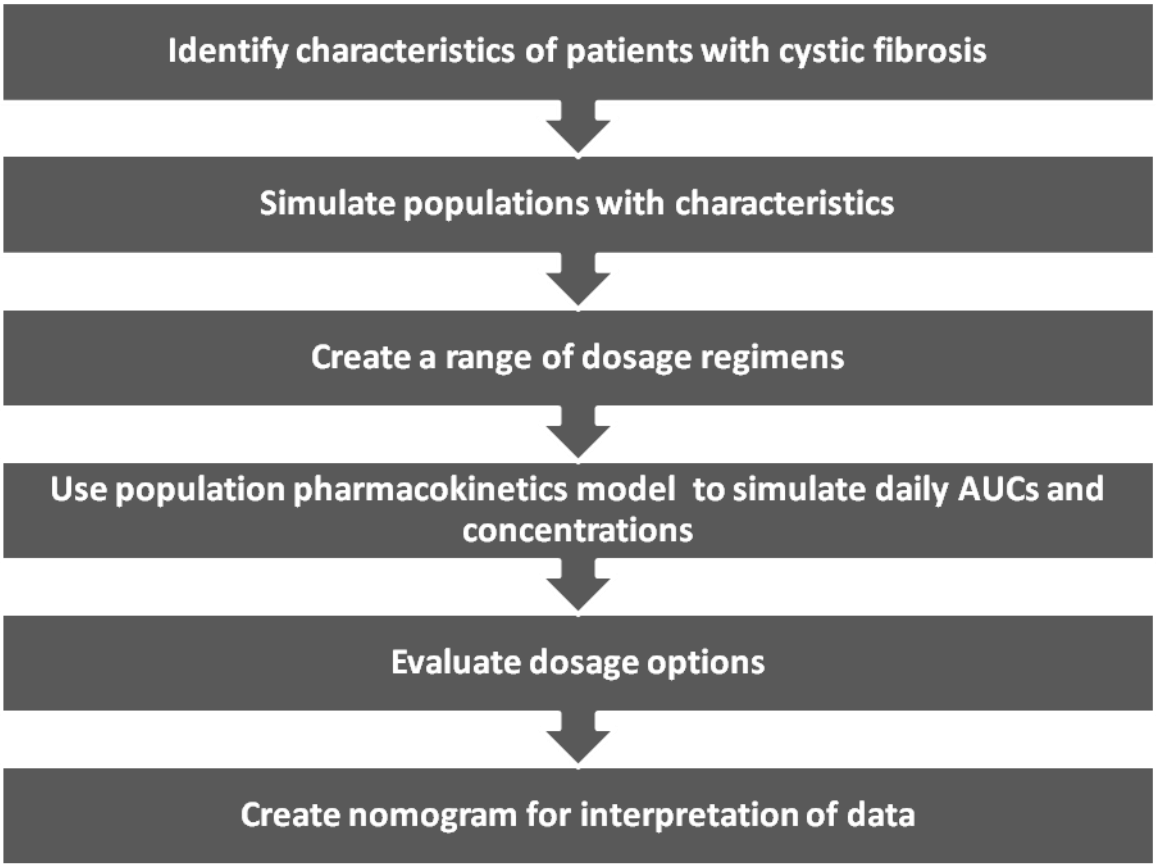
Dose per kg LBW = Median dose (mg)/40 kg = LBW dose mg/kg

Dose per cm height = Median dose (mg)/166 cm = Height dose mg/cm

Dose per m^2 BSA = Median dose (mg)/1.6 m^2 = BSA dose mg/m^2

Five data files and control files were prepared to generate 5000 simulated patients who were administered restricted and unrestricted weight, LBW, height and BSA scaled doses. Figure 6.2 shows the control file used to generate weight scaled dose (10 mg/kg) simulations and control files used to generate the other dose scaling factors including LBW, height and BSA are shown at APPENDIX VI (a, b and c). In addition, Figure 6.3 shows a summary of the simulation method presented as a workflow diagram.

Figure 6.3 The simulations workflow diagram to evaluate the different dosage regimens and develop dosage nomogram.



6.3.4 Analysis of peak and daily AUC estimates obtained with the simulated data

The predicted daily AUC was calculated for each patient from simulated daily dose/simulated individual CL estimate and compared with the target ranges determined from the literature and the analysis of real patient data. The daily doses and tobramycin CL estimates were compared among patients whose daily AUC estimates were below, within and above the target for each dose scaler. In addition the daily doses, daily AUC and peak concentration were compared for the examined dosage regimens. The comparison was performed using the non-parametric Kruskal-Wallis test implemented in Minitab® version 15, (Minitab Ltd, Coventry, UK). Statistical significance was set at $p < 0.05$. Simulated patients who achieved the target peak concentration of 20 – 30 mg/L 1 hour after starting the infusion were also determined.

The proportions of patients who achieved both the peak and daily AUC targets with the different scaling factors were compared with the proportions obtained with the current recommended dose (10 mg/kg with or without a maximum of 660 mg). The comparison was performed using the Chi-Square test in Minitab® version 15, (Minitab Ltd, Coventry, UK).

6.3.5 Development of nomograms for interpreting tobramycin concentrations

The nomogram was designed to identify patients who would fall outside the targets of peak concentration 20 – 30 mg/L, concentration < 1 mg/L at 18 hours post dose and daily AUC within the typical range identified from the real patient data. Concentration measurements from patients whose CrCL estimate was < 50 mL/min were excluded. The simulated patients whose concentrations achieved the target daily AUC and target peak (20 – 30 mg/L) ranges were selected from the currently recommended weight scaled dose and an alternative dosage regimen that achieved the highest proportion of patients within the target daily AUC and peak concentration ranges. From these data, the 97.5th, 95th, 2.5th and 5th percentiles of the simulated concentrations at each time point were plotted against time after start of therapy.

6.3.6 Validation of the nomogram for interpreting tobramycin concentrations

6.3.6.1 New dataset

A new, independent dataset comprising data from patients with cystic fibrosis who had been treated with tobramycin was received from a hospital laboratory in The Netherlands. The data were supplied in an Excel spreadsheet format exported from the software MWPharm (Mediware, Groningen, The Netherlands). Patients who had the same gender and date of birth were assumed to be the same individual. Courses of therapy that contained insufficient data or suspected errors were removed from the dataset. Courses administered to patients who were less than 14 years old were excluded. Patients in the new dataset were then compared with patients who were included in the model validation

dataset used in Chapter 4 and any who appeared in both datasets were removed from the new dataset.

The dataset comprised clinical and demographic data, the dosage regimen(s) and a list of measured concentrations. The original format was not suitable for nomogram validation since dosage information was required before each measured concentration. Consequently, some assumptions had to be made. These were as follows:

- "Peak" concentrations were assumed to be measured one hour after starting the infusion.
- If a measured concentration was recorded without information about the previous dose, the dose amount and time were assumed to be consistent with the previous dose and dosage interval.

Concentration measurements between 6 and 12 hours after the start of the infusion were plotted on the draft nomogram and the dosage recommendation noted. The recommendations arising from the nomogram were compared with the actual dosage decision that had been made from a MAP Bayesian (MWPharm) interpretation of the concentration measurements for the following dose. In addition, individual daily AUC was estimated using the administered dose and the individual clearance estimate (daily AUC= daily dose/ individual clearance) obtained following a POSTHOC analysis using the final empirical model with height parameter estimates. Data arising were analysed and plotted using Microsoft office Excel (Microsoft Office 2007), Minitab® version 15, (Minitab Ltd, Coventry, UK), and GraphPad Prism (version 6).

6.3.6.2 Glasgow and The Hague dataset

The Glasgow and The Hague datasets used for model development and validation were combined, and their weight and individual pharmacokinetic estimates predicted from the final model run were used to predict concentrations at 6, 9, 12 hours after start of therapy following a 10 mg/kg/day (maximum 660 mg/day) dose . The estimation step was omitted

by using the code MAXEVAL = 0. Then concentrations were plotted on the developed weight scaled nomogram to ensure that the developed nomogram is able to identify patients with low, within and above target AUC range.

6.4 RESULTS

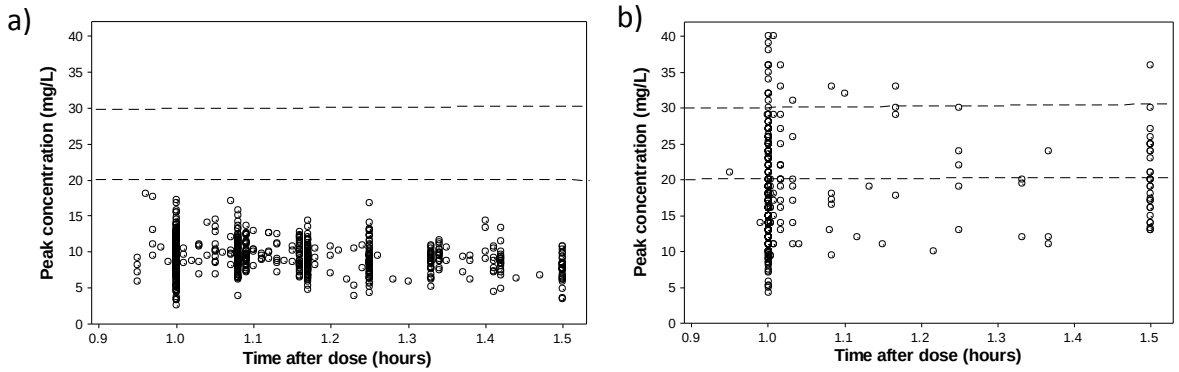
6.4.1 Daily exposure target and range using real data

The estimated target daily AUC from the TOPIC study was 106 mg.h/L. The daily doses and daily AUC estimates for the real datasets are listed in Table 6.1. The tobramycin doses given to the Glasgow patients were lower than doses administered to The Hague patients. Similarly, daily AUCs were lower for the Glasgow patients with up to 179 mg.h/L compared with daily AUCs from The Hague patients that were up to 268 mg.h/L. Patients in the Glasgow dataset who were administered doses between 9 and 11 mg/kg/day had a median daily AUC of 84.6 mg.h/L, which was lower than median value for patients from the Hague (106 mg.h/L). As shown in Figure 6.3 (a), none of the predicted peak concentrations in the Glasgow dataset achieved the target concentration and hence were excluded from further analysis. On the other hand, 120 out of 492 peak concentrations from The Hague achieved the target (20 -30 mg/L) as shown in Figure 6.4 (b).

Table 6.1 Median (range) daily doses and daily AUC estimates from the Glasgow and The Hague datasets.

	Glasgow dataset n = 1075 courses	**The Hague dataset n = 415 courses**
Daily dose (mg/day)	360 (120 - 660)	500 (120 – 880)
Daily dose (mg/kg/day)	7.17 (2.46 – 13.3)	8.64 (2.40 – 14.7)
Daily AUC (mg.h/L)	77.8 (36.9 – 179)	103 (42.2 – 267)
Daily AUC for courses of 9 – 11 mg/kg/day	n = 81 84.6 (56.5 – 128)	n = 165 106 (68.9 – 267)

Figure 6.4 Scatter plots of the measured peak concentrations in the (a) Glasgow and (b) The Hague datasets.

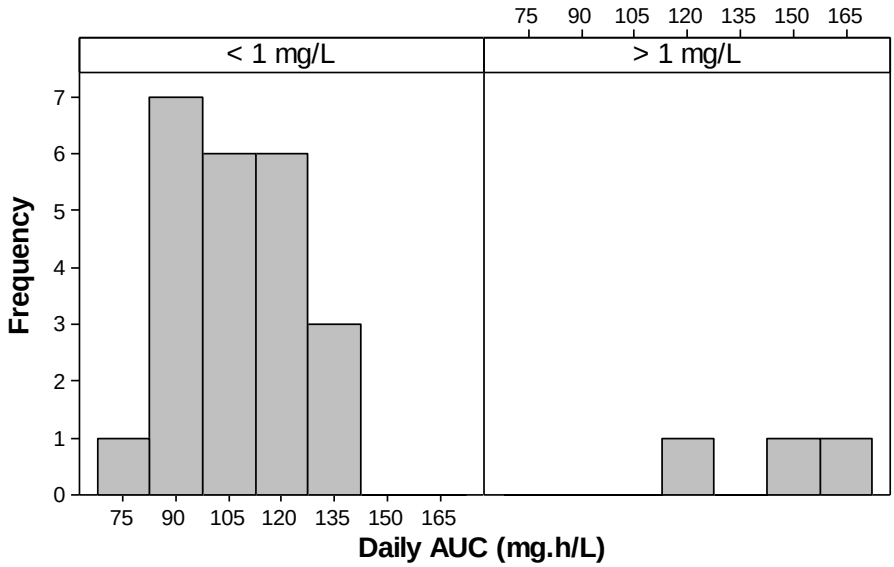


The distribution of daily doses and daily AUC values for The Hague patients who were administered once daily and extended interval tobramycin are shown in Table 6.2, in groups based on peak concentrations. In total, 202 peak concentrations were available from 77 patients (135 courses) of which 48% achieved the target peak concentration, 41 % were below and 11 % were above the target. The median daily AUC for patients who had peaks < 20 mg/L was 84.6 mg.h/L and hence 80 mg.h/L was chosen as a possible lower limit for the target daily AUC. Since the median daily AUC values for patients whose peak concentrations were within or above the target range were similar, it was difficult to identify an upper limit for daily AUC. There were 27 trough concentrations measured in 21 patients (25 courses) between 17.5 and 20 hours after the tobramycin dose. Three of these concentrations were above 1 mg/L and all were in patients who had low tobramycin CL estimates (2.37, 2.91 and 3.22 L/h). The daily AUC frequency histogram shown in Figure 6.5 for patients with troughs <1 mg/L suggested an upper daily AUC limit of 120 mg.h/L.

Table 6.2 Distributions of peak concentrations, dose and daily AUC values from patients in The Hague dataset who were administered 24 hourly doses (n = 385).

	Peak <20 mg/L n = 81	**Peak 20-30 mg/L n = 95**	**Peak >30 mg/L n = 21**
Dose (mg/kg/day)	5.0 (2.86 – 10.3)	8.67 (4.12– 11.4)	9.80 (7.74 – 11.9)
Daily AUC (mg.h/L)	84.6 (42.2 – 157)	102 (65.0 – 252)	113 (84.9 – 180)

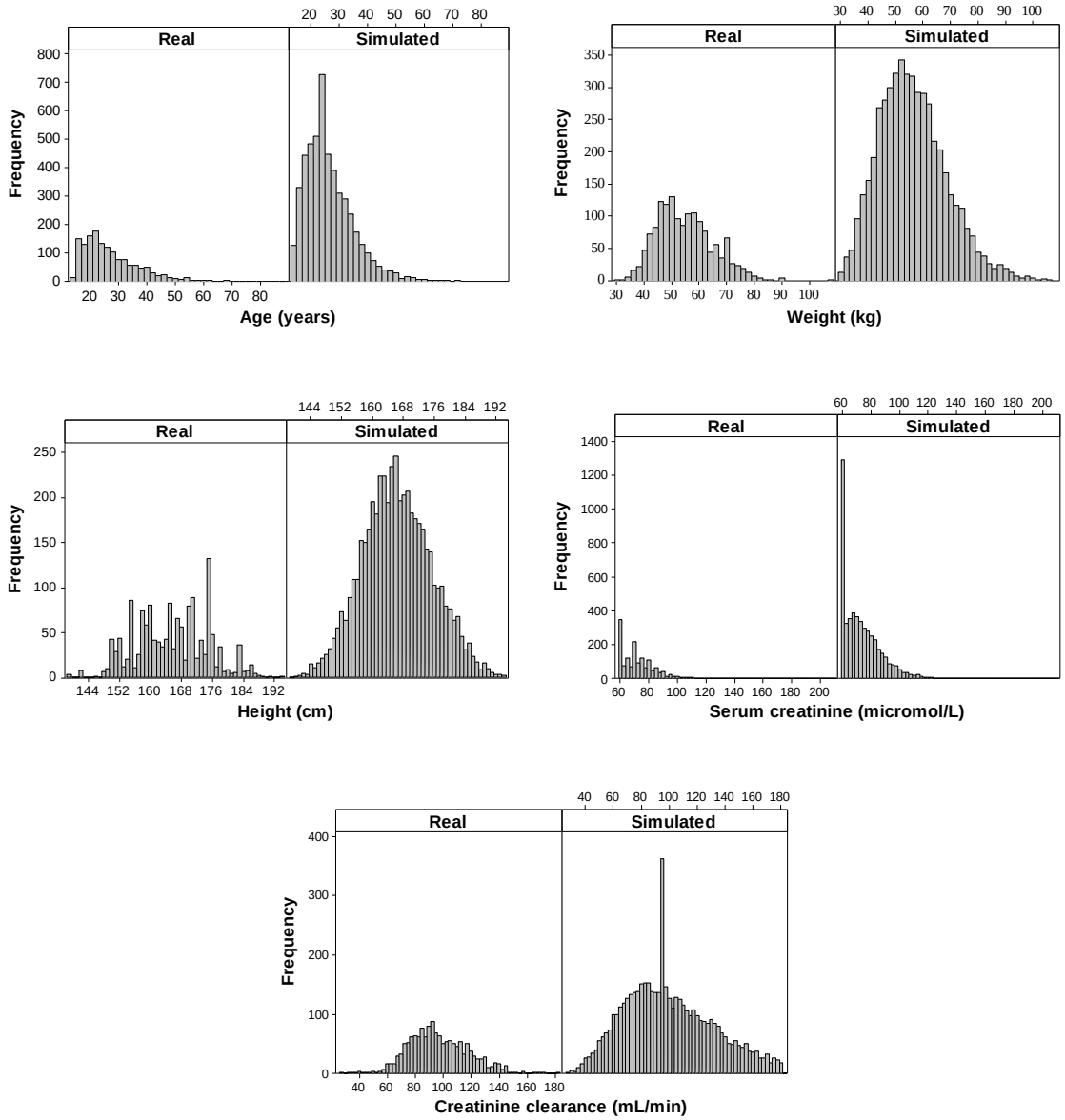
Figure 6.5 Frequency distribution of daily AUC split into patients whose 24 hour post dose trough concentrations were below or above 1 mg/L for The Hague dataset patients.



6.4.2 Creation of simulated patients

The distribution of patients' characteristics generated from the simulations was consistent with those of the real datasets, as shown in Figure 6.6. The simulation generated an excess of patients with the median values because the control file replaced simulated values that were above the upper limits and below the lower limits for each clinical characteristic with the median value. In addition, the lower limit for serum creatinine was fixed to 60 µmol/L and hence many simulated patients had this value.

Figure 6.6 Distributions of age, weight, height, creatinine concentration and creatinine clearance in the real and simulated datasets.



6.4.3 Development and evaluation of simulated datasets with a range of dosing scalers

Using the estimated target daily AUC based on the TOPIC (Touw DJ et al., 2007) study of 106 mg.h/L, the estimated median dose was 521.5 mg/day . The scaled doses were 13 mg/kg for LBW, 3 mg/cm for height and 326 mg/m^2 for BSA.

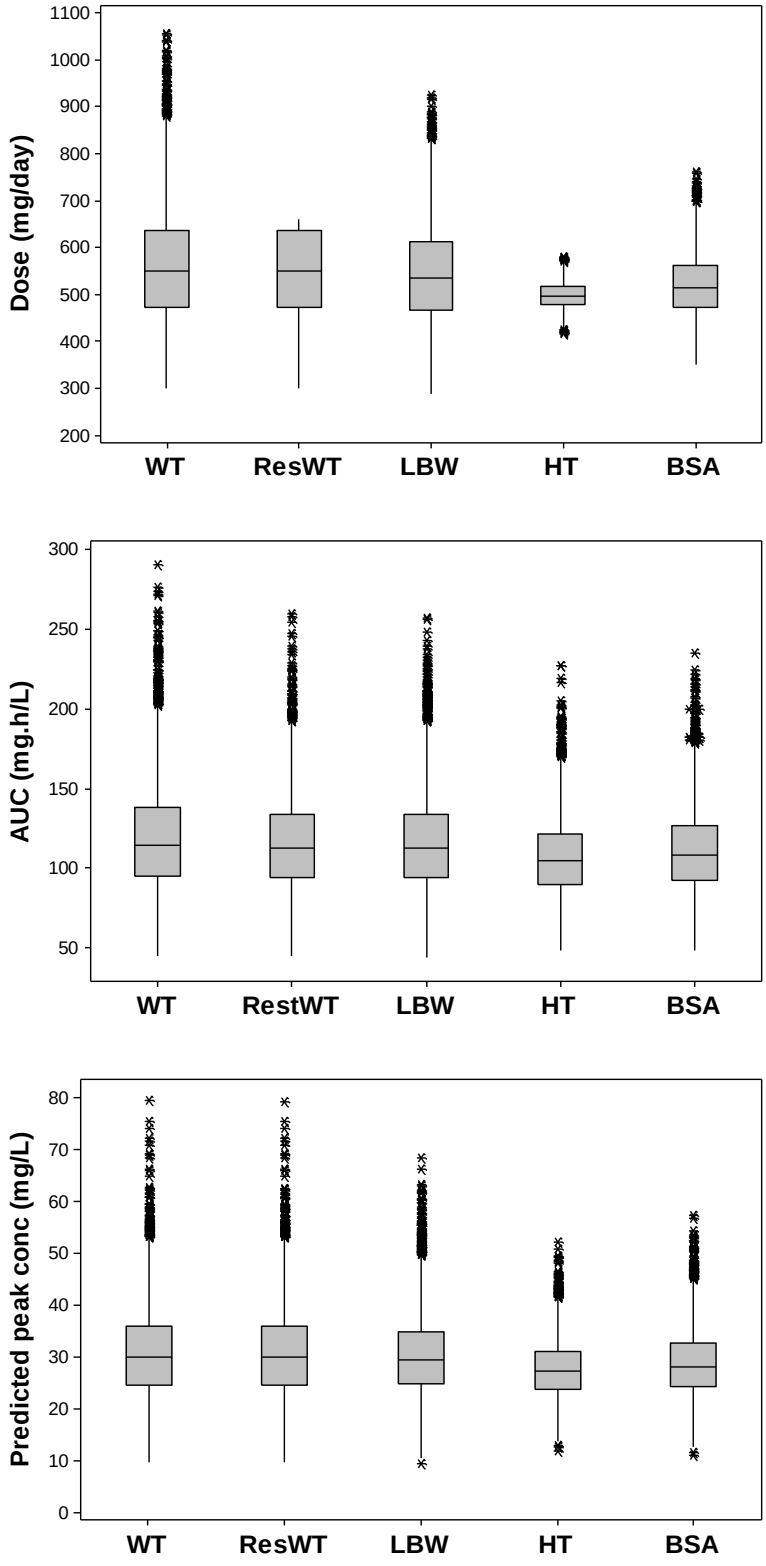
When the current weight scaled dose of 10 mg/kg was unrestricted, 477 (9 %) of the simulated patients had a daily AUC below 80 mg.h/L, 2392 (48 %) had a daily AUC within the target range and 2131 (43 %) had a daily AUC above the target range. Twenty per cent of the simulated patients had doses greater than 660 mg (range 661 to 1054 mg). Restricting the daily dose to 660 mg produced only a slight increase (to 50%) in the proportion of patients who achieved the target daily AUC and a slight reduction (to 40%) in the proportion above the target range. Patients whose daily AUC was estimated to be below the target had lower doses and had higher CL estimates than patients whose daily AUC estimates were within or above the target range ($p < 0.0001$ for 10 mg/kg with maximum of 660 mg). Conversely, patients whose daily AUCs were above the target, received higher doses and had lower CL estimates than patients whose daily AUC were below or within target ($p < 0.0001$ for 10 mg/kg with maximum of 660 mg).

When daily doses were unrestricted, the majority of patients (77%) whose daily AUC estimate was below the target were underweight (BMI ≤ 18.5 kg/m^2) compared with those within (45%) or above the target daily AUC (15%). No overweight or obese patients had a daily AUC below the target, whereas 26% were overweight or obese in the within and above target daily AUC groups, respectively. When the maximum dose was fixed, the values were slightly different. In the below target daily AUC group, 2.5 % of patients were overweight and none were obese, while 8 % were overweight and obese in within target daily AUC group. In the above the target daily AUC group, 18 % of patients were overweight or obese.

When the predicted peak concentrations were analysed, the percentages of patients whose concentrations were below (8%), within (41%) and above (51%) the target ranges were similar for both weight dosage groups. Results were also similar when the distributions of BMI were examined; 85 % of patients with low peaks were underweight, 15 % were normal weight and one patient was overweight.

Figure 6.7 shows the distributions of the doses, peak concentrations and daily AUC for the simulated weight (10 mg/kg with 660 mg/day maximum daily dose), LBW (13 mg/kg), height (3 mg/cm) and BSA (326 mg/m^2) scaled doses. A narrower range of doses were obtained when they were scaled according to height (up to 581 mg) compared with weight (up to 1054 mg) or restricted weight (up to 660 mg). The difference was statistically significant at $p < 0.0001$. The maximum daily AUC was lower when height was the dose scaling factor rather than weight, or restricted weight. Similarly, the maximum peak concentration was also lower when height was used as the dose scaling factor compared with weight and restricted weight.

Figure 6.7 The distributions of daily doses, daily AUC, and predicted peak concentration using doses scaled by weight (10 mg/kg unrestricted and limited to 660 mg/day), LBW (13 mg/kg), height (3 mg/cm) and BSA (326 mg/m^2).



Key: WT= weight, LBW= lean body weight, HT, height, BSA= body surface area

Figure 6.8 shows the number of simulated patients whose concentrations were below, within and above the target daily AUC range. A higher proportion of patients achieved the target daily AUC concentration with a height scaled dose (61%) compared with weight, LBW or BSA scaled doses (50 - 58%). This difference was statistically significant at $p < 0.0001$. Furthermore, a lower proportion of patients had their daily AUC above the target range (> 120 mg.h/L) with a height scaled dose (27%) compared with weight, LBW, or BSA (33 - 40%). Similar results were obtained with peak concentrations (Figure 6.9) where 63% achieved the target with a height-scaled dose compared to 41-56% with other size measurements. Only 30% of patients had their peak concentration greater than the target concentration (> 30 mg/L) with a height scaled dose compared with weight, LBW, or BSA (38 - 51%). This difference was statistically significant at $p < 0.0001$.

Figure 6.8 The numbers of simulated patients whose concentrations were below, within and above the target daily AUC range with for a range of dose scaling factors.

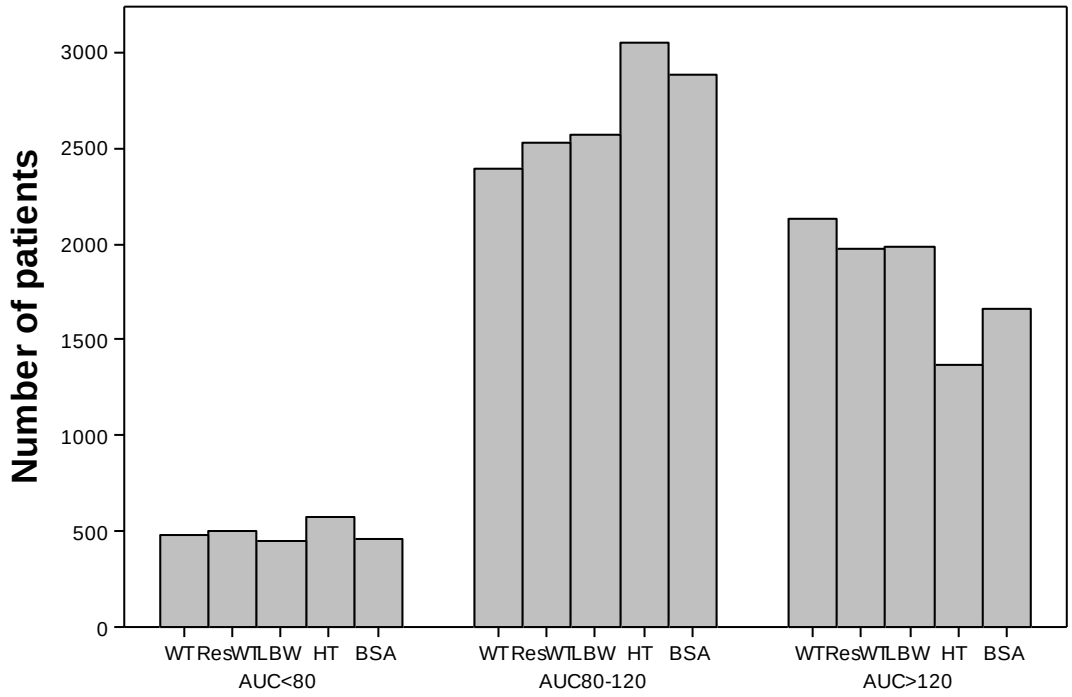


Figure 6.9 The distribution of simulated patients whose peak concentration were below, within and above the target peak concentration (20 - 30 mg/L) with the different dose scaling factors.

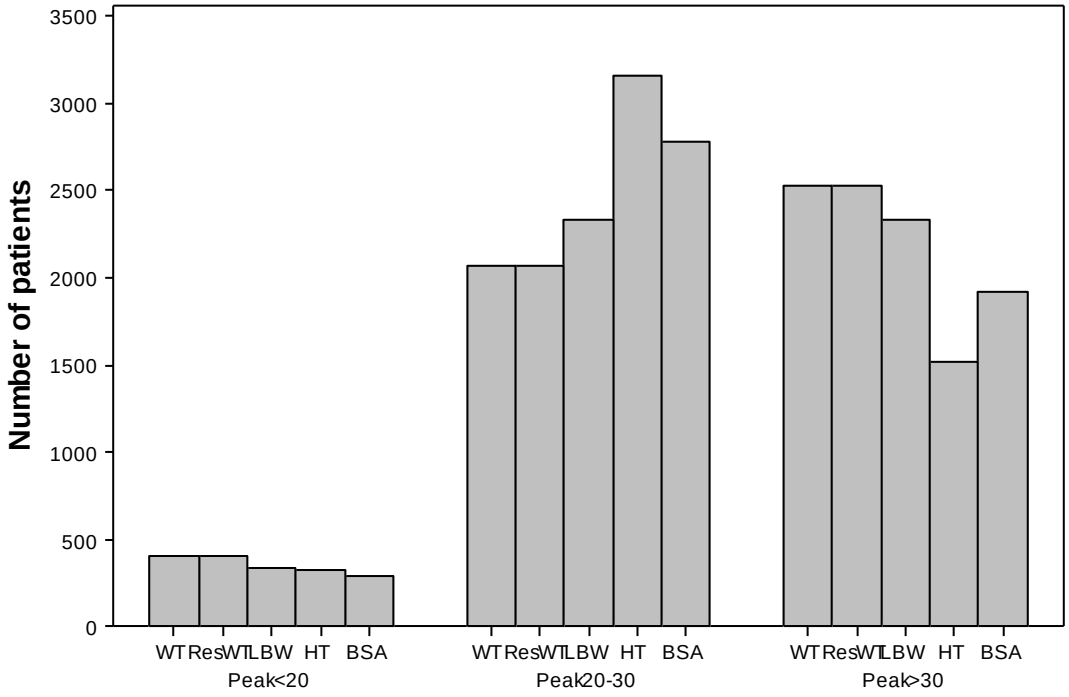


Figure 6.10 and 6.11 shows the distributions of tobramycin doses and CL estimates in the simulated patients, categorised by daily AUC range. There was no correlation between doses and daily AUCs when dose was scaled for height, whereas a clear correlation can be seen between the administered doses and expected daily AUC with doses scaled to weight, LBW and BSA. On the other hand, correlation between the expected daily AUC and tobramycin CL was observed regardless of the scaling factor in the different daily AUC groups. The distribution of doses was similar in all daily AUC groups when scaled according to height, and tobramycin clearance ranges were narrower compared with the other scaled doses. In general, patients who had daily AUC estimates above 120 mg.h/L had lower drug clearance estimates (median 3.67 L/h, range 1.99 - 4.79), while patients who had below the target daily AUC had high drug clearance (median 6.91L/h and ranged 5.61-10.5). As a result of the encouraging findings from the height scaled dose, it was chosen to develop a new tobramycin dosage adjustment nomogram in patients with cystic fibrosis.

Figure 6.10 The distribution of daily doses categorised by daily AUC estimate for (a) weight, (b) LBW, (c) Height and (d) BSA scaled doses.

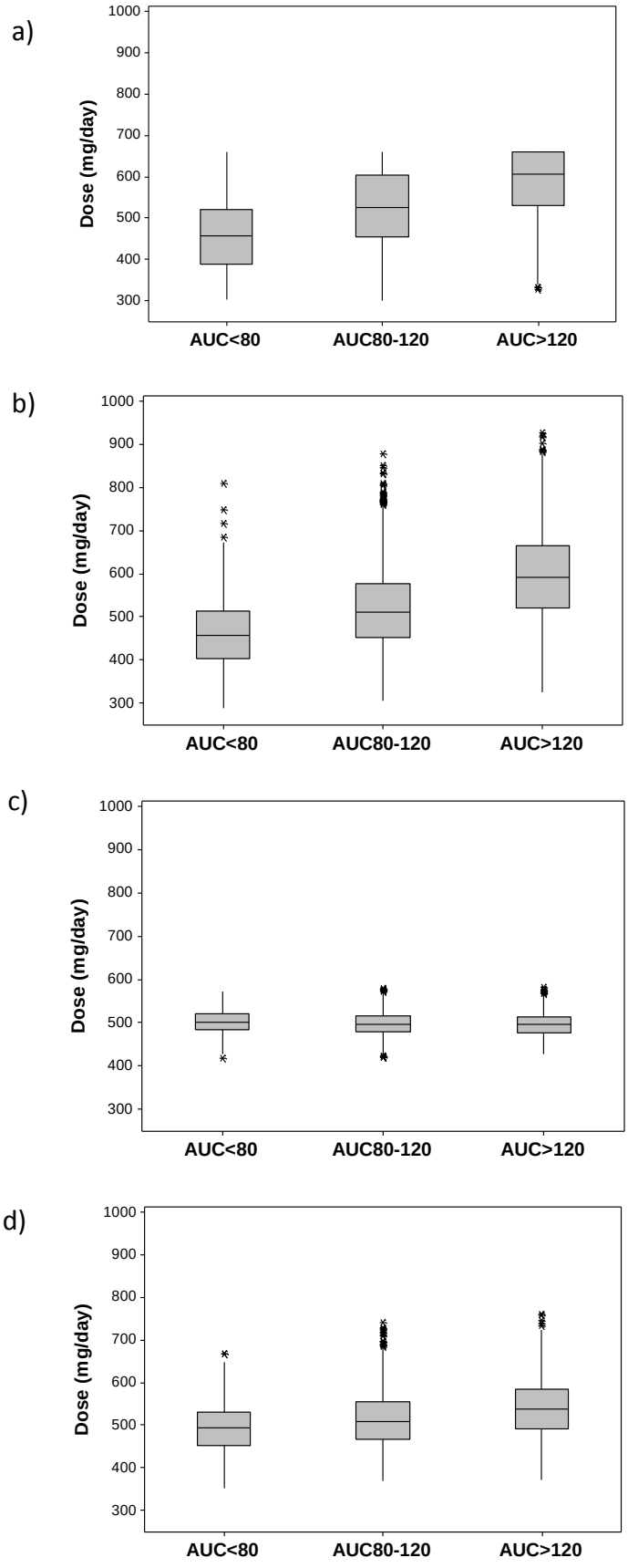
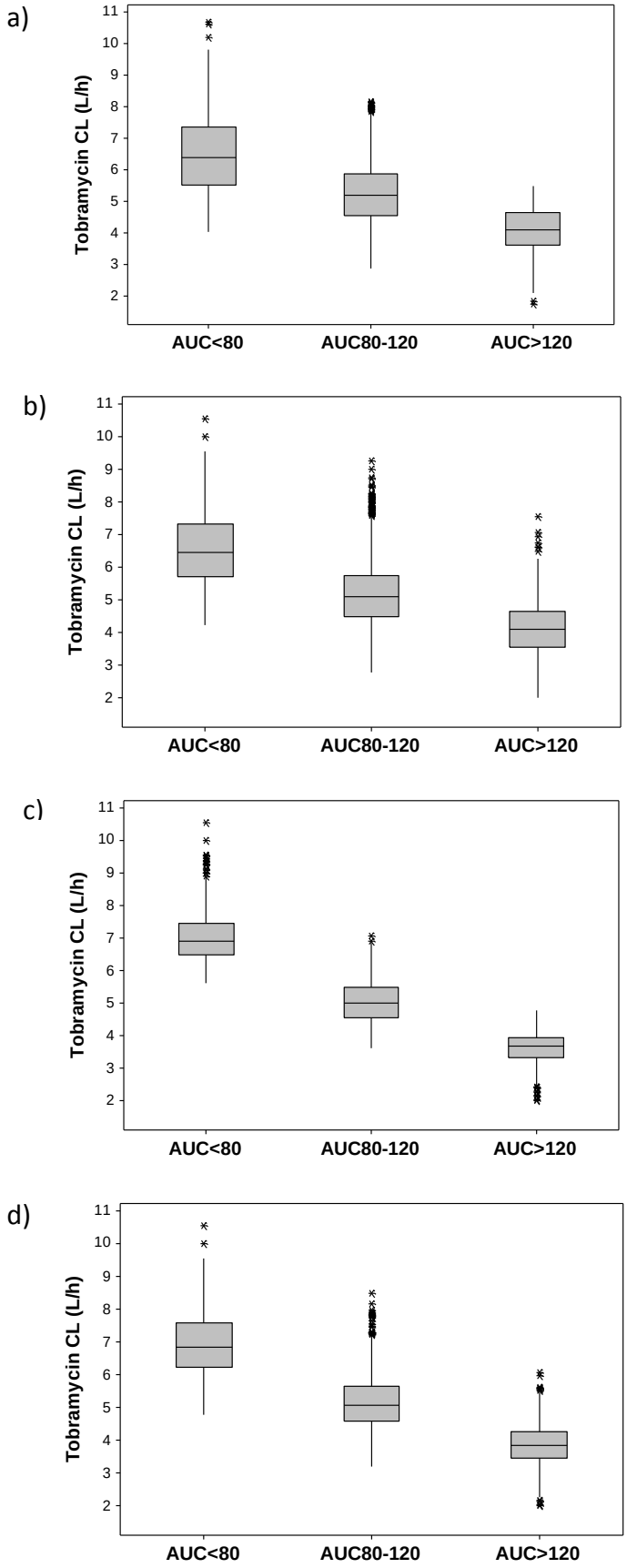


Figure 6.11 The distribution of tobramycin CL categorised by daily AUC estimate for (a) weight, (b) LBW, (c) Height and (d) BSA scaled doses.

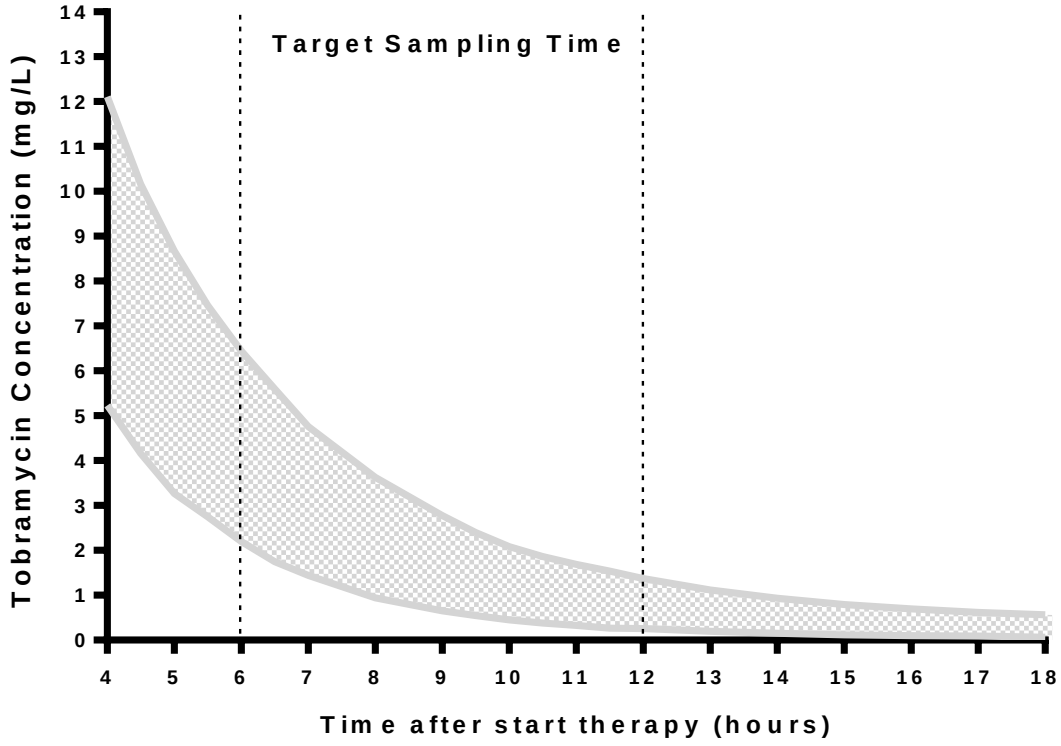


6.4.4 Development of dosage adjustment nomograms

6.4.4.1 Weight scaled dosage adjustment nomogram

The results obtained from the 10 mg/kg/day dose with a maximum set to 660 mg/day were examined and the data from the 1421 simulated patients who achieved both a daily AUC of 80 -120 mg.h/L and a peak of 20-30 mg/L were used to develop the nomogram. Figure 6.12 shows the proposed dosage adjustment nomogram. The lower nomogram bound represented the 2.5th percentile line for simulated patients with normal renal function who had a daily AUC of 80 -120 mg.h/L and a peak of 20-30 mg/L. A measured concentration below the lower bound shaded area represents a patient with a daily AUC less than 80 mg.h/L and/or peak tobramycin concentration less than 20 mg/L. On the other hand, the upper nomogram bound represented the 97.5th percentiles line for patients with normal renal function and whose daily AUC and peak concentrations were within the target range. A measured concentration above the shaded area is consistent with having daily AUC and/or peak concentration above the target. However, a measured concentration within the shaded area is consistent with being within the target daily AUC and/or peak concentration ranges.

Figure 6.12 The 2.5th and 97.5th and percentiles of all tobramycin concentration measurements at each time point for 10 mg/kg (maximum 660 mg).

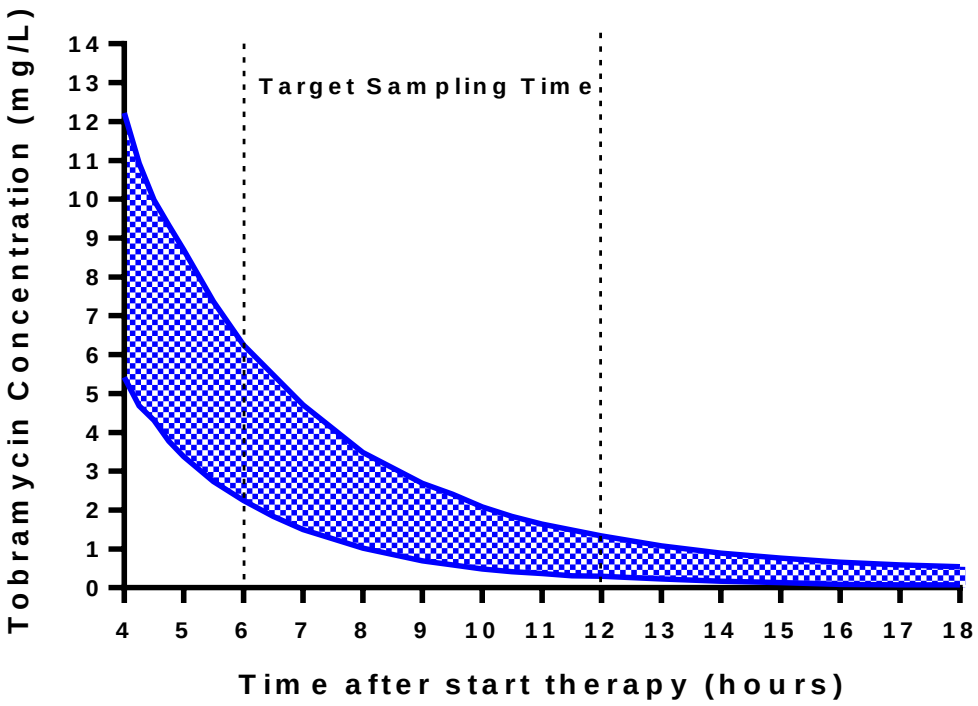


Key:
Below the shaded represents concentrations consistent with a daily AUC < 80 mg.h/L and/or a peak < 20 mg/L
Within shaded area represents concentrations consistent with a daily AUC of 80 -120 mg.h/L and a peak of 20 – 30 mg/L
Above the shaded area represents concentrations consistent with a daily AUC > 120 mg.h/L and/or a peak > 30 mg/L

6.4.4.2 Height scaled dosage adjustment nomogram

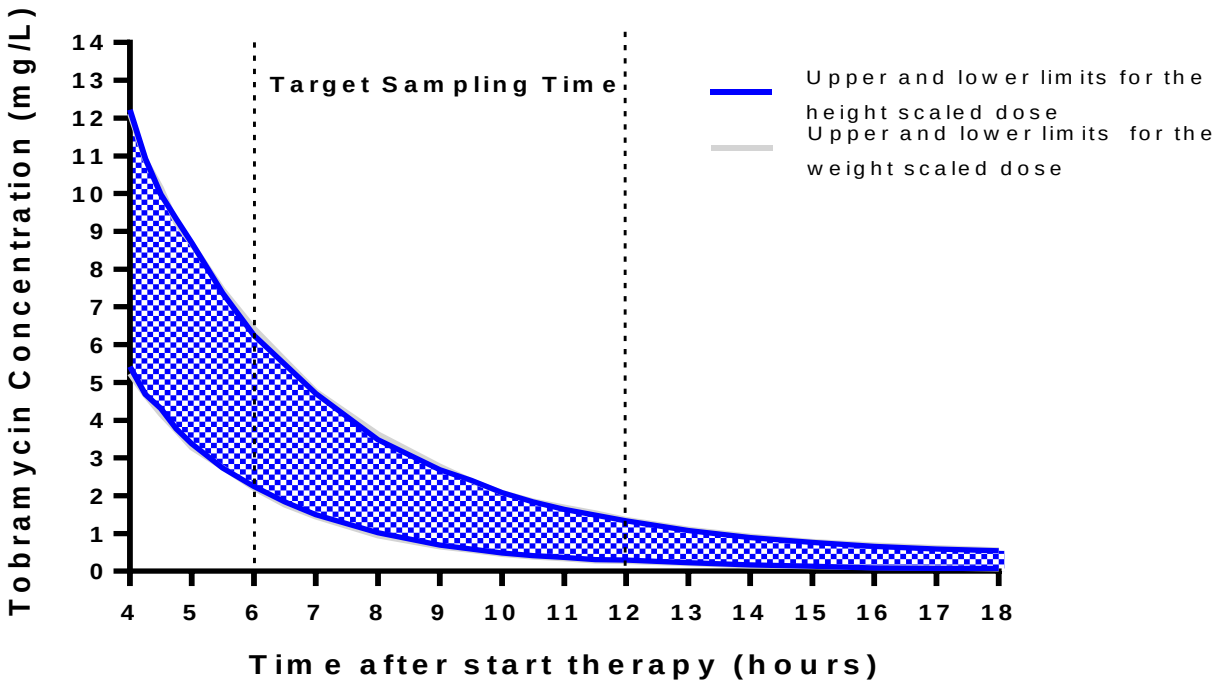
The concentration-time profiles obtained from the 3 mg/cm/day dose simulations were used to create the nomogram and the data from the 2065 patients who achieved both a daily AUC of 80-120 mg.h/L and a peak of 20-30 mg/L were used to develop the nomogram. Figure 6.13 shows the dosage adjustment nomogram with cut off daily AUC percentile. The lower bound was based on the 2.5th percentile from patients with normal renal function who had their daily AUC and peak concentration within the target range. The upper bound was based on the 97.5th percentile of patients with normal renal function who had their daily AUC and peak concentration within the target. The height scaled dosage adjustment nomogram and the weight scaled nomogram were plotted on the top of each other and are shown in Figure 6.14.

Figure 6.13 The 2.5th and 97.5th percentiles of all tobramycin concentration measurements at each time point for 3 mg/cm dose.



Key:
Below the shaded area represents concentrations consistent with a daily AUC < 80 mg.h/L and/or a peak < 20 mg/L
Within the shaded area represents concentrations consistent with a daily AUC of 80 -120 mg.h/L and a peak of 20 – 30 mg/L
Above the shaded area represents concentrations consistent with a daily AUC > 120 mg.h/L and/or a peak > 30 mg/L

Figure 6.14 The 2.5th and 97.5th and percentiles of all tobramycin concentration measurements at each time point for 10 mg/kg (max 660 mg/day) and 3 mg/cm dose.



6.4.5 Validation of the nomogram for interpreting tobramycin concentration measurements

6.4.5.1 New dataset

In total, data from 18 patients (10 males) with 25 different courses of tobramycin were available for nomogram validation. Table 6.3 summarises the clinical characteristics of the patients. Overall, they were young and had good renal function.

Table 6.3 Clinical characteristics of patients in the nomogram validation dataset.

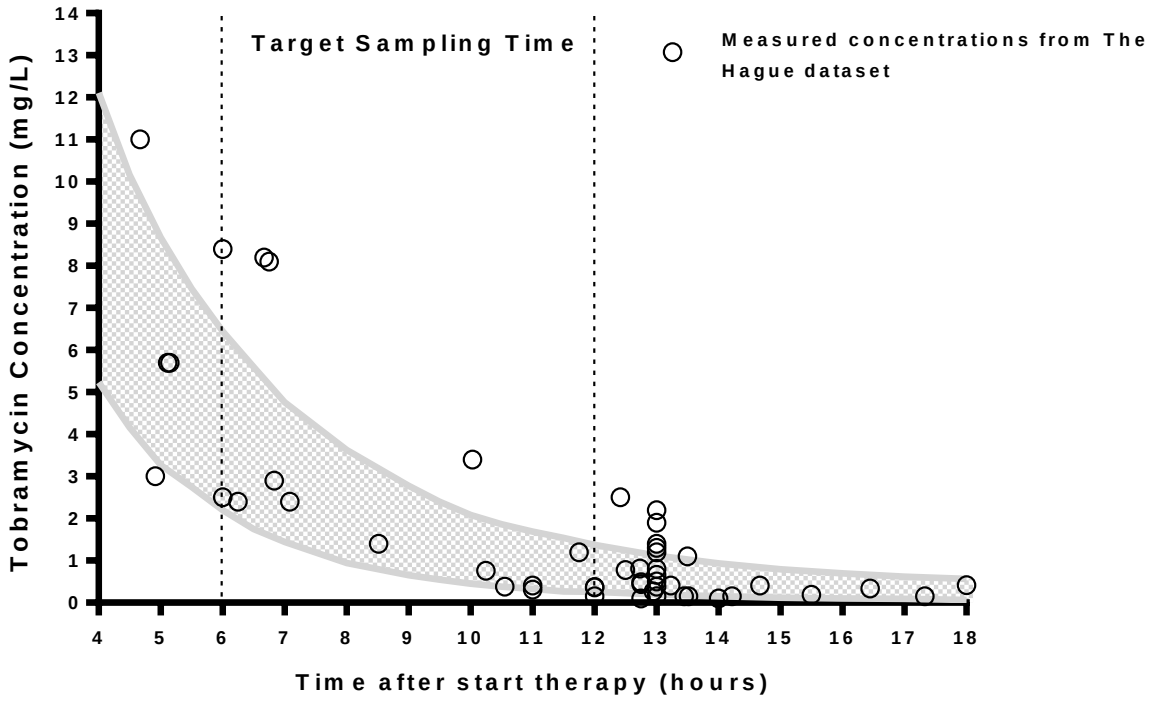
	Median	Range
Age (years)	19.0	14.1 – 78.4
Weight (kg)	57.0	41.3 – 84.2
Height (cm)	170	134 - 184
Serum creatinine (µmol/L)	54.0	26.0 – 83.0
Creatinine Clearance* (mL/min)	110	62.4 - 154
Dose (mg/day)	520	400 - 720
Dose (mg/kg/day)	9.46	7.62 – 10.3

Key: * Cockcroft and Gault equation (Cockcroft DW and Gault MH, 1976) with the lowest serum creatinine value fixed to 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998)

Eighty measured concentrations were available for analysis of which 12.5 % were peaks (1.00 – 1.50 hours after starting the infusion), 85 % were mid-dose concentrations (1.53 – 18.8 hours after starting the infusion) and 2.5 % were trough concentrations (21.8 -23.9 hours after starting the infusion). Concentrations obtained between 6 and 12 hours after the start of therapy were available from 11 patients and were selected to validate the nomogram (17 concentrations). Figure 6.15 shows the measured concentrations plotted on the weight scaled nomogram. Table 6.4 shows the distribution of measured concentrations categorised to the different nomogram areas, the interpretation of the results and the corresponding MWPharm interpretation. In 3 of the 4 samples that lay below the shaded area, 6 of 9 were within the shaded area and 1 of 4 were above the shaded area, there was agreement between the MWPharm and nomogram dose recommendations. In addition,

patients' daily AUCs were consistent with the daily AUC targets of 80 -120 mg.h/L. The median daily AUC for patients who had their concentrations below the shaded area was low, 77.4 mg.h/L, and for those who had their concentrations within shaded area the median value was within the target 85.5 mg.h/L. However, measurements that lay in area 3 were all from the same patient, and were high daily AUC value, 163 mg.h/L. Overall, 59 % of the nomogram recommendations matched the action taken.

Figure 6.15 Validation for the 10 mg/kg (maximum 660 mg) dosage adjustment nomogram using Dutch patients.



Key:
Below the shaded area represents concentrations consistent with a daily AUC < 80 mg.h/L and/or a peak < 20 mg/L
Within the shaded area represents concentrations consistent with a daily AUC of 80 -120 mg.h/L and a peak of 20 – 30 mg/L
Above the shaded area represents concentrations consistent with a daily AUC > 120 mg.h/L and/or a peak > 30 mg/L

Table 6.4 Comparison between The Hague and nomogram interpretations for concentrations obtained within nomogram target sampling time (6 and 12 hours after starting tobramycin infusion).

Nomogram area	Number of concs	MWPharm interpretations	Nomogram recommendation	Daily AUC mg.h/L	Matching nomogram recommendation
Below shaded area	4	• Increased subsequent dose (3) • No change (1)	• Increase subsequent dose	77.4 (73.5 – 81.8)	3 out of 4
within shaded area	9	• Increased subsequent dose (2) • No change (6) • Decreased subsequent dose (1)	• Continue with the same dose every 24 hours	85.5 (80.7 – 120)	6 out of 9
above shaded area	4	• Increased subsequent dose (1) • No change (2) • Decreased subsequent dose (1)	• Decrease the dose or extend dosage interval	163 (130 – 163)	1 out of 4

6.4.5.2 Glasgow and The Hague dataset

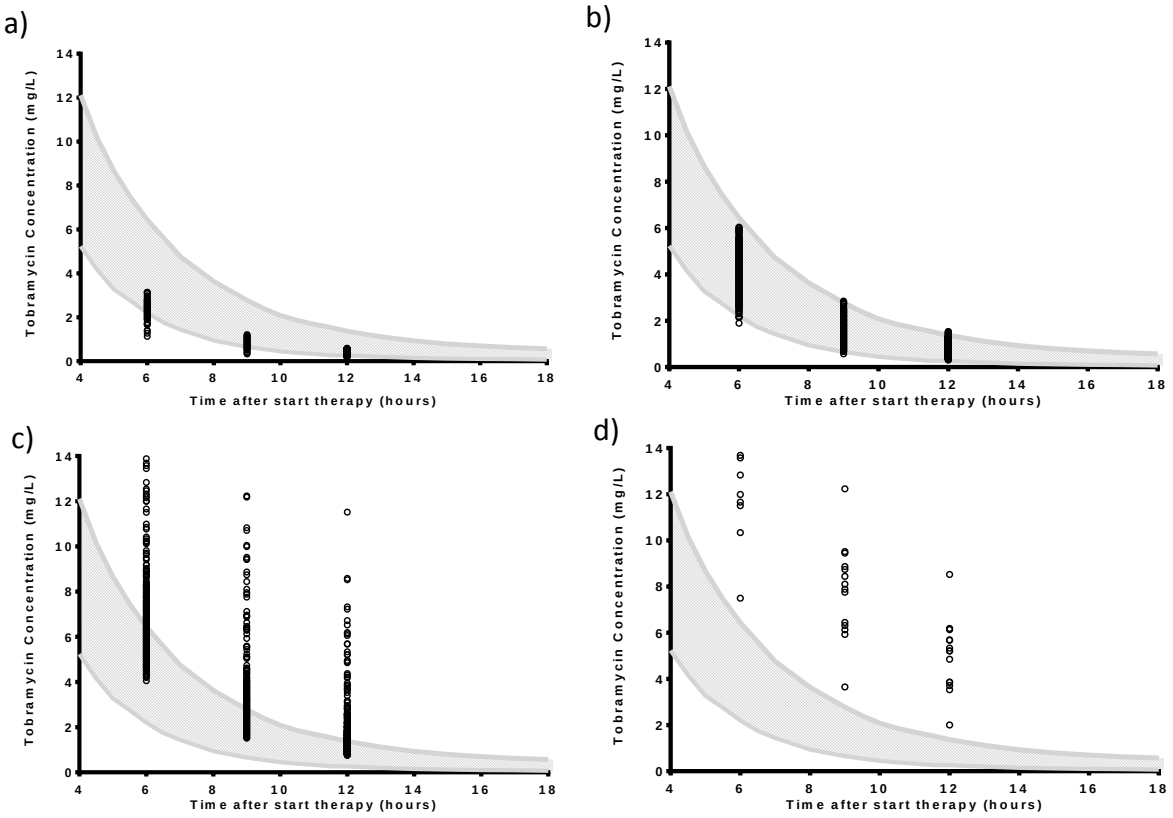
The combined dataset included 331 patients with 1490 course of aminoglycoside therapy. Thirty-eight patients had AUCs below, 239 patients had daily AUCs within and 163 patients had daily AUCs above the target daily AUC range of 80 – 120 mg.h/L. Patients whose AUCs were below the target had doses less than 600 mg/day and tended to have higher drug clearance (6.02 L/h) as shown in Table 6.5. Figure 6.16 (a-c) shows the predicted concentrations at the different time points plotted on the weight scaled nomogram for patients whose daily AUCs were below, within and above the target. The majority of patients who had below target daily AUCs had their concentrations within the shaded area but towards its lower end (Figure 6.16 (a)). These patients had a slightly lower drug clearance (5.62 L/h) compared with patients who had their concentrations below the shaded area (6.68 L/h). However, all patients who had their daily AUCs within target range had their concentrations within the shaded area.

In total 163 patients (433 courses) had daily AUC > 120 mg.h/L. However, not all concentrations were above the shaded area as shown in Figure 6.16 (c). Patients who had high daily AUC were sub-divided into four groups. Patients with daily AUCs between 120 to 130, 130 to 140, 140 to 150 and greater than 150 mg.h/L. Patients who had daily AUCs ranged 120 to 130 mg.h/L, had the highest CL values (median 4.81 L/h) as shown in Table 6.6. Patients whose daily AUC ranged from 120 to 140, had their concentrations within the shaded area as shown in Figure 6.17 (a and b). However, patients whose daily AUC was between 140 and 150 had their concentrations within and slightly above the shaded area for the nomogram as shown in Figure 6.17 (c). On the other hand, all patients who had their daily AUCs greater than 150 mg.h/L had their concentrations above the shaded area as shown in Figure 6.17 (d). Seven patients with 14 courses of therapy in the full dataset had creatinine clearances less than 50 mL/min. These patients had their concentrations above the nomogram shaded area, shown in Figure 6.16 (d), and were consistent with their high daily AUCs value (260 mg.h/L (148 – 353)).

Table 6.5 Doses and pharmacokinetic parameter estimates arising from simulated patients who received 10 mg/kg/day tobramycin, grouped according AUC range.

Variable	**< 80 mg.h/L** 38 patients (100 courses)	**80 – 120 mg.h/L** 239 patients (957 course)	**> 120 mg.h/L** 163 patients (433 courses)
Dose (mg/day)	432 (320 – 577)	514 (300 – 660)	610 (300 - 660)
V_1 (L)	13.7 (10.6 – 16.5)	13.4 (7.60 – 19.4)	13.8 (7.60 – 19.4)
CL (L/h)	6.02 (4.47 – 8.38)	5.18 (2.96 – 7.73)	4.14 (1.36 – 5.49)

Figure 6.16 Tobramycin dosage adjustment nomogram using 10 mg/kg (max 660 mg/day). The open circles are the predicted concentrations for patients who were administered 10 mg/kg and had AUCs a) below, b) within and c) above target range, and d) patients with CrCL less than 50 mL/min.

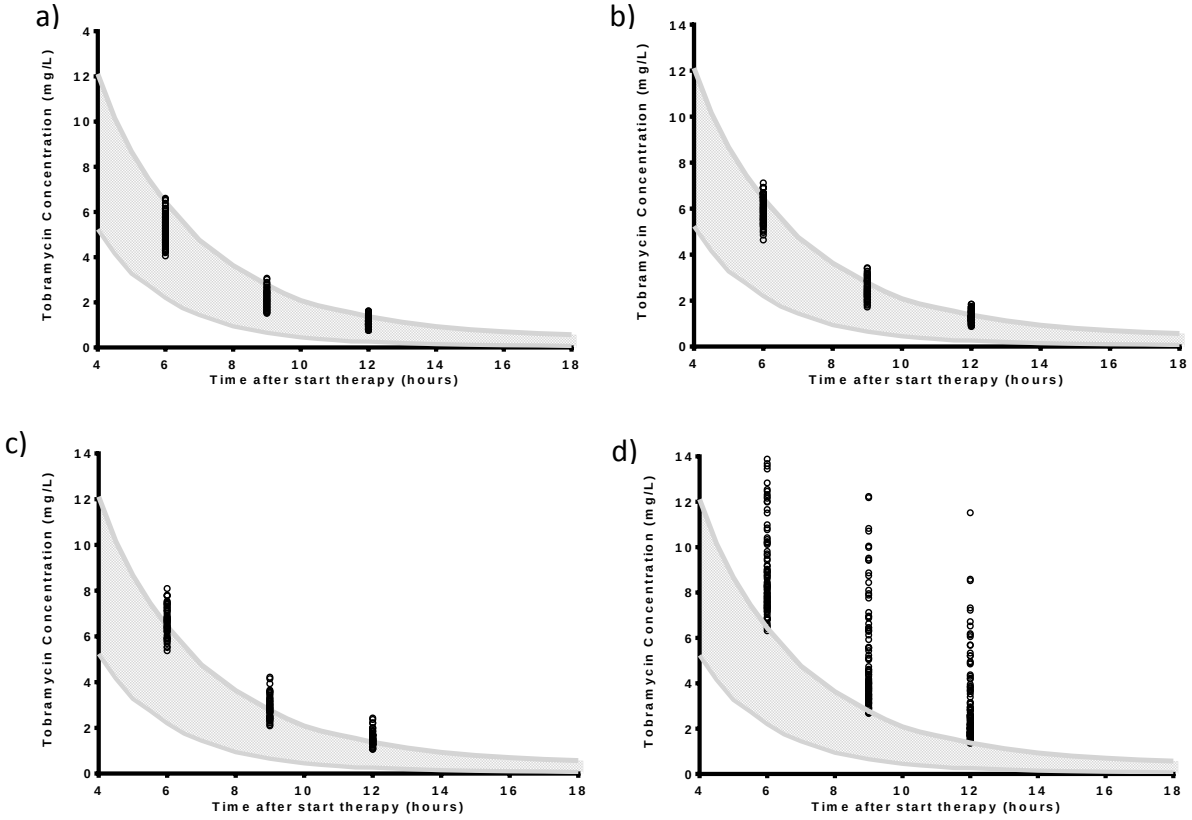


Key:
Below the shaded area represents concentrations consistent with a daily AUC < 80 mg.h/L and/or a peak < 20 mg/L
Within the shaded area represents concentrations consistent with a daily AUC of 80 -120 mg.h/L and a peak of 20 – 30 mg/L
Above the shaded area represents concentrations consistent with a daily AUC > 120 mg.h/L and/or a peak > 30 mg/L

Table 6.6 Doses and pharmacokinetic parameter estimates arising from simulated patients who received 10 mg/kg/day tobramycin and had a daily AUCs > 120 mg.h/L, grouped according AUC range.

Variable	AUC > 120 mg.h/L			
	AUC 120-130 (156 courses)	**AUC 130-140** (89 courses)	**AUC 140-150** (65 courses)	**AUC > 150** (123 courses)
Dose (mg/day)	600 (300 -660)	600 (336 – 660)	600 (420 – 660)	635 (418 – 660)
V_1 (L)	14.0 (7.60 – 19.4)	13.9 (8.00 – 18.2)	13.9 (9.30 – 18.4)	13.2 (9.3 – 16.6)
CL (L/h)	4.81 (2.48 – 5.49)	4.45 (2.56 – 5.07)	4.13 (2.85 – 4.71)	3.44 (1.36 – 4.37)

Figure 6.17 Weight based dose (10 mg/kg max 660 mg/day) nomogram. The open circle the predicted concentrations for patients who were administered 10 mg/kg and had a) AUC 120 -130 mg.h/L, b) AUC 130 -140 mg.h/L, c) 140 -150 mg.h/L, d) AUC > 150 mg.h/L.



Key:
Below the shaded area represents concentrations consistent with a daily AUC < 80 mg.h/L and/or a peak < 20 mg/L
Within the shaded area represents concentrations consistent with a daily AUC of 80 -120 mg.h/L and a peak of 20 – 30 mg/L
Above the shaded area represents concentrations consistent with a daily AUC > 120 mg.h/L and/or a peak > 30 mg/L

6.5 DISCUSSION

6.5.1 Introduction

This study aimed to examine whether a weight based dosing regimen is optimal in cystic fibrosis patients and to develop a tobramycin dosage adjustment nomogram for the recommended 10 mg/kg dose (max 660 mg/day) in patients with cystic fibrosis. The recommended monitoring strategy for such a high dose is a 30 minute peak concentration between 20 – 30 mg/L after a 30 minute infusion and trough (pre-dose) concentration less than 1 mg/L (Smyth A et al., 2005, Touw DJ et al., 2007). However, the definition of trough concentration was not clear regarding whether it was a real (24 hours) or 18 hour post dose trough. The typical daily AUC and ranges for the current tobramycin dose were 106 mg.h/L (80-120). In the current study, different weight dosage regimens were compared and the height based dosage regimen was found to achieve the highest peak concentration and daily AUC range.

There are a number of reasons why the measurement of trough or 18 hour post-dose concentrations is not the ideal approach when monitoring "once daily" dosing of tobramycin. Firstly, a 24 hour trough concentration would typically be less than 0.3 mg/L for a patient with normal renal function, which is too low to be detected by conventional aminoglycoside assays (Hennig S et al., 2007, Begg EJ et al., 1995). Furthermore, a limitation highlighted by Begg *et al* (1995) is that aminoglycosides have a multi-compartment disposition and therefore concentrations at 24 hours reflect the slow terminal elimination of the drug from deeper compartments ("gamma" phase) and not the "beta" phase, where most of the elimination of the drug from plasma occurs. Coulthard *et al* (2007) compared the daily AUC and the trough concentration (< 1 mg/L) monitoring approaches of tobramycin in adults and paediatric patients with cystic fibrosis. They confirmed that targeting a trough < 1 mg/L following a 10 mg/kg daily dose was inappropriate, because patients with either decreased or increased tobramycin clearance might be missed since both are likely to achieve trough concentrations less than 1 mg/L. Therefore, this approach might place these patients at risk of over- or under-exposure. Instead, they encouraged the use of an AUC approach with a target of 100 mg.h/L to monitor once daily tobramycin.

With aminoglycoside antibiotics, the rate and extent of bacterial killing is concentration-dependant and hence either C_{max} or AUC can be used as aminoglycoside monitoring parameters. Vogelman *et al* (1988a) found that AUC was the important pharmacokinetic parameter for aminoglycoside to ensure efficacy against a gram negative organism (*P.aeruginosa*) in an animal model. A recent *in vitro* study that evaluated the pharmacokinetic/pharmacodynamic indices for six antibacterial drugs, including gentamicin, found that the AUC/MIC ratio was better correlated with gentamicin antibacterial effect than the C_{max}/MIC ratio (Nielsen EI et al., 2011). Burkhardt *et al* (2006) investigated the relationship between once and three times daily tobramycin pharmacokinetic/pharmacodynamic parameters and clinical outcome in 33 adult patients with cystic fibrosis. They found good correlation between C_{max}/MIC and daily AUC/MIC ratios and lung function (Forced expiratory volume in 1 second, FEV_1%) in both once and three times daily dosing. However, it showed dosage regimen dependency where for equal values of daily AUC/MIC, the once daily tobramycin showed better improvement in lung function (Forced expiratory volume in 1 second, FEV_1%) than three times daily. In a similar context, Mouton *et al* (2005) looked at 13 paediatric and young adult patients with cystic fibrosis with an infectious exacerbation due to *P.aeruginosa*. They found that tobramycin pharmacodynamic index (daily AUC/MIC and C_{max}/MIC ratios) were correlated with effect (lung function (FEV_1% and FVC %)) and correlation was slightly better for daily AUC/MIC ratio.

Rybak *et al* (1999) examined the safety of twice vs once daily aminoglycoside therapy in 74 general medical patients and found that the daily aminoglycoside AUC was a predictor of nephrotoxicity. Similarly Croes *et al* (2012) examined simulations of aminoglycoside doses of 7 mg/kg/day administered using varying dosage regimens. They reported that the onset of nephrotoxicity could be predicted from daily AUC over the course of therapy regardless of the different dosage regimens used. In summary, all the previous evidence supports the use of daily aminoglycoside exposure as the monitoring approach for once daily dosing instead of the traditional peak and (especially) trough concentrations.

Begg *et al* (1995) suggested a target daily AUC range between 72 and 101 mg.h/L to monitor aminoglycosides in general medical patients. They defined the target daily AUC as the daily aminoglycoside exposure in a patient with a typical V of 0.25 L/kg and an elimination half-life of 2.5 hours for a dose range of 5 - 7 mg/kg/day (Begg EJ et al., 1995). This daily AUC range cannot be applied to cystic fibrosis patients because the recommended dose is much higher (10 mg/kg/day) and the target peak is also higher (20-30 mg/L vs 10-20 mg/L in general medical patients). Therefore, there was a need to define a target daily exposure and range for aminoglycosides in patients with cystic fibrosis. It has been reported that in the US, 20% of CF centres use a target of 100 mg.h/L (Prescott JrWA, 2011), which is estimated from two samples for daily tobramycin exposure, The origins of this value are not clear.

6.5.2 Daily exposure target and range using real data

The daily AUC values observed in clinical practice determined from Glasgow and The Hague datasets were compared with the target derived from the TOPIC study (Touw DJ et al., 2007) (106 mg.h/L). It was found that the median daily exposure for patients from The Hague who had their daily doses between 9 and 11 mg/kg/day was consistent with this target value.

None of the measured peak concentrations achieved the target concentration, because the target peak in the Glasgow dataset was low (8 -12 mg/L) and patients were administered low doses (median 360 mg/day). On the other hand, 120 peak concentrations achieved the target peak in The Hague dataset, where 95 of these concentrations were withdrawn from once daily dosing. Patients who had concentration measurements below the target peak concentration also had a lower daily exposure limit of 84.6 mg.h/L and hence 80 mg.h/L was chosen as the lower limit of the range. Looking at the median daily exposure values for patients who achieved or had their peak concentrations above the target did not help to decide on an upper limit, because the values were similar (102 and 113 mg.h/L). However, patients who had their trough concentrations greater than 1 mg/L had daily exposure greater than 120 mg.h/L and that those patients had a relatively low drug clearance. Therefore, 120 mg.h/L was chosen to be daily exposure upper limit. This range was slightly higher than the Australian ranges (72 and 101 mg.h/L) (Begg EJ et al., 1995). Since the dose

in patients with cystic fibrosis is higher than that for other medical conditions, higher daily tobramycin exposure was expected.

6.5.3 Weight-scaled dose simulations

In the TOPIC study (Smyth A et al., 2005) the daily dose was restricted to 660 mg, but there was no justification provided in the study for this restriction. Therefore, both an unrestricted dose of 10 mg/kg and with an upper limit of 660 mg were tested in the simulations. Because the pre-dose trough concentration is usually undetectable for patients with good renal function, an 18 hour trough concentration less than 1 mg/L was used to monitor daily tobramycin dose in the current study. A very slight improvement in the proportion of patients who would achieve the target daily AUC range was observed when restricting the dose but there was no difference in the proportion of patients achieving the target peak concentration. Similarly, dose restriction did not influence the proportion of patients who achieved satisfactory trough concentrations at 18 hours after the dose. Restricting the dose to 660 mg/day is more likely benefits patients with high body weight (> 66 kg) and those patients are few in the cystic fibrosis population. In the current study, only 20% of simulated patients had their weight 66 kg and above. This could be the reason behind the small influence of restricting the dose to 660 mg/day seen from the simulations.

Based on the current results, restricting the daily dose of tobramycin was appropriate because it was found that daily AUC increased as weight increased and this was a particular problem if the patient had a BMI above 25 kg/m^2. However, overweight and obesity are rare in the cystic fibrosis population. In the UK, the median and inter-quartile range of BMI in patients with cystic fibrosis (16 – 50^+ years old) has been reported as 21.9 kg/m^2 (19.9 – 24.3) (Cystic FibrosisTrust, 2013). In the present study, the median BMI was 20.0 kg/m^2 (17.5 – 22.5). The current simulations identified some limitations for using weight as the scaling factor to individualise doses in patients with cystic fibrosis. Firstly, weight scaled dose led to a very wide range of doses. Secondly, analysis of daily AUC estimates found that underweight patients (BMI ≤ 18.5 kg/m^2) were at risk for tobramycin under dosing, which might lead to treatment failure or the need to use tobramycin for longer than 14 days. In

term of safety, a high proportion of patients (40 %) had their daily exposure above the target and might be at risk of nephrotoxicity. These findings highlighted the need to identify an alternative approach to determining the daily dose of tobramycin.

6.5.4 Development of new dosage guidelines

The results obtained with weight-related dosing suggested that an alternative dosage regimen was required that would have less variation in doses and a higher probability of achieving the target daily AUC. Lean body weight, height and body surface area were tested as potential dose scaling factors and compared with the current weight scaled dosing. Using height as the scaling factor resulted in more patients achieving the target peak concentration and daily AUC ranges (63 % and 61 %) compared with using the existing weight scaled dosing (41 % and 50 %), estimated LBW (46 % and 51 %) or BSA (56 % and 58 %) scaled dosing. This made sense because height was less variable between patients and had a narrow range. In addition, it was evident from the early model development that height was a better descriptor of aminoglycoside clearances and volume of distribution of the central compartment. In a recent study of 24 paediatric and adult cystic fibrosis patients who were administered a 10 mg/kg tobramycin dose (VandenBussche HL and Homnick DN, 2012), the proportion of patients who achieved the target peak concentration was 42 %, which is similar to the present results (41 %).

Height scaled dosing resulted in a narrower range of daily doses with fewer daily AUC outliers. In contrast to doses scaled according to weight, LBW and BSA, the maximum daily dose when scaled for height was 581 mg/day and hence there was no need to define an upper limit. Interestingly, the distribution of doses, daily exposure and peak concentrations were similar for the weight and LBW scaling doses. Even when the maximum daily weight scaled dose was restricted to 660 mg, more patients had high predicted daily AUC or peak concentration values than with the height scaled dose (40 % compared to 27%). Since it has been shown that aminoglycoside exposure was correlated with nephrotoxicity (Croes S et al., 2012, Rybak MJ et al., 1999) the reduction in the proportion of patients with a daily AUC greater than 120 mg.h/L is an important advantage.

There was no relationship between the administered doses and daily exposure when doses were scaled for height but it was found that daily exposure increased with size when the scaling factor was weight or LBW and, to a lesser extent, BSA. On the other hand, a clear indirect relationship was found between tobramycin clearance and daily exposure for the four different doses. These observations could be explained by the narrow range of height scaled doses, which reduced the influence of amount administered and led to CL being the most important factor influencing daily AUC. This was confirmed by having more patients with impaired renal function (94 patients) achieving high daily AUC (>120 mg.h/L) compared with weight (52 patients), LBW (54 patients) and BSA (65 patients).

6.5.5 Development of weight and height scaled tobramycin dosage adjustment nomogram

The "Hartford nomogram" was the first graphical plot designed to interpret aminoglycoside concentration measurements. It was developed using a prospective evaluation of a 7 mg/kg dose in 20 patients who had at least two concentration measurements (Nicolau DP et al., 1995). However, there were several limitations in the development of Hartford nomogram (Nicolau DP et al., 1995). Firstly, the investigators used published pharmacokinetic parameters based on a one compartment model to determine the dose and the "peak" concentration was based on the predicted value at the end of a 60-minute infusion, without consideration of the distribution phase. Furthermore, they extrapolated the aminoglycosides, gentamicin and tobramycin, concentration-time profile from only small number of patients (20 patients) to develop the nomogram.

In patients with cystic fibrosis, a tobramycin dosage adjustment nomogram was developed by Massie and Cranswick (2006) for a 12 mg/kg daily dose using data from 44 paediatric and young adult patients (9 months to 20 years old). They predicted concentrations at different times after the dose using individual aminoglycoside pharmacokinetic parameter estimates derived from a post hoc analysis based on a one compartment model they had developed previously. The nomogram consisted of three lines: a central line that represented the mean predicted tobramycin plasma concentration and two lines that

represented a 20% variation around this line. Their target sampling time was between 1 and 6 hours. However, there was no validation study conducted for their nomogram.

In the current study, a tobramycin dosage adjustment nomogram was developed to identify potential under dosing, unexpected overdosing or to confirm that the dose is appropriate. The nomogram was developed using the 2.5th and 97.5th percentiles of the concentration profiles derived from 1421 simulated patients for weight scaled dose and 2065 simulated patients for height scaled dose who achieved the daily AUC and peak concentration targets. Interestingly the height scaled nomogram was identical to the weight scaled nomogram and although it was developed from a larger patient group. This observation was expected because simulated patients were generated from similar distribution and there was a large overlap in patients. Since the population model was not suitable for patients with renal impairment, simulated patients with CrCL of 50 mL/min and less were excluded from developing the nomogram. The target sampling time was chosen to be between 6 and 12 hours to avoid concentrations that might be lower than the limit of assay quantification, usually at the end of dosage interval. This wide sampling window offers flexibility when withdrawing samples and would be more practical for nursing staff.

The generated nomogram consists of three areas. If measured concentrations are below the shaded area, then daily AUC and peak concentration would be below the target and these patients might benefit from an increase in the dose. The majority of simulated patients (77 %) whose concentrations lay in this area were underweight and had high median tobramycin CL. VandenBussche and his colleagues (2012) documented a similar observation. They found that patients who had below the target peak concentration were young patients. If the measured concentrations are within the shaded area then they said to be within target daily AUC and/or peak concentrations, and those patients would be administered the same dose every 24 hours. However, measured concentrations above the shaded area indicated that daily AUC and/or peak concentration are above the target. These patients would benefit from reducing the administered dose, extending the dosage interval or withhold until the concentration falls below 1 mg/L.

6.5.6 Nomogram validation

A new independent dataset for cystic fibrosis patients administered 10 mg/kg/day tobramycin from The Hague was used to validate tobramycin dosage adjustment nomogram for the weight scaled dose. The nomogram validation showed reasonable agreement between the MWPharm interpretation and nomogram recommendation.

Moreover, the results from patients from Glasgow and The Hague who were administered the 10 mg/kg (maximum 660 mg/day) showed that the nomogram was able to predict patients whose daily AUCs were below, within and above the target daily AUC range from one sample point. Importantly, the nomogram was able to predict patients with poor renal function. However, some patients whose AUCs were greater than 120 mg.h/L were within the shaded area. Close examination of these patients showed that these patients had higher drug clearance and their AUCs were between 120 and 150 mg.h/L. Based on Croes *et al* work (2012), nephrotoxicty (a 50 % or more reduction in creatinine clearance) was observed when daily AUC was 150 mg.h/L. Therefore, we can say that a safe daily AUC upper limit is less than 150 mg.h/L, and hence the lower AUCs (120 -150 mg.h/L) are considered acceptable in the current analysis.

The strengths behind the current analysis are the use of pharmacokinetic parameter estimates from a large dataset comprising 331 patients from two independent sites to develop daily AUC range and generate the simulations. This provides wider applicability for the nomogram. In addition, using a simulation approach facilitated generation of a large sample size (5000 patients) with a distribution of clinical characteristics that mimicked the actual patient population. However, validation of the nomogram was conducted using retrospective concentration data and no clinical outcome data were available. In addition, the number of patients (11) and tobramycin concentrations (17) used to validate the nomogram were small, which considered a limitation. However, a second validation was conducted using the available full dataset using the known individual pharmacokinetics estimates predicted from the final model run developed in Chapter 3, and were administered the tested dose weight scaled dose (10 mg/kg). Moreover, there were 7

patients with poor renal function in the combined dataset, which helped to evaluate whether the nomogram was able to predict them using one sample.

Results from the current simulations confirmed the superiority of height over weight as dose scaling factor. For future work, validation of the derived target daily AUC and range is required and link them to clinical outcomes such as daily AUC/MIC ratio and/or lung function. In addition, comparison study between the proposed height scaled dose and the current dosage guideline is required.

6.5.7 Conclusion

In conclusion, a daily AUC range for tobramycin was proposed using actual concentration-time profile data. Evaluation of the current dosage guidelines (10 mg/kg/day) identified a high number of patients with daily AUCs >120 mg.h/L (40%). An alternative dosage guideline was therefore developed to overcome this problem, using height as a scaling factor. A tobramycin dosage adjustment nomogram for a dose of 10 mg/kg up to 660 mg/day was developed and validated using daily AUC and peak targets.

CHAPTER 7: PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS OF WEIGHT AND HEIGHT SCALED DOSAGE REGIMENS FOR ADULT PATIENTS WITH CYSTIC FIBROSIS

7.1 INTRODUCTION

Pharmacokinetic-pharmacodynamic (PK-PD) indices correlate pharmacokinetic parameters with response, which facilitate drug efficacy predictions. It is important to take into account patient to patient variability when predicting the probability of successful treatment. Drusano and colleagues (Drusano GL et al., 2001) were the first to apply Monte Carlo simulations to examine microbiological breakpoints. In this approach, population pharmacokinetic and microbiological susceptibility information are combined. The approach has become the standard methodology to set breakpoints and has been used by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) since 2002 (Mouton JW et al., 2012). One of the parameters that can be estimated using Monte-Carlo simulations is the probability of target attainment (PTA). PTA is the probability that at least a specific value of a pharmacodynamic index, usually the minimum inhibitory concentration, is achieved at a certain concentration (Mouton JW et al., 2012). Another parameter that can be predicted using Monte-Carlo simulations is the cumulative fraction of response (CFR). CFR is calculated from the proportion of the population achieving a certain PK-PD value, e.g. Peak/MIC ratio ≥ 10 based on simulations that consider the typical MIC distribution of the target microorganism. This value helps to assess the likelihood of antimicrobial treatment response.

Aminoglycosides are concentration-dependent antibiotics, and both Peak/MIC and daily AUC/MIC ratio are the PK-PD indices associated with their antibacterial effect. Their bactericidal activity increases at higher concentrations relative to the MIC and hence the ratio of peak concentration to MIC is the most important PK-PD parameter to predict response (Blaser J et al., 1987, Moore RD et al., 1987). In patients with cystic fibrosis, the main organism causing lung infections is *P. aeruginosa,* which accounts for 36.5 % of infections in all age groups and for 55.8 % in adults (Cystic FibrosisTrust, 2013). The present study uses the approach of Drusano *et al* (2001) to predict aminoglycoside dosage regimen breakpoint or treatment success in patients with cystic fibrosis. The likelihood of treatment success is examined using the current dosing aminoglycoside dosing regimen (Smyth A et al., 2005) (10 mg/kg restricted to 660 mg/day) and the new height based dosage regimen proposed in Chapter 6.

7.2 AIMS

- To determine the probability of target attainment for tobramycin for a Peak /MIC ratio ≥ 10 and a range of AUC/MIC ratios for organisms with different MICs in adult patients with cystic fibrosis.
- To determine the cumulative fraction of response for gram negative organisms encountered in patients with cystic fibrosis based on the current tobramycin and gentamicin weight scaled dosage regimen (10 mg/kg/day) and the new height scaled dosage regimen (3 mg/cm/day).

7.3 METHODS

The simulations performed in Chapter 6 were used to predict the peak concentration (at 1 hour) to MIC ratio and the daily AUC to MIC ratio. In brief, 5000 patients were simulated to be administered doses of 10 mg/kg/day (maximum 660 mg/day) and 3 mg/cm/day for both tobramycin and gentamicin. The following higher doses were also examined: 12 mg/kg/day; 3.5 mg/cm/day; and 4 mg/cm/day. Patients' demographic and biochemical data, including age, weight, height and serum creatinine were simulated. Log normal distributions for the variance of these data were used to generate new patients who mirrored the combined datasets. Simulations were created using NONMEM version 7.1 (Beal SL et al., 2009). Creatinine clearance was estimated by the Cockcroft and Gault equation (1976) using the simulated values of gender, age, weight and serum creatinine. The minimum serum creatinine value was fixed at 60 µmol/L (Duffull SB et al., 1997, Rosario MC et al., 1998). Simulated demographics and biochemical data were limited to the values observed in the combined data, i.e. weight 30 -108 kg; height 139 – 194 cm; age 14 – 88 years, serum creatinine 60 -209 µmol/L; creatinine clearance 26 -181 mL/min.

In the Glasgow dataset used to develop the population model, 96 % of patients were administered tobramycin and 4 % were administered gentamicin. However, aminoglycoside type had no influence on the model (for details see Chapter 3). Therefore, gentamicin and tobramycin pharmacokinetics were assumed to be similar.

7.3.1 Probability of target attainment

The probability of achieving the target pharmacokinetic – pharmacodynamic response was calculated for each patient. A target peak to MIC ratio ≥ 10 (Kashuba ADM et al., 1999) was chosen for the gram negative organisms e.g. *P.aeruginosa* and *H.influenzae*. The peak concentration was defined as a 30 minute post dose sample obtained after a 30 minute infusion. In addition, a target daily AUC to MIC ratio ≥ 100 was chosen, but also other published targets were examined, including ≥ 50 and 150 for gram negative organisms (Kashuba ADM et al., 1999, Mouton JW et al., 2005, Smith PF et al., 2001). The predicted one hour peak concentration to MIC and daily AUC to MIC ratios were calculated for each simulated patient at a wide range of MICs (0.002 to 512 mg/L) as per the EUCAST available MICs distributions. For each tobramycin regimen, the highest MIC at which the regimen achieved PTA ≥ 90% was defined as the PK-PD susceptibility breakpoint.

7.3.2 Cumulative fraction of response

Tobramycin and gentamicin MIC distributions against *P.aeruginosa* and *H.influenzae* were obtained from the EUCAST website (European Committee on Antimicrobial Susceptibility Testing). The cumulative fraction of response determined the expected overall response of each organism to tobramycin or gentamicin for each tested dosage regimen. The fraction of patients who were expected to achieve the target PK-PD index was multiplied by the fraction of the organism distribution at a particular MIC. The fraction of cumulative response was then calculated as the sum of all fraction products at each MIC value (Drusano GL et al., 2001). Table 7.1 shows an example of calculating the cumulative fraction of response against *P.aeruginosa* for the 10 mg/kg/day dose.

Table 7.1 An example of calculating the cumulative fraction of response against *P.aeruginosa* for the 10 mg/kg/day (maximum 660 mg/day) dose.

MIC mg/L	Number of isolates	Percentage of distribution at the indicated MIC	Fraction target attainment (Peak/MIC ≥10) at the MIC	Percentage products
0.002	0	0	1	0
0.004	0	0	1	0
0.008	0	0	1	0
0.016	3	0.0121	1	0.0121
0.032	6	0.0241	1	0.0241
0.064	12	0.0482	1	0.0482
0.125	383	1.54	1	1.54
0.25	2682	10.8	1	10.8
0.5	10473	42.1	1	42.1
1	7145	28.7	0.9998	28.7
2	2063	8.29	0.9818	8.14
4	540	2.17	0.3458	0.75
8	347	1.39	0.001	0.00139
16	451	1.81	0	0
32	335	1.35	0	0
64	225	0.904	0	0
128	74	0.297	0	0
256	36	0.145	0	0
512	116	0.466	0	0
Sum	**24891**	**100**	**-**	**92.1**

7.4 RESULTS

7.4.1 Simulated patients

Table 7.2 shows the median and ranges of daily doses, peak concentrations and daily AUCs for the weight and height scaled dosage regimens. For the 10 mg/kg/day doses, the maximum daily dose was restricted to 660 mg, according to standard clinical practice. However, a dose restriction was not applied to the higher weight scaled dose (12 mg/kg/day), and the maximum daily dose was 1296 mg. No restrictions were applied to the height scaled doses. The maximum daily dose for 3 mg/cm/day was 581 mg, 3.5 mg/cm/day was 679 mg and 4 mg/cm/day was 776 mg. The median peak concentration increased as the dose scalar increased; the highest peak value (98 mg/L) was achieved with the 12 mg/kg/day dosage regimen. However, for the highest height scaled dose of 4 mg/cm/day, the predicted maximum peak concentration was 68.7 mg/L. The maximum daily AUC for the 12 mg/kg/day dose was 410 mg.h/L and was greater than observed for the 4 mg/cm/day dose (303 mg.h/L).

Table 7.2 Median and ranges of daily doses, peak concentrations and daily AUC for a range of weight and height scaled dosage regimens.

	10 mg/kg/day	**12 mg/kg/day**	**3 mg/cm/day**	**3.5 mg/cm/day**	**4 mg/cm/day**
Daily Dose (mg/day)	550 (300 - 660)	656 (365 - 1296)	498 (418 - 581)	581 (487 - 679)	664 (556 – 776)
Peak Concentration (mg/L)	30.1 (9.74 -79.3)	35.6 (9.59-98.3)	27.2 (11.8-52.1)	31.7 (12.7-60.1)	36.2 (14.5-68.7)
Daily AUC (mg.h/L)	112 (45.1- 260)	137 (53.8- 410)	104 (48.1-227)	121 (52.6-265)	139 (60.1-303)

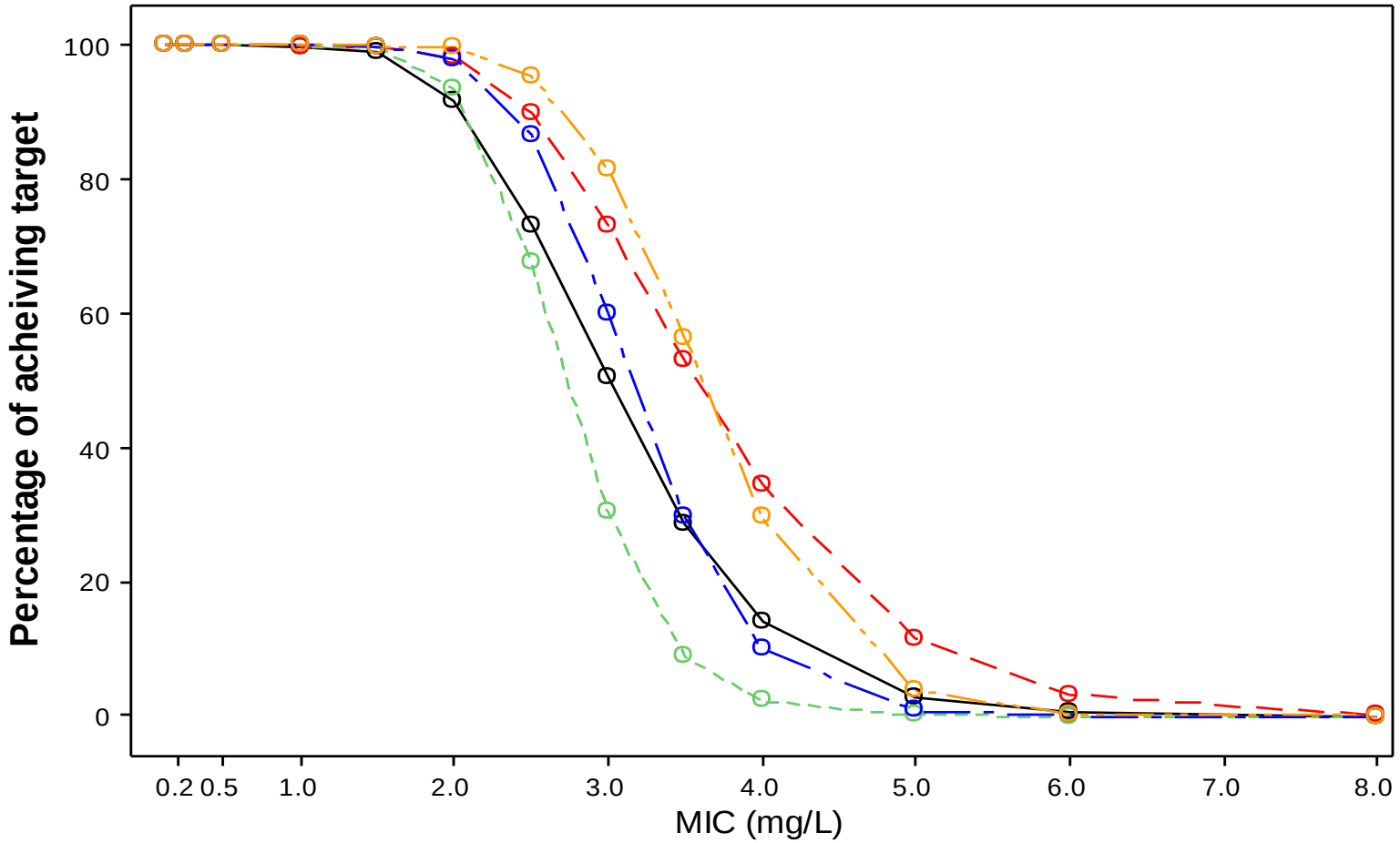
7.4.2 Probability of target attainment

The percentages of patients predicted to achieve Peak/MIC ratios ≥ 10 for the different tobramycin dosage regimens are shown in Figure 7.1. As observed from all PTA Figures, the PTA was close to 100 % at low MICs (less than 0.125 mg/L), and then decreased rapidly to 0

at high MICs (greater than 8 mg/L). Therefore, for simplicity, only the results from MICs 0.125 to 8 mg/L are shown in PTA Figures 6.1 and 6.2. For the current weight scaled dose, more than 90 % of patients were expected to achieve the target Peak/MIC if the MIC was 2 mg/L or less. If the MIC was 3 or 4 mg/L, only 50 % and 14 % of patients were expected to achieve the target. For the higher, weight scaled dose, at least 90 % of patients were expected to achieve the target Peak/MIC if the MIC was 2.5 mg/L or less. For higher MICs of 3 or 4 mg/L, the percentage of patients achieving the target decreased to 73 % and 35 % respectively.

Similarly for height scaled doses of 3 and 3.5 mg/cm/day, more than 90 % of patients were expected to achieve the target Peak/MIC ≥ 10 when the MIC was 2 mg/L or less and 30% and 60 %, respectively, of patients were expected to achieve the target when the MIC was 3 mg/L. At an MIC of more than 4 mg/L, then less than 2 % of patients given the 3 mg/cm/day dose and less than 10 % given the 3.5 mg/cm/day dose were expected to achieve the target. In contrast, with a dose of 4 mg/cm/day, more than 80 % of patients were expected to achieve the target if the MIC was 3 mg/L or less but only 30 % of patients if the MIC was 4 mg/L. Therefore, the PK-PD susceptibility breakpoint for the current tobramycin dose (10 mg/kg/day) and height scaled doses of 3 and 3.5 mg/cm/day is ≤ 2 mg/L and the PK-PD susceptibility breakpoint for the higher doses of 12 mg/kg/day and 4 mg/cm/day is ≤ 2.5 mg/L.

Figure 7.1 Percentage probability of achieving a target Peak/MIC ratio ≥ 10 with weight (10 and 12 mg/kg/day) and height (3, 3.5 and 4 mg/cm/day) scaled doses over a range of MIC values.

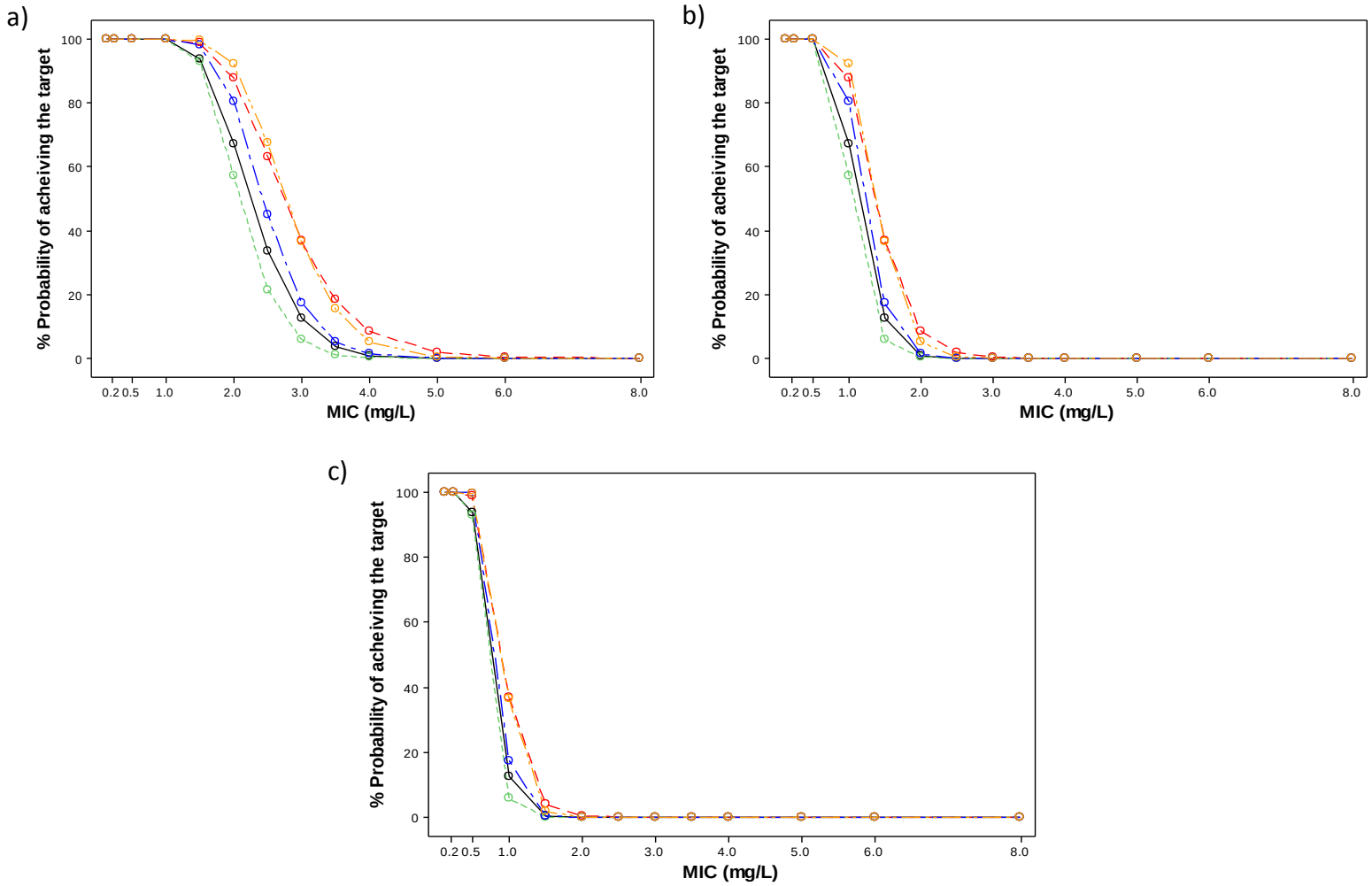


Key: The black line represents the 10 mg/kg/day dose, the red line represents the 12 mg/kg/day dose, the green line represents the 3 mg/cm/day dose, the blue line represents the 3.5 mg/cm/day dose, and the orange line represents the 4 mg/cm/day dose.

The percentages of patients predicted to achieve target daily AUC/MIC ratios for the different tobramycin dosage regimens are shown in Figure 7.2 a-c. For the weight scaled dosage regimens, more than 90% of patients were expected to achieve daily AUC to MIC ratios ≥ 50 when the MIC value was 1.5. For an MIC of 2 mg/L, only 67 % (10 mg/kg/day) and 88 % (12 mg/kg/day) of patients were expected to achieve a target daily AUC to MIC ≥ 50. If the MIC was 4 mg/L, only 0.8 % of patients were expected to achieve a daily AUC to MIC ≥ 50 for the 10 mg/kg/day dose, while 8.5 % of patients were expected to achieve the targets for the 12 mg/kg/day dose. For higher daily AUC to MIC targets (≥ 100 and ≥ 150), more than 90 % of patients were expected to achieve the target at MIC 0.5 mg/L for both weight scaled doses. When the MIC was greater than 2 mg/L, less than 10 % of patients were expected to achieve the target. Therefore the PK-PD breakpoints for both weight scaled doses were 1.5 mg/L for a ratio of ≥ 50, and 0.5 mg/L for ratios of ≥ 100 and ≥ 150.

More than 90% of patients were expected to achieve a daily AUC to MIC ratio ≥ 50 with doses of 3 and 3.5 mg/cm/day when the MIC was ≤ 1.5 mg/L. On the other hand, a dose of 4 mg/cm/day achieved this target when the MIC was ≤ 2 mg/L. For higher ratios (≥ 100 and 150), more than 90 % of patients were expected to achieve the target with the all doses if the MIC was ≤ 0.5 mg/L except for a dose of 4 mg/cm/day, where 90 % of patients achieved target daily AUC to MIC ratio ≥ 100 when MIC was ≤ 1 mg/L. As the MIC increased, fewer patients were expected to achieve the target even with an increase in dose. For example, when the MIC was ≥ 4 mg/L, <5% of patients were expected to achieve a ratio ≥ 50 for the three height scaled doses. However, when the MIC was ≥ 2 mg/L, less than 5 % of patients were expected to achieve ratios above 100 with all three height scaled doses and no patients were expected to achieve the target for ratios above 150. Therefore the PK-PD breakpoints for 3 and 3.5 mg/cm/day doses were 1.5 for a daily AUC/MIC ratio ≥ 50, and 0.5 mg/L for ratios of ≥ 100 and ≥ 150. The PK-PD breakpoints for 4 mg/cm/day were 2 mg/L for a daily AUC/MIC ratio ≥ 50, 1 mg/L for a ratio ≥ 100, and 0.5 mg/L for ratios ≥ 150.

Figure 7.2 Percentage probability of achieving the target daily AUC/MIC ratios (a) > 50, (b) > 100, (c) > 150, with weight (10 and 12 mg/kg/day) and height (3, 3.5 and 4 mg/cm/day) scaled doses over a range of MIC values.



Key: The black line represents the 10 mg/kg/day dose, the red line represents the 12 mg/kg/day dose, the green line represents the 3 mg/cm/day dose, the blue line represents the 3.5 mg/cm/day dose, and the orange line represents the 4 mg/cm/day dose.

The gap between the PTA lines for the 10 mg/kg/day and 3 mg/cm/day doses shown in Figure 7.1 indicates that more patients who were administered the 10 mg/kg/day dose achieved a Peak/MIC ratio greater than 10 than patients who were administered the 3 mg/cm/day dose. This gap started clearly at an MIC of 2.5 mg/L. Patients' who had a Peak/2.5 ratio less than 10 and those whose Peak/2.5 was greater than 10 were then examined in more detail. As shown in Figure 7.3, 26 % of patients whose Peak/2.5 was greater than 10 and were administered the 10 mg/kg/day dose had higher doses (up to 660 mg/day) which was associated with 69 % of patients had peak concentrations above 30 mg/L. On the other hand, there was no difference in doses administered based on height with 45 % had peak concentrations greater than 30 mg/L. Similar observations arise from Figure 7.2, where a better achievement of daily AUC to MIC above 50, 100, and 150 was observed for weight over height scaled dose that started clearly at MIC of 1 mg/L and was also related to the amount of dose administered. To illustrate the difference and derive an explanation for these findings, patients who had daily AUC/1 below and above 100 were examined in more details. Figure 7.4 shows that 25 % of patients whose daily AUC/1 was greater than 100 and were administered the 10 mg/kg/day regimen had daily doses up to 660 mg/day with and 59 % of these patients had a daily AUC above the upper limit of 120 mg.h/L, and with no difference in doses administered based on height. These results indicated that the greater chance of efficacy seen from the weight based dose occurred at a higher risk of toxicity.

Figure 7.3 Box plots of doses (top) and peak concentrations (bottom) categorised according to Peak/2.5 mg/L MIC ratios < 10 and > 10 for (a) weight (10 mg/kg/day) and (b) height (3 mg/cm/day) scaled dosage regimens.

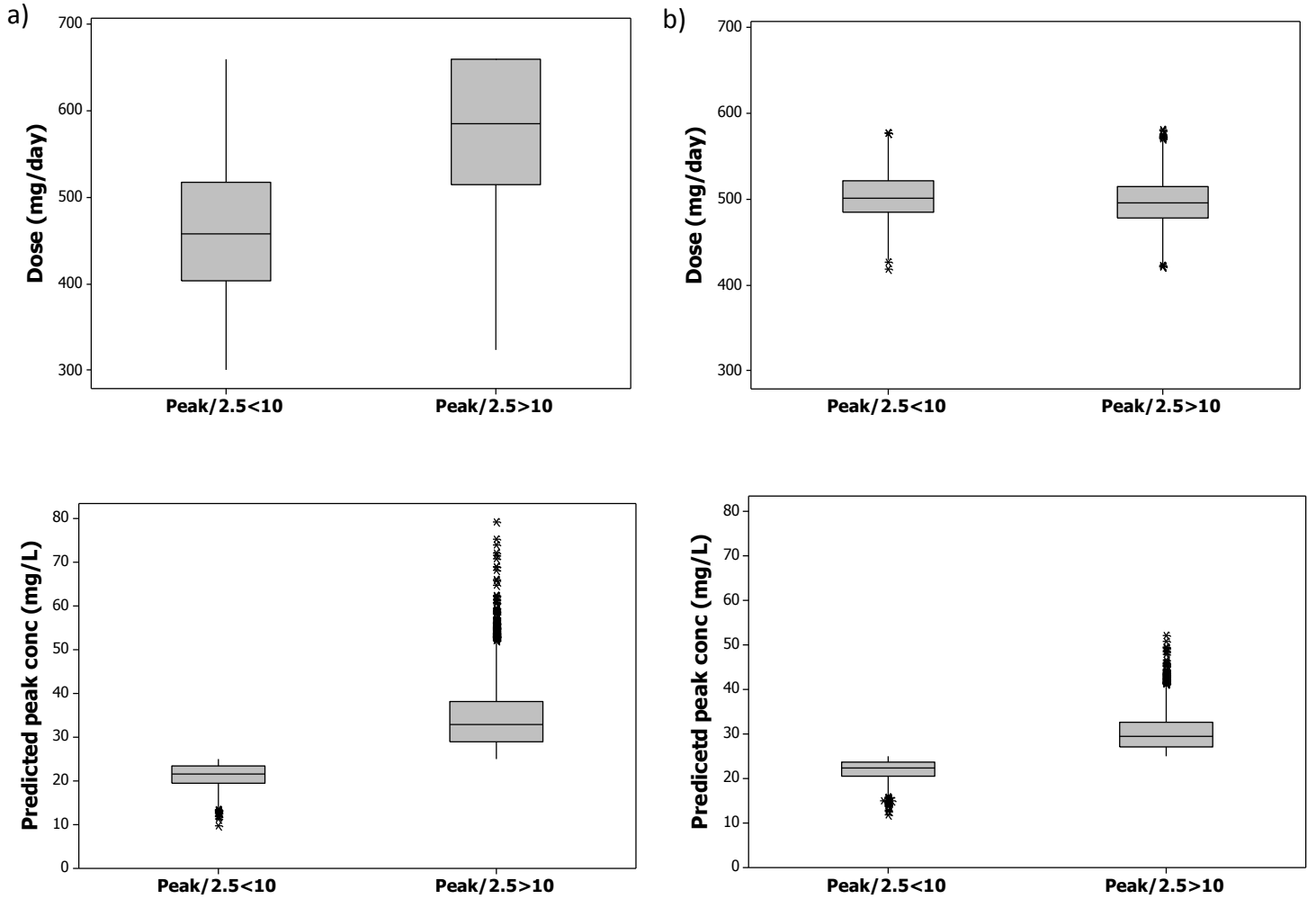
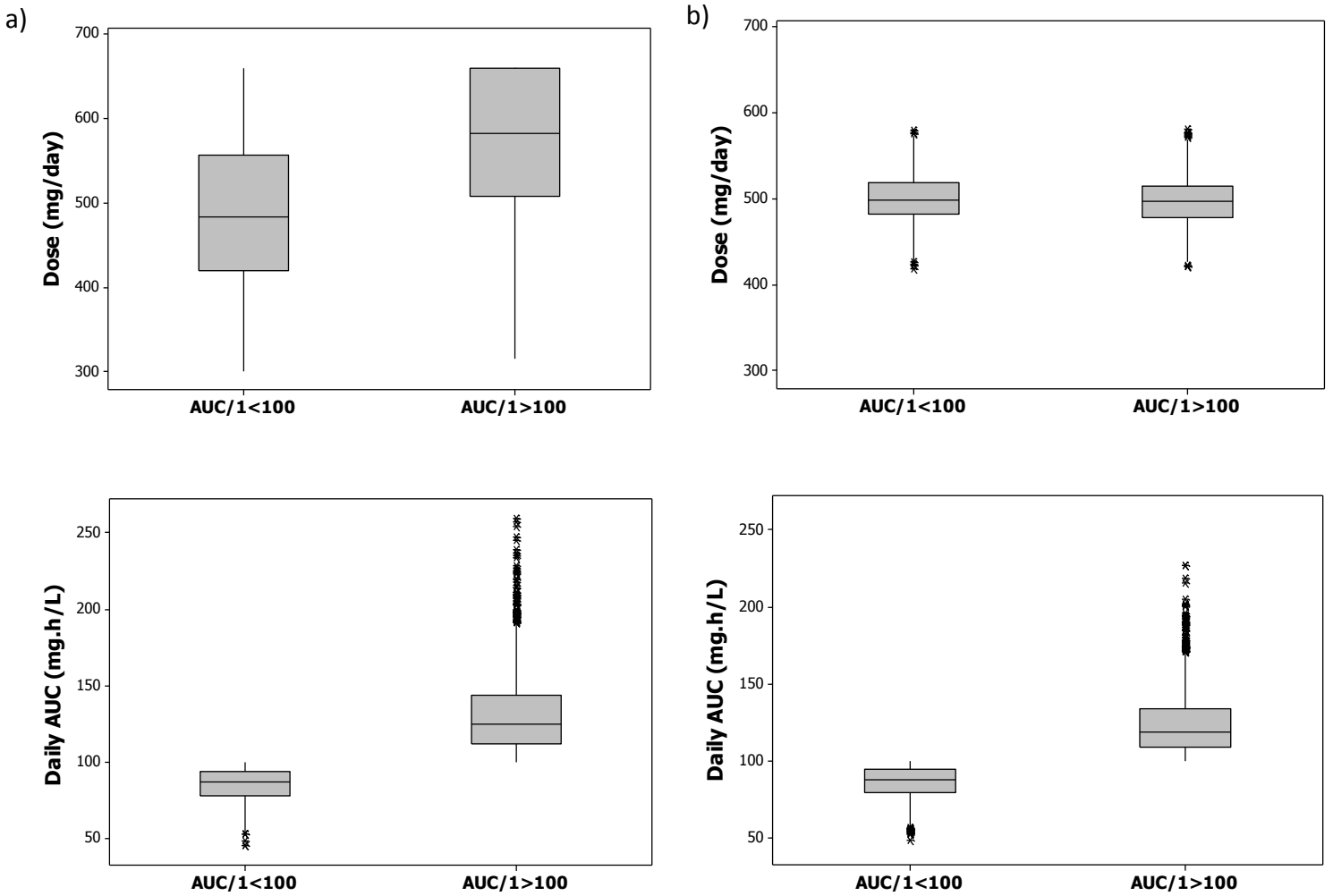


Figure 7.4 Box plots of doses (top) and daily AUCs (bottom) categorised according to daily AUC/1 mg/L MIC ratios < 100 and > 100 for (a) weight (10 mg/kg/day) and (b) height (3 mg/cm/day) scaled dosage regimens.



7.4.3 Cumulative fraction of response

Table 7.3 shows the cumulative fractions of response for the weight and height scaled dosage regimens against a range of gram negative organisms, assuming a target Peak to MIC ratio ≥ 10. All dosage regimens were expected to achieve more than 90 % of target response against *P.aeruginosa.* There was no difference in the predicted response for the current and developed regimens (91 % for 10 mg/kg/day and 3 mg/cm/day) against *P.aeruginosa*. When the tobramycin dose was increased to 12 mg/kg/day and 4 mg/cm/day, the CFR value increased slightly to 92 % for both regimens against *P.aeruginosa*. However, the CFR value was less than 64 % for all dosage regimens against *H.influenzae*.

Table 7.3 Cumulative fraction of response to achieve a Peak/MIC ratio of at least 10 for weight and height scaled dosage regimens of **tobramycin** against *P.aeruginosa* and *H.influenzae* (the MIC distribution data were obtained from the EUCAST database).

Organisms	Target Peak/MIC Ratio	Cumulative fraction of response (%)				
		10 mg/kg/day	12 mg/kg/day	3 mg/cm/day	3.5 mg/cm/day	4 mg/cm/day
P.aeruginosa	10	91	92	91	91	92
H.influenzae	10	51	64	46	51	62

Table 7.4 shows the cumulative fraction of response to achieve a Peak to MIC ratio ≥ 10 for gentamicin against gram negative pathogens. All gentamicin dosage regimens were expected to achieve a less than 71 % response for *P.aeruginosa*. The lowest CFR value occurred with the 3 mg/cm/day dose and the highest with the 12 mg/kg/day and 4 mg/cm/day doses. Similarly, all gentamicin dosage regimens were expected to achieve more than 80% response for *H.influenzae*. Overall, the predicted responses for all weight and height based dosage regimens were similar.

Table 7.4 Cumulative fraction of response to achieve target Peak/MIC ratio for weight and height scaled dosage regimens of **gentamicin** against *P.aeruginosa* and *H.influenzae* (the MIC distribution was obtained from the EUCAST database).

Organisms	Target Peak/MIC ratio	Cumulative fraction of response (%)				
		10 mg/kg/day	12 mg/kg/day	3 mg/cm/day	3.5 mg/cm/day	4 mg/cm/day
P.aeruginosa	10	65	71	63	67	71
H.influenzae	10	88	92	87	89	92

Table 7.5 shows the cumulative fraction of response to achieve the target daily AUC to MIC ratios for the different gram negative pathogens at the tested dosage regimens for tobramycin. More than 80 % of patients were expected to achieve target daily AUC to MIC ratios ≥ 50 against *P.aeruginosa* for the five tested dosage regimens. However, this probability decreased as the ratio increased. For a ratio ≥ 100, more than 70 % of patients were expected to achieve the target against *P.aeruginosa* for both weight and height scaled doses. However for the higher ratio of ≥ 150, less than 70 % of patients were expected to achieve the target against *P.aeruginosa* for the five tested dosage regimens with the lowest probability of 53 % for the 3 mg/cm/day dose. The predicted response for the five tested tobramycin dosage regimens against *H.influenzae* was low (≤ 46 %) even with the higher doses.

Table 7.6 shows daily AUC to MIC ratio results for gentamicin. The predicted response for weight and height scaled dosage regimens against *P.aeruginosa* was low (≤ 63 %) for all five dosage regimens at the different daily AUC/MIC ratios. However, the response was higher (≥ 80 %) against *H.influenzae* when the ratio was ≥ 50 for both weight scaled doses (10 and 12 mg/kg/day) and for the higher height scaled doses (3.5 and 4 mg/cm/day) but lower for the 3 mg/cm/day dose (77 %). The response decreased as the ratio increased. In general, the predictive response for the current weight scaled dose was slightly higher than the new height scaled dose (10 mg/kg/day vs 3 mg/cm/day). However, increasing the height scale dose produced higher predicted responses against the tested organisms.

Table 7.5 Cumulative fraction of response to achieve target AUC/MIC ratio for weight and height scaled dosage regimens of **tobramycin** against *P.aeruginosa* and *H.influenzae* based on MIC distributions from the EUCAST database.

Organisms	Target AUC/MIC ratio	Cumulative fraction of response (%)				
		10 mg/kg/day	12 mg/kg/day	3 mg/cm/day	3.5 mg/cm/day	4 mg/cm/day
P.aeruginosa	50	89	90	88	90	91
H.influenzae	50	35	42	31	40	47
P.aeruginosa	100	74	79	71	78	81
H.influenzae	100	7.9	10	6.8	9.3	12
P.aeruginosa	150	55	60	53	59	65
H.influenzae	150	2.6	3.6	1.9	3.1	4.8

Table 7.6 Cumulative fraction of response to achieve target AUC/MIC ratio for weight and height scaled dosage regimens of **gentamicin** against *P.aeruginosa* and *H.influenzae,* (MIC distribution was obtained from the EUCAST database).

Organisms	Target AUC/MIC ratio	Cumulative fraction of response (%)				
		10 mg/kg/day	12 mg/kg/day	3 mg/cm/day	3.5 mg/cm/day	4 mg/cm/day
P.aeruginosa	50	53	60	49	58	63
H.influenzae	50	80	85	77	84	87
P.aeruginosa	100	22	25	20	24	28
H.influenzae	100	49	56	45	55	61
P.aeruginosa	150	10	13	9.1	12	15
H.influenzae	150	26	31	23	29	36

7.5 DISCUSSION

The aim of the current work was to estimate and evaluate the probability of achieving the PK-PD indices with the current tobramycin dosage regimen of 10 mg/kg/day (The UK Cystic Fibrosis Trust Antibiotic Working Group, 2009, Smyth A et al., 2005) and the developed height scaled dose of 3 mg/cm/day in adults with cystic fibrosis. Additional studies examined the impact of increasing the weight based dose to 12 mg/kg/day and the height based dose to 3.5 or 4 mg/cm/day.

In the current study, the maximum daily dose for the weight scaled dose (10 mg/kg/day) was restricted to 660 mg as per the TOPIC study recommendation (Smyth A et al., 2005). The maximum daily dose following the higher dose of 12 mg/kg/day was 1296 mg/day and was associated with excessive exposure with some daily AUC estimates above 400 mg.h/L and some peak concentrations greater than 90 mg/L. This problem particularly occurred with overweight and obese patients. Other studies have suggested that patients who are categorised as being overweight (BMI= 25-29.99 mg/m^2) or obese (BMI > 30 mg/m^2) (World Health Organisation, 2011) can be dosed based on ideal body weight (Devine method) (Devine BJ, 1974) instead of their actual body weights. Alternatively, restricting daily doses for the weight based doses has been used to avoid having very high doses and subsequently high daily exposure and/or peak concentrations. However, the appropriate upper limit to use is unclear. In The Hague dataset used for model validation in Chapter 4, six patients were administered doses greater than 660 and up to 800 mg/day for once daily tobramycin (9 -11 mg/kg/day). These were associated with daily exposures of 112 and 161 mg.h/L and a very high daily exposure of 252 mg.h/L was associated with one patient whose creatinine clearance was less than 50 mL/min. However, no evidence of renal function deterioration was available from The Hague dataset.

Moreover, tobramycin doses above 10 mg/kg/day have been used in previous studies in patients with cystic fibrosis. No ototoxicity or nephrotoxicity was documented when a dose of 15 mg/kg/day was administered over 30 or 60 minute infusion (Vic P et al., 1998, Bragonier R and Brown NM, 1998, Master V et al., 2001, Bates RD et al., 1997). These

findings indicate that a 660 mg/day restriction might not be an appropriate limit for higher weight scaled dose. Since the maximum daily dose of 660 mg/day is related to a weight of 66 kg, if we assume similar maximum weight, an 800 mg/day dose might be a suitable limit for the 12 mg/kg/day dose. On the other hand, no restriction would be required when the dose was scaled according to height because the maximum daily dose was less than 800 mg/day for all three height scaled doses that were examined. Furthermore, there was no difference in the height based dose administered for overweight and obese patients, and underweight and normal weight patients. The explanation for this finding might be that because tobramycin is a water soluble drug it distributes mainly into plasma and not to any great extent into fat. Since in the current study none of the patients were obese, these finding cannot be extrapolated to obese patients.

Higher peak concentrations were achieved with the dose of 12 mg/kg/day (up to 98 mg/L) compared with the dose of 4 mg/cm/day (up to 69 mg/L). From previous studies, tobramycin peak concentrations > 30 mg/L (up to 56 mg/L) were well tolerated in most patients with no evidence of oto- or nephrotoxicity (Vic P et al., 1998, Bragonier R and Brown NM, 1998, Master V et al., 2001). These studies included in total 42 paediatric and adolescent patients with cystic fibrosis (age 5 – 19 years old) who were administered tobramycin dose of 8 -15 mg/kg/day over 5 , 15 or 30 minutes (Bragonier R and Brown NM, 1998, Vic P et al., 1998, Master V et al., 2001). However, a few patients experienced ototoxicity in the form of dizziness and tinnitus, which responded to an increase in the infusion time, a reduction of the dose or stopping the antibiotic (Master V et al., 2001, Bragonier R and Brown NM, 1998, VandenBussche HL and Homnick DN, 2012). VandenBussche *et al* (2012) found that female patients were more susceptible to aminoglycoside ototoxicity and suggested using different weight-based doses for older female patients. Similar suggestions were made by Lam *et al* (2007). They found that female patients older than 14 years old had high peak concentrations and suggested a lower dose of 7 mg/kg/day with a target peak concentration of 25 - 35 mg/L (Lam W et al., 2007). No information on ototoxicity was available in the current study.

VandenBussche and his colleagues (VandenBussche HL and Homnick DN, 2012) suggested increasing the target peak concentration range up to 35 mg/L for a tobramycin dose of 12 mg/kg/day. In the current study, if the accepted maximum tobramycin peak concentration was increased to 35 mg/L, 53 % of patients who were administered 12 mg/kg/day would have peak concentrations above the target compared with 73 % of patients if the limit was set at 30 mg/L. Similarly, with a dose of 4 mg/cm/day, 57 % of patients would have a peak concentration above 35 mg/L compared with 81 % above 30 mg/L. Such high peaks would also maximise the tobramycin pharmacodynamic effect against gram negative pathogens. For the current 10 mg/kg/day and 3 mg/cm/day doses, peak ranges of 20 - 30 mg/L are acceptable. However, for higher doses (12 mg/kg/day and 4 mg/cm/day) a higher peak range (25 – 35 mg/L) might be more appropriate.

The maximum daily AUC for the 12 mg/kg/day dose was 410 mg.h/L and greater than for the 4 mg/cm/day dose (303 mg.h/L). Limited information is available on the safety of such high daily aminoglycoside AUC values. Croes *et al* (2012) found that following the administration of 7 mg/kg/day aminoglycoside, creatinine clearance tended to decrease by day 10 of a 14 day course. This was linked with a daily AUC greater than 100 mg.h/L. They defined nephrotoxicity as a 50 % or more reduction in creatinine clearance, which was observed on day 14 of therapy when the daily AUC was 150 mg.h/L. These results suggest that a safe daily AUC upper limit is less than 150 mg.h/L. Since daily AUC ranges of 80 - 120 mg.h/L are acceptable for the current weight and developed height scaled doses, higher daily AUC ranges, such as 85 – 125 mg.h/L, might be more appropriate for the higher doses. For the 12 mg/kg/day dose, there was only a small reduction in the percentage of patients whose daily AUC above the target when the daily exposure upper limit was increased from 120 mg.h/L (69 %) to 125 mg.h/L (63 %). Similar observations occurred with the 4 mg/cm/day dose, where 67 % of patients would have had a daily AUC above 125 mg.h/L compared with 74 % with 120 mg.h/L. Looking at these daily exposure percentages might falsely indicate that the high weight based dose is safer than the high height based dose, particularly for daily exposure greater than 120 mg.h/L. However, a sub-analysis of patients whose predicted daily exposure was greater than 120 indicated that 54 % of them had more than 150 mg.h/L whereas 49 % had more than 150 mg.h/L with the high height based dose.

As previously discussed and according to Croes *et al* (2012), daily exposure between 120 and 150 mg.h/L were considered acceptable in the present study.

7.5.1 Probability of Target Attainment

Aminoglycosides are active against aerobic gram-negative bacilli including *Escherichia coli*, *Proteus*, *Enterobacter*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Serratia*, and *Providencia* species (Schentag et al., 2006). According to the UK Cystic Fibrosis Trust annual report (2013), *P.aeruginosa* infections account for 36.5 % for all lung infections at all age group and for 55.8 % in adults , followed by *S.aureus* infections (gram positive infection) 15.7 % and to a least percentage *H.influenzae* (gram negative) infection which accounts for 6.2 %. Therefore in the current study, the gram negative, *P.aeruginosa and H.influenzae* organisms were chosen to evaluate the effect of aminoglycosides against them.

The rate and extend of killing is highly dependent on the concentration of an aminoglycoside antibiotic. Mouton *et al* (2005) examined the relationship between the pharmacodynamic index and the effect of aminoglycosides in 13 paediatric and young adult patients with cystic fibrosis. They reported that the maximum effect of tobramycin was achieved with a peak to MIC ratio of 5 for tobramycin administered 8 hourly. This Peak/MIC ratio is lower than ratios documented from other researchers who reported values ranging from 8 to 10 against gram negative pathogens (Kashuba ADM et al., 1999, Blaser J et al., 1987, Moore RD et al., 1987). Mouton *et al* (2005) derived this low ratio from a multiple dosage regimen of tobramycin rather than once daily (3.3 mg/kg three times a day), and hence it was not used in the current study. However, they reported a daily AUC to MIC ratio ≥ 50 to achieve the maximum effect of tobramycin for a daily tobramycin dose of 9.9 mg/kg. In contrast, others reported higher daily AUC/MIC ratios of ≥ 100 and ≥ 150 to predict tobramycin treatment success, regardless of the infection site or pathogen type (Smith PF et al., 2001, Kashuba ADM et al., 1999). The very high AUC/MIC ratio reported by Kashuba *et al* (1999) was derived from isolates obtained from patients with nosocomial pneumonia with MIC ranges of 1 to 4 mg/L.

The approach of Drusano *et al* (2001) was applied in the present study to predict aminoglycoside dosage regimen breakpoint or treatment success in patients with cystic fibrosis. Previous PK-PD studies in patients with cystic fibrosis usually defined an MIC breakpoint and examine whether the tested dosage regimen was able to achieve that breakpoint (Beringer PM et al., 2000, VandenBussche HL and Homnick DN, 2012). However, Drusano *et al* (2001) used simulation approach and examined the distribution of a wide range of MICs against particular organism to determine breakpoints and treatment success.

In this study, the PK-PD susceptibility breakpoint for a Peak/MIC ratio ≥ 10 for the current tobramycin dose (10 mg/kg/day) and two of the height scaled doses (3 and 3.5 mg/cm/day) was ≤ 2 mg/L against gram negative pathogens. However, the PK-PD susceptibility breakpoint for the higher doses (12 mg/kg/day and height 4 mg/cm/day) was ≤ 2.5 mg/L. Vic and his colleagues (1998) found that a tobramycin MIC of 2 mg/L was required to inhibit the growth of 50 % of *P.aeruginosa* isolates. Based on those findings, the dosage regimens examined in the current study should be able to inhibit the growth of 50 % *P.aeruginosae.* However, Milne *et al* (2010) examined 315 *P.aeruginosa* isolates from 76 patients with cystic fibrosis, and reported a higher MIC breakpoint value for tobramycin of ≥ 3 mg/L. Both MIC breakpoints reported previously, 2 and 3 mg/L (Milne KEN and Gould IM, 2010, Vic P et al., 1998), were lower than the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the British Society for Antimicrobial Chemotherapy (BSAC) breakpoint value for *Pseudomonas* spp. for tobramycin of ≤ 4 mg/L (British Society for Antimicrobial Chemotherapy, 2013, European Committee on Antimicrobial Susceptibility Testing). Similarly, Lam and his colleagues (2007) reported that the majority of *P.aeruginosa* isolates had MICs ≤ 4 mg/L and around 80 % had an MIC ≤ 2 mg/L. The reason behind the slight variation in MIC breakpoints for *P.aeruginosa* seen in previous publications might be the formation of biofilms in the studied sample. Lopes *et al* (2012) studied the MIC breakpoint for *P.aeruginosa* in addition to other organisms including *Inquilinus limosus* and *Dolosigranulum pigrum* in patients with cystic fibrosis. They found that *P.aeruginosa* biofilms were more resistant to antibiotics, including tobramycin and gentamicin, than *Inquilinus limosus* and *Dolosigranulum pigrum* biofilms. The presence of *Inquilinus limosus* and *Dolosigranulum pigrum* in addition to *P.aeruginosa* could enhance antibiotic resistance.

Recent evidence showed that *P.aeruginosa* isolated from patients with cystic fibrosis were more resistant to tested antibiotics, including aminoglycosides, β-lactams, ciprofloxacin and colistin than isolates from patients without cystic fibrosis (Milne KEN and Gould IM, 2010, Rao P et al., 2012).

For the daily AUC/MIC ratios, the MIC breakpoints for both weight scaled (10 and 12 mg/kg/day) and height scaled (3 and 3.5 mg/cm/day) dosage regimens were 1.5 mg/L for a ratio ≥ 50, and 0.5 mg/L for ratios ≥ 100, and for the 4 mg/cm/day the breakpoint was 2 mg/L for ratios ≥ 50, 1 mg/L for ratios ≥ 100, and 0.5 mg/L for ratios ≥ 150. Although the effect was less pronounced, more patients were predicted to achieve PTA target with the weight scaled dose compared with the height scaled at MIC greater 2 and less than 8 mg/L, which could be related to the amount of dose administered. This observation was seen when analysing Peak/MIC and daily AUC/MIC ratios results. These breakpoints are lower than the EUCAST and BSAC breakpoints against *P.aeruginosa* (MIC ≤ 4 mg/L), which indicated that if the daily AUC/MIC is used then high tobramycin dosage regimen is required (4 mg/cm/day) is required to achieve MIC ≤ 2 mg/L. In addition, more patients in the weight scaled dose had above target peak concentration of 30 mg/L (51 % versus 30 %) and daily AUC of 120 mg.h/L (40 % versus 27 %) compared with the height scaled dosage regimen. These results indicated a greater chance of efficacy but with higher risk of toxicity associated with the weight scaled dose.

7.5.2 Cumulative Fraction of Response

Infection with *P.aeruginosa* accounts for 36.5 % for all lung infections at all age groups (Cystic FibrosisTrust, 2013) and was found by Lam *et al* (2007) to account for the majority of isolates (48 %) in 102 paediatric and young adult patients with cystic fibrosis. These isolates were susceptible to tobramycin. Therefore, the current analysis supported the use of tobramycin in cystic fibrosis patients suffering from lung infections regardless of age with the current weight or developed height scaled dosage regimens. In the current study, all examined tobramycin dosage regimens were expected to achieve more than a 90 % response for a Peak/MIC ratio ≥ 10 against *P.aeruginosa.* At low daily AUC/MIC ratios of 50,

tobramycin CFR value was between 88 - 90 % against *P.aeruginosa* for all examined dosage regimens. However, at high daily AUC/MIC ratios (greater than 100 and 150) tobramycin CFR value was lower and between 50 - 80 % for all dosage regimens examined against *P.aeruginosa*. However, the treatment of success for tobramycin against *H.influenzae* was low for all dosage regimens, less than 64 % for target Peak/MIC ≥ 10 and less than 50 % for target daily AUC/MIC ratios.

On the other hand, *H.influenzae* accounts for 8.7 % of infections in paediatric patients with cystic fibrosis (Cystic FibrosisTrust, 2013). However, in adulthood *H.influenzae* accounts for only 4.4 % of the causative pathogens for lung infections (Cystic FibrosisTrust, 2013). Hence gentamicin can be used for highly sensitive strains of *H.influenzae*. The simulation results showed that gentamicin is not the antibiotic of choice to treat infections caused by *P.aeruginosa,* because the CFR value was less than 70 % for a Peak/MIC ratio ≥ 10 and less than 63% at all AUC/MIC ratios were above the target for all dosage regimens. Milne and his colleagues (Milne KEN and Gould IM, 2010) found that colistin was the most effective antimicrobial against *P.aeruginosa*, followed by tobramycin, whereas gentamicin had the lowest susceptibility. On the other hand, the treatment of success for gentamicin against *H.influenzae* for the examined dosage regimens was high for a Peak/MIC ratio ≥ 10, ranging from 87 % to 92 %. However, the percentage was lower using daily AUC/MIC ratios of 50, which achieved less than 87 %. This percentage reduced to 60 % and less at daily AUC/MIC ratios of 100 or more.

7.5.3 Conclusion

Overall, the current tobramycin dose of 10 mg/kg/day and the developed height scaled dose of 3 mg/cm/day had similar MIC breakpoints of ≤ 2 mg/L to achieve a Peak/ MIC ratio ≥ 10 and ≤ 0.5 mg/L for a daily AUC/ MIC ratio ≥ 100 mg.h/L against *P.aeruginosa*. However, they were lower than the susceptibility breakpoints for the EUCAST and BSAC against gram negative pathogens (≥ 4 mg/L). Both dosage regimens were able to achieve more than a 90 % treatment success against *P.aeruginosa* using the EUCAST MIC distributions. When the tobramycin dose was increased, breakpoints and treatment success increased slightly.

However, high weight scaled dose was associated with high peak and daily exposure and might result in more toxicities compared with the high height scaled dosage regimen.

CHAPTER 8: GENERAL CONCLUSIONS AND FUTURE WORK

8.1 GENERAL CONCLUSIONS

The aim of this thesis was to use population pharmacokinetic methodology to examine how aminoglycoside pharmacokinetic parameters change over time in patients with cystic fibrosis who receive multiple courses of therapy and how this would affect future dosage recommendations. The conducted analysis in 166 adult patients with cystic fibrosis with 1075 courses of aminoglycoside therapy identified that aminoglycoside pharmacokinetics were influenced by height and creatinine clearance. Even though height and creatinine clearance were the best descriptors, they had small effect on between-subject variability with a reduction from 23 % to 18 % for clearance and 14 % to 12 % for the volume of distribution of the central compartment. This reflects the narrow range of patient characteristics within patients with cystic fibrosis. In addition, the relationship between creatinine clearance and clearance was unexpectedly poor for a drug like aminoglycoside, which is known to be cleared renally. These finding raises the question about the adequacy of using creatinine based methods to estimate renal function in patients with cystic fibrosis where underweight is common and hence had low creatinine production, and the use of an alternative method of estimating renal function might be required. In the Glasgow dataset, 40 % of patients were underweight of whom 26 % had low serum creatinine. These findings were confirmed using two methods of estimation, the parametric FOCE I implemented in NONMEM and when a larger dataset with 331 adult patients and 1490 courses of aminoglycosides were analysed using the nonparametric NPAG implemented in Pmetrics. Although the data were collected over 15 years, within-subject variability was low at 11 %, which indicated that aminoglycoside pharmacokinetics is stable in this group of patients. This indicated that the patients can be started with the previous individualised tobramycin dosage regimen if a new therapy is required. The results also showed that multiple courses of therapy did not influence aminoglycoside clearance over time. In addition, the population model was able to predict pharmacokinetics well using another population from The Hague. This is quite important particularly if the population model would be used to develop dosage guidelines.

In this thesis, a target daily area under the concentration-time curve value of 106 mg.h/L, range 80-120 mg.h/L was established for the standard 10 mg/kg/day dosage regimen, which is the first time this target has been defined in this patient group. High proportion of patients on the 10 mg/kg/day dose had their daily AUC above the target 80 - 120 mg.h/L (40 %), which raises concern about safety of such dose particular the nephrotoxic effect of aminoglycoside (tobramycin). A new dosage guideline was developed which used height as a scaling factor (3 mg/cm/day). The proposed height based dosage guideline enable more patients to achieve the target concentration (63 vs 41 %) and daily AUC target (61 vs 50 %) compared with the weight scaled dosage regimen (10 mg/kg/day). In addition, the height scaled dosage regimen was expected to be less toxic compared with weight scaled dosage regimen because fewer patients (27 %) had their daily AUC greater than 120 mg.h/L compared with the weight scaled dosage regimen (40 %).

A nomogram for interpreting tobramycin concentrations was also developed for the first time for both the weight (10 mg/kg/day) and height (3 mg/cm/day) scaled dosage regimens. The nomogram was able to predict patients with low, within and above the target daily area under the concentration-time curve using the Glasgow and The Hague patients who were simulated to be administered the 10 mg/kg/day dose and using new patients from The Hague who was on 10 mg/kg/day dosage regimen. In addition, the nomogram was able to identify patients with poor renal function. The results from using patients from The Hague showed that the recommendations generated from the nomogram were in agreement with the clinical decisions that had actually been made for these patients during routine clinical use.

For the first time, the susceptibility breakpoints for the 10 mg/kg/day dose and 3 mg/cm/day dose were determined against gram negative organisms in this group of patients. The results from the pharmacokinetic-pharmacodynamic analysis showed that both the 10 mg/kg/day and 3 mg/cm/day dosage regimens had MIC breakpoints of ≤ 2 mg/L to achieve a Peak/ MIC ratio ≥ 10 against gram negative organism (*P.aeruginosa*), which was lower than the clinical breakpoint of MIC ≤ 4 mg/L for the EUCAST and BSAC. In addition,

the analysis showed that both regimens are effective against *P.aeruginosa* with more than 90 % of treatment response. However, when the tobramycin dose was increased to 12 mg/kg/day and 4 mg/cm/day, breakpoints and treatment success increased slightly. However, the 12 mg/kg/day dosage regimen was associated with high peaks and daily exposures and might result in more toxicity compared with the 4 mg/cm/day dosage regimen.

The proposed height based dosage guideline (3 mg/cm/day) achieved adequate concentration and daily AUC and provided a good overall treatment success against gram negative organism (*P.aeruginosa*). The use of height is an advantage of the model and the dosage guideline as it would enable reduction of calculations error. In addition, height is usually stable for adult patients, which reduce the need to measure each time the patient required aminoglycoside (tobramycin) therapy and is consistence with the finding that these patients had stable pharmacokinetics and patients can be started with the previous dosage regimen. In addition, the use of height is not surprising for dosing aminoglycosides, the dose of aminoglycosides is usually based on ideal body weight, which is based on height. Therefore the proposed guideline would eliminate the need to estimate ideal body weight and doses directly using patient's height.

8.2 FUTURE WORK

The proposed height based dosage regimen (3 mg/cm/day) can be used in clinical practice and be monitored by the developed tobramycin dosage adjustment nomogram to advise on the subsequent dose and dosage interval. In addition, a prospective study would be a nice extension of the work to evaluate both the height dosage guideline and the dosage adjustment nomogram.

In the present study, between-subject variability in V_2 and Q were difficult to characterise because of the sparse nature of data. Therefore, the population model can be used to determine optimal design for future studies. The main aim of the optimal design for

population pharmacokinetic studies is to maximise parameter estimates precision and minimise the amount of information required. The optimal study design is a useful technique to determine the required number of subjects and number and times of samples to be obtained from each subject, which is expected to contain the most information. Another area that could be explored further is to look at the effect of cystic fibrosis genotype mutation as a covariate and how it influence aminoglycoside handling in patients with cystic fibrosis.

REFERENCES

AL-ALOUL M, MILLER H, ALAPATI S, STOCKTON PA, LEDSON MJ & WALSHAW MJ 2005. Renal impairment in cystic fibrosis patients due to repeated intravenous aminoglycoside use. *Pediatric Pulmonology,* 39**,** 15-20.

AMINIMANIZANI A, BERINGER PM, KANG J, TSANG L, JELLIFFE RW & SHAPIRO BJ 2002. Distribution and elimination of tobramycin administered in single or multiple daily doses in adult patients with cystic fibrosis. *Journal of Antimicrobial Chemotherapy,* 50**,** 553-559.

ANDERSON BJ & HOLFORD NHG 2009. Mechanistic basis of using body size and maturation to predict clearance in humans. *Drug Metabolism and Pharmacokinetics,* 24**,** 25-36.

ANDRIEUX A, HARAMBAT J, BUI S, NACKA F, IRON A, LLANAS B & FAYON M 2010. Renal impairment in children with cystic fibrosis. *Journal of Cystic Fibrosis,* 9**,** 263-268.

BARCLAY ML & BEGG EJ 2001. Aminoglycoside adaptive resistance: importance for effective dosage regimens. *Drugs,* 61**,** 713-721.

BARZA M, IOANNIDIS JP, CAPPELLERI JC & LAU J 1996. Single or multiple daily doses of aminoglycosides: a meta- analysis. *British Medical Journal,* 312**,** 338-344.

BATES RD, NAHATA MC, JONES JW, MCCOY K, YOUNG G, COX S & BARSON WJ 1997. Pharmacokinetics and safety of tobramycin after once-daily administration in patients with cystic fibrosis. *Chest,* 112**,** 1208-1213.

BAUER LA, PIECORO JJ, WILSON HD & BEOUIN RA 1983. Gentamicin and tobramycin pharmacokinetics in patients with cystic fibrosis. *Clinical Pharmacy,* 2**,** 262-264.

BAYARD DS, MILMAN MH & SCHUMITZKY A 1994. Design of dosage regimens: a multiple model stochastic control approach. *International Journal of Bio-Medical Computing,* 36**,** 103-115.

BEAL SL, SHEINER LB, BOECKMAN A & BAUER RJ 2009. NONMEM User's Guides (1989-2009). Ellicott City, MD, USA: Icon Development Solutions.

BEGG EJ, BARCLAY ML & DUFFULL SB 1995. A suggested approach to once-daily aminoglycoside dosing. *British Journal of Clinical Pharmacology,* 39**,** 605-609.

BERGSTRAND M, HOOKER AC, WALLIN JE & KARLSSON MO 2011. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *The AAPS Journal,* 13**,** 143-151.

BERINGER PM, VINKS AATMM, JELLIFFE RW & SHAPIRO BJ 2000. Pharmacokinetics of tobramycin in adults with cystic fibrosis: Implications for once-daily administration. *Antimicrobial Agents and Chemotherapy,* 44**,** 809-813.

BERNARD B, GARCIA-CAZARES SJ, BALLARD CA, THRUPP LD, MATHIES AW & WEHRLE PF 1977. Tobramycin: maternal-fetal pharmacology. *Antimicrobial Agents and Chemotherapy.,* 11**,** 688-694.

BERTENSHAW C, WATSON AR, LEWIS S & SMYTH A 2007. Survey of acute renal failure in patients with cystic fibrosis in the UK. *Thorax,* 62**,** 541-545.

BLASER J, STONE BB, GRONER MC & ZINNER SH 1987. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrobial Agents and Chemotherapy,* 31**,** 1054-1060.

BONDAREVA IB, JELLIFFE RW, GUSEV EI, GUEKHT AB, MELIKYAN EG & YB, B. 2006. Population pharmacokinetic modelling of carbamazepine in epileptic elderly patients: implications for dosage. *Journal of Clinical Pharmacy and Therapeutics,* 31**,** 211-221.

BONDAREVA IB, JELLIFFE RW, SOKOLOV AV & TISCHENKOVA IF 2004. Nonparametric population modeling of valproate pharmacokinetics in epileptic patients using routine serum monitoring data: implications for dosage. *Journal of Clinical Pharmacy and Therapeutics,* 29**,** 105-120.

BRAGONIER R & BROWN NM 1998. The pharmacokinetics and toxicity of once-daily tobramycin therapy in children with cystic fibrosis. *Journal of Antimicrobial Chemotherapy,* 42**,** 103-106.

BRENDEL K, COMETS E, LAFFONT C & MENTRÉ F 2010. Evaluation of different tests based on observations for external model evaluation of population analyses. *Journal of Pharmacokinetics and Pharmacodynamics,* 37**,** 49-65.

BRENDEL K, DARTOIS C, COMETS E, LEMENUEL-DIOT A, LAVEILLE C, TRANCHAND B, GIRARD P, LAFFONT CM & MENTRÉ F 2007. Are population pharmacokinetic and/or pharmacodynamic models adeqately evaluated?: a survey of the literature from 2002 to 2004. *Clinical Pharmacokinetics,* 46**,** 221-234.

BRITISH SOCIETY FOR ANTIMICROBIAL CHEMOTHERAPY. 2013. *BSAC methods for antimicrobial susceptibility testing* [Online]. Available: http://bsac.org.uk/wp-content/uploads/2012/02/Version-12-Apr-2013_final.pdf [Accessed 03 June 2013].

BURKHARDT O, LEHMANN C, MADABUSHI R, KUMAR V, DERENDORF H & WELTE T 2006. Once-daily tobramycin in cystic fibrosis: better for clinical outcome than thice-daily tobramycin but more resistance development. *Journal of Antimicrobial Chemotherapy,* 58**,** 822-829.

BUSTAND A, TERZIIVANOV D, LEARY R, PORT R, SCHUMITZKY A & JELLIFFE R 2006. Parametric and nonparametric population methods: their comparative performance in analysing a clinical dataset and two monte carlo simulation studies. *Clinical Pharmacokinetics,* 45**,** 365-383.

CAMPBELL D, THOMSON AH & STACK B 1999. Population pharmacokinetics of aminoglycoside antibiotics in patients with cystic fibrosis. *Therapeutic Drug Monitoring,* 21**,** 281-288.

CARLSSON KC, VAN DE SCHOOTBRUGGE M, ERIKSEN HO, MOBERG ER, KARLSSON MO & HOEM NO 2009. A population pharmacokinetic model of gabapentin developed in nonparametric adaptive grid and nonlinear mixed effects modeling. *Therapeutic Drug Monitoring,* 31**,** 86-94.

CHOW A, HECHT R & WINTERS R 1971. Gentamicin and carbenicillin penetration into the septic joint. *New England Journal of Medicine,* 285**,** 178-179.

COCKCROFT DW & GAULT MH 1976. Prediction of creatinine clearance from serum creatinine. *Nephron,* 16**,** 31-41.

COMETS E, BRENDEL K & MENTRÉ F 2008. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R. *Computer Methods and Programs in Biomedicine,* 90**,** 154-166.

CONIL J-M, GEORGES B, RUIZ S, RIVAL T, SEGUIN T, COUGOT P, FOURCADE O, PHARMD GH & SAIVIN S 2010. Tobramycin disposition in ICU patients receiving a once daily regimen: population approach and dosage simulations. *British Journal of Clinical Pharmacology,* 71**,** 61-71.

CONTOPOULOS-IOANNIDIS, D., GIOTIS ND, BALIATSA DV & IOANNIDIS JPA 2004. Extended interval aminoglycoside administration for children: a meta-analysis *Pediatrics,* 114**,** e111-e118.

COREY M, GASKIN K, DURIE P, LEVISON H & FORSTNER G 1984. Improved prognosis in CF patients with normal fat absorption. *Journal of Pediatric Gastroenterology and Nutrition,* 3**,** S99-105.

COULTHARD KP, PECKHAM DG, CONWAY SP, SMITH CA, BELL J & TURNIDGE J 2007. Therapeutic drug monitoring of once daily tobramycin in cystic fibrosis- caution with trough concentrations. *Journal of Cystic Fibrosis,* 6**,** 125-130.

CRAIG WA 1993. Postantibiotic effects in expirmental infection models: relationship to in vitro phenomena and to treatment of infections in man. *Journal of Antimicrobial Chemotherapy,* 31 149-158.

CRAIG WA, REDINGTON J & EBERT SC 1991. Pharmacodynamics of amikacin in vitro and in mouse thigh and lung infections. *Journal of Antimicrobial Chemotherapy,* 27**,** 29-40.

CROES S, KOOP AH, VAN GILS SA & NEEF C 2012. Efficacy, nephrotoxicity and ototoxicity of aminoglycosides, mathematically modelled for modelling-supported therapeutic drug monitoring. *European Journal of Pharmaceutical Sciences,* 45**,** 90-100.

CYSTIC FIBROSIS TRUST, U. K. 2012. *UK Cystic fibrosis registry annual data report 2010* [Online]. London: Cystic Fibrosis Trust Available: https://www.cysticfibrosis.org.uk/media/108230/CR_Annual_Data_Report_2010_Dec_11.pdf [Accessed 01 February 2013 2012].

CYSTIC FIBROSIS TRUST, U. K. 2013. *UK Cystic fibrosis registry annual data report 2011* [Online]. London: Cystic Fibrosis Trust Available: https://www.cysticfibrosis.org.uk/media/82506/CR_Annual_Data_Report_2011_Jan_13.pdf [Accessed 03 June 2013 2013].

DAIKOS GL, JACKSON GG, LOLANS VT & LIVERMORE DM 1990. Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. *Journal of Infectious Disease,* 162**,** 414-420.

DAIKOS GL, LOLANS VT & JACKSON GG 1991. First exposure adaptive reistance to aminoglycoside antibiotics in vivo with meaning for optinal clinical use *Antimicrobial Agents and Chemotherapy,* 35**,** 117-123.

DE BROE ME, VERBIST L & VERPOOTEN GA 1991. Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. *Journal of Antimicrobial Chemotherapy,* 27**,** 41-47.

DE JAGER P & VAN ALTENA R 2002. Hearing loss and nephrotoxicity in long-term aminoglycoside treatment in patients with tuberculosis. *International Journal of Tuberculosis and Lung Disease,* 6**,** 622-627.

DEE TH & KOZIN F 1977. Gentamicin and tobramycin penetration into synovial fluid. *Antimicrobial Agents and Chemotherapy,* 12**,** 548-549.

DEN HOLLANDER JG, MOUTON JW, VAN GOOR MP, VLEGGAAR FP & VERBRUGH HA 1996. Alteration of postantibiotic effect during one dosing interval of tobramycin, simulated in an *in vitro* pharmacokinetic model. *Antimicrobial Agents and Chemotherapy.,* 40**,** 784-786.

DEVINE BJ 1974. Gentamicin Therapy. *Drug Intelligence and Clinical Pharmacy,* 8**,** 650-655.

DODGE JA, MORISON S, LEWIS PA, COLES EC, GEDDES D, RUSSELL G, LITTLEWOOD JM & SCOTT MT 1997. Incidence, population, and survival of cystic fibrosis in the UK, 1968–95. *Archives of Disease in Childhood,* 77**,** 493-496.

DÖRING, G., FLUME, P., HEIJERMAN, H. & ELBORN JS 2012. Treatment of lung infection in patients with cystic fibrosis: current and future strategies. *Journal of Cystic Fibrosis,* 11**,** 461-479.

DRUSANO GL & LOUIE A 2011. Optimization of aminoglycoside therapy. *Antimicrobial Agents and Chemotherapy,* 55**,** 2528-2531.

DRUSANO GL, PRESTON SL, HARDALO C, HARE R, BANFIELD C, ANDES D, VESGA O & CRAIG WA 2001. Use of preclinical data for selection of a phase II/III dose for evernimicin and identification of a preclinical MIC breakpoints. *Antimicrobial Agents and Chemotherapy,* 45**,** 13-22.

DUFFULL SB, KIRKPATRICK CMJ & BEGG EJ 1997. Comparison of two Bayesian approaches to dose-individualization for once-daily aminoglycoside regimens. *British Journal of Clinical Pharmacology,* 43**,** 125-135.

EFRON B 1979. Bootstrap methods: another look at the Jackknife. *The Annals of Statistics,* 7**,** 1-26.

EFRON, B. & TIBSHIRANI, R. 1993. *An introduction to the bootstrap*, Chapman and Hall.

EMERSON, J., ROSENFELD, M., MCNAMARA, S., RAMSEY, B. & GIBSON, R. 2005. Pseudomonas aeruginosa and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatric Pulmonology,* 34**,** 91-100.

EQUI A, BALFOUR-LYNN IM, BUSH A & ROSENTHAL M 2002. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. *Lancet,* 360**,** 978-984.

ETTE EI 1997. Stability and performance of a population pharmacokinetic model *Journal of Clinical Pharmacology,* 37**,** 486-495.

EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING. *Data from the EUCAST MIC distribution website* [Online]. Available: http://mic.eucast.org/Eucast2/ [Accessed 12 April 2013].

FABRE J, RUDHARDT M, BLANCHARD P & REGAMEY C 1976. Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. *Kidney International,* 10**,** 444-449.

FANTIN B, EBERT S, LEGGETT J, VOGELMAN B & CRAIG WA 1991. Factors affecting duration of in vivo postantibiotic effect for aminoglycosides against gram-negative bacilli. *Journal of Antimicrobial Chemotherapy,* 27**,** 829-836.

FISCHEL-GHODSIAN N, PREZANT TR, CHALTRAW WE, WENDT KA, NELSON RA, ARNOS KS & FALK RE 1997. Mitochondrial gene mutation is a significant predisposing factor in aminoglycoside ototoxicity. *American Journal of Otolaryngology,* 18**,** 173-178.

FLUME PA, O'SULLIVAN BP, ROBINSON KA, GOSS CH, MOGAYZEL PJ, WILLEY-COURAND DB, BUJAN J, FINDER J, LESTER M, QUITTELL L, ROSENBLATT R, VENDER RL, HAZLE L, SABADOSA K & MARSHALL B 2007. Cystic fibrosis pulmonary guidelines. *American Journal of Respiratory and Critical Care Medicine,* 176**,** 957-969.

GALVEZ R, LUENGO C, CORNEJO R, KOSCHE J, ROMERO C, TOBAR E, ILLANES V, LLANOS O & CASTRO J 2011. Higher than recommended amikacin loading doses achieve pharmacokinetic targets without associated toxicity. *International Journal of Antimicrobial Agents,* 38**,** 146-151.

GAN K-H, VEEZE HJ, VAN DEN OUWELAND AMW, HALLEY DJJ, SCHEFFER H, VAN DER HOUT A, OVERBEEK SE, DE JONGSTE JC, BAKKER W & HEIJERMAN HGM 1995. A cystic fibrosis mutation associated with mild lung disease. *The New England Journal of Medicine,* 333**,** 95-99.

GAN SH, ISMAIL R, WAN ADNAN WA, ZULMI W & JELLIFFE RW 2004. Population pharmacokinetic modelling of tramadol with application of the NPEM algorithms. *Journal of Clinical Pharmacy and Therapeutics,* 29**,** 455-463.

GARDNER JC, GOLIATH R, VILJOEN D, SELLARS S, CORTOPASSI G, HUTCHIN T, GREENBERG J & BEIGHTON P 1997. Familial streptomycin ototoxicity in a south african family: a mitochondrial disorder. *Journal of Medical Genetics,* 34**,** 904-906.

GASTONGUAY MR & EL-TAHTAWY A 2005. Effect of NONMEM minimization status and number of replicates on bootstrap parameter distributions for population pharmacokinetic models: A case study. *Clinical Pharmacology and Therapeutics,* 77**,** P2.

GIBIANSKY L, GIBIANSKY E & BAUER R 2012. Comparison of NONMEM 7.2 estimation methods and parallel processing efficiency on a target-mediated drug disposition model. *Journal of Pharmacokinetics and Pharmacodynamics,* 39**,** 17-35.

GILL MA & KERN JW 1979. Altered gentamicin distribution in ascitic patients. *The American Journal of Hospital Pharmacy,* 36**,** 1704-1706.

GILLELAND LB, GILLELAND HE, GIBSON JA & CHAMPLIN FR 1989. Adaptive resistance to aminoglycoside antibiotics in *Pseudomonas aeruginosa Journal of Medical Microbiology,* 29**,** 41-50.

GORDON RC, REGAMEY C & KIRBY WMM 1972. Serum protein binding of the aminoglycoside antibiotics. *Antimicrobial Agents and Chemotherapy.,* 2**,** 214-216.

GUTHRIE OW 2008. Aminoglycoside induced ototoxicity. *Toxicology,* 249**,** 91-96.

GYSELYNCK AM, FORREY A & CUTLER R 1971. Pharmacokinetics of gentamicin: distribution and plasma and renal clearance. *Journal of Infectious Disease,* 124**,** S70-S76.

HALACOVA M, KOTASKA K, KUKACKA J, VAVROVA V, KUZELOVA M, TICHA J & PRUSA R 2008. Serum cystatin C level for better assessment of glomerular filtration rate in cystic fibrosis patients treated by amikacin. *Journal of Clinical Pharmacy and Therapeutics,* 33**,** 409-417.

HARDMAN JG, LIMBIRD LE & GILMAN AG 2001. *The pharmacological basis of therapeutics,* London, UK, McGraw-Hill.

HATALA R, DINH T & COOK DJ 1996. Once-daily aminoglycoside dosing in immunocompetent adults: a meta-analysis. *The Annals Internal Medicine,* 124**,** 717-725.

HENDELES L, LAFRATE RP, STILLWELL PC & MANGOS JA 1987. Individualizing gentamicin dosage in patients with cystic fibrosis: limitation to pharmacokinetic approach. *Journal of Pediatrics,* 110**,** 303-310.

HENNIG S, NORRIS R & KIRKPATRICK CMJ 2007. Target concentration intervention is needed for tobramycin dosing in paediatric patients with cystic fibrosis- a population pharmacokientic study. *British Journal of Clinical Pharmacology,* 65**,** 502-510.

HENNIG S, STANDING JF, STAATZ CE & THOMSON AH 2013. Population pharmacokinetics of tobramycin in patients with and without cystic fibrosis. *Clinical Pharmacokinetics,* 52**,** 289-301.

HENWOOD, C., LIVERMORE, D., JAMES, D., WARNER, M. & THE *PSEUDOMONAS* STUDY GROUP 2001. Antimicrobial susceptibility of *Pseudomonas aeruginosa*: Results of a UK survey and evaluation of the British society for antimicrobial chemotherapy disc susceptibility test. *The Journal of Antimicrobial Chemotherapy,* 47**,** 789-799.

HILL ID, MACDONALD WBG, BOWIE MD & IRELAND JD 1988. Cystic fibrosis in Cape town. *South African Medical Journal,* 73**,** 147-149.

HOLFORD, N. 2005. The visual predictive check-superiority to standard diagnostic (Rorschach) plots. *PAGE 14.* Pamplona, Spain.

HOLFORD N, KIRKPATRICK C & DUFFULL S 2006. NONMEM termination status is not an important indicator of the quality of bootstrap parameter estimates. *PAGE 15.* Bruges, Belgium.

HOOKER AC, STAATZ, C. & KARLSSON MO 2007. Conditional weighted residuals (CWRES): a model diagnostic for the FOCE method. *Pharmaceutical Research,* 24**,** 2187-2197.

HU DN, QUI WQ, WU BT, FANG LZ, ZHOU F, GU YP, ZHANG QH, YAN JH, DING YQ & WONG H 1991. Genetic aspects of antibiotic induced deafness: mitochondrial inheritance. *Journal of Medical Genetics,* 28**,** 79-83.

HUNT BE, WEBER A, BERGER A, RAMSEY B & SMITH AL 1995. Macromolecular mechanisms of sputum inhibition of tobramycin activity. *Antimicrobial Agents and Chemotherapy.,* 39**,** 34-39.

JACKSON GG, LOLANS VT & DALKOS GL 1990. The inductive role of ionic binding in the bactericidal and postexposure effects of aminoglycoside antibiotics with importance of the ratio of peak concentration to minimal inhibitory concentration *Journal of Infectious Disease,* 162**,** 408-413.

JANMAHASATIAN S, DUFFULL SB, ASH S, WARD LC, BYRNE NM & GREEN B 2005. Quantification of lean bodyweight. *Clinical Pharmacokinetics,* 44**,** 1051-1065.

JELLIFFE R, BAYARD D, MILMAN M, VAN GUILDER M & SCHUMITZKY A 2000. Achieving target goals most precisely using nonparametric compartment models and "multiple model" design of dosage regimens. *Therapeutic Drug Monitoring,* 22**,** 346-353.

JELLIFFE RW 1991. The USC*PACK PC programs for population pharmacokinetic modeling, modeling of large kinetic/dynamic systems, and adaptive control of drug dosage regimens. *Procceedings Annual Symposium on Computer Applications in Medical Care***,** 922-924.

JONSSON, E. & KARLSSON, M. 1999. Xpose-An S-PLUS based population pharmacokientic/pharmacodynamic model building aid for NONMEM. *Computer Methods and Programs in Biomedicine,* 58**,** 51-64.

KAHLMETER G & DAHLAGER JI 1984. Aminoglycoside toxicity – a review of clinical studies published between 1975 and 1982. *Journal of Antimicrobial Chemotherapy,* 13**,** 9-22.

KAPUSNIK JE, HACKBARTH CJ, CHAMBERS HF & AL, E. 1988. Single, large, daily dosing versus intermittent dosing of tobramycin for treating experimental *Pseudomonas pneumonia*. *Journal of Infectious Disease,* 158**,** 7-12.

KARLSSON M & HOLFORD N 2008. A tutorial on visual predictive checks. *PAGE 17.* Marseille, France.

KARLSSON M & SAVIC RM 2007. Diagnosing model Diagnostics. *Clinical Pharmacology & Therapeutics,* 82**,** 17-20.

KARLSSON MO & SHEINER LB 1993. The importance of modeling interoccasion variability in population pharmacokinetic analyses. *Journal of Pharmacokinetics and Biopharmaceutics,* 21**,** 735-750.

KASHUBA ADM, NAFZIGER AN, DRUSANO GL & BERTINO JS 1999. Optimizing aminoglycoside therapy for nosocomial pneumonia caused by gram-negative bacteria. *Antimicrobial Agents and Chemotherapy,* 43**,** 623-629.

KAZAZIAN HH 1994. Population variation of common cystic fibrosis mutations. *Human Mutation,* 4**,** 167-177.

KEARNS GL, HILMAN BC & WILSON JT 1982. Dosing implications of altered gentamicin disposition in patients with cystic fibrosis. *The Journal of Pediatrics,* 100**,** 312-318.

KELMAN, A., WHITING, B. & BRYSON, S. 1982. OPT: a package of computer programs for parameter optimisation in clinical pharmacokinetics. *British Journal of Clinical Pharmacology,* 14**,** 247-256.

KEREM B, ROMMENS JM, BUCHANAN JA, MARKIEWICZ D, COX TK, CHAKRAVARTI A, BUCHWALD M & TSUI L-C 1989. Identification of the cystic fibrosis gene: genetic analysis. *Science,* 245**,** 1073-1080.

KEREM E, COREY M, KEREM B-S, ROMMENS J, MARKIEWICZ D, LEVISON H, TSUI L-C & DURIE P 1990. The relation between genotype and phenotype in cystic fibrosis — Analysis of the most common mutation (ΔF508). *New England Journal of Medicine,* 323**,** 1517-1522.

KEREM E, REISMAN J, COREY M, CANNY GJ & LEVISON H 1992. Prediction of mortality in patients with cystic fibrosis. *New England Journal of Medicine,* 326**,** 1187-1191.

KHUU T, BAGDASARIAN G, LEUNG J, NGUYEN N, LAM LD, HAN EE & AMBROSE PJ 2010. Estimating aminoglycoside clearance and creatinine clearance in underweight patients. *American Journal of Health-System Pharmacy,* 67**,** 274-279.

KIRBY WM, CLARKE JT, LIBKE RD & REGAMEY C 1976. Clinical pharmacology of amikacin and kanamycin. *Journal of Infectious Diseases,* 134**,** S312-S315.

KIRKPATRICK CMJ, DUFFULL SB & BEGG EJ 1999. Pharmacokinetics of gentamicin in 957 patients with varying renal function dosed once daily. *British Journal of Clinical Pharmacology,* 47**,** 637-643.

KNOWLTON RG, COHEN-HAGUENAUER O, VAN CONG N, FREZAL J, BROWN VA, BARKER D, BRAMAN JC, SCHUMM JW, TSUI L-C, BUCHWALD M & DONIS-KELLER H 1985. A polymorphic DNA marker linked to cystic fibrosis is located on chromosome 7. *Nature,* 318**,** 380-382.

KRISTIDIS P, BOZON D, COREY M, MARKIEWICZ D, ROMMENS J, TSUI L-C & DURIE P 1992. Genetic determination of exocrine pancreatic function in cystic fibrosis. *American Journal of Human Genetics,* 50**,** 1178-1184.

KUHN E & LAVIELLE M 2005. Maximum likelihood estimation in nonlinear mixed effects models. *Computational Statistics & Data Analysis,* 49**,** 1020-1038.

LACEY LF, KEENE ON, PRITCHARD JF & BYE A 1997. Common noncompartmental pharmacokinetic variables: are they normally or log-normally distributed? *Journal of Biopharmaceutical Statistics,* 7**,** 171-178.

LAM W, TJON J, SETO W, DEKKER A, WONG C, ATENAFU E, BITNUN A, WATERS V, YAU Y, SOLOMON M & RATJEN F 2007. Pharmacokinetic modelling of a once-daily dosing regimen for intravenous tobramycin in paediatric cystic fibrosis patients. *Journal of Antimicrobial Chemotherapy,* 59**,** 1135–1140.

LEARY RH, JELLIFFE R, SCHUMITZKY A & VAN GUILDER M 2002. A unified parametric/nonparametric approach to PK/PD population modeling. *PAGE 11.* Paris.

LEVY J 1986. Antibiotic activity in sputum. *Journal of Pediatrics,* 108**,** 841-846.

LEVY J, SMITH AL, KOUP JR, WILLIAMS-WARREN J & RAMSEY B 1984. Disposition of tobramycin in patients with cystic fibrosis: a prospective controlled study. *The Journal of Pediatrics,* 105**,** 117-124.

LINDBOM L, RIBBING J & JONSSON EN 2004. Perl-speaks-NONMEM (PsN)- a Perl module for NONMEM related programming. *Computer Methods and Programs in Biomedicine,* 75**,** 85-94.

LINDBOM, L., PIHLGREN, P. & JONSSON, N. 2005. PsN-Toolkit--A collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Computer Methods and Programs in Biomedicine,* 79**,** 241-257.

LOPES SP, CERI H, AZEVEDO NF & PEREIRA MO 2012. Antibiotic resistance of mixed biofilms in cystic fibrosis: impact of emerging microorganisms on treatment of infection. *International Journal of Antimicrobial Agents,* 40**,** 260-263.

LUFT FC & KLEIT SA 1974. Renal parenchymal accumulation of aminoglycoside antibiotics in rats. *Journal of Infectious Disease,* 130**,** 656-659.

MACDONALD NE, ANAS NG, PETERSON RG, SCHWARTZ RH, BROOKS JG & POWELL KR 1983. Renal clearance of gentamicin in cystic fibrosis. *Journal of Pediatrics,* 103**,** 985-990.

MANDEMA JW, VEROTTA D & SHEINER LB 1992. Building population pharmacokinetic-pharmacodynamic models; I. Models for covariate effects. *Journal of Pharmacokinetics and Biopharmaceutics,* 20**,** 511-528.

MANN HJ, CANAFAX DM, CIPOLLE RJ, DANIELS CE, ZASKE DE & WARWICK WJ 1985. Increased dosage requirements of tobramycin and gentamicin for treating *pseudomonas pneumonia* in patients with cystic fibrosis. *Pediatric Pulmonology,* 1**,** 238-243.

MARSH DCJR, MATHEW EB & PERSELLIN RH 1974. Transport of gentamicin into synovial fluid. *The Journal of American Medical Association,* 228**,** 607.

MASSIE J & CRANSWICK N 2006. Pharmacokinetic profile of once daily intravenous tobramycin in children with cystic fibrosis. *Journal of Paediatric and Child Health,* 42**,** 601-605.

MASTER V, ROBERTS GW, COULTHARD KP, BAGHURST PA, MARTIN A, ROBERTS ME, ONISHKO CR, MARTIN AJ, LINKE RJ, HOLMES M, JARVINEN A, KENNEDY D, COLEBATCH KA, HANSMAN D & PARSONS DW 2001. Efficacy of once-daily tobramycin monotherapy for acute pulmonary exacerbations of cystic fibrosis: a preliminary study. *Pediatric Pulmonology,* 31**,** 367-376.

MATTHEWS, I., KIRKPATRICK, C. & HOLFORD, N. 2004. Quantitative justification for target concentration intervention-parameter variability and predictive performance using population pharmacokinetic models for aminoglycosides. *British Journal of Clinical Pharmacology,* 58**,** 8-19.

MAYER-HAMBLETT N, KRONMAL RA, GIBSON RL, ROSENFELD M, RETSCH-BOGART G, TREGGIARI MM, BURNS JL, KHAN U, RAMSEY BW & FOR THE EPIC INVESTIGATORS 2012. Initial Pseudomonas aeruginosa treatment failure is associated with exacerbations in cystic fibrosis. *Pediatric Pulmonology,* 47**,** 125-134.

MCKONE EF, EMERSON SS, EDWARDS KL & AITKEN ML 2003. Effect of genotype on phenotype and mortality in cystic fibrosis: a retrospective cohort study. *Lancet,* 361**,** 1671-1676.

MCKONE EF, GOSS CH & AITKEN ML 2006. *GFTR* genotype as a predictor of prognosis in cystic fibrosis. *Chest,* 130**,** 1441-1447.

MENDELSON J, PORTNOY J & SIGMAN H 1973. Pharmacology of gentamicin in the biliary tract of humans. *Antimicrobial Agents and Chemotherapy,* 4**,** 538-541.

MENTRÉ F & ESCOLANO S 2006. Prediction discrepancies for the evaluation of nonlinear mixed-effects models. *Journal of Pharmacokinetics and Pharmacodynamics,* 33**,** 345-367.

MILNE KEN & GOULD IM 2010. Combination testing of multidrug-resistant cystic fibrosis isolates of *Pseudomonas aeruginosa*: use of a new parameter, the susceptible breakpoint index. *Journal of Antimicrobial Chemotherapy,* 65**,** 82-90.

MOORE RD, LIETMAN PS & SMITH CR 1987. Clinical response to aminoglycoside therapy: Importance of the ratio of peak concentration to minimal inhibitory concentration. *Journal of Infectious Diseases,* 155**,** 93-99.

MOSTELLER RD 1987. Simplified calculation of body surface area. *The New England Journal of Medicine,* 317**,** 1098.

MOUTON JW, BROWN DFJ, APFALTER P, CANTO´N R, GISKE CG, IVANOVA M, MACGOWAN AP, RODLOFF A, SOUSSY C-J, STEINBAKK M & G, K. 2012. The role of pharmacokinetics/pharmacodynamics in setting clinical MIC breakpoints: the EUCAST approach. *Clinical Microbiology and Infection,* 18**,** E37-E45.

MOUTON JW, JACOBS N, TIDDENS H & HORREVORTS AM 2005. Pharmacodynamic of tobramycin in patients with cystic fibrosis. *Diagnostic Microbiology and Infectious Disease,* 52**,** 123-127.

MULHERAN M, HYMAN-TAYLOR P, TAN KH-V, LEWIS S, STABLEFORTH D, KNOX A & SMYTH A 2006. Absence of cochleotoxicity measured by standard and high-frequency pure tone Audiometry in a trial of once- versus three-times-daily tobramycin in cystic fibrosis patients. *Antimicrobial Agents and Chemotherapy,* 50**,** 2293-2299.

MUNCKHOF WJ, GRAYSON ML & TURNIDGE JD 1996. A meta-analysis of studies on the safety and efficacy of aminoglycosides given either once daily or as divided doses. *Journal of Antimicrobial Chemotherapy,* 37**,** 645-663.

MYERS DR, DEFEHR J, BENNETT WM, PORTER GA & OLSEN GD 1977. Gentamicin binding to serum and plasma proteins. *Clinical Pharmacology and Therapeutics,* 23**,** 356-360.

NEELY M & JELLIFFE R 2008. Practical therapeutic drug management in HIV-infected patients: use of population pharmacokinetic models supplemented by individualized Bayesian dose optimization. *Journal of Clinical Pharmacology,* 48**,** 1081-1091.

NEELY MN, VAN GUILDER MG, YAMADA WM & SCHUMITZKY A 2012. Accurate detection of outliers and subpopulations with Pmetrics, a nonparametric and parametric pharmacometric modeling and simulation package for R. *Therapeutic Drug Monitoring,* 34**,** 467-476.

NICOLAU DP, FREEMAN CD, BELLIVEAU PP, NIGHTINGALE CH, ROSS JW & QUINTILIANI R 1995. Experience with a once-daily aminoglycoside program administered to 2,184 adult patients. *Antimicrobial Agents and Chemotherapy,* 39**,** 650-655.

NIELSEN EI, CARS O & FRIBERG LE 2011. PK/PD indices of antibiotics predicted by a semi-mechanistic PKPD model- a step towards model-based dose optimization. *Antimicrobial Agents and Chemotherapy,* 55**,** 4619-4630.

PITT HA, ROBERTS RB & JOHNSON WDJR 1973. Gentamicin levels in the human biliary tract. *Journal of Infectious Disease,* 127**,** 299-302.

PLAN EL, MALONEY A, MENTRÉ F, KARLSSON MO & BERTRAND J 2012. Performance comparison of various maximum likelihood nonlinear mixed-effects estimation methods for dose–response models. *The AAPS Journal,* 14**,** 420-432.

PLANTIER J, FORREY AW, O'NEILL MA, BLAIR AD, GHRISTOPHER TG & CUTLER RE 1976. Pharmacokinetics of amikacin in patients with normal or impaired renal function: radioenzymatic acetylation assay. *Journal of Infectious Disease,* 134**,** S323-S330.

POOLE K 2005. Aminoglycoside resistance in *pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherpay,* 49**,** 479-487.

PRÉMAUD A, WEBER LT, TÖNSHOFF B, ARMSTRONG VW, OELLERICH M, URIEN S, MARQUET P & ROUSSEAU A 2011. Population pharmacokinetics of mycophenolic acid in pediatric renal transplant patients using parametric and nonparametric approaches. *Pharmaceutical Research,* 63**,** 216-224.

PRESCOTT JRWA 2011. National survey of extended-interval aminoglycoside dosing in pediatric cystic fibrosis pulmonary exacerbations. *Journal of Pediatric Pharmacology and Therapeutics,* 16**,** 262-269.

PRESTIDGE C, CHILVERS MA, DAVIDSON AGF, CHO E, MCMAHON V & WHITE CT 2011. Renal function in pediatric cystic fibrosis patients in the first decade of life. *Pediatric Nephrology,* 26**,** 605-612.

PREZANT TR, AGAPIAN JV, BOHLMAN MC, BU X, OZTAS S, QIU W-Q, ARNOS KS, CORTOPASSI GA, JABER L, ROTTER JI, SHOHAT M & FISCHEL-GHODSIAN N 1993. Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nature Genetics,* 4**,** 289-294.

PRINS JM, WEVERLING GJ, DE BLOK K, VAN KETEL RJ & SPEELMAN P 1996. Validation and nephrotoxicity of a simplified once-daily aminoglycoside dosing schedule and guidelines for monitoring therapy. *Antimicrobial Agents and Chemotherapy,* 40**,** 2494-9.

PROOST JH & MEIJER DKF 1992. MW/Pharm, an integrated software package for drug dosage regimen calculation and therapeutic drug monitoring. *Computers in Biology and Medicine,* 22**,** 155-163.

R CORE TEAM 2012. R: A language and environment for statistical computing. 2.15.1 ed. Vienna, Austria: R Foundation for Statistical Computing.

RAMPHAL R, LHERMITTE M, FILLIAT M & ROUSSEL P 1988. The binding of antipseudomonal antibiotics to macromolecules from cystic fibrosis sputum. *Journal of Antimicrobial Chemotherapy,* 22**,** 483-490.

RAO P, MCCAUGHAN J, MCCALMONT M, GOLDSMITH CE, HALL V, MILLAR BC, MCCANN M-A, DOWNEY DG, RENDALL JC, ELBORN JS & MOORE JE 2012. Comparison of antibiotic susceptibility patterns in *Pseudomonas aeruginosa* isolated from adult patients with cystic fibrosis (CF) with invasive *Pseudomonas aeruginosa* from non-CF patients. *Journal of Cystic Fibrosis,* 11**,** 349-352.

RATJEN F, MUNCK A, KHO P, ANGYALOSI G & FOR THE ELITE STUDY GROUP 2010. Treatment of early Pseudomonas aeruginosa infection in patients with cystic fibrosis: the ELITE trial. *Thorax,* 65**,** 286-291.

REY E, TRELUYER J-M & PONS G 1998. Drug disposition in cystic fibrosis. *Clinical Pharmacokinetics,* 35**,** 313-329.

RIORDAN JR, ROMMENS JM, KEREM B, ALON N, ROZMAHEL R, GRZELCZAK Z, ZIELENSKI J, LOK S, PLAVSIC N, CHOU JL, DRUMM, M., IANNUZZI MC, COLLINS FS & TSUI L-C 1989. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science,* 245**,** 1066-1073.

RODRIGUEZ V, STEWART D & BODEY GP 1970. Gentamicin sulfate distribution in body fluids. *Clinical Pharmacology and Therapeutics,* 11**,** 275-281.

ROSARIO MC, THOMSON AH, JODRELL DI, SHARP CA & ELLIOTT HL 1998. Population pharmacokinetics of gentamicin in patients with cancer. *British Journal of Clinical Pharmacology,* 46**,** 229-236.

ROTH SM, WILLIAMS SM, JIANG L, MENON KS & JEKA JJ 2008. Susceptibility genes for gentamicin-induced vestibular dysfunction. *Journal of Vestibular Research,* 18**,** 59-68.

RYAN G, SINGH M & DWAN K 2011. Inhaled antibiotics for long-term therapy in cystic fibrosis. *Cochrane Database of Systematic Reviews*.

RYBAK MJ, ABATE BJ, KANG SL, RUFFING MJ, LERNER SA & DRUSANO GL 1999. Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. *Antimicrobial Agents and Chemotherapy,* 43**,** 1549-1555.

SAIMAN L, ANSTEAD M, MAYER-HAMBLETT N, LANDS LC, KLOSTER M, HOCEVAR-TRNKA J, GOSS CH, ROSE LM, BURNS JL, MARSHALL BC, RATJEN F & GROUP, F. T. A. A. S. 2010. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA,* 303**,** 1707-1715.

SAIMAN L, MARSHALL BC, MAYER-HAMBLETT N, BURNS JL, QUITTNER AL, CIBENE DA, COQUILLETTE S, FIEBERG AY, ACCURSO FJ, CAMPBELL PW & GROUP, F. T. M. S. 2003. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa*: a randomized controlled trial. *JAMA,* 290**,** 1749-1756.

SANCHEZ-NAVARRO A & SANCHEZ RECIO MM 1999. Basis of anti-infective therapy: pharmacokinetic-pharmacodynamic criteria and methodology for dual dosage individualisation. *Clinical Pharmacokinetics,* 37**,** 289-304.

SCHEENSTRA RJ, HEIJERMAN HGM, ZUUR CL, TOUW DJ & RIJNTJES E 2010. No hearing loss after repeated courses of tobramycin in cystic fibrosis patients. *Acta Oto-Laryngologica,* 130**,** 253-258.

SCHENTAG JJ, CERRA FB & PLAUT ME 1982. Clinical and pharmacokinetic characteristics of aminoglycoside nephrotoxicity in 201 critically ill patients. *Antimicrobial Agents Chemotherpy,* 21**,** 721-726.

SCHENTAG JJ, GENGO FM, PLAUT ME, DANNER D, MANGIONE A & JUSKO WJ 1979. Urinary casts as an indicator of renal tubular damage in patients receiving aminoglycosides. *Antimicrobial Agents and Chemotherapy.,* 16**,** 468-474.

SCHENTAG JJ, JUSKO WJ, VANCE JW, CUMBO TJ, ABRUTYN, E., DELATTRE M & GERBRACT LM 1977. Gentamicin disposition and tissue accumulation on multiple dosing. *Journal of Pharmacokinetics and Biopharmaceutics,* 5**,** 559-577.

SCHENTAG JJ & PLAUT ME 1980. Patterns of urinary beta 2-microglobulin excretion by patients treated with aminoglycosides. *Kidney International,* 17**,** 654-661.

SCHENTAG, J. J. & JUSKO, W. 1977. Gentamicin Persistance in The Body. *The Lancet,* 1**,** 486.

SCHENTAG, J. J., MEAGHER, A. K. & JELLIFFE, R. W. 2006. Aminoglycosides. *Applied Pharmacokinetics and Pharmacodynamics: Principles of Therapeutic Drug Monitoring.* Philadelphia, United State of America: Lippincott Williams and Wilkins.

SCHUMITZKY A 1991. Nonparametric EM algorithms for estimating prior distributions. *Applied mathematics and computation,* 45**,** 143-157.

SELBY NM, SHAW S, WOODIER N, FLUCK RJ & KOLHE NV 2009. Gentamicin-associated acute kidney injury. *QJM,* 102**,** 873-880.

SHEINER LB & BEAL SL 1981a. Evaluation of methods for estimating population pharmacokinetic parameters II: bioexponential model and experimental pharmacokinetic data. *Journal of Pharmacokinetics and Biopharmaceutics,* 9**,** 635-651.

SHEINER LB & BEAL SL 1981b. Some suggestions for measuring predictive performance. *Journal of Pharmacokinetics and Biopharmaceutics,* 9**,** 503-512.

SMITH PF, BALLOW CH, BOOKER BM, FORREST A & SCHENTAG JJ 2001. Pharmacokinetics and pharmacodynamics of aztreonam and tobramycin in hospitalized patients. *Clinical Therapeutics,* 23**,** 1231-1244.

SMITHIVAS T, HYAMS PJ & RAHAL JJ 1971. Gentamicin and ampicillin in human bile. *Journal of Infectious Disease,* 124**,** S106-S108.

SMYTH A, LEWIS S, BERTENSHAW C, CHOONARA I, MCGAW J & WATSON A 2008. Case-control study of acute renal failure in patients with cystic fibrosis in the UK. *Thorax,* 63**,** 532-535.

SMYTH A, TAN KH-V, HYMAN-TAYLOR P, MULHERAN M, LEWIS S, STABLEFORTH D, KNOX A & FOR THE TOPIC STUDY GROUP 2005. Once versus three-times daily regimens of tobramycin treatment for pulmonary exacerbations of cystic fibrosis - the TOPIC study: a randomised controlled trial. *Lancet,* 365**,** 573-578.

SOBEL ML, MCKAY GA & POOLE K 2003. Contribution of the MexXY multidrug transporter to aminoglycoside resistance in Pseudomonas aeruginosa clinical isolates. *Antimicrobial Agents and Chemotherapy,* 47**,** 3202-3207.

SOULSBY N, GREVILLE H, COULTHARD K & DOECKE C 2010. What is the best method for measuring renal function in adults and children with cystic fibrosis? *Journal of Cystic Fibrosis,* 9**,** 124-129.

STEINKAMP G, LÜTGE M, WURSTER U, SCHULZ-BALDES JG, GRÖNE HJ & EHRICH JHH 1986. Renal function in cystic fibrosis: proteinuria and enzymuria before and after tobramycin therapy. *European Journal of Pediatrics,* 145**,** 526-531.

STUTMAN HR, LIEBERMAN JM, NUSSBAUM E & MARKS MI 2002. Antibiotic prophylaxis in infants and young children with cystic fibrosis: A randomized controlled trial. *Journal of Pediatrics,* 140**,** 299-305.

SUNDIN DP, SANDOVAL R & MOLITORIS BA 2001. Gentamicin inhibits renal protein and phospholipid metabolism in rats: implications involving intracellular trafficking. *Journal of The American Society of Nephrology,* 12**,** 114-123.

SWEILEH WM 2009. A prospective comparative study of gentamicin- and amikacin- induced nephrotoxicty in patients with normal baseline renal function. *Fundamental and Clinical Pharmacology,* 23**,** 515-520.

TAKAMOTO K, KAWADA M, USUI T, ISHIZUKA M & IKEDA D 2003. Aminoglycoside antibiotics reduce glucose reabsorption in kidney through down-regulation of SGLT1. *Biochemical and Biophysical Research Communications,* 308**,** 866-871.

TATARINOVA T, NEELY M, BARTROFF J, VAN GUILDER M, YAMADA W, BAYARD D, JELLIFFE R, LEARY R, CHUBATIUK A & SCHUMITZKY A 2013. Two general methods for population pharmacokinetic modeling: non-parametric adaptive grid and non-parametric Bayesian. *Journal of Pharmacokinetics and Pharmacodynamics,* 40**,** 189-199.

THE UK CYSTIC FIBROSIS TRUST ANTIBIOTIC WORKING GROUP. 2009. *Antibiotic Treatment for Cystic Fibrosis* [Online]. London: UK Cystic Fibrosis Trust. Available: https://www.cysticfibrosis.org.uk/media/82010/CD_Antibiotic_treatment_for_CF_May_09.pdf [Accessed 03 June 2013].

TOUW DJ, KNOX AJ & SMYTH A 2007. Population pharmacokientics of tobramycin administered thice daily and once daily in children and adults with cystic fibrosis. *Journal of Cystic Fibrosis,* 6**,** 327-333.

TOUW DJ, VINKS AATMM, JACOBS F, HEIJERMAN HGM & BAKKER W 1996. Creatinine clearance as predictor of tobramycin elimination in adult patients with cystic fibrosis. *Therapeutic Drug Monitoring,* 18**,** 562-569.

TOUW DJ, VINKS AATMM & NEEF C 1997. Pharmacokinetic modelling of intravenous tobramycin in adolescent and adult patients with cystic fibrosis using the nonparametric expectation maximization (NPEM) algorithm. *Pharmacy World and Science* 19**,** 142-151.

TOUW DJ, VINKS ATMM, HEIJERMAN HGM, HERMANS J & BAKKER W 1994. Suggestions for the optimization of the initial tobramycin dose in adolescent and adult patients with cystic fibrosis. *Therapeutic Drug Monitoring,* 16**,** 125-131.

TREGGIARI MM, RETSCH-BOGART G, MAYER-HAMBLETT N, KHAN U, KULICH M, KRONMAL R, WILLIAMS J, HIATT P, GIBSON RL, SPENCER T, ORENSTEIN D, CHATFIELD BA, FROH DK, BURNS JL, ROSENFELD M, RAMSEY BW & FOR THE EARLY PSEUDOMONAS INFECTION CONTROL (EPIC) INVESTIGATORS 2011. Comparative efficacy and safety of 4 randomized regimens to treat early Pseudomonas aeruginosa infection in children with cystic fibrosis. *Archives of Pediatrics and Adolescent Medicine,* 165**,** 847-856.

TSUI L.-C, ZENGERLING S, WILLARD HF & BUCHWALD M 1986. Mapping of the cystic fibrosis locus on chromosome 7. *Cold Spring Harbor Symposia on Quantitative Biology,* 51**,** 325-335.

VANDENBUSSCHE HL & HOMNICK DN 2012. Evaluation of serum concentrations achieved with an empiric once-daily tobramycin dosage regimen in children and adults with cystic fibrosis. *Journal of Pediatric Pharmacology and Therapeutics,* 17**,** 67-77.

VERPOOTEN GA, GUILLIANO RA, VERBIST L, EESTERMANS G & DE BROE ME 1989. Once-daily dosing decreases renal accumulation of gentamicin and netilimicin. *Clinical Pharmacology and Therapeutics,* 45**,** 22-27.

VIC P, ATEGBO S, TURCK D, HUSSON MO, LAUNAY V, LOEUILLE GA, SARDET A, DESCHILDRE A, DRUON D & ARROUET-LAGANDE C 1998. Efficacy, tolerance, and pharmacokinetics of once daily tobramycin for pseudomonas exacerbations in cystic fibrosis. *Archives of Disease in Childhood,* 78**,** 536-539.

VOGELMAN B, GUDMUNDSSON S, LEGGETT J, TURNIDGE J, EBERT S & CRAIG WA 1988a. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *Journal of Infectious Diseases,* 158**,** 831-847.

VOGELMAN B, GUDMUNDSSON S, TURNIDGE J, LEGGETT J & CRAIG WA 1988b. In vivo postantibiotic effect in thigh infection in neutropenic mice. *Journal of Infectious Disease,* 157**,** 287-298.

WADE JR, BEAL SL & SAMBOL NC 1994. Interaction between structural, statistical, and covariate models in population pharmacokinetic analysis. *Journal of Pharmacokinetics and Biopharmaceutics,* 22**,** 165-177.

WARD DT, MCLARNON SJ & RICCARDI D 2002. Aminoglycosides increase intracellular calcium levels and ERK activity in proximal tubular OK cells expressing the extracellular calcium-sensing receptor. *Journal of The American Society of Nephrology,* 13**,** 1481-1489.

WOLTER J, SEENEY S, BELL S, BOWLER S, MASEL P & MCCORMACK J 2002. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax,* 57**,** 212-216.

WORLD HEALTH ORGANISATION. 2011. *BMI classification* [Online]. Geneva: World Health Organisation. Available: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html [Accessed 03 June 2013].

WRIGHT SW & MORTON NE 1968. Genetic studies on cystic fibrosis in Hawaii. *Journal of Pediatrics,* 20**,** 157-169.

YOSHIOKA H, MOMMA T & MATSUDA S 1972. Placental transfer of gentamicin. *Journal of Pediatrics,* 80**,** 121-123.

APPENDICES

APPENDIX I: ETHICAL APPROVAL

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Western Infirmary
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NHS
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23 July 2009

Dr Alison Thomson
Area Pharmacy Specialist in Clinical Pharmacokinetics
Clinical Pharmacokinetics Unit
Pharmacy Department
Western Infirmary
Glasgow
G11 6NT

Dear Dr Thomson

REC reference number:	**09/S0709/50**
Protocol number:	**2**
Study Title:	**Population pharmacokinetics of aminoglycoside antibiotics in patients with cystic fibrosis**

The Research Ethics Committee reviewed the above application at the meeting held on 21 July 2009. Thank you for attending to discuss the study.

Ethical Opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical Review of Research Sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the Favourable Opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Continued……./

23 July 2009

Letter to Dr Alison Thomson, Western Infirmary.....continued/

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved Documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter	-	18 June 2009
Protocol	2	18 June 2009
Investigator CV	-	15 June 2009
Application	2.0	03 July 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of Compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After Ethical Review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Continued...../

23 July 2009

Letter to Dr Alison Thomson, Western Infirmary.....continued/

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/S0709/50	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

Evelyn Macfadyen

for **Dr R Soutar**
Alternate Vice-Chair

Enclosures: List of names and professions of members who were present at the meeting
"After ethical review – guidance for researchers"
Site approval form (SF1)

Copy to: Dr Melissa McBride, R&D Office, Tennant Institute, Western Infirmary

APPENDIX II: NONMEM CONTROL FILE INCLUDING WITHIN-SUBJECT VARIBILITY

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$PROB NEW CYSTIC DATA OCT 2009
$INPUT ID TIME AMT RATE DV OCC EVID AGE GEN WT HT BMI BSA LBW CREA
EGCL FGCL CGCL CODE=DROP DRUG
$DATA  C:\CFNONMEM\CFdata362010.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$ABBREVIATED DERIV2= NOCOMMON
$ABBREVIATED DERIV2= NO
$PK       IF (AMT.GT.0)THEN
          TDOSE=TIME
          TAD=0.0
          ENDIF
          IF(AMT.EQ.0)TAD=TIME-TDOSE
          OCC1=0
          OCC2=0
          OCC3=0
          OCC4=0
          OCC5=0
          OCC6=0
          OCC7=0
          OCC8=0
          OCC9=0
          OCC10=0
          OCC11=0
          OCC12=0
          OCC13=0
          OCC14=0
          OCC15=0
          OCC16=0
          OCC17=0
          OCC18=0
          OCC19=0
          OCC20=0
          OCC21=0
          OCC22=0
          OCC23=0
          OCC24=0
          OCC25=0
          OCC26=0
          OCC27=0
          OCC28=0
          IF(OCC.EQ.1) OCC1=1
          IF(OCC.EQ.2) OCC2=1
          IF(OCC.EQ.3) OCC3=1
          IF(OCC.EQ.4) OCC4=1
          IF(OCC.EQ.5) OCC5=1
          IF(OCC.EQ.6) OCC6=1
          IF(OCC.EQ.7) OCC7=1
          IF(OCC.EQ.8) OCC8=1
          IF(OCC.EQ.9) OCC9=1
          IF(OCC.EQ.10) OCC10=1
          IF(OCC.EQ.11) OCC11=1
          IF(OCC.EQ.12) OCC12=1
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          IF(OCC.EQ.13) OCC13=1
          IF(OCC.EQ.14) OCC14=1
          IF(OCC.EQ.15) OCC15=1
          IF(OCC.EQ.16) OCC16=1
          IF(OCC.EQ.17) OCC17=1
          IF(OCC.EQ.18) OCC18=1
          IF(OCC.EQ.19) OCC19=1
          IF(OCC.EQ.20) OCC20=1
          IF(OCC.EQ.21) OCC21=1
          IF(OCC.EQ.22) OCC22=1
          IF(OCC.EQ.23) OCC23=1
          IF(OCC.EQ.24) OCC24=1
          IF(OCC.EQ.25) OCC25=1
          IF(OCC.EQ.26) OCC26=1
          IF(OCC.EQ.27) OCC27=1
          IF(OCC.EQ.28) OCC28=1
          IOCL=ETA(1)*OCC1+ETA(2)*OCC2+ETA(3)*OCC3+ETA(4)*OCC4
          IOCL=IOCL+ETA(5)*OCC5+ETA(6)*OCC6+ETA(7)*OCC7
          IOCL=IOCL+ETA(8)*OCC8+ETA(9)*OCC9+ETA(10)*OCC10
          IOCL=IOCL+ETA(11)*OCC11+ETA(12)*OCC12+ETA(13)*OCC13
          IOCL=IOCL+ETA(14)*OCC14+ETA(15)*OCC15+ETA(16)*OCC16
          IOCL=IOCL+ETA(17)*OCC17+ETA(18)*OCC18+ETA(19)*OCC19
          IOCL=IOCL+ETA(20)*OCC20+ETA(21)*OCC21+ETA(22)*OCC22
          IOCL=IOCL+ETA(23)*OCC23+ETA(24)*OCC24+ETA(25)*OCC25
          IOCL=IOCL+ETA(26)*OCC26+ETA(27)*OCC27+ETA(28)*OCC28
          TVCL=THETA(1)*HT+THETA(7)*(CGCL-92))
          TVV1=THETA(2)*(1+THETA(8)*(HT-163))
          TVV2=THETA(3)
          TVQ=THETA(4)
          CL=TVCL*EXP(ETA(29)+IOCL)
          V1=TVV1*EXP(ETA(30))
          V2=TVV2
          Q=TVQ
          S1=V1
$ERROR    IPRED=F
$ERROR    W=SQRT(THETA(5)**2+THETA(6)**2*F**2)
          IRES=DV-IPRED
          IWRES=IRES/W
          Y=IPRED+W*ERR(1)
$THETA   (0,0.05) (0,14) (0,7) (0,0.5) 0.2 0.2 0.005 0.01
$OMEGA BLOCK(1) 0.02  ;IOV CL    1
$OMEGA BLOCK(1) SAME ;2
$OMEGA BLOCK(1) SAME ;3
$OMEGA BLOCK(1) SAME ;4
$OMEGA BLOCK(1) SAME ;5
$OMEGA BLOCK(1) SAME ;6
$OMEGA BLOCK(1) SAME ;7
$OMEGA BLOCK(1) SAME ;8
$OMEGA BLOCK(1) SAME ;9
$OMEGA BLOCK(1) SAME ;10
$OMEGA BLOCK(1) SAME ;11
$OMEGA BLOCK(1) SAME ;12
$OMEGA BLOCK(1) SAME ;13
$OMEGA BLOCK(1) SAME ;14
$OMEGA BLOCK(1) SAME ;15
$OMEGA BLOCK(1) SAME ;16
```

```
$OMEGA BLOCK(1) SAME ;17
$OMEGA BLOCK(1) SAME ;18
$OMEGA BLOCK(1) SAME ;19
$OMEGA BLOCK(1) SAME ;20
$OMEGA BLOCK(1) SAME ;21
$OMEGA BLOCK(1) SAME ;22
$OMEGA BLOCK(1) SAME ;23
$OMEGA BLOCK(1) SAME ;24
$OMEGA BLOCK(1) SAME ;25
$OMEGA BLOCK(1) SAME ;26
$OMEGA BLOCK(1) SAME ;27
$OMEGA BLOCK(1) SAME ;28
$OMEGA BLOCK(2) 0.04 0.02 0.02
$SIGMA 1 FIX
$ESTIMATION MAX=9999 METHOD=1 INTERACTION SIG=3 PRINT=5 NOABORT
$COVAR
$TABLE ID TIME TAD IPRED IRES IWRES NOPRINT ONEHEADER FILE=SDTAB433
$TABLE ID CL V1 V2 Q ETA(1) ETA(29) ETA(30) TVCL TVV1 TVV2 TVQ
NOPRINT ONEHEADER FILE=PATAB433
$TABLE ID AGE WT HT BMI BSA LBM CREA EGCL FGCL CGCL NOPRINT
ONEHEADER FILE=COTAB433
$TABLE ID GEN DRUG NOPRINT ONEHEADER FILE=CATAB433
$TABLE ID TIME TAD AMT RATE DV OCC EVID AGE GEN WT CREA DRUG
TVCL TVV1 TVV2 TVQ CL V1 V2 Q ETA(1) ETA(29) ETA(30) IPRED IRES
IWRES NOPRINT ONEHEADER FILE=433.TAB
$SCAT OMIT
```

APPENDIX III: THE MECHANISTIC MODEL NONMEM CONTROL FILE

```
$PROB NEW CYSTIC DATA OCT 2009
$INPUT ID TIME AMT RATE DV OCC EVID AGE GEN WT HT LBW BSA CREA ACREA
CGCL EGCL FGCL DRUG
$DATA  C:\Mechanisticmodel\CFdata6.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$PK      IF (AMT.GT.0)THEN
         TDOSE=TIME
         TAD=0.0
         ENDIF
         IF(AMT.EQ.0)TAD=TIME-TDOSE

; THE CODING ARE FROM ANDERSON & HOLFORD, DRUG METAB PK 2009

CL_FFAT=0 ;FRACTION OF FAT MASS FOR CL
NFMCL=LBW+CL_FFAT*(WT-LBW); NORMAL FAT MASS CL
FSZCL=(NFMCL/70)**0.75 ; ALLOMETRIC SIZE CL

V_FFAT=1 ;FRACTION OF FAT MASS FOR V
NFMV=LBW+V_FFAT*(WT-LBW);NORMAL FAT MASS V
FSZV=NFMV/70 ;ALLOMETRIC SCALED V

CPR_FFAT=1 ;FRACTION FAT MASS CREATININE PRODUCTIOM RATE (CPR)
NFMCPR=LBW+CPR_FFAT*(WT-BW) ; NORMAL FAT MASS CPR
FSZCPR=NFMCPR/70 ; ALLOMETRIC SIZE CPR

GFR_FFAT=0.211 ;FRACTION FAT MASS FOR GFR
NFMGFR=LBW+GFR_FFAT*(WT-LBW); NORMAL FAT MASS GFR
FSZGFR=(NFMGFR/70)**0.75 ; ALLOMETRIC SIZE GFR
GFRNRM=7.26*FSZGFR; 7.26 IS GFR STD IN L/h/70kg

IF(CREA.GT.60)THEN
IF(GEN.EQ.1.04)THEN; FEMALE
FSEX=THETA(9) ;0.82
ELSE
FSEX=1
ENDIF
FCPR=(THETA(10)-AGE)*FSEX/(THETA(10)-40) ;112
ELSE; LOW CREA (<60 MOCROMOL/L)
FCPR=(THETA(11)-AGE)*THETA(12)/(THETA(11)-40);119 , 0.7
ENDIF

CPR=516*FCPR*FSZCPR ; 516=STD CPR IN MICROMOL/L IN 70 KG ADULT ;AGED
40 YRS

CRCL=CPR/CREA ; L/h

RF=CRCL/GFRNRM ;RENAL FUNCTION

TVCL=THETA(1)*FSZCL+THETA(2)*RF
TVV1=THETA(3)*(1+THETA(4)*FSZV)
TVV2=THETA(5)
TVQ=THETA(6)
CL=TVCL*EXP(ETA(1))
```

```
V1=TVV1*EXP(ETA(2))
V2=TVV2*EXP(ETA(3))
Q=TVQ*EXP(ETA(4))
S1=V1
$ERROR   IPRED=F
$ERROR   W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
         IRES=DV-IPRED
         IWRES=IRES/W
         Y=IPRED+W*ERR(1)
$THETA (0,2) (0,1) (0,14) (0,0.005) (0,7.5) (0,0.5) (0,0.2) (0,0.2)
(0,0.802) (0,73.4) (0,197) (0,1.07)
$OMEGA BLOCK(2) 0.005 0.002 0.05
$OMEGA 0.05 0.05
$SIGMA 1 FIX
$ESTIMATION MAX=4000 METHOD=1 INTERACTION SIG=3 PRINT=5 NOABORT
$COVAR
$TABLE ID TIME TAD IPRED IRES IWRES CWRES NOPRINT ONEHEADER
FILE=SDTAB285Y2
$TABLE ID CL V1 V2 Q ETA(1) ETA(2) ETA(3) ETA(4) TVCL TVV1 TVV2 TVQ
NOPRINT ONEHEADER FILE=PATAB285Y2
$TABLE ID AGE WT HT FFM BSA LBM4 CREA CGCL NOPRINT ONEHEADER
FILE=COTAB285Y2
$TABLE ID GEN DRUG NOPRINT ONEHEADER FILE=CATAB285Y2
$TABLE ID TIME AMT RATE TAD DV OCC EVID AGE GEN WT FFM FSZCL FSZV
FSZCPR GFRNRM FCPR CPR CRCL CGCL RF CREA
TVCL TVV1 TVV2 TVQ CL V1 V2 Q IPRED NOPRINT ONEHEADER FILE=285Y2.TAB
$SCAT OMIT
```

APPENDIX IV: NONMEM CONTROL FILE TO GENERATE SIMULATIONS FOR NPDE

```
$PROB NPDE simulations
$INPUT ID TAD TIME AMT RATE DV MDV OCC EVID AGE GEN WT HT BSA LBW
CREA CGCL DRUG
$DATA  C:\npde\Simdata.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$PK
         TVCL=THETA(1)*HT+THETA(2)*(CGCL-92)
         TVV1=THETA(3)*(1+THETA(4)*(HT-163))
         TVV2=THETA(5)
         TVQ=THETA(6)
         CL=TVCL*EXP(ETA(1)+ETA(2))
         V1=TVV1*EXP(ETA(3))
         V2=TVV2
         Q=TVQ
         S1=V1

$ERROR   IPRED=F
$ERROR   W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
         IRES=DV-IPRED
         IWRES=IRES/W
         Y=IPRED+W*ERR(1)
         FSIM=Y

$THETA   0.0285 0.0114 13.3 0.0113 6.62 0.452 0.086 0.148
$OMEGA  0.0129  ;IOV CL
$OMEGA BLOCK(2) 0.0325 0.014 0.0134;IIV CL BLOCK MATRIX IIV V1
$SIGMA 1 FIX
$SIMULATION (06032012) (909 UNIFORM)ONLYSIM SUBPROBLEMS=1000
$TABLE ID TAD FSIM IPRED NOPRINT NOHEADER NOAPPEND
FILE=SimData10.tab
```

APPENDIX V: NONMEM CONTROL FILE TO RUN THE EXTERNAL VALIDATION

```
$PROB External validation
$INPUT ID OCC OLD=DROP ID=DROP TIME AMT RATE DV EVID SS II II2 WT
CREA AGE GEN HT CGCL LBW BMI
$DATA  C:\R436bvalidation\CFVALIDATION.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$ABBREVIATED DERIV2= NOCOMMON
$ABBREVIATED DERIV2= NO
$PK       IF (AMT.GT.0)THEN
          TDOSE=TIME
          TAD=0.0
          ENDIF
          IF(AMT.EQ.0)TAD=TIME-TDOSE

          TVCL=THETA(1)*HT+THETA(2)*(CGCL-92)
          TVV1=THETA(3)*(1+THETA(4)*(HT-163))
          TVV2=THETA(5)
          TVQ=THETA(6)
          CL=TVCL*EXP(ETA(1)+ETA(2))
          V1=TVV1*EXP(ETA(3))
          V2=TVV2
          Q=TVQ
          S1=V1
$ERROR    IPRED=F
$ERROR    W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
          IRES=DV-IPRED
          IWRES=IRES/W
          Y=IPRED+W*ERR(1)
$THETA    0.0285 0.0114 13.3 0.0113 6.62 0.452 0.086 0.148
$OMEGA 0.0129  ;IOV CL    1
$OMEGA BLOCK(2) 0.0325 0.014 0.0134
$SIGMA 1 FIX
$ESTIMATION MAX=0 POSTHOC
$COVAR OMIT
$TABLE ID TIME TAD IPRED IRES IWRES CWRES NOPRINT ONEHEADER
FILE=SDTAB436bV
$TABLE ID CL V1 V2 Q ETA(1) ETA(2) ETA(3) TVCL  TVV1 TVV2 TVQ
NOPRINT ONEHEADER FILE=PATAB436bV
$TABLE ID AGE WT HT LBM4 CREA CGCL NOPRINT ONEHEADER FILE=COTAB436bV
$TABLE ID GEN NOPRINT ONEHEADER FILE=CATAB436bV
$TABLE ID OCC II2 TIME AMT RATE TAD DV EVID AGE GEN WT CREA
TVCL TVV1 TVV2 TVQ CL V1 V2 Q ETA(1) ETA(2) ETA(3) IPRED IRES IWRES
NOPRINT ONEHEADER FILE=436bV.TAB
$SCAT OMIT
```

APPENDIX VI: NONMEM CONTROL FILE TO GENERATE SIMULATIONS FOR THE DOSAGE GUIDELINE

a) USING LEAN BODY WEIGHT AS THE SCALING FACTOR

```
# $PROB SIMULATION ;13 mg/LBW
$INPUT ID TIME AMT RATE EVID AGE WT HT LBW GEN CREA CGCL DV
$DATA  C:\Nomogramsimulations\LBWscaledose.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$PK

SIMHT=HT*EXP(ETA(4))
IF(SIMHT.LT.139)SIMHT=HT
IF(SIMHT.GT.194)SIMHT=HT


IF(ICALL.EQ.4.AND.NEWIND.NE.2) THEN
GEN1=0 ;MALE
CALL RANDOM(2,R)
GENDER=R
IF(GENDER.LT.0.50) THEN
GEN1=1 ;FEMALE
ENDIF
ENDIF

SIMAGE=AGE*EXP(ETA(5))
IF(SIMAGE.LT.14)SIMAGE=AGE
IF(SIMAGE.GT.88)SIMAGE=AGE

SIMCREA=CREA*EXP(ETA(6))
IF(SIMCREA.LT.60)SIMCREA=60
IF(SIMCREA.GT.209)SIMCREA=CREA

SIMWT=(-67.1+1.09 *GEN1+0.731*SIMHT)*EXP(ETA(7))
IF(SIMWT.LT.30)SIMWT=WT
IF(SIMWT.GT.108)SIMWT=WT

BMI=SIMWT/(SIMHT/100)**2

IF(GEN1.EQ.1) THEN
SIMLBW=(9270*SIMWT)/(8780+(244*BMI))
ELSE
SIMLBW=(9270*SIMWT)/(6680+(216*BMI))
ENDIF

F1=SIMLBW/LBW ; DOSING ADJUSTMENT FACTOR
AMT2=AMT*F1

IF(GEN1.EQ.1) THEN
SIMCGCL=((1.04*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ; LOGCRCL
VARAINACE
ELSE
SIMCGCL=((1.23*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ;LOGCRCL
VARIANCE
ENDIF
```

```
IF(SIMCGCL.LT.26.3)SIMCGCL=CGCL
IF(SIMCGCL.GT.181.4)SIMCGCL=CGCL


          TVCL=THETA(1)*SIMHT+THETA(2)*(SIMCGCL-92)
          TVV1=THETA(3)*(1+THETA(4)*(SIMHT-163))
          TVV2=THETA(5)
          TVQ=THETA(6)
          CL=TVCL*EXP(ETA(1)+ETA(2))
          V1=TVV1*EXP(ETA(3))
          V2=TVV2
          Q=TVQ
          S1=V1
          AUC=AMT2/CL

$ERROR    IPRED=F
$ERROR    W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
          IRES=DV-IPRED
          IWRES=IRES/W
          Y=IPRED+W*ERR(1)

$THETA    0.0285 0.0114 13.3 0.0113 6.62 0.452 0.086 0.148
$OMEGA    0.0129  ;IOV CL
$OMEGA BLOCK(2) 0.0325 0.0140 0.0134;IIV CL BLOCK MATRIX IIV V1
$OMEGA    0.003 0.112 0.042 0.035 0.05;LOG NORMAL DISTRIBUTION
VARIANE VALUE FOR ETA 4 5 6 7 8
$SIGMA 1 FIX
$SIMULATION (22032012) (812 UNIFORM)ONLYSIM SUBPROBLEMS=5000
$TABLE ID TIME AMT2 EVID DV GEN1 SIMAGE SIMWT SIMHT BMI SIMLBW
SIMCREA SIMCGCL CL AUC V1 V2 Q NOPRINT ONEHEADER FILE=mgperLBW.TAB
```

b) USING HEIGHT AS THE SCALING FACTOR

```
$PROB NOMOGRAM DEVELOPMENT BY SIMULATION ;3mg/cm
$INPUT ID TIME AMT RATE EVID AGE WT HT GEN CREA CGCL DV
$DATA  C:\Nomogramsimulations\COMBINEDNOMOGRAM2.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$PK

SIMHT=HT*EXP(ETA(4))
IF(SIMHT.LT.139)SIMHT=HT
IF(SIMHT.GT.194)SIMHT=HT
F1=SIMHT/HT ; DOSING ADJUSTMENT FACTOR
AMT2=AMT*F1

IF(ICALL.EQ.4.AND.NEWIND.NE.2) THEN
GEN1=0 ;MALE
CALL RANDOM(2,R)
GENDER=R
IF(GENDER.LT.0.50) THEN
GEN1=1 ;FEMALE
ENDIF
ENDIF

SIMAGE=AGE*EXP(ETA(5))
IF(SIMAGE.LT.14)SIMAGE=AGE
IF(SIMAGE.GT.88)SIMAGE=AGE

SIMCREA=CREA*EXP(ETA(6))
IF(SIMCREA.LT.60)SIMCREA=60
IF(SIMCREA.GT.209)SIMCREA=CREA

SIMWT=(-67.1+1.09 *GEN1+0.731*SIMHT)*EXP(ETA(7))
IF(SIMWT.LT.30)SIMWT=WT
IF(SIMWT.GT.108)SIMWT=WT


IF(GEN1.EQ.1) THEN
SIMCGCL=((1.04*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ; LOGCRCL
VARAINACE
ELSE
SIMCGCL=((1.23*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ;LOGCRCL
VARIANCE
ENDIF

IF(SIMCGCL.LT.26.3)SIMCGCL=CGCL
IF(SIMCGCL.GT.181.4)SIMCGCL=CGCL


          TVCL=THETA(1)*SIMHT+THETA(2)*(SIMCGCL-92)
          TVV1=THETA(3)*(1+THETA(4)*(SIMHT-163))
          TVV2=THETA(5)
          TVQ=THETA(6)
          CL=TVCL*EXP(ETA(1)+ETA(2))
          V1=TVV1*EXP(ETA(3))
          V2=TVV2
```

```
          Q=TVQ
          S1=V1
          AUC=AMT2/CL

$ERROR    IPRED=F
$ERROR    W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
          IRES=DV-IPRED
          IWRES=IRES/W
          Y=IPRED+W*ERR(1)

$THETA    0.0285 0.0114 13.3 0.0113 6.62 0.452 0.086 0.148
$OMEGA    0.0129  ;IOV CL
$OMEGA BLOCK(2) 0.0325 0.0140 0.0134;IIV CL BLOCK MATRIX IIV V1
$OMEGA    0.003 0.112 0.042 0.035 0.05;LOG NORMAL DISTRIBUTION
VARIANE VALUE FOR ETA 4 5 6 7 8
$SIGMA 1 FIX
$SIMULATION (22032012) (812 UNIFORM)ONLYSIM SUBPROBLEMS=5000
$TABLE ID TIME AMT2 EVID DV GEN1 SIMAGE SIMWT SIMHT SIMCREA SIMCGCL
TVCL TVV1 TVV2 TVQ CL AUC V1 V2 Q NOPRINT ONEHEADER
FILE=Simcombine2A.TAB
```

c) USING BODY SURFACE AREA AS THE SCALING FACTOR

```
 $PROB SIMULATION ;326 mg/BSA
$INPUT ID TIME AMT RATE EVID AGE WT HT BSA GEN CREA CGCL DV
$DATA  C:\Nomogramsimulations\BSAscaledose.CSV IGNORE=#
$SUBROUTINE ADVAN3 TRANS4
$PK

SIMHT=HT*EXP(ETA(4))
IF(SIMHT.LT.139)SIMHT=HT
IF(SIMHT.GT.194)SIMHT=HT


IF(ICALL.EQ.4.AND.NEWIND.NE.2) THEN
GEN1=0 ;MALE
CALL RANDOM(2,R)
GENDER=R
IF(GENDER.LT.0.50) THEN
GEN1=1 ;FEMALE
ENDIF
ENDIF

SIMAGE=AGE*EXP(ETA(5))
IF(SIMAGE.LT.14)SIMAGE=AGE
IF(SIMAGE.GT.88)SIMAGE=AGE

SIMCREA=CREA*EXP(ETA(6))
IF(SIMCREA.LT.60)SIMCREA=60
IF(SIMCREA.GT.209)SIMCREA=CREA

SIMWT=(-67.1+1.09 *GEN1+0.731*SIMHT)*EXP(ETA(7))
IF(SIMWT.LT.30)SIMWT=WT
IF(SIMWT.GT.108)SIMWT=WT

SIMBSA=SQRT(SIMHT*SIMWT/3600)
F1=SIMBSA/BSA ; DOSING ADJUSTMENT FACTOR
AMT2=AMT*F1

IF(GEN1.EQ.1) THEN
SIMCGCL=((1.04*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ; LOGCRCL
VARAINACE
ELSE
SIMCGCL=((1.23*(140-SIMAGE)*SIMWT)/SIMCREA)*EXP(ETA(8)) ;LOGCRCL
VARIANCE
ENDIF

IF(SIMCGCL.LT.26.3)SIMCGCL=CGCL
IF(SIMCGCL.GT.181.4)SIMCGCL=CGCL


          TVCL=THETA(1)*SIMHT+THETA(2)*(SIMCGCL-92)
          TVV1=THETA(3)*(1+THETA(4)*(SIMHT-163))
          TVV2=THETA(5)
          TVQ=THETA(6)
```

```
          CL=TVCL*EXP(ETA(1)+ETA(2))
          V1=TVV1*EXP(ETA(3))
          V2=TVV2
          Q=TVQ
          S1=V1
          AUC=AMT2/CL

$ERROR    IPRED=F
$ERROR    W=SQRT(THETA(7)**2+THETA(8)**2*F**2)
          IRES=DV-IPRED
          IWRES=IRES/W
          Y=IPRED+W*ERR(1)

$THETA    0.0285 0.0114 13.3 0.0113 6.62 0.452 0.086 0.148
$OMEGA    0.0129  ;IOV CL
$OMEGA BLOCK(2) 0.0325 0.0140 0.0134;IIV CL BLOCK MATRIX IIV V1
$OMEGA    0.003 0.112 0.042 0.035 0.05;LOG NORMAL DISTRIBUTION
VARIANE VALUE FOR ETA 4 5 6 7 8
$SIGMA 1 FIX
$SIMULATION (22032012) (812 UNIFORM)ONLYSIM SUBPROBLEMS=5000
$TABLE ID TIME AMT2 EVID DV GEN1 SIMAGE SIMWT SIMHT SIMBSA SIMCREA
SIMCGCL TVCL TVV1 TVV2 TVQ CL AUC V1 V2 Q NOPRINT ONEHEADER
FILE=mgperBSA.TAB
```