Development of a population pharmacokinetic model to determine the optimal doses of amikacin in the treatment of neonatal sepsis

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“Not everything that can be counted counts and not everything that counts can be counted.”
Albert Einstein (attributed)

A la meva família,

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Contents

Abbreviations

2-DOS	2-deoxystreptamine
AGA	Adequate for Gestational Age
AIC	Akaike Information criteria
AUC	Area Under the Curve
BHC	Birth head circumference
BHGT	Birth height
BLQ	Below the limit of quantification
BSA	Body Surface Area
BPV	Between patient variability
BWGT	Birth weight
CI	Confidence Intervals
CL	Clearance
CLCR	Creatinine Clearance
C_{max}	Maximum Concentration
MIC	Minimum Inhibitori Concentration
Cpeak	Peak concentration
CREA	Creatinine serum concentrations
Ctrough	Trough concentration
CSF	Cerebral spinal fluid
CWRES	Conditional weighted residuals
DV	Observed data
EBE	Empirical Bayes Estimates
EMEA	European Medicines Agency
FDA	Food and Drug Administration
FO	First-order estimation method
FOCE	First-order Conditional Estimation method
FOCE I	First-order Conditional Estimation method with Interaction
FPIA	Fluorescence polarization immunoassay
GA	Gestational age
GAM	Generalised Additive Modelling
GOF	Goodness-of-fit plots
HABA	Dihydroxyaminobutyric acid
HC	Head Circumference
HGT	Height
IPRED	Individual model predictions
IWRES	Individual weighted residuals
LLOQ	Lower limit of quantification
ME	Median prediction error
MIC	Minimum Inhibitory Concentration
NONMEM	NONlinear Mixed Effects Model

NPC	Numerical predictive check
NPDE	Normalised prediction distribution errors
OFV	Objective Function Value
PCA	Postconceptional age
pc-VPC	Predicted corrected Visual Predictive Check
PD	Pharmacodynamic
PDA	Patent arterial duct
PI	Prediction intervals
PK	Pharmacokinetic
PMA	Postmenstrual age
PNA	Postnatal age
PPC	Posterior Predictive Check
PRED	Typical population predictions
Q	Distributional clearance
RE	Residual error
RES	Residual concentrations
RMSE	Root median prediction error
RNA	Ribosomal ribonucleic acid
RSE	Relative standard error
SGA	Small for Gestational Age
SS	Sum of squares
TAD	Time after the last dose
TIME	Time
UREA	Urea serum concentrations
V1	Central compartment distribution volume
V2	Peripheral compartment distribution volume
VPC	Visual Predictive Check
WGT	Weight
WRES	Weighted residuals

ε	Residual variability
θ	Typical value of the parameter
η	Interindividual variability
σ	Standard deviation of the residual variability
ω	Standard deviation of the interindividual variability

Abstract

The aim of this study was to establish the population pharmacokinetics of amikacin in newborns from serum concentration data obtained during the routine therapeutic drug monitoring and to explore the influence of patient covariates on drug disposition. To validate the developed model in into a external dataset, belonging to the same population as the development group, to evaluate the current dose regimen and to optimize the first dose recommendations, were also aims of the study. Data were retrospectively collected for a study in newborns with postnatal age less than 90 days admitted in the neonatal unit of Vall Hebron (July 2000 to July 2006) who were treated with amikacin and with at least two serum concentration data of the aminoglycoside. Amikacin was administered as an i.v. infusion over 30 or 60 min. Blood samples were collected just before ("through") and 1h after start of the infusion ("peak"). Demographic, clinical and amikacin dosing and concentration data were collected. Amikacin serum concentration measurements were done using fluorescence polarization immunoassay (TDx; Abbott Laboratories). Population PK analysis was performed from 149 newborns using the non linear mixed-effect approach (NONMEM version VII). The First order conditional estimation method (FOCE) with interaction was used throughough all the model bulding process.The PK of amikacin after iv administration was best described by a two-compartment linear disposition model. Between-patient variabilities expressed as coefficient of variation (CV%) were associated to total plasma clearance (CL) (16.39%) , central compartment distribution volume (V1) (25.23%) and distributional clearance (Q) (40.08%). Residual variability, modelled as a combined error model (proportional + additive), was 6.97% and 15.37%, respectively. Creatinine Clearance (CLCR) and body weight (WGT) were the most influential covariates in CL, and WGT was in V1. The final population model is: $TVCL=0.133\cdot(CLCR/31.97)^{0.649}\cdot x(WGT/1880)^{0.752}$ and $TVV1=0.837\cdot(WGT/1880)^{1.09}$. The external validation as well as th internal validation either through bootstrapping, or by Visual Predictive Check, prediction-corrected visual predictive check, posterior predictive check, or by normalised prediction distribution errors, suggested a good predictive ability for the developed model.

The several simulations based on the final pharmacokinetic estimates of the model showed the influence of the covariates identified as significant in the serum amikacin concentrations, demonstrating the ability of the model to stablish optimal initial doses of amikacin for the treatment of neonatal sepsis. Due to the possibility of including the model in clinical pharmacokinetic software, the use of this model could improve the design of initial amikacin dosage in neonate populations and provide feedback adjustments of dosage regimens to achieve desired serum concentrations.

1. INTRODUCTION

1.1 PHARMACOLOGY OF AMIKACIN

1.1.1 Background

Aminoglycoside antibiotics were the first drugs discovered by systematic screening of natural product sources for antibacterial activity. The aminoglycoside antibiotics comprise a large group of naturally occurring or semi-synthetic polycationic compounds. Streptomycin was the first aminoglycoside identified, in 1944 by Waksman's group, as a natural product of a soil bacterium, *Streptomyces griseus*. This was followed by the discoveries of neomycin by the same group in 1949, kanamycin by Umezawa et al in 1957, and later tobramycin. Gentamicin, first reported in 1963 by Weinstein, netilmycin and sisomycin were isolated from different species of *Micromonospora*. Thereafter, the research was focused on chemical modification of known compounds in order to increase its antibacterial activity and reduce its associated toxicity, rather than on the discovery of new antibiotics from soil microbes (1). Amikacin and dibekacin are derivative compounds of kanamycin through chemical modifications, while netilmycin is a semi-synthetic derivative of sisomicin.

The chemical structure of all natural aminoglycosides includes an aminocyclitol moiety of 2-deoxystreptamine (2-DOS), where amino sugars are linked glycosidically to aminocyclitol at positions 4-, 5- or 6- (Figure 1.1). According to these substitutions, aminoglycosides can be classified into three different groups, of which the two first are the most important (2):

- 2-DOS (4,5-disubstituted). Including neomycin B, one of the oldest aminoglycosides still used today, but limited to topical application because of its potential toxicity.
- 2-DOS (4,6-disubstituted). The largest group, which includes gentamicin, tobramycin and amikacin.
- 2-DOS (4-monosubstituted). Apramycin, which use is limited to clinical veterinary, is the only compound known of this group.

The chemical differences between these compounds are particularly important in determining differences related with the antibacterial activity. Streptomycin and spectinomycin are not strictly 2-DOS derivatives, because its central moiety is a streptidine derivative (with and hidroxil- group at position 2), while spectinomycin sometimes is not classified as an aminoglycoside, because it does not has the aminocyclitol moiety of 2-DOS. Nonetheless, the group of compounds derived from streptidin, 2-DOS aminoglycosides derivatives and Spectinomicyn, are all known as Aminocyclitols.

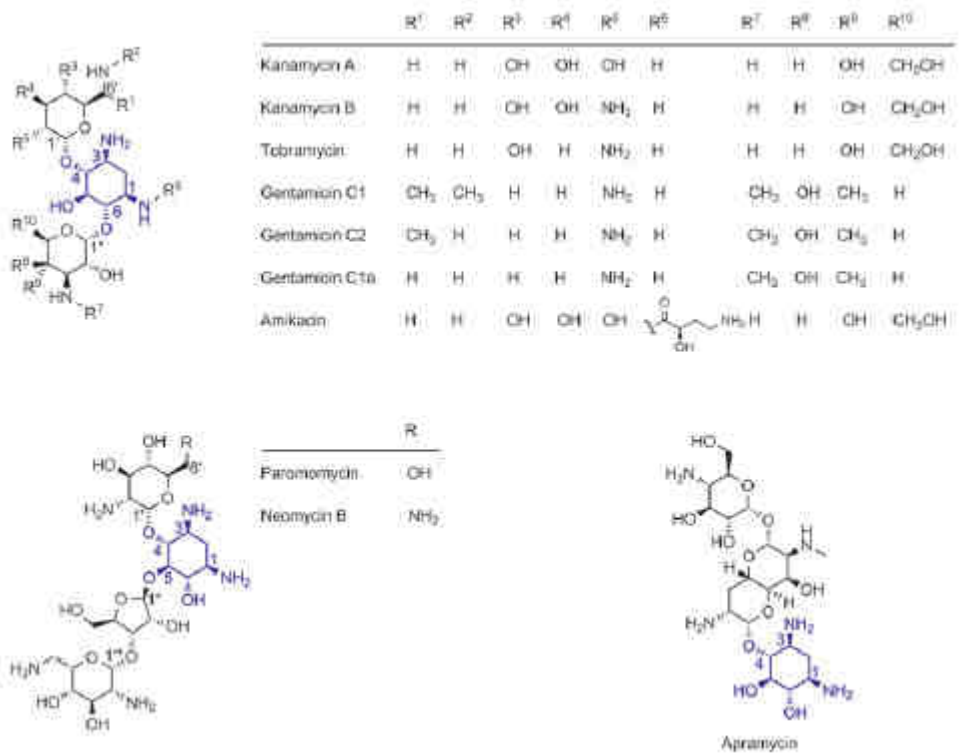


Figure 1.1. Structural families of aminoglycoside antibiotics

Physicochemically, amikacin $(C_{22}H_{43}N_5O_{13})$ is a white, crystalline, basic and water-soluble antibiotic with a molecular weight of 585.60 g/mol, a melting point of 201º-204ºC, and a specific optical rotation from +97º to 105º (3). It is a semi-synthetic derivative of kanamycin A developed in order to protect it from the inactivating mechanisms of resistant organisms. Amikacin differs in its chemical structure from kanamycin by the acylation with dihydroxyaminobutyric acid (HABA) at the C-1 amino group of the 2-DOS nucleus. The addition of this HABA moiety confers upon amikacin its unique properties, which include a broadening of its antibacterial spectrum over that of kanamycin, and resistane to most plasmid mediated inactivating enzymes (4).

1.1.2 Mechanism of action and Resistance

Amikacin, like other aminoglycoside antibiotics, shows a concentration-dependent bactericidal activity (its effectiveness is related to the maximum concentration achieved). Its diffusion across the cell membranes is very limited because of its polar characteristics, being carried out through aqueous channels in the outer membrane of gram-negative bacteria into the periplasmic space, and being then actively transported across the cytoplasmic membrane to the site of action (1). Therefore, intracellular accumulation of this group of antibiotics occurs by active transport. Once transported across the bacterial cell membrane, the aminoglycosides are attached to 30S and 50S ribosomal units, inhibiting protein synthesis by three mechanisms: (i) blockage of the start of protein synthesis, (ii) premature blockage of translation process by separating

the ribosomic complex 30S-50S, with the consequent misreading of the genetic code and production of defective proteins, and (iii) incorporation of incorrect amino acids, resulting in abnormal polypeptides synthesis. Other possible mechanisms contributing to the overall antimicrobial effect of aminoglycoside antibiotics can be the modification of cytoplasmic membrane, which results in the release of intracellular components, and the alteration of metabolism and cell respiration (5). The rate of transport of aminoglycosides across the bacterial cell membranes can be altered significantly by the presence of divalent cations (Mg^{2+}, Ca^{2+}), the pH of the environment, and the amount of oxygen present. This observation explains the significant decrease in antimicrobial activity in anaerobic or acidic environments (4).

Resistance to aminoglycosides can be developed mainly by the following three mechanisms (5):

i. **Enzymatic inactivation.** Microbial enzymes are the main cause of resistance within this class of antibiotics. Once the aminoglycoside reaches the periplasmic space, it is susceptible to enzymatic action, which is mediated by plasmids, by phosphorylation, adenylation or acetylation. Although it does not inactivate the antibiotic directly, it alters the transport mechanism across the cell, inactivating its union to ribosomes. Eleven different responsible enzims of this action have been described. The degree of susceptibility of the antibiotics to enzymatic inactivation is location and time-dependent. Amikacin has been reported to be the most stable (being susceptible only to two out of the 11 enzymes), because of the presence of molecular side chains that protect amikacin from the enzyme action. The enzymatic inactivation of amikacin occurs mainly by acetylation of the amino group at C6' position.
ii. **Loss of permeability of the bacterial cell to the drug.** This mechanism is related to chromosomal mutations that affects gene responsible of the amynoglicosides transport by blocking the transport of the drug inside the cells.
iii. **Ribosomal alterations.** A very specific resistance mechanism of streptomycin triggered by an alteration of the 30S ribosomal subunit of the receptor.

The main mechanism of resistance associated to amikacin seems to be related with a decreased penetration of the drug into the bacterial cell. This type of resistance seems to be non-specific, and this vulnerability is shared by all other amynoglicosides. As a consequence, the strains resistant to gentamicin or tobramycin by enzymatic inactivation remain sensitive to amikacin, but strains resistant by the non-enzymatic inactivation are generally cross-resistant to all three aminoglycosides (4).

1.1.3 Spectrum of activity

Aminoglycosides are indicated primarily for the treatment of infections caused by aerobic gram-negative bacilli. The spectrum of activity of aminoglycosides includes *Citrobacter* spp, *Enterobacter* spp, *Escherichia Coli*, *Klebsiella pneumoniae*, *Proteus* spp, *Providencia* spp and *Pseudomonas aeruginosa*. Other aerobic gram-negative bacilli are also susceptible to aminoglycosides but are rarely indications for their clinical use, as *Neisseria gonorrhea*, *Neisseria meningitidis*, and *Haemophilus influenza*. The usefulness of aminoglycosides against gram-positive organisms is greatly restricted to *Staphylococus aureus* and *Staphylococus epidermidis*. The oxygen-dependent mechanism of action explains its inactivity against anaerobic bacteria (1).

Several antibiotic groups have been demonstrated to have synergistic activity with aminoglycosides. This is especially true for the β-lactam antibiotics, including penicillins and cephalosporins. One of the proposed mechanisms of synergy is the increase in porosity of the bacterial cell wall caused by the β-lactam antibiotic, allowing a higher penetration of the aminoglycoside into the bacterial cell. *Enterococcus* and some *Streptococcus* species are sensitive to the aminoglycoside action by this synergistic effect of the aminoglycosides with penicillins (5).

The “in vitro” activity of the aminoglycosides against several microorganisms can be assessed based on the Minimum Inhibitory Concentration (MIC), which values differ depending on the antibiotic and the microorganism. Table 1.1 shows MICs of some aminoglycosides against aerobic gram-negative bacilli according to the standards of the National Committee for Clinical Laboratory Standards (NCCLS) (6)

Table 1.1. Classification of MICs of the aminoglycosides against aerobic gram-negative bacilli

Aminoglycoside	**MICs interpretation (mg/L)**		
	S	**I**	**R**
Gentamicin	≤4	8	≥16
Amikacin	**≤16**	**32**	**≥64**
Tobramycin	≤4	8	≥16
Kanamycin*	≤16	32	≥64
Netilmycin	≤8	16	≥32

S: Sensitive. I: Intermediate. R: Resistant
** Only valid for Enterobacteriaceae spp.*

1.1.4 Clinical Pharmacokinetics

1.1.4.1 Absorption

Like all aminoglycosides, amikacin is a large, highly polar and basic compound, which explain its low absorption from the gut. When given orally, less than 1% can reach the blood stream. For this reason, these drugs are not administered orally, but by parenteral injection. Amikacin is well absorbed after intramuscular injection, and it achieves peak levels within 30-90 minutes. However, the absorption may be delayed in alterations of tissue perfusion, such as in cases of shock or hypoxemia, leading to a high interindividual variability. When hypoxemia occurs, the intravenous route of administration is preferable to the intramuscular (7). Aminoglycosides can be administered intravenously by 30 to 60 minutes intermittent infusions, by continuous intravenous infusion, or by slow bolus injection (5). The intermittent infusion is the most frequently administration way used, mainly with infusion periods of 60 minutes, because of pharmacokinetic and practicability reasons. Some authors have been reported a three-compartment pharmacokinetic behaviour with first order elimination kinetics after intravenous bolus administration, although two- and one-compartment models have also shown to fit the time-concentration profiles reasonably well (8). Differences in sampling schedule (dense or sparse data) is the main reason of the different pharmacokinetic behaviours described.

Direct administration of amikacin via inhalation has also been used, especially in cystic fibrosis patients with infections caused by *Pseudomonas aeruginosa* (9). Subconjunctival injection gives adequate levels in the aqueus humor, but neither parenteral injection nor subconjunctival administration give effective levels in vitreous humor. So, in the case of endophtalmitis, the use of intravitreal administration is required.

Other ways of administration less recommended are: subcutaneous administration, that although it has a similar kinetic to intramuscular administration it has a risk of skin necrosis; administration into pleural and peritoneal cavities, because of the possibility of rapid absorption and the subsequent toxicity (10); and topic administration, due to its transdermal absorption, caution should be taken when administered for long periods of time on burns, wounds and skin ulcers, particularly if the patient shows a compromised renal function, because of the risk of toxicity.

1.1.4.2 Distribution

The volume of distribution of aminoglycosides decreases from birth to 5 years, when reaches a value similar to that of adulthood, between 0.2-0.3 L/Kg. Protein binding of almost all aminoglycosides is less than 10%.

Their polar nature is determining in its body distribution. Firstly, due to its polar characteristics, the distribution of amikacin to adipose tissue is very limited. This should be taken into account in obese patients, in which case dose tailoring adjustment should be performed based on lean body weight or ideal weight rather than on total weight. Their polarity also determines the intracellular body distribution, being usually confined to the extracellular fluid compartment. Besides, the pass through membranes is poor, not only because of its size but also due to its positive charge. As a consequence, the concentrations achieved in most tissues are really low, with the exception of the inner ear and renal proximal tubule, which have active transport systems for aminoglycosides. It could explain the nephrotoxicity and ototoxicity seen with this class of antibiotics; by contrast, amikacin concentrations in cerebral spinal fluid (CSF), in the absence of inflammation, are less than 10% of plasma concentrations. For this reason, to achieve therapeutic concentrations in CSF, these antibiotics must be administered by intrathecal or intraventricular routes; in the case of newborns, however, the penetration into the CSF is higher than in adults. These agents can cross the placenta, achieving fetal serum concentrations that are 21 to 37% of maternal serum concentrations. Adequate antibiotic concentrations are achieved in most other body fluids, including synovial, peritoneal, ascetic and pleural fluids (5).
There are several factors that can contribute to the interpatient variability observed in the amikacin distribution volume, such as the presence of edema or ascites, that are responsibles not only of the high interpatient variability, but also of the so called “third space”, increasing the distribution volume and decreasing plasma concentrations (1).

1.1.4.3 Metabolism and Elimination

Aminoglycosides are primarily eliminated unchanged by the kidney via glomerular filtration (85-95%), being a linear correlation between creatinine clearance and the clearance of aminoglycosides (5). However, a non linear relationship has been reported between filtration rate and gestational age (11,12). Until 34 weeks of gestation, filtration rate values are low and constant, coinciding with the completion of the glomerulus formation. From 34 to 36 weeks, the filtration rate increases with age, reflecting a significant maturation of renal function that could be attributable to morphological changes of the glomerulus. Besides, extraction renal rates of the drug around 50% can be achieved in patients undergoing renal dialysis. For this reason, aminoglycosides can be safely used in patients with renal impairement. Contrary, in peritoneal dialysis, the extraction rate is between two and three times less effective.

The amikacin serum half-life, in adults under normal renal function, ranges from 2 to 3 hours. However, several factors either physiological or pathological can alter these values .i.e., in the case of newborns clearly higher half-life values can be found compared to those of the childhood and adulthood, or lower values can be found either during pregnancy or in patients with cystic fibrosis.

1.1.5 Pharmacodynamics

The efficacy of overall effect of aminoglycosides is the result of: (i) the concentration-dependent bactericidal activity, (ii) the adaptive resistance, and (iii) the occurrence of a post-antibiotic effect (10).

Due to its *concentration-dependent bactericidal activity,* also characteristic of quinolones, the rate and magnitude of the bactericidal action increases with increasing antibiotic concentrations above the minimum bactericidal concentration until achieving a maximum effect. Various empirical pharmacokinetic/pharmacodynamic (PK/PD) indices have been proposed to predict the success or failure of therapy (13). In concentration-dependent antibiotics, both Maximum Concentration/Minimum Inhibitory Concentration (C_{max}/MIC) and Area Under the Curve/Minimum Inhibitory Concentration (AUC/MIC) ratios are the main PK/PD parameters correlating with efficacy. In animal infection models, the 24h AUC/MIC ratio demonstrated to be a better predictor of therapeutic efficacy than the C_{max}/MIC ratio, whereas the opposite was true in human clinical trials (14). It has been reported that to obtain a clinical response of ≥ 90% and reduce the risk of emergence resistance, C_{max}/MIC needs to be around 8-10 (15). This is easily achieved in individuals with normal renal function by the administration of a single day large dose of aminoglycosides, which also minimizes the consequences of adaptive resistance (16). *Adaptive resistance* is a phenotypic and reversible increase in MIC associated with a temporary lack of drug transport into the bacterial cells. *The post-antibiotic effect*, defined as the residual bactericidal activity of the antibiotic persisting some time after the drug concentrations have reached values below the MIC, persists up to around 2-4 hours in the case of aminolgycosides. So, the efficiency of spaced administration of large doses of aminoglycosides is related to their prolonged and concentration-dependent post-antibiotic effect, which prevents bacterial regrowth when serum levels fall below the MIC.

The overall combination of the concentration-dependent bactericidal activity, the adaptive resistance and the post-antibiotic effect provides the theorical basis supporting the use of aminoglycosides at high doses and wide dosage intervals (once daily dose administration). So that, the administration as a single dose maximizes the ratio C_{max}/MIC, increasing and prolonging the bactericidal action, preventing the development of bactericidal resistance and reducing potential toxicity.

1.1.6 Adverse effects

The administration of aminoglycosides through intramuscular and intravenous routes is usually well tolerated without causing local inflammatory reactions. However, all of them, with the exception of spectinomycin, exhibit a relatively high potential risk of renal and otic toxicity, and rarely neuromuscular blockade, that are an important limitation for their use. However, the incidence of toxicity is difficult to be established,

because there are several factors that should be considered: the uniformity in the denifition of toxicity applied, the measurement methods used, the type of target population, the duration of the treatment and the route of administration, among others. In adults it has been well reported that the toxicity of aminoglycosides is usually associated with maintained high predose serum concentrations (trough concentrations > 5 mg/L, or > 10 mg/L in some cases). No previous data exist about the consequences of maintaining high trough concentrations in newborns. Otherwise, the few available data obtained from controlled studies that have evaluated the incidence of toxicity of aminoglycosides in pediatry suggest that the risk of toxicity in this population is lower than in adults (16(18).

1.1.6.1 Nephrotoxocity

Aminoglycoside-induced nephrotoxicity can be attributed to its uptake by proximal tubular cells and to its long-term retention in the renal cortex (19). Aminoglycosides can gradually accumulate in the lysosomes of these cells and induce morphological changes. After multiple dosing, lysosomes can increase in size and ultimately burst. Above a critical threshold, mitochondrial damage and cell necrosis occur. In most cases, the nephrotoxicity is manifested as non-oliguric acute renal failure, usually reversible, that appears some days after starting the treatment.

Both animal and clinical studies have shown that, at therapeutic doses, amikacin causes less lysosomal overloading than other aminoglycosides, with the exception of netilmycin. Otherwise, the incidence of nephrotoxicity is difficult to be established (5-25%) because it depends not only on the aminoglycoside considered but also on other aspects. One of them is related to the definition of nephrotoxocity taken into account. The most frequently used definition is based on serum creatinine variations; generally, serum creatinine is monitored routinely to detect changes in renal function before the onset of substantial damage, manifested by clinically apparent symptoms. The presence of nephrotoxicity is considered to be appeared when increases of at least 15% to 50% from the baseline serum creatinine concentrations exist. It should be noted, however, that serum creatinine is primarily a reflection of glomerular damage and, as such, is a less than optimal means of monitoring aminoglycoside-induced nephrotoxicity. Moreover, both animal and human studies have shown that nephrotoxicity depends on the time of the day is given (20), being more likely administered at inactivity hours (night) versus active hours (day). Other factors influencing the occurrence of nephrotoxicity are the concentration and the duration of treatment. For these reasons, once daily administrations have been postulated as the best to decrease the risk of nephrotoxocity (21,22). Finally, there are many other factors to consider, such as the type of target population or the simultaneous administration of other potentially nephrotoxic drugs.

No clear relationship between serum concentration of aminoglycosides and presence of nephrotoxicity has been previously established. Nevertheless, high trough concentrations are generally related to its appearance, being an indicator of renal clearance of the drug but not a predictor of nephrotoxicity by themselves (23).

1.1.6.2 Ototoxicity

Aminoglycosides can cause irreversible ototoxicity, which occurs in a dose-dependent and idiosyncratic way (24). The mechanism of selective toxicity of aminoglycosides for auditory cells is only poorly understood, but experimental evidence in animals has pointed reactive oxygen species as possible responsibles of the development of aminoglycoside ototoxicity (25). Otherwise, the idiosyncratic mechanism of this adverse effect has also been linked to genetic predisposition, relating it to an inheritable mutation (A1555G) in the mitochondrial 12S ribosomal RNA (24,26). For this reason, preventive screening of mitochondrial 12S rRNA mutations has been suggested as a way to decrease the incidence of aminoglycoside-induced hearing loss (27,28). The severity of the toxicity, usually bilateral, is higher in prolonged treatments, since repeated dosing with aminoglycosides produce a cumulative lesion (cochlear cells previously destroyed cannot regenerate). On the other hand, the risk of ototoxicity increases with the co-administration of other drugs showing the same kind of potential toxicity (loop diuretics, vancomycin, etc...) (5).

Ototoxicity has two types of expression: auditive alterations (hearing loss), and vestibular symptoms. Auditive alterations result from the destruction of the outer hair cells of the organ of Corti. This auditive damage initially affects to high frequencies, but it can progress until affecting intermedium or low frequencies. Vestibular manifestations (vertigo, nausea, dizziness) comes from the destruction of the hair cells of the semicircular ducts. The kind of toxicity expressed depends on which aminoglycoside is used. Amikacin mainly causes auditive alterations. Similarly to nephrotoxicity, the relationship between serum concentrations of aminoglycosides and presence of ototoxicity has not been well stablished, but high peak levels (> 38.5 mg/L, in the case of amikacin) have been reported to be probable responsibles of this kind of toxicity, when considering the use of conventional dosing regimens.

1.1.6.3 Neuromuscular blockade

In rare situations, aminoglycosides can produce neuromuscular blockade, that can lead to death. It is manifested as a respiratory muscle weakness, flaccid paralysis and midriasis (8). It can occur associated to diseases or drugs that interfere with neuromuscular transmission (carrying botulism, myasthenia gravis or treatment with curaritzants) and being related with quick intravenous perfusions of large amounts of aminoglycosides.

1.1.7 Therapeutic use

Aminoglycosides are primarily indicated for the treatment of infections caused by aerobic gram-negative bacilli (including septicaemia, neonatal sepsis, osteomelitis, septic arthritis, respiratory and urinary tract infections), mainly *Enterobacteries* spp and *Pseudomonas aeruginosa*. Their spectrum activity makes them the best choice against *Enterococcus faecalis* (associated to ampicilin or vancomycin); *Enterococus faecium* (associated to vancomycin and rifampicin), *Pseudomonas aeruginosa* and *Yersinia enterocolitica* (associated to a third generation cephalosporine), *Serratia marcenses*, *Providencia, Hafnia*, *Francisella tularensis* and *Yersinia pestis*, among others. Otherwise, aminoglycosides are usually used in empirical treatments (when the treatment is started before a diagnosis is confirmed). The use of amikacin is generally limited to the treatment of infections caused by gram-negative bacilli resistant to other aminoglycosides (29). Several antibiotic groups have been demonstrated to have synergistic activity with aminoglycosides. This is particularly true for the β-lactam antibiotics, being active for the treatment of endocarditis caused by some gram-positive bacteria, such as *Streptococcus* spp, *Enterococcus* spp and *Staphylococcus* spp (5).

Antimicrobial therapy is widely used during the neonatal period. Specifically, aminoglycosides are very important in cases of sepsis by aerobic gram-negative bacilli, either confirmed or suspected. The main indications of aminoglycosides during neonatal period are:

i. Neonatal sepsis. Referred to the clinical situation derived from the invasion and proliferation of bacteria, fungi or viruses in the bloodstream of the newborn manifested during the first 28 days of life. According to the transmission mechanism, it can be distinguished *sepsis of vertical transmission* (caused by germs located in the maternal genital canal, where the transmission is produced via ascending or by direct contact with contaminated secretions during delivery); and *sepsis of nosocomial transmission*, which is caused by microorganisms located on Neonatal Services that colonize the newborn through medical staff and/or diagnostic material. Amikacin is mainly used in nosocomial infections, where the treatment of choice is based on the association of an antibiotic against Staphylococcus coagulase-negative (vancomycin or teicoplanin) and another against gram-negative bacteria (an aminoglycoside, usually gentamicin or amikacin).

ii. Necrotizing enterocolitis. Of unknown etiology, it is the most frequent and severe digestive pathology during the neonatal period that can results into intestine necrosis. There are some determining factors, such as ischemia, bacterial overgrowth and systemic inflammatory response. Aminoglycoside antibiotics, gentamicin or amikacin, are used as a part of the treatment.

iii. Meningitis. As neonatal sepsis, neonatal meningitis can be classified as one of *vertical transmission* (when clinical manifestations appear before 3 days of life

and early nosocomial infection is discarded), and as *nosocomial meningitis* (when clinical manifestations appear after 72 hours of life and late vertical infection is discarded). In both, the empirical treatment is based on the association of ampicilin, to the double dose used in sepsis, with a third generation cephalosporin. Once the microorganism has been identified, some aminoglycoside is associated. The pathogens related to each one are slightly different; vertical meningitis are usually caused by *Streptococcus haemolytic B*, *E. Coli* and *L. Monocytogenes*, and less frequently by gram-negative bacilli. Contrary, around 45% of the nosocomial meningitis are caused by gram-negative bacilli, mainly by *E. Coli*.

iv. Antibiotic prophylaxis. Aminoglycosides are given intravenously, associated to other antibiotics, as prophylactic treatment before and after many surgical interventions. Among them, gentamicin is the most used.

1.1.8 Dosage

Initially, the dosing regimen of aminoglycosides consisted on divide the total dose to be administered into two equal parts. However, the concept of once-daily dosing was gradually introduced, firstly for the treatment of urinary tract infection (30,31), and later for systemic infections. There is currently a large number of meta-analysis that support the safe use of aminoglycosides in once-daily doses (32-37). All of these meta-analysis have shown either equivalence or superiority for once-daily dosing in clinical and bacteriologic efficacy, and also in nephrotoxicity. None of them have shown differences related to ototoxicity or mortality rates.

The once-daily dosing is widely accepted in adults, but not in the neonatal period. During this period, the existence of reduced values of renal clearance compared to infants has been recognized for many years, and twice daily regimens have consequently become the standard. In the 70s, the first recommendation of amikacin dosing was based on an initial dose of 7.5 or 10 mg/Kg every 12 hours (38,39), which was widely accepted. Later, it was observed that this dosage was not appropriate in all cases, and new protocols arised based on postnatal age (PNA). Cookson et al (40) suggested a dosing regimen of 10 mg/Kg every 12 hours during the first month of life, and every 8 hours from then. Ortherwise, Prober et al (41) proposed the administration of 7.5 mg/Kg every 12 hours during the first month of life. Nevertheless, the use of these recommendations led to a rather large percentage of ineffective concentrations. From then to now, several studies that support the utility and safety of the administration of aminoglycosides as a single daily dose have been published, demonstrating that the conventional dosing regimens are not well adapted to the newborn population, and so resulting in serum concentrations not always in agreement with the expected, particularly in very low birth weight individuals (42). In the particular case of amikacin, there are currently several specific studies that support it (35,43,44).

Table 1.2 summarizes some of the several guidelines for amikacin dosing in neonates. Most of them propose the use of once-daily dosing, and in detriment of it, dosing intervals higher than 24 hours. Otherwise, the current product sheet (45) does not recommend it, establishing guidelines similar to those of 70s. Its recommendations are, for the first 2 weeks of life: a dose of 7.5 mg/Kg every 12 hours for premature newborns; for full-term neonates, the same regimen but adding an initial dose of 10 mg/Kg. From 2 weeks of life, dosing recommendation is of 7.5 mg/Kg every 12 hours or 5 mg/Kg every 8 hours.

Table 1.2. Guidelines for amikacin dosing in neoantes

Neofax (29)

GA (weeks)	PNA (days)	Dose (mg/Kg)	Interval (hours)
≤ 29*	0-7	18	48
	8-28	15	36
	≥ 29	15	24
30-34	0-7	18	36
	≥ 8	15	24
≥ 35	Tots	15	24

** or significant asphixia, PDA or treatment with indometacine*

Spanish Association of Pediatrics (AEP) (46)

WEIGHT (g)	PNA (days)	Dose (mg/Kg)	Interval (hours)
<1200	≤ 28	7.5	12
1200-2000	0-7	7.5	12
	>7	7.5	8
>2000	0-7	10	12
	>7	10	8

Sherwin et al (47)

GA (weeks)	PNA (days)	Dose (mg/Kg)	Interval (hours)
<29	≤9	15	36
29-36		14	24
>36		15	24

Allegaert et al (48)

GA (weeks)	PNA (days)	Dose (mg/Kg)	Interval (hours)
<28	<29	20	42
28-30		20	36
31-33		18.5	30
34-37		17	30
>37		15.5	24

GA: gestational age; PNA: postnatal age; PDA: patent arterial duct

1.1.9 Therapeutic monitoring of aminoglycosides

Conventionally, aminoglycosides have been monitored during therapy in an effort to reduce the risk of toxicity, being particularly useful when the treatment is extended more than 48 hours. This process requires two blood samples. One, extracted 60 minutes after the start of the intravenous administration that is called maximum or peak concentration (Cpeak), but not always corresponds with the highest drug concentration. The other, extracted just before the administration of the next dose, corresponds to the minimum or trough concentration (Cthrough).

Studies in adults suggest that, using conventional dosing schedules, amikacin toxicity is minimal with peak concentrations below 38.5 mg/L and trough concentrations lower than 5 mg/L (in some cases, trough concentrations below 10 mg/L) (49). Therefore, peak concentrations between 20 and 30 mg/L and trough concentrations below than 5 mg/L are intended to be reached. Recently, Shwervin et al (47) revised the target concentrations in neonates using the results of the PD analysis, and adjusting the optimum target for Cpeak from 20–30 mg/L to 24-35 mg/L. But there is not enough data in pediatrics, so the adult threshold is used. Using the once-daily dosing, peak concentrations achieved are generally higher, and, although therapeutic ranges have not been defined, it is recommended to be around 8 to 10 times the MIC value.

Three groups of monitoring methods have been developed:

i. Single-level methods. This type of monitoring involves the determination of a single concentration value in the elimination phase, usually between 6 and 14 hours after the end of the infusion. The first of the single-level methods developed was the Hartfort method (50). A variation of this method was developed at the Barnes-Jewish Hospital in St. Louis. A third single-method, promulgated in Australia, was based on the administration of a varying dose depending on age. All of the single-dose methods assume that patients show normal distribution volumes, although this is not always true. Moreover, because peaks are not measured, reduced peaks are not recognized. Nevertheless, these methods are simple to apply and less costly.

ii. Area-under-the curve (AUC) methods. Two methods have been developed (Christchurch and Aladdin methods), in which two concentrations are measured and the AUC estimated by means of a simplified monoexponential model.

iii. Bayesian methods. Bayesian methods applied to aminoglycoside monitoring have been used for a long time (51,52). These methods usually use population pharmacokinetic values to generate prior probabilities, and then, from one or two measured levels, perform dosage tailoring adjustment. The more used computer programs for this purpose are ABBOTTBASE and SeBA-GEN.

1.2 PHARMACOKINETICS IN NEONATES

The adequate use of drugs supposes not only the knowledge of the drug but also of the individual to whom it will be administered, as well as the factors and conditions that can modify the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics. One of these factors is age. Consistent definitions are required to describe the length of gestation and age in neonates and to compare data. The terms “gestational age” (GA), “postmenstrual age” (PMA), “corrected age” and “postconceptional age” (PCA) have frequently been defined unconventionally (53*)*, or left undefined. For this reason, a standard terminology is required (54) (Figure 1.2):

- “Gestational age” (or “menstrual age”) is the time elapsed between the first day of the last normal menstrual period and the day of delivery. Gestational age is conventionally expressed as completed weeks.
- “Postnatal age” is the time elapsed after birth. It is usually described in days, weeks, months, and/or years.
- “Postmenstrual age” is the time elapsed between the first day of the last menstrual period and birth (gestational age) plus the time elapsed after birth (postnatal age). Postmenstrual age is usually described as the number of weeks and is most frequently applied during the perinatal period beginning after the day of birth.
- “Conceptional age” is the time elapsed between the day of conception and the day of delivery.

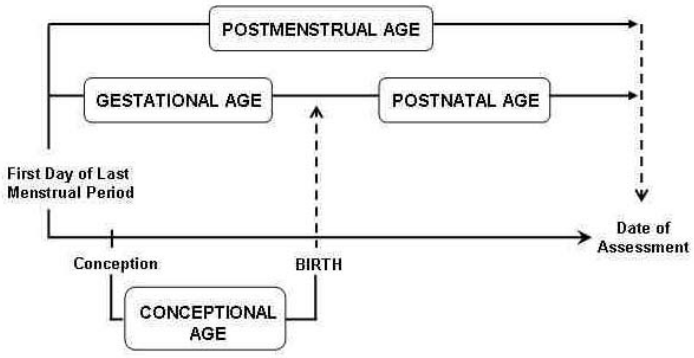


Figure 1.2. Standard terminology for neonate ages (*Modified from Engle WA (53)*

Pharmacokinetic characteristics in pediatrics are greatly different from adult and even among the childhood (neonates versus infancy and children). The first year of age is associated with major changes in processes affecting the absorption, distribution, metabolism and excretion of drugs. The rate of development of these processes is maximum from birth to one month of age but changes also occur rapidly during infancy

(1 month to 1 year), as well as in children (1 year through to puberty). During postnatal age, the maturation process takes place at several rates and patterns, giving a great pharmacokinetic variability within neonates as a reflex of the immaturity of various organs involved in drug disposition. So that, nowadays is widely accepted that the newborn infant cannot be considered as a "small adult". Maturation of pharmacokinetic processes occurs gradually until equalling those of adults, early (gastric emptying and tubular secretion maturation take place during the first 6 months of life) or later (compartmental volumes and metabolic activity achieved total maturity around 15 years of life). Table 1.3 summarizes some of the most important differences of pharmacokinetics between the preterm and born at term neonates compared to adult, and the derived pharmacokinetic consequences.

Table 1.3. Differences of pharmacokinetics between newborns and adults and derived pharmacokinetic consequences.

Pharmacokinetic parameter	**Age group**		**Pharmacokinetic consequence**	**Examples**
	Preterm neonate	**At term neonate**		
Absorption	↓	↔	↓ AUC	Penicillins, sulfonamides
Distribution				
Body water	↑↑↑	↑↑	↓ Cmax of water-soluble drugs	Gentamicin, digoxin
Body fat	↓↓	↓	Minimal clinical effect	-
Metabolism				
Hidroxilation	↓↓↓	↓↓	↓ Clearance	Diazepam
N-demethylation	↓↓↓	↓		Theophiline, caffein
Acetilation	↓	↓		Sulfonamides
Glucuronidation	↓↓	↓		Chloramphenicol
Renal excretion				
Glomerular filtration	↓↓	↓	↑ AUC ↑ $t_{1/2}$	Gentamicin
Tubular secretion	↓↓	↓	↑ AUC ↑ $t_{1/2}$	Gentamicin

↑: increased; ↓: decreased; ↔: unaltered.
Source: George W. Rylance (55)

Systemic action of a drug depends on its concentration, that is related to pharmacokinetic processes. For this reason, the knowledge of pharmacokinetic characteristics and of all potential physiological and pathological modifiers is of great importance in order to optimize pharmacological treatments. In addition to the physiologic complexity during the first days of life, there are several factors that contribute to the lack of knowledge in pediatric clinical pharmacology. First of all, the ethical-legal considerations related to pediatric studies (being sometimes a great barrier to do them). Secondly, the lack of micromethods suitable for drug assay in small serum and plasma samples. And finally, the refusal to accept routine drug monitoring as an approach capable of generating scientific and valuable data (54).

1.2.1 Drug absorption

The gastro-intestinal absorption of drugs is mainly done by passive difusion. It depends on several factors, changing from individual to individual, but mainly on pH and gastric emptying time. Both factors affect the solubility and the degree of ionization of drugs, as well as gastro-intestinal motiliy (56). Moreover, these factors are markedly different between neonates and those present in older children and adults.

At birth, gastric pH is usually neutral (between ph 6 and 8) because of the presence of amniotic fluid. But it transitionally falls within the first 24 hours to values of 1 to 3. Subsequently, until day 8 to 10 of life there is practically no acid secretion, with a condition of more or less relative achlorhydria. From this moment, there is a gradual acidification that equals pH to adult values around 3 years of life, coinciding with the maturation of the acid secretion mechanisms (54). Gastric pH differences affect the absorption of several drugs, usually producing an increase of the absorption of basic, and a decrease of those with weak acid nature. Since most orally administered drugs are absorbed in the small intestine, the rate of gastric emptying is an important determinant of the rate and extent of drug absorption. The rate of gastric emptying decreases from birth until equalling those of adults, approximately around 6 to 8 months of life, depending on several factors, as gestational age (it is greater in preterm than in neonates borned at term) (57).

Also irregular and unpredictable gastrointestinal motility explain the slower absorption of neonates compared with older infants. Table 1.4 displays some drugs showing modified or unchanged bioavailability in neoates compared with adult.

Table 1.4. Bioavailability of some drugs during neonatal period compared to adult.

Decreased absorption	Unchanged absorption	Increased absorption
Nalidixic acid	Co-trimoxazole	Amoxicillin
Phenytoin	Diazepam	Ampicillin
Phenobarbital	Digoxin	Penicillin G
Paracetamol	Eritromicin	
Rifampicin	Phenilbutazone	
	Sulfonamides	

Absorption following intramuscular administration depends mainly on the regional blood flow. So, situations with a low peripheric perfusion, such as in low heart rate or in the distress respiratory syndrome, can compromise the absorption if using this route of administration. In the neonate, the intramuscular absorption pattern may considerably change during the first 2 weeks of extrauterine life. But there are some cases (aminoglycosides, ampicillin and carbenicillin), in which the time to achieve maximum concentrations after intramuscular administration is comparable among neonates, pediatrics and adults (58). Percutaneous absorption, inversely related to the thickness

of the stratum corneum and directly related to skin hydration, is clearly increased in neonates. So that, chemical drugs applied to the skin of a premature infant may result in an inadvertent poisoning.

1.2.2 Drug distribution

Distribution of drugs is mainly affected by the quantitative and qualitative availability of plasma proteins and by the relative size of body compartments. All these factors are clearly different among neonates, pediatrics and adults (59).

Plasma protein binding of drugs depends on the amount of proteins, the affinity constant of the drug for them and the presence of patho-physiological conditions and/or compounds capable of modifying the drug-protein interaction. In the newborn, the concurrence of several factors leads to a decreased plasma protein binding compared to older children and adults (54): (i) a lower plasma protein concentration, not being equal to adult until 10-12 months of life, (ii) a qualitatively different albumin (fetal albumin shows a lower affinity for drugs), and (iii) endogenous competing substrates, such as bilirrubin and free fatty acids, that compete for protein union. Reduced protein binding will change the relationship between total and free (presumably active) amikacin concentrations, and will also result in amikacin being distributed more widely through the body, increasing the apparent volume of distribution of the it. Table 1.5 shows some examples of drugs with different distribution volumes between neonates and adults as a result of the different protein binding.

Table 1.5. Distribution parameters of some drugs in neonates and adults.

Drug	% Protein binding		Vd (L/Kg)	
	Neonates	Adults	Neonates	Adults
Gentamicin	<10	<10	0.77-1.62	0.30-0.67
Theophylline	32-48	53-65	0.20-2.80	0.44-0.50
Diazepam	84	94-98	1.40-1.82	2.20-2.60
Phenytoin	75-84	89-92	1.20-1.40	0.60-0.67

** Source:Rebecca L Milsap, William J. Jusko* (59*)*

The distribution volume of drugs is also influenced by body composition, which depends on both gestational and postnatal ages (Table 1.6). In preterm, total body water comprises nearly 92% of bodyweight, with the extracellular fluid volume accounting for 65% of bodyweight. Increasing postnatal age, percentage of total body water decreases and body fat and intracellular volume increases, at the same time. Water-soluble drugs, mainly distributed to the water compartment, will be the most affected, showing distribution volumes in the neonate higher than in adult. That is the case of aminoglycosides, whose differences in the distribution volume is due to only differences in body composition, as their protein binding is really low (60).

Table 1.6. Body composition at different ages

	Body water (%)			Body fat (%)
	Extracellular fluid	Intracellular fluid	Total body water	
Preterm	65	25	92	<1%
Neonate	45	35	75	15%
Adult	20	40	60	30%

Because of the expanded apparent volume of distribution in neonates, for a given dose, maximum concentrations will be lower than in adults.

1.2.3 Metabolism

During the neonatal period, both hepatic enzymatic and blood esterase activities are decreased (57). Enzymatic microsomal system is already present at birth, but its activity is quite lower than that of older children and adults. Therefore, at birth a maturation process begins, which increases with both gestational and postnatal ages, being slower in preterms than in born at term (61). Phase I metabolism processes (hydroxylation, deacetylation and oxidation) develop quickly, equalling to that of adult around the 6 months of life. Phase II reactions (conjugation) are usually reduced or do not exist, as gluco-conjugation reactions, exclusives of adults. During the neonatal period, only glycine or sulphate conjugations are present. This is why theophylline is only metabolized to caffeine in neonates (62). The immediate consequence of the hepatic metabolism immaturity is a more prolonged elimination half-life value. Several mechanisms to compensate the decreased enzymatic function are possible, either general, as the relative higher size of the liver and the higher hepatic flux compared with adult, or specifics, such as the case of phenobarbital, whose decreased conjugation is compensated by the elimination of the inaltered drug and its conjugated metabolite by urine. For acetaminophen, a sulphuric conjugation occurs as an effort to compensate the lack of conjugation with glucuronic acid (63).

Blood esterases activity is also decreased, mainly in preterm, in which activity levels similars to those of born at term are reached around 10 or 12 months of postnatal age. The decrease of blood esterases activity, joint to the characteristic low distribution volume of the anesthesics in neonates, could be the reason of the prolonged effect observed in this drugs at birth.

Other functions also decreased during the neonatal period can affect the disposition of some drugs. The incomplete development of biliar function can modify the disposition of those drugs undergoing gluco-conjugation and enterohepatic circulation. Pancreatic enzyme activity can also be incomplete at birth, being lower in premature than in full term neonates, but at 1 week of postnatal age, is greater in preterm than in full term neonates (64).

Prolonged elimination half-life values in neonates involve either lower doses or wider dosage intervals to achieve drug concentrations within the therapeutic range. Table 1.7 summarizes the differences in the elimination half-life values of some drugs between neonates and adults.

Table 1.7. Comparative elimination half-life values of some drugs during neonatal period and adulthood

Drug	Elimination half-life (hours)	
	Neonates	Adults
Diazepam	25-100	15-25
Digoxin	60-107	30-60
Phenytoine	30-60	12-18
Phenobarbital	100-500	64-140
Paracetamol	2.2-5	1.9-2.2

**Source: P.L. Morselli* (54)

1.2.4 Renal excretion

At birth, renal function is greatly reduced, both glomerular filtration (approximately around 30-40% of that in the adult) and tubular secretion (around 30-40% of that in the adult). The maturation of these processes of renal function takes place at different rates and according to gestacional and postnatal ages, achieving adult values around 6-12 months of life. The drug filtration process depends mainly on (i) the drug protein binding, (ii) the renal blood flow and (iii) the area and filter characteristics (glomerular membrane). Renal blood flow increases with age as a result of an increase in cardiac output and a reduction in peripheral vascular resistance. Neonate kidney can only get around 5-6% of the cardiac output, compared with the 15-20% of the adult. Renal plasma flow averages 12 mL/min at birth and increases to 140 mL/min by one year of age. The most important consequences of the maturation of this process affects to those drugs that are mainly eliminated by glomerular filtration, such as aminoglycosides.

At birth, in the full-term newborn, the postnatal renal maturation involves only elongation and maturation of tubules, while in the premature there is also a deficiency in glomerullus (65,66). Tubular function is also influenced by several factors, such as the reduced capacity to concentrate urine, the lack of a diurnal circadian rhythm and the low urinary pH (it can affect mainly the excretion of acid compounds, giving an increase of the re-absorption rate).

The clinical consequences of renal maturation become apparent when considering drugs that are mainly eliminated unchanged through the kidneys (penicillins, cephalosporines, digoxin, aminoglycosides…). In those cases, the high elimination half-life in neonates will be a potential toxicity risk in this population. Among neonate

population, in order to achieve the same concentrations at steady state, preterms should have lengthened dosing intervals or decreased individual doses, compared with full term.

1.2.5 Physio-pathological factors on neonatal pharmacokinetics

The design of dosage regimens in neonates leads to consider the most important physiological factors, such as age or weight, affecting drug pharmacokinetics. Other common pathological conditions should also be taken into account. Table 1.8. summarizes the most frequent pathological conditions and the corresponding pharmacokinetic processes affected.

Table 1.8. Pathological conditions in neonates affecting pharmacokinetic processes.

Pathology	**Phisiological change**	**Pharmacokinetic alteration**
Gastroenteritis Inflammatory bowel disease	Increase of intestinal motility	↓ Absorption
Intestinal obstruction	Delay on gastric empty	Delay on absorption
Hepatic cholestasi Biliar obstruction	↓ Biliary salts excretion	↓ Absorption of water-soluble compounds
Hyperbilirrubinemia	Displacement of binding plasma proteins	↑ Free fraction
Respiratory distress syndrome Ductus arteriosus Cardiac insufficiency Perinatal asphyxia Septic shock	↓ Splenic area perfusion ↓ Cerebral perfusion ↓ Hepatic perfusion	↓ Oral and im BDP* ↓ Tissular distribution and cross of BBB* ↓ Enzymatic microsome activity
Intrauterine growth retardation	↓ Albumin concentration Delay on gastric empty ↓ Intestinal area surface	↑ Free fraction ↓ Absorption
Necrotizing enterocolitis Respiratory distress syndrome Gastroenteritis	↓ Binding plasma protein ↑ Tissular distribution ↑ No-ionized fraction for weak-acids	↑ Volume of distribution
Edema	↑ Intracellular water Delay on gastric empty	↑ Volume of distribution ↓ Absorption

**BDP: bioavailability; BBB: blood brain barrier*

The most influencing factors of the absorption process are the rate of gastric emptying, intestinal motility and blood flow in splenic area. Several clinical situations can affect this process: diarrhoea (gastroenteritis or inflammatory bowel disease) by an increase of gastro-intestinal transit, malnutrition by a delay on gastric emptying and a decrease of the intestinal area surface, and edemas by a delay on gastric emptying (57). Pathologies related to biliar obstruction can also cause a reduction of the absorption of the drugs undergoing enterohepatic recirculation. When the obstruction goes with hyperbilirrubinemia, the high affinity of bilirrubin for serum albumin can either be displaced by several drugs, resulting in kernicterus, or even bilirrubin can displace other drugs from their binding site, such as phenytoine and phenobarbital.

The distribution volume can also be modified by several factors as the acid environment caused by some conditions (necrotizing enterocolitis, respiratory distress syndrome) and the edema (shock, hypoxia, renal diseases, cardiac insufficiency). The edema can cause an increase of the volume of distribution of water-soluble drugs by increasing extracellular volume. Conditions involving hypoxia not only affect the volume of distribution but also can produce a decrease of the renal excretion of some drugs due to a lack of control of the filtration process. There are two common situations on the neonate that results in hypoxia: respiratory distress syndrome and meconium aspiration syndrome.

Renal clearance can also be modified by several pathological conditions, such as shock and sepsis, which cause a decrease of the cardiac output by decreasing systemic venous return, and consequently the blood renal, hepatic and visceral flow. It will be important mainly for those drugs whose clearance depends on the blood renal flow, such as vancomycin, digoxin, beta-lactam antibiotics and aminoglycosides. Finally, all pathologies than can modify renal hemodynamics can also delay renal maturation or decrease its functional capacity, resulting in a decrease of the clearance of drugs eliminated through the kidneys.

1.3 PHARMACOMETRICS

The term of pharmacometrics first appeared in the literature in 1982 in the Journal of Pharmacokinetics and Biopharmaceutics (67). Since this time, the importance of pharmacometrics in optimizing pharmacotherapy and drug development has been recognized, and increasing rate of literature focused on this field has been observed. Pharmacometrics is therefore the science of developing and applying mathematical and statistical methods to (i) caractherize, understand and predict a drug's pharmacokinetic and phamacodynamic behaviour; (ii) quantify uncertainty of information about that behaviour; and (iii) rationalize data-driven decision making in the drug development process and pharmacotherapy. In effect, pharmacometrics is the science of quantitative pharmacology (68). Due to the current importance of this field, guidelines for industry have been published by the FDA (1999) (69) and by the EMEA (2007) (70).

Pharmacometrics begins with pharmacokinetics (PK), which can be defined as the study of various biological processes affecting the rate of disposition of the drug in the body: dissolution, absorption, distribution, metabolism and elimination. The counterpart to pharmacokinetics is pharmacodynamics (PD), which in short is the study of the biological effects induced by drugs on the body. PK/PD modelling provides the integration of PK and PD models to obtain a good understanding of the dose-exposure-response relationship.

Every drug has a so-called "therapeutic window" (Figure 1.3). It means that for every drug there is a certain range of concentrations that promise successful treatment, while at the same time have a sufficiently low chance for adverse events. The probability of treatment failure is acceptable large with concentrations below and upper the "therapeutic window". This concentration range exists for the majority of drugs, being quite wide for some and extremely narrow for others. It is in particular for the ones with the narrow window that pharmacokinetic monitoring is of benefit.

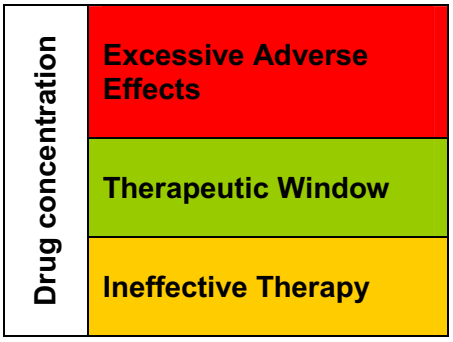
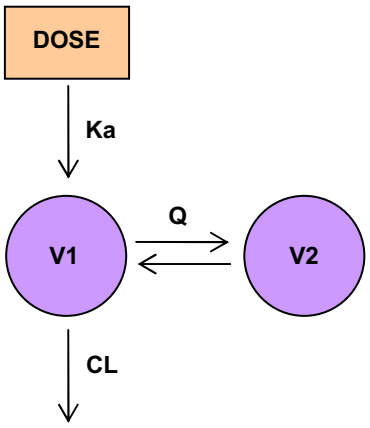


Figure 1.3. Concentration ranges

To achieve drug concentrations within the “therapeutic range”, there are prestablished dose regimens for almost all drugs, but even in these cases, monitoring drug concentrations sometimes is required to optimize efficacy and reduce the toxicity of the treatment.

In order to be applied to clinical practice, pharmacokinetics requires the use of models that describe the evolution of serum drug concentrations over time, from which it can be estimated the pharmacokinetic parameters that characterize the drug behaviour in the individual and allow to predict serum concentrations that will be achieved after the administration of a given dose. Organisms are complex systems in which it is difficult to establish quantitative relashionships between dose, route of administration and drug concentration in different body regions as a function of time. For the description of the temporal evolution of drug levels, mathematical models are used. Among all the models, compartmental are the most used in pharmacokinetics (71). A compartment represents a fraction of biological material in which the drug is supposed to be uniformly distributed and has the same kinetic properties. Obviously, the body contains no such distinct compartments, but through this approach drug plasma concentrations can be predicted satisfactorily. The body is a very complex system and could theorically be divided into a large number of compartments. However, this would require a complex mathematical treatment and a large number of observations. For this reason, in practice it is used a simplification where the body is considered to be constituted by the minimum number of compartments with which is possible to describe the kinetics of the drug (one-compartmental, two-compartmental or a maximum of three compartments). Figure 1.4 shows the schematic of a two-compartment model for a drug which is absorbed into the central compartment. From there, the drug can be either eliminated or distributed to the peripheral compartment. There is also transport back from the peripheral compartment and into the central one once distribution has started out.

Given the complexity of the human body, usually it is not feasible to measure the concentrations of drugs or metabolites at every location, or compartment, in the body and so, to achieve the concentrations of the active drug at the site of action. In most cases, one only has plasma-samples to work with, and has to extrapolate concentrations in other locations, correlating this concentration with the success or failure of treatment. The integration of pharmacokinetic and physiopatological data of a specific population of interest allows to obtain a more rational and effective individualized therapeutic strategy (72).



Ka: absortion constant
V1: distribution volume into central compartment
V2: distribution volume into peripheral compartment
Q: inter-compartment distribution
CL: total clearance

Figure 1.4. Schematic of a two-compartment model

The estimation of pharmacokinetic parameters can be considered from two approximations. Initially, pharmacokinetic studies were designed to obtain the maximum information about the disposition of the drug in an individual, without considering the associated interindividual variability of the pharmacokinetic parameters. It was the *individual pharmacokinetics.* Later, the attention was focused into the estimation of the population pharmacokinetic parameters in order to define the typical kinetic behaviour of a drug in a target population, but also the interindividual variability associated. It was the so called *population pharmacokinetics,* whose is the individual dosing considering all physiological and patological factors that explains part of the associated variability (73).

1.3.1 Individual pharmacokinetics

This approach allows to study the kinetic of a drug in a particular individual, not focusing on the intersubject variability of the pharmacokinetic parameters in the target population. The estimation of the individual pharmacokinetic parameters is performed after fitting given mathematical models to the concentration vs time data. The availability of the individual pharmacokinetic parameters allows to calculate appropriate dose regimens for each subject. Several methods can be applied to study individual pharmacokinetics.

1.3.1.1 Linear Regression

The linear regression analysis allows to fit a linear regression equation to the drug concentration vs time data in order to be used for concentration predictions. In pharmacokinetics, the relationship between variables is generally not linear, so the application of this analysis requires the prior linealization of the function (logarithmic transformation). From transformed plasma concentrations, it is calculated the straight

that minimizes the sum of squares (SS) of the differences between the observed and the predicted concentrations by the regression line (1.1).

$$ss = \sum_{i=1}^{n} [Y_i - f(X_i)]^2 \quad (1.1)$$

where Y_i refers to the experimental values of dependent variable and $f(X_i)$ the predicted values by the selected equation.

The linear regression is a simple and quick technique with several advantages: (i) the reduced number of blood samples required to estimate the pharmacokinetic parameters, (ii) the ease of implementation (it does not require any computer), and (iii) the greater accuracy of the predictor algorithms. However, it has some drawbacks: (i) it does not allow to use all available information of an individual. It uses only the concentrations values from a dose interval, discarding all previous information of the individual. For this reason, the estimation of the pharmacokinetic parameters depends on the clinical situation in which it is the individual at this time (renal function, hydration status, weight, etc…), and so, the results obtained are less consistent along the time and with a limited posterior predictive value for future plasma levels; (ii) the error associated to the previous linearization of the functions (not possible to do for variances or its associated errors), causing errors in the estimation of parameters, and (iii) it ignores any available prior population information about the pharmacokinetics of the drug (74).

1.3.1.2 Non-linear Regression

The non-linear methods do not require previous linearization of data. The estimation of model parameters is done by the use of iterative algorithms that start from an initial value (initial estimates) and search for the combination of these values that minimizes a previously defined objective function. The most used iterative algorithms are the direct search (Simple, Nelder-Mead) and the gradient search (Steepest Descent, Mardkart, Gauss-Newton).

Among the large number of non-linear regression methods, Ordinary Least Squares and Expanded Least Squares are the most importants. The latter is the most widely used in clinic, and the function to be minimized is (1.2):

$$ss = \sum_{i=1}^{n} W_i [C_i, t - f(P_m, t)]^2 \quad (1.2)$$

where *n* is the number of serum concentrations, *Ci* is the drug concentration at time *t*, P_m are the parameters to be estimated, $f(P_m,t)$ is the equation of the model to fit the data, and W_i is a statistical weighting factor. The weighting factor into the dependent variable indicates that not all the experimental data either have the same degree of reliability or is not affected by the same degree of error (it depends on the variance of the experimental data).

In general, non-linear regression methods provide good pharmacokinetic estimations (eliminate the errors associated with linearization). There are several advantages associated to the use of these methods: (i) they allow to use all available information (old data) and properly weighted data, (ii) they also allow model updating adjustments by introducing some clinical descriptors based on physiological and clinical characteristics of the individual that can vary along the treatment (i.e. weight, surface area, creatinine clearance), and (iii) they do not require concentrations in steady state to be applied (75). The most important limitation of this approach is the low reliability and accuracy of the obtained parameters when the number of concentration-time data is low, a situation very common in the clinical practice. Some other drawbacks are (i) its required, at least, the same number of serum concentration levels as the PK parameters being estimated, being of little use at the beginning of the treatment, (ii) for its use they require a computer and enough experience, and (iii) they ignore the possible knowledge of the population pharmacokinetic behaviour of the drug.

Gentamicin and amikacin pharmacokinetic parameters in the newborn have been obtained and compared (76) by non-linear regression using program MULTI2(BAYES) (77).

1.3.1.3 Bayesian methods

In clinical pharmacokinetics is common to have a very limited number of concentration-time data, resulting in a lost of precision of the parameters determined individually. It can lead to the estimation of individual parameters far from the parameters of the population with similar characteristics to the monitored patient. In order to solve what represents the major limitation of the non-linear regression methods, an alternative method based on Thomas Bayes (1702-1761) theory (78) was introduced. This theory allows to calculate the likelihood of an event based on the initial probability and the contribution of new data.

Unlike methods described above, Bayesian method allows to incorporate into the model not only experimental information (concentration-time data) obtained for a given individual, but also priori known information about the pharmacokinetic behavior of the drug in a population with similar characteristics to those of the individual (population information). So, the application of Bayes theory allows, knowing the probability that an

even occurs, modify its value when we have new information. It describes the quantitative relationship between the *a priori* probability that the individual has a defined PK parameters before knowing its plasma concentration and the resulting *a posteriori* probability to obtain similar parameters once analytical results are known.

In this method, the objective function to minimize, using non-linear regression is (1.3):

$$SS = \sum_{j=1}^{p} \frac{\left(P_j^* - P_i\right)^2}{\sigma^2{}_{Pj}} + \sum_{i=1}^{n} \frac{\left(C_i, t - f\left(P_m, t\right)^2\right)}{\sigma_{ci}^2} \qquad (1.3)$$

where, P^*_j and P_i are the mean population parameters and the estimated for the individual, $\sigma^2 P_j$ the interindividual variance of P^*_j, $C_i t$ the observed drug concentration at time t, $f(P_m,t)$ the predicted concentration at time t, and $\sigma^2 C_i$ the variance of C_i (analytical error and interindividual variance). This function can be divided into two parts, one relative to the individual information, and the other to the population. Increasing individual information (greater number of serum concentrations), the individual contribution to the objective function increases, and the solution is close to that obtained by non-linear regression. However, at the begining of the treatment there are little serum concentrations of the individual, so the population term contribution is higher than the individual. In this case, the schedule dosing is based, mainly, on the population information.

Bayesian method has some advantages: (i) the information required is minimum (only n≥1 during the monitorization, but being able to predict the first optimal dose without any drug concentration data), what is of great utility in therapeutic drug monitoring, where usually there is limited available information, (ii) having a good population model, the predicitive function of Bayesian method is higher than the convencional methods (74). The most important disadvantage related to Bayesian methodology is the need to have prior good information of the pharmacokinetic parameters. The suitability of the prior population information influence on the fiability and predictive capacity of the bayesian methods, particularly when the individual information is limited.

1.3.2 Population pharmacokinetics

Population pharmacokinetics can be defined as the study of variability in plasma drug concentrations in a population representative of the individual of interest. This type of study focuses on certain demographical, pathophysiological, physiological, therapeutical, and other kinds of features that vary between individuals, and that are known to possibly be responsibles of some of the differences in the achieved drug concentrations (i.e, obese patients have an increased body-mass, going to a higher

distribution volume. A given amount of drug will therefore result in a lower apparent concentration than in a patient with normal weight).

Unlike traditional pharmacokinetic studies, population pharmacokinetics is greatly concerned about the identification and measurement of the sources of variability responsibles of some of the differences in drug concentrations. Taking it into account, the dose given to each patient could be modified ensuring optimal therapeutic concentration (68). Generally, variability is divided into interindividual and residual variability. The interindividual variability is a biological imperative, and stems from the simple fact that every person is biologically different from practically all others. This leads in most cases to variations in plasma drug concentrations which can be quite large. Residual variability is a combination of sources of variation, such as intraindividual differences, interoccasional differences, and errors made in measurement, dosing and modelling. Although population pharmacokinetics try to explain and measure most of all the variabilities, there will almost always be a remaining unexplained variability, both interindividually and intraindividually. This may be because of time-dependent pharmacological variations within each patient, errors during sampling, or possibly other unforeseen events. It is important for the optimal treatment of patients to have a sense of understanding for how these unexplained differences behave, and the magnitude of them.

A great advantage of population pharmacokinetics is that it allows to gain quite extensive and integrated information on pharmacokinetics from sparse data, being also usable on dense data, and even mixed sparse/dense data. This makes it possible to analyze and gain information from studies of unbalanced design, and also some that would otherwise have been excluded because they do not normally lend themselves to pharmacokinetic analysis.

The establishment of a good population model is really important not only to apply Bayesian method effectively, and then to be able to predict individuals serum concentrations, but also to create dose regimens when there is not previous information about individual pharmacokinetic parameters (72). The use of population approach has been a major development in pharmacometrics. A brief summary of the different population methodologies is given in the following sections.

1.3.2.1 Naïve pooled data approach

This method, together with Naïve Averagin Data, belongs to the group of *"Simple or Naïve Methods"*. As the name implies, the Naïve Pooled Data (NPD) is a method that treat all the data from the different patients as if it came from a single patient.

The main advantages associated to this method are that, (i) it is easy to use and requires only little computational power, and (ii) it can be used on a variety of data, from experimental to routine pharmacokinetic data. But there are also some drawbacks that lie mainly in the simplification process: (i) by pooling, there is a lost of detailed information of each subject and of variation between individuals. So that, this can lead to the mistake of believe that the data is neat and simple, or also possible trends in individual curves, smothered in the multitude, can become unrecognizable. For this reason, the method works specially well when there are low variations between individuals, what is rarely in the case of humans, so that limiting its usefulness; (ii) although this approach is interesting because of its simplicity, it performs poorly in terms of parameter estimation, and (iii) also sources of variability are confused because no separation of interindividual and residual variability is possible.

The utility of this method to the clinical practice is really limited, because dose adjustment is done without taking into account physiological and clinical changes that may be occur in an individual.

1.3.2.2 The two-stage approach

The two-stage approach is a traditional pharmacokinetic analysis, designed to be used in the data-rich environment. As the name indicates, mean population parameters are obtained through two stages. The first part entails using nonlinear regression to estimate individual pharmacokinetic parameters from the concentration-time data gathered. These estimates are then used in the second step to calculate statistics such as mean parameter estimates, variance and covariance of these parameters estimates.

Whilst this approach allows the separation of interindividual and residual variability, it tends to over-estimate either interindividual or residual variabilities. Besides, it requires that data are rich enough so that all parameters can be estimated for each individual. So, being a method easy to use, it gives good parameters estimations of the population when it is done with a large number of individuals and with rich data (79).

1.3.2.3 Nonlinear mixed-effects modelling

In the mixed-effects modeling, data from all individuals are used to simultaneously estimate the typical population parameters and the variability (interindividual and residual) associated. Even so, during this process the individual is not lost in the masses, and it is possible to make predictions regarding individual patients through the estimates of population parameters and their variability (and not least the covariates that influence these parameters) (80).

This approach is based on the principle that individual pharmacokinetic parameters of a population came from the distribution of this parameter. This distribution can be described by the mean parameter population and its interindividual variance. So, each individual pharmacokinetic parameter can be expressed as mean population plus a typical deviation for this individual. This deviation is the difference between the parameter population mean and the individual parameter.

The term “mixed-effects” comes from the fact that a model built this way will contain a fixed structure and a randomness block (81):

- The **fixed structure**, quantify mean pharmacokinetic parameters values of a drug in a population, and the relation among pharmacokinetic parameters.
- The **randomness block**, quantify the magnitude of interindividual and intraindividual (residual) variability.

The use of nonlinear mixed-effects modeling offers some advantages: (i) sparse or rich data can be analysed, (ii) data do not have to be balanced, (iii) samples do not need to be taken at the same time for all subjects, and (iv) rich and sparse data can be analysed simultaneously.

The use of nonlinear mixed-effects modelling on population pharmacokinetics makes possible to design less extensive studies than what is needed for a two-stage approach, and still gives valid results. These designs are less restrictive to the patients, which make it easier both for the participants and the conductors. Mixed-effects modelling is currently the method of choice for analysing pharmacokinetic data arising from clinical studies.

Nonlinear mixed-effects modelling can be performed by two approximations:

i. *Parametric:* It assumes that the pharmacokinetic parameters of the studied population belong to a known distribution (normal or log- normal), characterized by a mean and its dispersion measures. The most used computer program for data treatment is NONMEM (“Nonlinear Mixed-Effects Model”) (82).

ii. *Non-parametric:* This approach arises from the impossibility to characterize adequately the population parameters of samples with non-parametric distributions. It is based on the probability (density function) that some of the studied parameters explain the process to be studied (80). There are two approximations: Nonparametric maximum likelihood method (NPML) (83) and Nonparametric expectation maximisation method (NPEM) (84). Both have the inappropriate that do not allow to separate the sources of inter- and intraindividual variability, and also that it is not possible to quantify the confidence limits of the distributions obtained.

During the last decades several softwares that implements the non-linear mixed-effects modelling (WinNonmix (85), NONMEM (82), Monolix (86)) have been developed. NONMEM was the first such program that stands for nonlinear mixed-effects modelling. This program allows to analyze large quantities of pharmacokinetic data, and the most important advantage of NONMEM is the possibility to use pharmacokinetic data from clinical practice, even though when there are not many samples by individual and they have been obtained in different circumstances. It has other several advantages: (i) the estimation of parameters is more efficient, (ii) it can estimate conficence intervals of the parameters obtained, and (iii) it is possible to evaluate statistically the fit of the model (79,87). The main obstacle is that it is based on a complicated theory and it is not a particularly user-friendly program.

The development of a model with NONMEM takes into account both fixed structure and randomness block. The model built of the fixed structure has two stages:

i. Development of a ***base model*** (which describes observed concentrations without relating pharmacokinetic parameters with individual characteristics), that includes a structural part, *pharmacokinetic model* (one-, two- and three-compartmental models are the most usual), and a random variability part, *statistical model* (defines both interindividual and residual variability).
ii. Introduction of covariates into the base model to obtain the ***final model***.

The randomness block of the model will quantify the magnitude of all kind of variabilities:

Inter-individual variability is the result of the simple fact that we are not all alike, even physiologically. Represent the difference (η) between the individual pharmacokinetic parameter and the population pharmacokinetic parameter (typical value of the parameter into the population). The interaction between η and the typical value can be modelled as:

- Additive model: η is added to the population typical value of the parameter. In this case, parameter variance is constant along independent variable range (1.4).

$$\theta_{1i} = \theta_{1pop} + \eta_{1i} \qquad (1.4)$$

  θ_{1i} is the *1-th* pharmacokinetic parameter for the *i-th* individual, θ_{1pop} is the population "typical value" of the *1-th* parameter, η_{1i} is a random variable for the *i-th* individual on the *1-th* parameter.

- Proportional model: η is multiplied to the population typical value of the parameter. In this case, parameter variance increases with the increase of parameter value (1.5 and(1.6). It can be modelled as:

$$\theta_{1i} = \theta_{1pop} \times (1 + \eta_{1i}) \quad (1.5)$$

$$\theta_{1i} = \theta_{1pop} \times (e^{\eta_{1i}}) \quad (1.6)$$

Equation (1.5) assumes a normal distribution, whereas equation (1.6) assumes a log-normal distribution.

Both models have a distribution of 0 and a variance of ω_{ij}^2. The variance-covariance matrix Ω includes variances $\omega^2_{1....n}$ (n is the number of estimated pharmacokinetic parameters) and possible covariances that characterize interindividual variability of the pharmacokinetic parameters.

Residual variability is the "noise", or the associated errors, as well as intraindividual variance. Represent the difference (ε) between observed concentrations and predicted by the structural model combined with the interindividual variability model. The interaction between ε and the typical value can be modelled as:

- Additive model: ε is added to the function that describes the individual pharmacokinetic profile ($f(pk_i,D_i,t_{ij})$). Parameter variance is constant along independent variable range (1.7).

$$C_{ij} = f(pk_i, D_i, t_{ij}) + \varepsilon_{ij} \quad (1.7)$$

  C_{ij} is the observed concentration of the drug in the individual *i* at *j* time; *f* the pharmacokinetic selected model; pk_i the group of pharmacokinetic parameters estimated for the individual *i*; D_i the dose administered to the individual *i;* t_{ij} the independendent variable time, and ε_{ij} the residual error.

- Proportional model: ε is multiplied to the function that describes the individual pharmacokinetic profile ($f(pk_i,D_i,t_{ij})$). In this case, parameter variance increases with the increase of parameter value (1.8).

$$C_{ij} = f(pk_i, D_i, t_{ij}) \times (1 + \varepsilon_{ij}) \quad (1.8)$$

- Combined model: is the combination of additive and proportional model, having two components of residual variability, one additive and another proportional (1.9).

$$C_{ij} = f(pk_i, D_i, t_{ij}) \times (1 + \varepsilon_{ij}) + \varepsilon_{2ij} \quad (1.9)$$

All of them have a distribution of 0 and a variance of σ^2. The variance-covariance matrix Σ includes variances $\sigma^2_{1....n}$ (n is the number of estimated pharmacokinetic parameters) and possible covariances that characterize residual variability.

1.3.3 Model performance

A fundamental principle of modeling is that a model can never be proven, only disproved. Thus, model evaluation attempts to disprove a model by applying a series of evaluation tests to a model and its predictions. The more tests a model passes, the greater credibility the model will have. The degree of model evaluation will ultimately depend on the model objectives. Strategies for model evaluation have been object of intense research recently, and currently many advances in pharmacometrics are related to this area.

It is now recognized that there is not a single statistic or graphic that allows selecting and evaluating a population PK model, therefore several diagnostics should be used together to evaluate a model performance. Commonly used diagnostics are presented making a distinction between numerical and graphical and simulation based diagnostics.

1.3.3.1 Numerical diagnostics

The Objective Function Value (OFV) measures the difference between observed and predicted values for a group of patients, describing how good a model is at fitting the observed data. It does this by assuming that the model is correct, and asks how probable is it to get data like that which has been observed if the model is true. It employs the -2log likelihood, or -2LL equation (1.10):

$$-2\log(L) = n\log(2\pi) + \sum_{i=1}^{n}\left(\log\sigma_i^2 + \frac{\left(y_i - \hat{y}\right)^2}{\sigma_i^2}\right) \qquad (1.10)$$

By minimizing this value, one increases the likelihood of the model being a good fit for the data. To minimize -2LL one cannot do anything about the part nlog(2π), seeing as this is a constant. However it is possible to minimize the second part, also known as the “extended least squares” objective function. NONMEM looks for parameter estimates that will give the smallest possible -2LL. NONMEM can minimize the objective function by different estimation methods. The most used are:

i. FO (First-order estimation method): all η take the value of 0 (mean population of η is 0) during the estimation process. This is the default estimation method.

ii. FOCE (First-order Conditional Estimation method): all η are considered during the estimation process. FOCE with INTERACTION is the most used version (it assumes that there is interaction between ε and η).

However, the -2LL value does not say anything of interest by itself. It allows to compare models trying to describe the same data. By subtracting the lowest OFV from two models, one can see if one is significantly better than the other. The likelihood ratio test is a common test for statistical significance. If there are two models, one of which is nested within the other (a nested model is where one model can be written as a simplification of another model), we can test the significance of the parameter which differs between the two models. The difference between -2LL values follows a chi squared distribution, with the degrees of freedom being the difference in the number of parameters. For example, with a probability of 0.05, and 1 degree of freedom, the value of the chi distribution is 3.84. Thus, if the difference in -2LL values (i.e., the difference in NONMEM objective function) for two models that differ by only 1 parameter exceeds 3.84, then the parameter is significant at $p<0.05$ level. In fact, the level of significance to accept/reject extra parameters in the model depends on the type of data analyzed, and the estimation method used (88,89). In the case of non-nested models, the log-likelihood test does not apply, and other criteria such us the Akaike Information criteria have to be used to compare between models (90).

The uncertainty in the parameters is an indicator of the reliability of the model. Ette et al 2004 (91), indicates that the standard error for structural model parameters and random effects parameters should not exceed 25% and 50%, respectively. The standard errors for the model parameters can be obtained directly from NONMEM. From the standard errors provided, confidence intervals (CIs) can be computed under the assumption that CIs are symmetric around the point estimate of the parameter. In some cases the standard errors are not accurately estimated from the NONMEM output or it is not possible to estimate them. In those situations, CIs can be obtain with other methods such as log-likelihood profiling and parametric or nonparametric bootstrap.

Correlation between parameters and conditional number, calculated from the eigen values, are additional useful information to get insight around model over-parameterization.

1.3.3.2 Graphical diagnostics

Goodness-of-fit plots (GOF) have been used in the past to show how different aspects of the population data are described by the selected model. GOF plots are created to detect potential bias or problems in the structural model and/or the random effects

models, and are generated based on the (i) typical population predictions (PRED), (ii) individual model predictions (IPRED), (iii) observations (DV), (iv) residuals (RES), (v) weighted residuals (WRES), (vi) conditional weighted residuals (CWRES) (92) and time (TIME).

Some authors have reviewed the several evaluation methods more often applied in the literature (93). A useful display is the observed data (DV) and the individual (IPRED) and population (PRED) predictions plotted versus time after dose (TAD). If IPRED vs TAD and PRED vs TAD are satisfactory, meaning that they look similar as DV vs TAD, it indicates that the model is improving.

These plots are good for obtaining a general impression of the performance of the model. For getting more detailed look at how the predictions mate the observations, we can plot them against each other. The first one is to plot DV vs PRED (94), which is appealing in its simplicity and in that each individual's data are not involved in making the prediction, except as being part of the data defining the population parameters. The most common manner of displaying this diagnostic is as a plot of observations versus population predictions (the latter often denoted "PRED"). A line of identity, and sometimes also a regression line, is included to illustrate how well the observations and predictions agree. This diagnostic may give a useful impression of the extent of variability in the data that is explained by the structural and covariate components of the model. However, a drawback is that there is no expected pattern for this plot. One possible solution to this is to generate a mirror plot, that is to create a PRED vs DV plot, where DV in this case corresponds to model based simulated observations and look for similarities in the trends between both plots. The second one, the plot showing DV vs IPRED, which is based on individual parameters estimates, is also very common. However, for this diagnostic to be informative on model misspecification the individual data has to be informative on the parameters that are estimated in the individual fit. Otherwise an overfit will occur and even a misspecified model can give agreement between observation and predictions. The ε-shrinkage (1.11) is used to quantify how informative this plot is, and thus will increase from zero to one as data becomes less informative. In Equation (1.11), SD(IWRES) is the standard deviation of IWRES.

$$\varepsilon\text{ - shrinkage} = 1 - SD\,(IWRES) \tag{1.11}$$

The DV vs IPRED plots have been reported to lose their power and become meaningless around shrinkage values of 20-30% and higher (95). Individual parameters are based on individual values of η, which are obtained from the estimated elements of the Ωmatrix. Individual η are often used for covariate selection. The concept of shrinkage is also applied to η, and a high η-shrinkage is associated with less reliable individual parameters. (1.12) shows how η-shrinkage can be calculated;

where SD(η) is the standard deviation of the empirical Bayes estimates of the interindividual random effects (η) and ω the population model estimate of the η.

$$\eta_x - shrinkage = 1 - \left(\frac{SD(\eta_X)}{\omega} \right) \quad (1.12)$$

To get a more detailed impression of the differences between the predictions and the observations and how these differences are distributed over the independent variable, the plot IWRES vs TAD can be useful. An alternative, especially in the case when the data per individual is sparse, is to plot the WRES instead of IWRES. However, even when FOCE is employed to estimate the population parameters, the WRES is computed using the first-order (FO) approximation in NONMEM. It is not clear what statistical properties the WRES should have when using the FOCE approximation. In this case, has been proposed to use the conditional WRES (CWRES), when the FOCE approximation is used (92).

1.3.3.3 Model evaluation

Most of the used evaluation methods are based on simulation diagnostics. Simulation is defined as the use of a model and its parameters to predict possible outcomes. External and Internal methods can be distinguished.

External validation is the most stringent type of validation. It can be done when both input data to estimate and develop the model, and output data on which the model can be tested exist. It consists on the application of the developed model to a new data set. When a model is validated externally, it provides the strongest evidence for transportability.

Internal validation has several approaches, which include data splitting, resampling techniques and simulation-based diagnostics, as described below:

Bootstraping is a resampling method suggested by Bradley Efron in 1979 (96). Resampling has been defined as a method of repeatedly generating pseudosamples distributed according to the same distribution as the original sample. The procedure of interest is then carried out on each pseudosample and then the results of the application of these procedures to the pseudosamples are summarized. This methodology also allows to estimate the precision of the parameters estimations through the calculation of the confidence intervals associated to each.

"Predictive check" is the name given to the multiple simulations that are made from the model and reference distributions created for features of the observed data. Within these diagnostics, the *visual predictive check (VPC)* refers to the plot of the time

course of the observations together with the time course of prediction intervals for the simulated values, this approach being a diagnostic of both the fixed and random effects parts of the PK/PD model (97). The *numerical predictive check (NPC)* is a related statistic derived from the simulated data used for the VPC (98). Heterogeneity in the design and in the model has to be low for a VPC to be informative (94). One way to account for this situation is to stratify VPCs by the variable that is varying (for example to stratify by dose or even a given covariate), but this can lead to few information per plot and therefore uninformative plots. Recently, in an effort to account for this situation, the *predicted corrected VPCs (pc-VPCs)* have been proposed (99). The pc-VPC normalizes the observations and the model predictions by the typical model prediction in each bin of the independent variable.

Posterior Predictive Check (PPC) was suggested by DB Ruffin in 1984 (100) as a tool for constructing inferential procedures in modern statistical data analysis. In this approach a model is estimated directly from the index data, and then a new set of data is generated through the simulation of the resulting model. The simulated data set is compared with the index data to see if the model's deficiencies have a noticeable effect on the substantive inferences.

Normalised prediction distribution errors (NPDE) are a relatively new metric designed to allow the evaluation of non-linear mixed-effect models (101(102). Briefly, prediction discrepancies are obtained as the quantile of each observation within its predicted distribution. A model describes the data well when the predicted discrepancies are evenly distributed.

The current model evaluation standards can only be handled in a practical and efficient manner with the help of tools specifically designed to aid in the developing process of population PK/PD models, such as PsN (http://psn.sourceforge.net/), Xpose (http://xpose.sourceforge.net/), Census (http://census.sourceforge.net/) and Pirana (http://pirana.sourceforge.net/). Also, software for data manipulation, statistical calculation and graphical display such as R (http://cran.r-project.org) and S-PLUSR (Copyright 1988, 2002 Insightful Corp) are widely used in the field of pharmacometrics.

2. AIMS

The main objectives of this study were:

1. To develop a population pharmacokinetic model for amikacin from data collected in the neonatal and pediatric units of Vall d'Hebron University Hospital during routine clinical monitoring of a neonatal population with postnatal ages ≤90 days, and to evaluate the effect of several phisiologyical and pathological factors on amikacin pharmacokinetics, to identify potential predictive factors for dosage individualization.

2. To verify the predicitive performance of the final population pharmacokinetic model into a external dataset, belonging to the same population as the development group.

3. To evaluate the current dose regimen, in order to achieve amikacin concentrations within the therapeutic range, preventing under- or over-exposure in the target population, by comparing with the amikacin concentrations achieved after applying the population pharmacokinetic model developed.

4. To optimize the initial dose recommendations, according to the individual characteristics identified as best predictors of between-patient variability in amikacin pharmacokinetics, in order to achieve therapeutic concentrations in the target population.

3. METHODS

3.1 STUDY DESIGN AND PATIENT CHARACTERISTICS

3.1.1 Study design

Amikacin serum concentration-time data from therapeutic drug monitoring were retrospectively collected from patients belonging to the neonatal intensive care and pediatric units of Vall Hebron University Hospital (Barcelona, Spain) between July 2000 and July 2006, that accomplished the following inclusion and exclusion criteria:

Inclusion criteria:

- Available patient demographic characteristics.
- Dose regimen and blood sampling times known.
- Available at least 2 samples per patient.
- Postnatal age ≤ 90 days at the time of serum amikacin concentration determination.

Exclusion criteria:

- Acute or chronic renal failure requiring extra-renal purification techniques for the maintenance of homeostasis (hemodialysis, hemofiltration). Of note, the presence of unestable renal function, defined as fluctuations in serum creatinine values higher than 0.5 mg/dl during the treatment, was not considered as exclusion criterion (103(104).
- Serum concentrations determined at no steady state conditions.

Good Clinical Practice (GCP) and the Declaration of Helsinki agreements were fulfilled. No additional blood samples were requested other than those strictly necessary for classical therapeutic drug monitoring of aminoglycosides. Hence, no informed consent from parents was needed, according to Spanish laws.

The patients included in the study were randomly distributed into two groups. The first one, called *"Model building dataset"*, was used to build the population pharmacokinetic model. The second one, the *"External evaluation dataset"*, was used to verify the predictive performance of the final model with an external group of patients belonging to the same population as the target one.

3.1.2 Patient characteristics

Data recording was performed through the datasheet included in Appendix 1, specifically designed for this study. The following data were recorded for each patient:

- Initials, medical record and date of birth.
- Gender. For this categorical variable, the value of 1 was assigned to males and of 0 to females.
- Main diagnosis.
- History of dose regimen and serum concentrations: date and time of start of the amikacin treatment, dosing schedule (dose and interval), duration of perfusion, exact times of amikacin administration and blood sampling. All these data were recorded from nursing data.
- Perinatal data:
  - Apgar evaluation. Apgar test is a medical test used in neonatology. It allows the assessment of the neonate according to five parameters (skin colour, heart rate, reflexes, muscular tone and breathing). A score between 0 and 2 was assigned to each parameter, so that the final test score results from the sum of the individual ones. This test should be performed at one (APGAR1) and five (APGAR5) minutes after birth to be considered as valid, with an expected value ranging from 8 to 9.
  - Number of gestation.
  - Twin pregnancy.
- Age measurements:
  - Gestational age (GA) (weeks), that allowed to classify neonates into: (i) *Premature neonates*, those with a GA less than 37 weeks (within this group *"Extremely prematures*" were considered when GA was less than 32 weeks), and (ii) *Term neonates*, when the GA was equal or greater than 37 weeks.
  - Postnatal age (PNA) (days).
  - Postmenstrual age (PMA) (days).
- Anthropometric characteristics:
  Height (cm) (BHGT), weight (Kg) (BWGT) and head circumference (cm) (BHC) were recorded at the day of delivery, but also at the same day of blood sampling or alternatively within the closest $\pm$ 2 days (HGT, WGT and HC). All of these data were collected from nursing graphs.
  Body Surface Area (BSA) was calculated according to DuBois nomogram (105), as follows (3.1):

$$BSA(m^2) = \frac{Weight\ (Kg)^{0.425} * Height\ (cm)^{0.725} * 71.84}{10^4} \qquad (3.1)$$

According to BWGT, neonates could be classified in three groups: (i) Low birth weight (< 2500 g), (ii) Very low birth weight (<1500 g), and (iii) Extremely low birth weight (<1000g). Moreover, an infant was considered to be *Small for Gestational Age (SGA)* when BWGT and GA values were below the 3th percentile according to the fetal growth weight pattern of the neonatal unit of Vall Hebron University Hospital (Appendix 2 and 3). Otherwise, the infant was considered to be *Adequate for Gestational Age (AGA).*

- Urea (UREA) (mg/dl) and creatinine (CREA) (mg/dl) serum concentrations, indicative of renal function, were measured at the same day of blood sampling or alternatively within the closest $\pm$ 2 days.
  Additionally, creatinine clearance (CLCR) was calculated according to Schwartz nomogram as follows (106) (3.2):

$$CL_{CR}(mL/min/1.73^2) = \frac{K * HGT(cm)}{CREA} \quad (3.2)$$

where *K* was equal to 0.33 for prematures and SGA neoantes, and equal to 0.45 for term neonates.

SPSS ver.19 for statistical analysis (107) was used for the descriptive analysis (mean, median, standard deviation, maximum, minimum, etc…).

3.2 AMIKACIN DOSING AND BLOOD SAMPLING

Amikacin dosing was done according to the established protocol of the neonatal intensive care and pediatric units of Vall Hebron University Hospital, based on Neofax recommendations (29) (Table 3.1). But in some cases, Neofax guide was not applied strictly. Amikacin administration was done either after the identification of the microorganism or as empirical treatment.

Table 3.1 Dosage protocol

GA (weeks)	PNA (days)	Dose (mg/Kg)	Interval (hours)
≤ 29*	0 to 7	18	48
	8 to 28	15	36
	≥ 29	15	24
30 a 34	0 to 7	18	36
	≥ 8	15	24
≥ 35	ALL	15	24

** or significant asphyxia, patent arterial duct, or treatment with indomethacin*

Amikacin was administered by intravenous infusion over a period of time ranging from 30 to 60 minutes. Blood samples were collected at the steady state. Trough or predose (just before the following dose) and peak or postdose (1 hour after initiation of administration) samples were obtained in all the cases. Any deviation from these theorical times was registered to be considered in the subsequent pharmacokinetic analysis. From each sample, serum was obtained by centrifugation for subsequent amikacin concentration analytical quantification.

3.3 DRUG ANALYSIS AND BIOCHEMICAL DETERMINATIONS

Drug analysis

Serum amikacin concentrations were determined by a fluorescence polarization immunoassay (FPIA) method using the TDX system from Abbott Laboratories. This assay was linear up to 50 mg/L, the intra- and interassay precision values were lower than 5% and the lowest limit of quantification (LLOQ) was 0.1 mg/L (108).

Fluorescence polarization is a competitive assay where the substance to be measured in the sample competes with the fluorescent-labeled compound (tracer) for a limited number of binding sites on the antibody. So, the greater is the concentration of analyte, the smaller is the fraction of tracer that is bound, and hence, smaller the polarization detected. The relationship between fluorescence polarization and compound concentration is established by measuring a set of calibrations of known concentration.

Biochemical determinations

Serum urea concentrations were determined by a kinetic procedure with urease and glutamate dehydrogenase, based on the method of Talke and Schubert (1965) according to reactions given by equation (3.3 and equation 3.4. This method was optimized for use in automatic analyzers and the Modular Analytics system from Roche Laboratories® was used in our case

$$Urea + 2H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} \quad (3.3)$$

$$NH_4^+ + 2\text{-}oxoglutarat + NADH \xrightarrow{GLDH} L\text{-}glutamate + NAD^+ + H_2O \quad (3.4)$$

The ammonia obtained in the first step reacts with 2-oxoglutarat in the presence of glutamate dehydrogenase and the coenzyme NADH to produce L-glutamate. In this reaction, two moles of NADH to NAD^+ are oxydized per mol of urea hydrolysed. The NADH concentration, that decreases proportionally to the concentration of urea in the sample, is photometrically measured. This assay is linear up to 400 mg/dL , the intra- and interassay precision values are lower than 3.5% and the LLOQ is 5 mg/dL (109).

Serum creatinine concentrations were determined by the method of Jaffé with sample-blank based on the following reaction (3.5).

$$Creatinine + picric\ acid \xrightarrow{ph\ alcali} yellow\ -\ red\ complex \quad (3.5)$$

The production rate of the colorant (yellow intensity) that is directly proportional to the concentration of creatinine in the sample, is photometrically measured. The *"rate-blanking"* method minimizes interferences by bilirrubin. Since serum and plasma samples contain proteins that react unspecifically with Jaffé method, the results are corrected to obtain accurate results. The Modular Analytics system from Roche Laboratories® was used. This method is linear up to 24.9 mg/dL, the intra- and interassay precisions values are lower than 3.5% and the LLOQ is 0.17 mg/dL (110).

3.4 POPULATION PHARMACOKINETIC (PPK) ANALYSIS

The PK analysis was performed with the nonlinear mixed-effects modeling (NONMEM) software, version 7.2 (82). Psn 3.5.2 (pearl speak for nonmem) (111) was used for generation of visual predictive checks and bootstrap analysis. R software ver.2.14 (112) and the R package Xpose ver. 4.2 (113) were used for post-processing of data, graphical analysis of model outputs, and for generalized additive modelling during the exploratory covariate analysis. SPSS ver.19 was used for statistical analyzes performed during the initial data exploration (107).

The modelling process consisted on the following steps:

i. Data exploration.
ii. Model development. Including base model development, covariate selection process and final model development.
iii. Final model evaluation and qualification.

3.4.1 Data exploration

Prior to modelling, exploratory graphs and tables of the data to be analysed were generated to gain understanding of the data to be modelled, to look for trends in data, to identify potential outliers or erroneous data values, to check for errors in data coding and to verify model assumptions. A graphical exploration of the data to be analyzed was performed according to the following steps:

- Histograms of all the quantitative continuous covariates (age, weight, etc..).
- Scatterplots of all the continuous covariates, in order to investigate the potential correlations existing among them.

- Plots corresponding to the amikacin concentrations vs time data in order to investigate the general trend of the data and to identify potential outliers.

Numerical exploration by descriptive statistics (mean, median, standard deviation, maximum, minimum, etc…) of demographic and biochemical characteristics, doses given and blood sampling times, was also considered.

3.4.2 Model development

All amikacin serum concentration-time data were analyzed simultaneously by the nonlinear mixed-effects approach implemented in NONMEM software. The first-order conditional estimation (FOCE) and the first-order conditional estimation with interaction (FOCEI) methods were tested throughout the model building process.

3.4.2.1 Base model development

Handling of below the limit of quantification (BLQ) data

The model building process was done according to three different approaches:

i. Simultaneous analysis of concentration vs time data including concentration values below the limit of quantification (<BLQ), all of them treated as continuous data. The pharmacokinetic analysis was performed including the concentrations below the limit of quantification reported as a value of 0.09 mg/L, just below the lower limit of quantification (LLOQ=0.1 mg/L).
ii. Simultaneous analysis of concentration vs time data after removing data below the lowest limit of quantification.
iii. Simultaneous analysis of concentration vs time data including data below the lowest limit of quantification, that were treated as censored data (the Method 3 reported by Bergstrand and Karlsson) (114-(117). The Laplacian estimation method was applied in this case.

In all the cases, the structural and the statiscial models were developed as follows:

Structural model development

One-, two and three-open compartment models with linear elimination process and zero order input were tested using the following subroutines:

- One-compartment model: ADVAN1 TRANS2, parameterized in terms of distribution volume (Vd) and total drug clearance (CL).
- Two-compartment model: ADVAN3 TRANS4, parameterized in terms of central compartment distribution volume (V1), total drug clearance (CL), distributional clearance (Q) and peripheral compartment distribution volume (V2).
- Three compartment model: ADVAN11 TRANS4, parameterized in terms of central compartment distribution volume (V1), total drug clearance (CL), distributional clearance corresponding to compartment 2 (Q2), peripheral

compartment distribution volume corresponding to compartment 2 (V2), distributional clearance corresponding to compartment 3 (Q3) and peripheral compartment distribution volume corresponding to compartment 3 (V3).

Statistical model development

The between-patient variabilities (BPV), were modeled exponentially, assuming a log-normal distribution. The diagonal and full variance-covariance matrices were tested. Additive, proportional and combined models were compared to assess the residual error (RE) in amikacin serum concentrations. The inter-occasion variability modelling was also tested.

Initial estimates of parameters

The initial estimates of fixed parameters were in accordance with those previously reported in the literature (118). The lower limit of all pharmacokinetic parameters (θ) was fixed at 0 in order to ensure a positive value. The initial values for the variances associated to the distributions of between-patient variability (ω^2 for etas) or residual variability random effects (σ^2 for epsilons), were selected considering an associated error of 50%, expressed as coefficient of variation. Since the coefficient of variation was the square root of the variance parameter, the initial estimates of ω^2 or σ^2 (when modelled proportionally) were set to 0.25 (3.6).

$$\omega^2 = (0.5)^2 = 0.25 \qquad 3.6$$

The initial estimate considered for the the additive residual error was the limit of quantification of the analytical method (0.1 mg/L).

3.4.2.1.1 Model Discrimination

The evaluation of the base models developed and their comparison was performed according to the recommendations of the *"European Medicines Agency (2007)"* (70) as follows:

- Statistical criteria:
  To statistically assess the differences between nested models, the likelihood ratio test, based on the reduction of the OFV was used (ΔOFV: -2 log likelihood (-2LL), approximate χ^2 distribution). A significance level of p<0.005 corresponding to a ΔOFV= -7.879 for 1 degree of freedom was considered. For non-hierarchical models, the most parsimonious model with the lowest OFV according to the Akaike Information Criterion (AIC) calculated as -2LL+2·Np, where *Np* is the number of model parameters, was used instead (90).

- Plausibility and precision of the parameters estimates:
  Plausibility of the pharmacokinetic parameter values from a physiological point of view was taken into account. The precision of the parameters estimates expressed as relative standard error (RSE%), that is the the ratio between the standard error, obtained from the covariance step in NONMEM, and the parameter estimate, was also evaluated (3.7).

$$RSE\% = \left(\frac{Standard\ error}{Parameter\ value} \right) * 100 \qquad (3.7)$$

- Goodness-of-fit-plots:
  The following graphs were investigated:
  - Plots of observed (DV) vs population predicted and individual predicted (PRED and IPRED) concentrations including the identity line and an smoothed line representing the general trend of the data. Data points should be distributed closely and symmetrically to the line of identity, if the data were adequately described by the model. Comparing both plots, DV vs IPRED plot should adjusted better than DV vs PRED, as IPRED incorporated between-patient variability.
  - Plots of weighted residuals (WRES) or conditional weighted residuals (CWRES) vs population predicted concentrations with a zero horizontal line and smoothed trend line included. These plots allowed the evaluation of the suitability of the residual error model. Residuals should be spread randomly and closely around the zero horizontal line, without any specific trend.
  - Plots of weighted residuals (WRES) or conditional weighted residuals (CWRES) (when FOCE method was applied) vs time (expressed either as time after the last dose or as time from the start of the study) with a zero horizontal line and smoothed trend line included. These plots allowed to assess the general model fit, mainly the structural model. Residuals should be spread randomly and closely around the zero horizontal line withouth any specific trend.
  - Plots of absolute individual weighted residuals (IWRES) vs individual predicted concentrations (IPRED) with a trend line included.
  - Plots of superimposed observed (DV), individual predicted (IPRED), and population predicted (PRED) concentrations vs. time in order to check if IPRED and PRED values described adequately the observed concentrations for each individual.
  - Other plots provided by the Xpose software were also explored, as:

- Histograms or QQ-plot of weighted residuals in order to check if they were distributed normally.
- Histograms of between-patient variability random effects in order to check if they were symmetrically distributed.
- Scatter plots of individual random effects associated to the pharmacokinetic parameters in order to identify potential correlations among them.

- Reductions in model residual error in each step of the modelling process were also assessed.

The model with the lowest -2LL value (for nested models) or AIC value (for non-nested models), with the best plausibility of the parameter estimates and an acceptable parameter precision supported by the goodness of fit plots was finally selected.

3.4.2.1.2 Shrinkage evaluation

To assess the informativeness of the data in terms of BPV parameters, shrinkage was calculated before the covariate selection and final model development. High shrinkage values (>20%) could lead to unreliable diagnostics for covariate versus empirical Bayes estimates. Hence, it is desirable to report the extent of η- and ε-shrinkage to assess the relevance of diagnostics employing EBEs, IPRED and IWRES. η-shrinkage and ε-shrinkage of the parameter estimates were computed as indicated by equation 3.8 and equation 3.9, respectively:

$$\eta_x - shrinkage = 1 - \left(\frac{SD(\eta_x)}{\omega} \right) \quad (3.8)$$

$$\varepsilon - shrinkage = 1 - SD(IWRES) \quad (3.9)$$

Where SD(η) was the standard deviation of the empirical Bayes estimates of the interindividual random effects (η), ω was the population estimate of that parameter and SD(IWRES) was the standard deviation of IWRES (94,(95).

3.4.2.2 Covariate selection and final model development.

Once the base model had been developed, the influence of all covariates physiologically reasonable on PK parameters was investigated. Before the inclusion of the covariates, an initial exploration was performed including:

i. Identification of potential correlations among covariates by visual inspection of correlation plots. For high correlated covariates, clinical relevance criteria were used for selection of the most appropriate.

ii. Identification of potential statistically significant covariates, by:
   - Plots of individual random effects associated to each PK parameter, estimated from the base model, vs covariates.
   - Plots of CWRES vs covariates.
   - Multivariant analysis using the "Stepwise Generalised Additive Modelling" (GAM), a technique not restricted to linear models, implemented in Xpose. The GAM allowed to identify statistically significant relationships, between Bayesians estimates of the individual pharmacokinetic parameters and covariates, according to the Akaike Information Criterion (AIC) (119).

According to the information obtained from the initial exploration, all the covariates physiologically plausible were tested in NONMEM on any of the model pharmacokinetic parameters. The impact of continuous covariates was tested in their respective parameters as allometric (Equation 3.10) or linear relationships.

$$TVP_j = \theta_1 \cdot (COV / COV_{median})^{\theta_{COV}} \quad (3.10)$$

where the population typical value of the pharmacokinetic parameter was defined by θ_1 as the typical value of the j^{th} pharmacokinetic parameter for a patient, COV_{median} as the median covariate value in the population, and θ_{COV} as the change in $lnTVP_j$ per unit change in $ln(COV/COV_{median})$.

The categorical covariates (i.e. gender, number of gestation) were tested in their respective parameters as indicated by equation 3.11:

$$\begin{aligned} TVP_j &= \theta_1 \quad for \quad Z = 0 \\ TVP_j &= \theta_1 \cdot \theta_2 \quad for \quad Z = 1 \end{aligned} \quad (3.11)$$

where *Z* values represent each level of the categorical covariate. Specifically, in the case of gender, *TVPj* was the typical value of the j^{th} pharmacokinetic parameter for females, and θ_2 was the fractional change in θ_1 by males.

Covariates were firstly tested univariately in the model and then by the cumulative stepwise forward inclusion/backward elimination procedures. Multiplicative equations were used to describe the combined effect of multiple covariates on the same parameter. Those that had been significant on each of the pharmacokinetic parameters were sequencially combined following the descending order according to the decrease on the OFV they had produce. If the addition of a covariate produced a significant decrease in the objective function, it was retained in the model. If not, the covariate was removed. Once the intermediate or covariate model was established, the

retrospective covariate exclusion (backward) until reaching the base model was carried out.

The following selection criteria were considered during the covariate model building:

- Statistical criteria given by changes in OFV (-2 log likelihood). Significance levels of 5% (ΔOFV=-3.841 units) and 0.1% (ΔOFV=10.8 units) were considered during the forward addition and backward elimination steps, respectively.
- Parameter precision estimates expressed as relative standard error (RSE%).
- Reductions in BPV associated with pharmacokinetic parameters on which the covariate resulted to be statistically significant.
- Reductions in residual error.
- Model completion status. Visual inspection of the goodness-of-fit plots before described (3.4.2.1.1.), together with the additional following graphs:
  - Plots of the random effects vs the covariates in the final model.
  - Histograms of the Bayesian estimates of the parameters or random effects associated (η).
- Clinical relevance of the covariate given by changes in the pharmacokinetic parameter value of at least 10%.

3.4.3 Final Model evaluation and qualification

Once the final model had been selected, additionally to the diagnostic plots used for the evaluation during the model building development, external and internal evaluations were applied in order to check the performance of the final covariate model.

3.4.3.1 External validation techniques

The predictive performance of the developed model was assessed in 53 new patients (External evaluation dataset) that belonged to the same population as those of the "Model building dataset" using the posterior Bayesian estimates of amikacin concentrations. The observed concentrations of the new dataset were compared with the corresponding predictions given the final model. This performance was evaluated in terms of bias (median prediction error (ME)) and precision (root median squared prediction error (RMSE)) using the equations 3.12 and 3.13, according to the method proposed by Sheiner and Beal (120). Both the bias and precision were calculated from the observed versus population predicted concentrations (PRED) and from the observed versus individual predicted concentrations (IPRED), either for trough or peak concentrations.

$$ME = \frac{1}{N}\sum_{i=1}^{N}(C_{Obs} - C_{Pred}) \quad (3.12)$$

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(C_{Obs} - C_{Pred})^2} \quad (3.13)$$

In equation 3.14, each value therein were first squared. In both equations, *Cpred* were either the trough or peak predicted concentrations (PRED or IPRED) and *Cobs* were either the trough or peak observed concentrations.

3.4.3.2 Internal validation techniques

The prediction ability of the model was further evaluated by several internal evaluation techniques as bootstrap, visual predictive check (VPC), prediction-corrected visual predictive check (pcVPC), posterior predictive check (PPC) and normalised prediction distribution errors (NPDE).

Bootstrap

The bootstrap method (96) with replacement was used to assess the robustness of the final model and to construct the prediction intervals (PIs) of the parameters estimated using PsN-Toolkit version 3.5.2. One thousand data sets of the same size as the original were reconstructed by resampling from the original data. The final model was fitted to each replicate data set, and the parameter estimates were obtained for each one. Then, the mean values of the parameters obtained were calculated and the percentages of difference with respect to those estimated from the original data were calculated according to the equation 3.14.

$$\%\ difference = \frac{Population\ mean\ estimate - bootstrap\ mean\ value}{Population\ mean\ estimate} \cdot 100 \quad (3.14)$$

The population mean estimate of each parameter should be included within the prediction intervals given by the bootstrap method.

Visual Predictive check (VPC) and Prediction-corrected visual predictive check (pcVPC)

For the VPC (97), one thousand individual profiles as those of the original dataset were simulated from the final model and then, the 95% confidence intervals for the median, and the 5th and 95th percentiles of the predicted data, were calculated and plotted together with the median, and the 5th and 95th percentiles of the observed data. If the model described data adequately, the lines corresponding to the median, the 5th and 95th percentiles of the observed data should fall in the respective 95% confidence intervals of the predicted data. By using pcVPC (99), both the observations and model predictions were normalized for the typical model predictions in each bin of independent variables.

Then, the 95% confidence intervals for the median, and the 2.5th and 97.5th percentiles of the predicted data were calculated and plotted together with the median, the 2.5th and 97.5th percentiles of the observed data. If the model described the data adequately the lines corresponding to the median, and the 2.5th and 97.5th percentiles of the observed data should fall in the respective 95% confidence intervals of the predicted data.

Posterior Predictive Check (PPC)

In the PPC (100), investigation of how well the model predicted trough and peak concentration values of amikacin by simulating 1000 data sets as the original dataset was performed. Then, distributions of simulated trough and peak concentrations were compared with those of trough and peak observed concentrations. The trough and peak concentrations were selected for evaluation because these parameters were, as described before, the most relevant surrogate markers of efficacy and toxicity for amikacin, and were expected to vary with dose and relevant covariates.

Normalized Prediction Distribution Errors (NPDE)

One thousand individual profiles as those of the original dataset were simulated from the final model (101,(102). Then, the differences between each observation and simulations were calculated and they were then normalized by using the inverse of the cumulative density function. If the model fitted data adequately, NPDEs should result in a normal distribution with a mean of zero and variance of 1.

3.5 MODEL-BASED SIMULATIONS

Once the final model was achieved, simulations based on the final pharmacokinetics estimates were performed in order to:

i. **Assess the influence of the covariates that were identified as statistically significant, on amikacin trough and peak concentrations**.

   For that purpose, and for different cut-offs of body weights and estimated CLCR values of the original dataset (Table 3.2), simulations of trough and peak concentrations for 1000 virtual patients of the same characteristics and having received the same dosage as those of the original data set were performed.

   The resulting simulated concentrations vs time data for each cut-off were used to calculate the percentages of patients with trough concentrations < 5 mg/L (within the therapeutic range), < 10 mg/L (the threshold considered as potentially toxic) and peak concentrations < 20 mg/L (marker of inefficacy). The medians and 2.5% and 97.5% percentiles of the trough and peak concentrations achieved in all the cases were also calculated.

Table 3.2. Cut-offs of CLCR and WGT values taken from the original dataset for simulations

CLCR (mL/min)	WGT (g)		
	<1199	1200-1999	≥ 2000
<15	SIM1	SIM2	SIM3
15-30.99	SIM4	SIM5	SIM6
31-59.99	SIM7	SIM8	SIM9
≥60	SIM10	SIM11	SIM12

Besides, the overall resulting simulated concentrations vs time data were stratified by the same age groups as those considered by the Neofax guide (Table 3.1). For each group, the percentages of patients with trough concentrations < 5 mg/L (within the therapeutic range), < 10 mg/L (the threshold considered as potentially toxic) and peak concentrations < 20 mg/L (marker of inefficacy) achieved in all the cases were calculated and compared with the corresponding percentages achieved with the previously indicated CLCR/weight cutoffs (Table 3.2).

ii. Establish initial dose recommendations, in view of the efficacies and toxicities given by serum amikacin concentrations.

For that purpose, the parameter estimates from the final pharmacokinetic model were used to simulate amikacin concentration-time profiles for different dosing regimens. Simulations were performed for differents cut-offs of WGT and CLCR values (Table 3.3). The selection of cut-offs was performed by covering as much as possible the entire range of WGT and CLCR values of the target population.

Table 3.3. Values of CLCR and WGT taken into acocunt for the simulations corresponding to first dose recommendations

CLCR (mL/min)	WGT (g)					
	500	1000	1200	1500	2000	2500
10	SIM13	SIM14	SIM15	SIM16	SIM17	SIM18
20	SIM19	SIM20	SIM21	SIM22	SIM23	SIM24
30	SIM25	SIM26	SIM27	SIM28	SIM29	SIM30
50	SIM31	SIM32	SIM33	SIM34	SIM35	SIM36
60	SIM37	SIM38	SIM39	SIM40	SIM41	SIM42
80	SIM43	SIM44	SIM45	SIM46	SIM47	SIM48

In a first step, a prospective analysis of trough and peak concentrations achieved after doses ranging from 2.5 to 50 mg with dosing intervals of 12, 24, 36 or 48 hours, was performed for all the CLCR/WGT cut-offs. Based on these results, the best recommended dose was selected for each pair of CLCR and WGT values,

aiming to achieve peak concentrations around 30mg/L and through concentrations below or between 1.5 and 3 mg/L. The resulting simulated concentrations vs time data were used to calculate the percentages of patients with trough concentrations < 5 mg/L (within the therapeutic range) and peak concentrations < 20 mg/L, within the therapeutic range (20-30 mg/L) and > 30 mg/L. The 2.5% and 97.5% percentiles of the trough and peak concentrations achieved in all the cases were calculated, and also the 50% percentile of the peak concentrations.

4. RESULTS

4.1 PATIENT CHARACTERISTICS

A retrospective chart review was performed that included all newborns treated with amikacin in the neonatal intensive care and pediatric units at Vall Hebron University Hospital from July 2000 to July 2006. Data were collected from 202 newborns who had at least two amikacin concentrations recorded, and accomplished the above mentioned inclusion criteria (3.1.1). These 202 subjects were randomly distributed into two groups, so that 149 were assigned to the *"Model building dataset "*, and the other 53, to the *"External evaluation dataset"*. Table 4.1 summarizes the main demographic and biochemical characteristics of both groups.

Table 4.1. Demographic and biochemical characteristics of patients included into the *"Model building dataset "* and into the *"External evaluation dataset"*.

Characteristics	**Units**	**"Model building dataset"**	**"External evaluation dataset"**
		Median (Range)	**Median (Range)**
Gender (male/female)	N	86/63 *	34/19 *
Gestational age (GA)	weeks	31.8 (24.3 – 41)	32.5 (24.3 – 42)
Postnatal age (PNA)**	days	28 (4 – 86)	26 (5 – 89)
Postmenstrual age (PMA)**	days	248 (183 – 358)	257 (178 – 374)
Birth weight (BWGT)	Kg	1.64 (0.45 – 3.89)	1.76 (0.37 – 3.82)
Current weight (WGT)**	Kg	1.88 (0.52 – 4.62)	2.09 (0.44 – 5.54)
Birth height (BHGT)	cm	40.01 (27 – 55)	41.09 (26 – 56)
Current height (HGT)**	cm	42.01 (28.5 – 57)	43.83 (28.5 – 57)
Current head circumference (HC)	cm	29.46 (20 – 38)	30.28 (22 – 38.5)
Body Surface Area (BSA)	m^2	0.14 (0.06 – 0.26)	0.15 (0.06 – 0.27)
Serum urea (UREA)**	mg/dL	26.69 (3 – 138)	28.23 (2 – 97.49)
Serum creatinine (CREA)**	mg/dL	0.59 (0.19 – 2.50)	0.54 (0.20 – 1.90)
Creatinine clearance (CLCR)**	mL/min	31.97 (5.87 – 121.5)	36.78 (8.7 – 110.25)

**number in each group*
***Parameters determined at the beginning of treatment*

4.2 AMIKACIN DOSING AND SERUM CONCENTRATIONS

4.2.1 Amikacin dosing

A total of 2443 doses were administered to the 149 newborns belonging to the "*Model building dataset"*. The infusion rate could not be accurately recorded so that it had to be assumed a mean value of 45 minutes in all the cases. The number of doses given by individual ranged from 3 to 61.The median value of amikacin administered doses was 20 mg, ranging from 2.5 mg to 125 mg. The distribution of administered doses normalized by bodyweight is showed in Figure 4.1. The most common administered doses were 10 mg/kg (14.3%), 7 mg/Kg (13%), 15 mg/Kg (11.3%) and 8 mg/Kg (10.7%). Doses over 50 mg/Kg only represent 1.1% of the total and were given in very few occasions and always to the same patient. The dosing intervals were whether 8, 12, 18, 24, 36 or 48 hours depending on the patient.

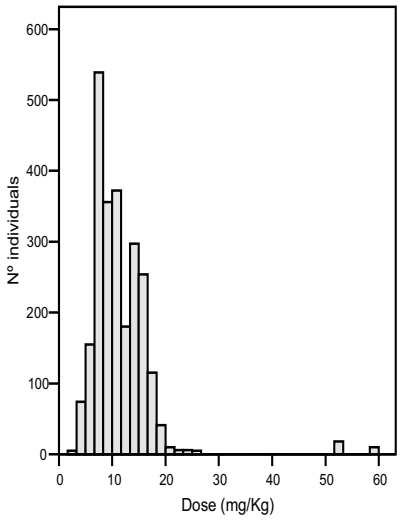


Figure 4.1 Distribution of amikacin doses (mg/kg) administered to the patients of the "Model building dataset"

Amikacin dosing protocol, based on Neofax recommendations, should be done according to the age groups specified in Table 3.1. Table 4.2. summarizes the percentages of patients that received doses below (< Protocol) and over (> Protocol) than those recommended by the Neofax guide for each one of the groups defined (29). Percentages of patients treated with each one of the dosing intervals from 8 to 48 hours are also summarized in this table. Bold numbers correspond to the percentages of patients with doses or dosing intervals coinciding with those recommended by Neofax.

Table 4.2. Amikacin dosing in the "Model building dataset" according to age groups of the Neofax guide.

		GroupA	GroupB	GroupC	GroupD	GroupE	GroupF
GA (weeks)			**≤ 29**		**30-34**		**≥ 35**
PNA (days)		**0–7**	**8–28**	**≥29**	**0–7**	**≥8**	**All**
Dose (mg/Kg)	**< Protocol**	*48.7%*	*78%*	*67.8%*	*84.8%*	*77.2%*	*75.2%*
	Protocol	**43.9%**	**5.7%**	**9.3%**	**10.9%**	**13%**	**14.4%**
	> Protocol	7.4%	16.3%	22.9%	4.3%	9.8%	10.4%
Interval (hours)	**8**	-	-	10.3%	-	9.2%	*36%*
	12	-	3.6%	*48.9%*	5.3%	*47.1%*	21.1%
	18	-	-	1.1%	31.6%	4.7%	2.1%
	24	*57.9%*	*72.1%*	**30.3%**	*42.1%*	**32.3%**	**35.9%**
	30	-	-	-	-	0.4%	-
	36	13.2%	**16.3%**	6%	**15.7%**	3.6%	2.7%
	48	**28.9%**	8%	3.4%	5.3%	2.7%	2.2%

In bold, values in accordance with Neofax guide. In cursive, the most frequent values among each group

As Table 4.2 shows, neither dose nor interval recommendations were followed strictly in any group. The most frequent administered doses were always below the guide recommendations. For dosing intervals, those recommended by Neofax were not the most frequent applied in any group. The 48 hours interval was mainly applied to the youngest group (Group A), as it was the recommended by Neofax, but it was rarely applied to the rest of the groups. The Group B was mainly dosed using a 24 hours interval schedule. In Group C, dosing intervals of 12 and 24 hours were almost equally applied meanwhile, surprisingly, in Group E the 18 hours interval in place of that of 12 hours was used. The oldest group (Group F) was the only one that used the 8 hours interval with similar frequency to those of the 12 and 24 intervals.

Taking into account both doses and intervals, the lowest gestational and postnatal age group (Group A) showed the greatest percentage of dosing regimens applied according to Neofax guide (22%). The lowest gestational age with postnatal ages between 8 and 28 days group (Group B) showed the least percentage of coincident dosing regimens according to Neofax guide (2.1%). In the remaining groups, similar precentages of patients dosed according to Neofax were found (from 5.7% to 9.7%).

4.2.2 Amikacin serum concentrations

A total of 446 concentration-time values (203 trough concentrations and 243 peak concentrations) from 149 patients were simultaneously analysed. Among all the concentrations analyzed, 8.8% corresponded to data below the limit of quantification (0.1 mg/L), which were recorded as 0.09 mg/L. Figure 4.2 displays the amikacin serum concentrations (trough and peak concentrations) vs time (after the last dose) profiles including the smoothed line representing the general trend of the data, in the target

population. It should be noted that each patient was sampled between 2 and 11 times. While trough concentrations were taken just before the following dose; i.e. 8 (9.4%), 12 (20.2%), 18 (1.9%), 24 (46.8%), 36 (14.3%) or 48 (7.4%) hours post-dosing (depending on the dosing interval), the sampling times of so-called peak concentrations ranged from 1 to 3.25 hours after initiation of administration.

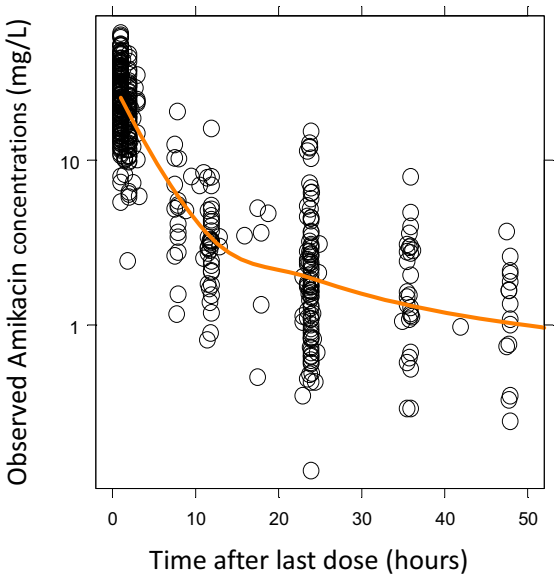


Figure 4.2. Amikacin serum concentrations (mg/L) versus time after the last dose for the target population. *Orange solid line: smoothed line showing the general trend of the data*

For the entire population, mean ± SD values of trough and peak amikacin concentrations were 3.09 ± 3.01 mg/L and 23.97 ± 11.04 mg/L, respectively. The 83.2% of trough concentrations were within the therapeutic range (lower than 5 mg/L), and the 94.6% were lower than 10 mg/L, the threshold considered potentially toxic. Regarding peak concentrations, the 40.7% were below the value considered as effective (20 mg/L).

Trough and peak concentrations achieved were analysed according to the Neofax age groups (Table 4.3). Percentages of trough concentrations within the therapeutic range (< 5 mg/L) decreased from groups A-C to groups D followed by E and then by F. Among the latter (D-F) only Groups E and F had potentially toxic through concentrations (> 10 mg/L), 10.3% and 7.9% respectively. Regarding, percentages of peak concentrations below 20 mg/L, the Group C was that achieved the highest percentage (52.2%) of ineffective peak concentrations, whereas values ranging from 33.3% to 43.2% were found in the other groups. Group A was only represented by one individual so that percentages in this group were not calculated.

Table 4.3 Percentages of through and peak concentrations within the therapeutic range

Neofax age group	Through concentrations < 5 mg/L	Through concentrations < 10 mg/L	Peak concentrations < 20 mg/L
Group A	100%	100%	-*
Group B	95.9%	98%	36.2%
Group C	100%	100%	52.2%
Group D	80%	100%	33.3%
Group E	78.1%	89.7%	43.2%
Group F	72.2%	92.1%	37.3%

** Not calculated because only one individual was available for this group*

Group A: GA<29 weeks + PNA 0-7 days; Group B: GA<29 weeks + PNA 8-28 days; Group C: GA<29 weeks + PNA>29 days; Group D: GA:30-34 weeks + PNA 0-7 days; Group E:GA 30-34 weeks + PNA>8; Group F: GA>35 weeks

Furthermore, patients were splited in two classes, i.e. those dosed according to Neofax recommendations (Class I) and those patients that were not (Class II). Figure 4.3 shows the distribution of percentages of trough concentrations < 5 mg/L for Classes I and II according to Neofax age groups. A trend to lower percentages of trough concentrations within the therapeutic range (< 5 mg/L) in patients of Class II vs Class I was observed for GA from 30 to upwards (groups D, E, F) meanwhile similar values were found in the remaining groups (A-C) The lowest percentage of trough concentrations < 5 mg/L was found in patients not dosed according to Neofax (Class II) of Group D (GA between 30-34 weeks and PNA within 0-7 days).

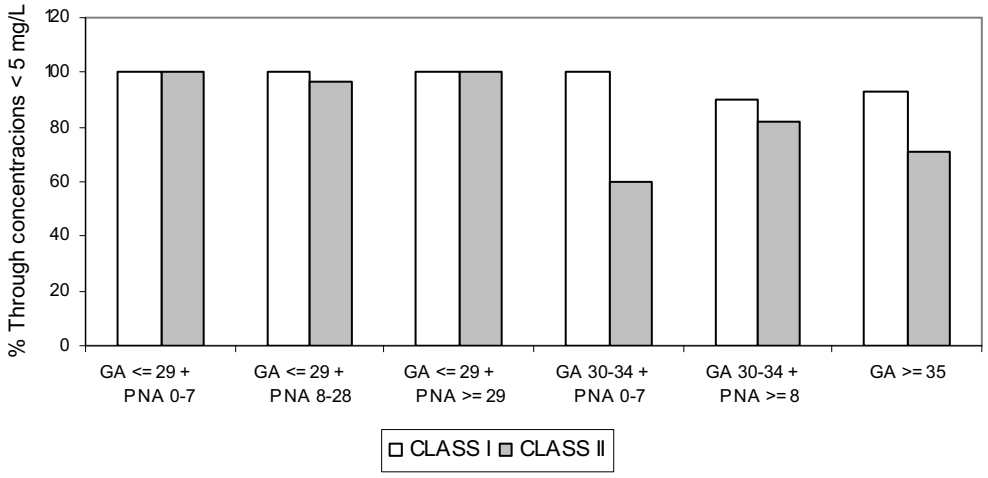


Figure 4.3. Percentages of amikacin trough concentrations < 5 mg/L for Class I and Class II according to Neofax age groups.

Figure 4.4 shows the distribution of percentages of trough concentrations < 10 mg/L among all the Neofax age groups. Only Groups E and F of both Classes I and II showed trough concentrations above 10 mg/L, considered as potentially toxic.

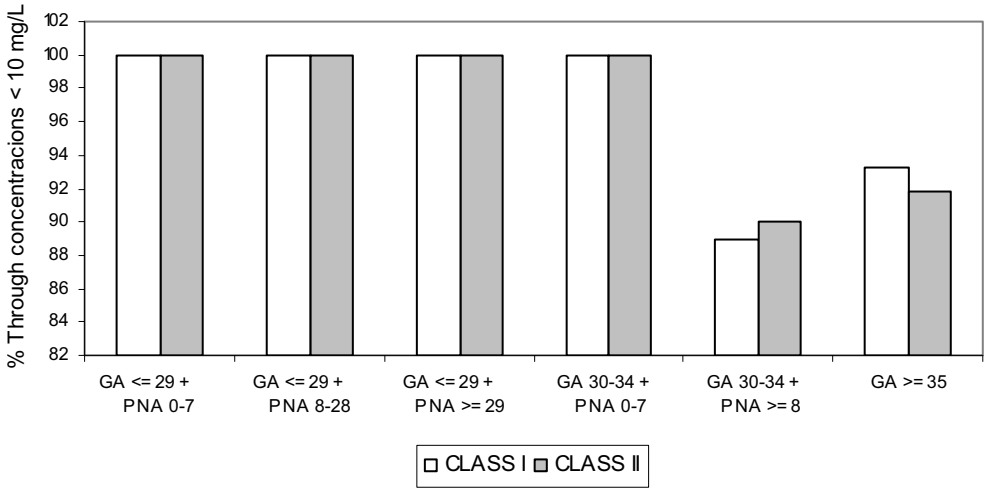


Figure 4.4. Percentages of amikacin trough concentrations lower than the limit considered potencially toxic (10 mg/L) for Class I and Class II according to Neofax age groups.

Figure 4.5 shows the distribution of percentages of peak concentrations < 20 mg/L among all the Neofax age groups. Patients of Class II showed higher percentages of peak concentrations below the value considered as effective (< 20 mg/L) than those of Class I, regardless of GA and PNA. The Group A, in both Class I and Class II, did not show any peak concentration lower than 20 mg/L, but only two peak levels had been analysed on both cases. From the remaining, the most effective dosing regimen was that of groups B, D and F for patients dosed as Neofax, with no ineffective peak concentrations. The highest percentage of peak concentrations < 20 mg/L were found in Group C (GA ≤ 29 weeks and PNA ≥ 29 days) both for patients of Class I and II.

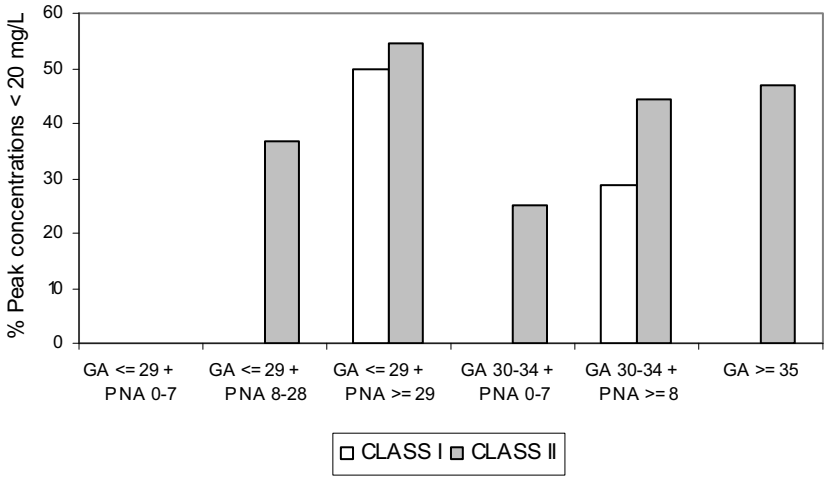


Figure 4.5. Percentages of amikacin peak concentrations < 20 mg/L for Class I and Class II according to Neofax age cut-offs.

4.3 POPULATION PHARMACOKINETIC ANALYSIS

4.3.1 Data exploration

Exploratory data process was based not only on descriptive analysis of demographic and biochemical characteristics of the individuals (see section 4.1), and amikacin dosing and serum concentrations (see section 4.2), but also on the exploratory graph analysis of the registered covariates, that is presented in the following paragraphs.

4.3.1.1 Age covariates

Among the 149 subjects of the *"Model building dataset",* 112 (75.2%) of them were premature newborns, with gestational ages lower than 37 weeks, while the remaining 37 (24.8%) borned at term, with gestational ages greater than or equal to 37 weeks. Regarding to postnatal ages, the 64% of data recorded at the time of amikacin concentration measurement belonged to subjects under 30 days of life, and the 93% to subjects under 60 days of life. Figure 4.6 shows the statistical distributions for GA, PNA and PMA. According to the Kolmogorov-Smirnov test, GA showed a normal distribution ($p>0.05$), while no normality could be proved for PNA and PMA ($p<0.05$).

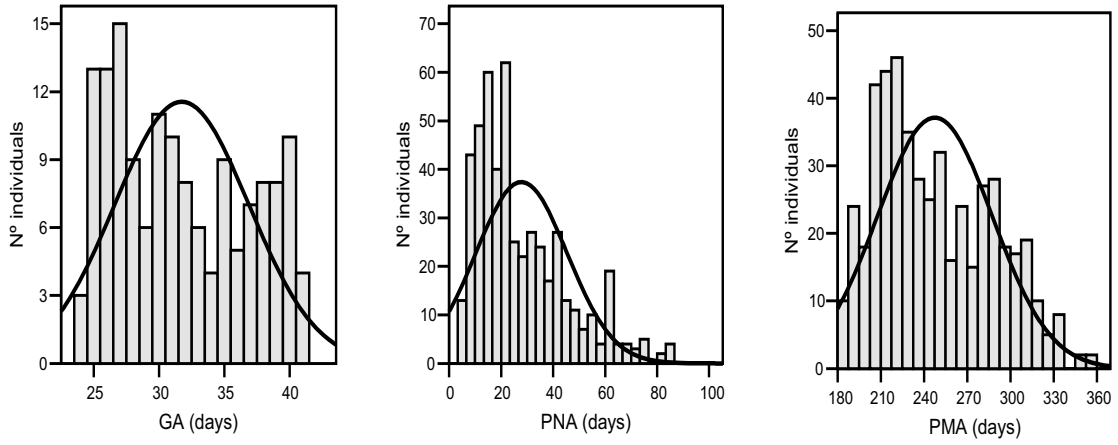


Figure 4.6. Histograms of the distributions of GA, PNA and PMA

When the relationships among these variables (GA, PNA and PMA) were investigated, a strong linear correlation was found between PMA and GA (Pearson correlation coefficient=0.898, $p<0.01$) while it was medium between PMA and PNA ($r=0.399$, $p<0.01$). However, no linear (Pearson correlation coefficient, $r=-0.046$) or any other kind of correlation was found between PNA and GA. Figure 4.7 shows the graphic representation of these relationships.

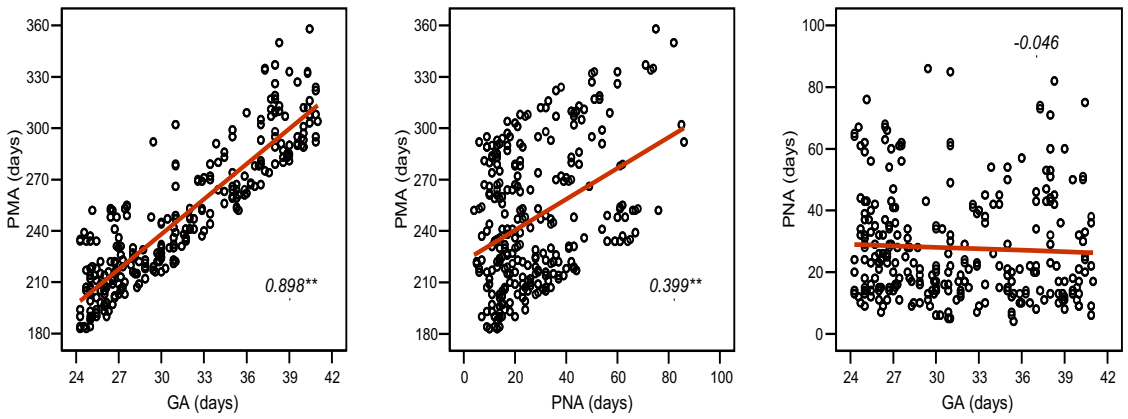


Figure 4.7. Relationships between age covariates *(Pearson correlation coefficient, at p=0.01 significant level**).*

4.3.1.2 Covariates related to body size

According to the Kolmogorov-Smirnov test, the assumption of normality was rejected for all the covariates related to body size. Figure 4.8 shows the histograms corresponding to the statistical distributions of each one of these covariates.

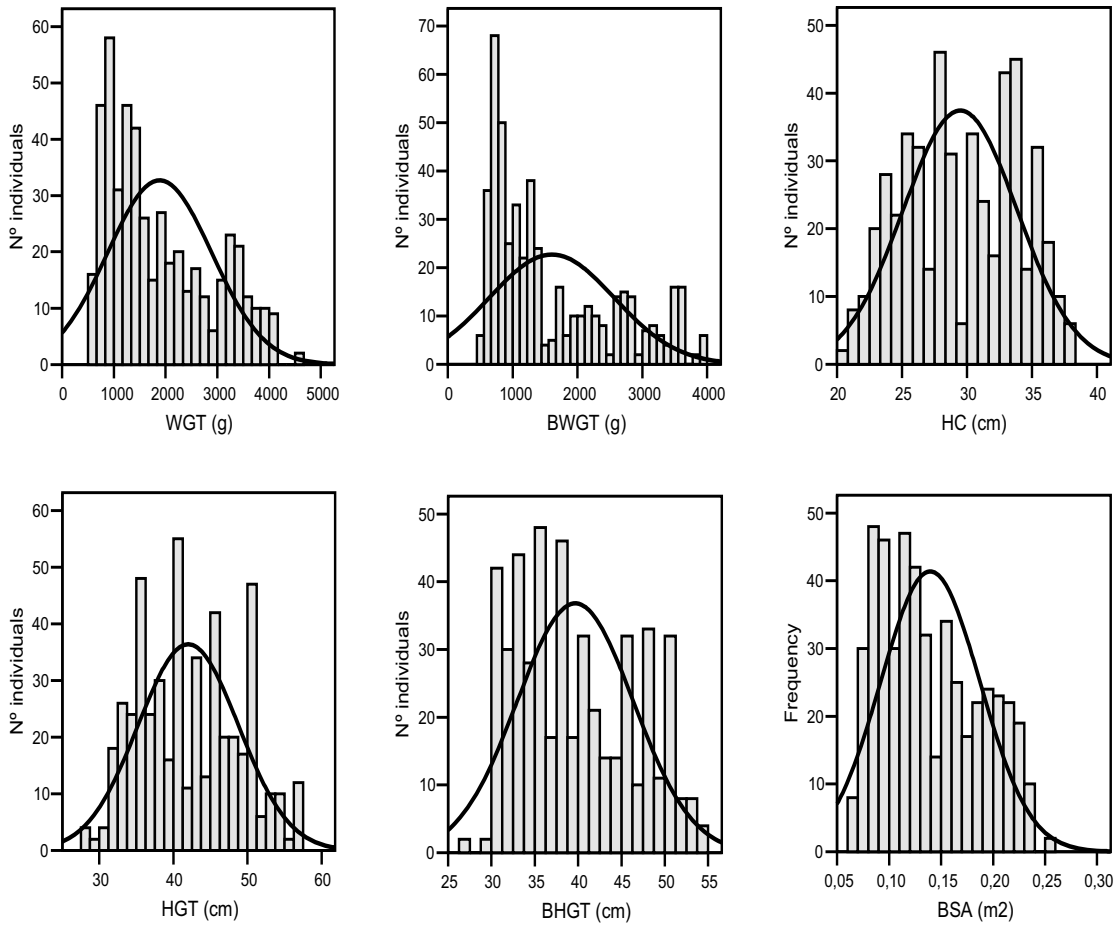
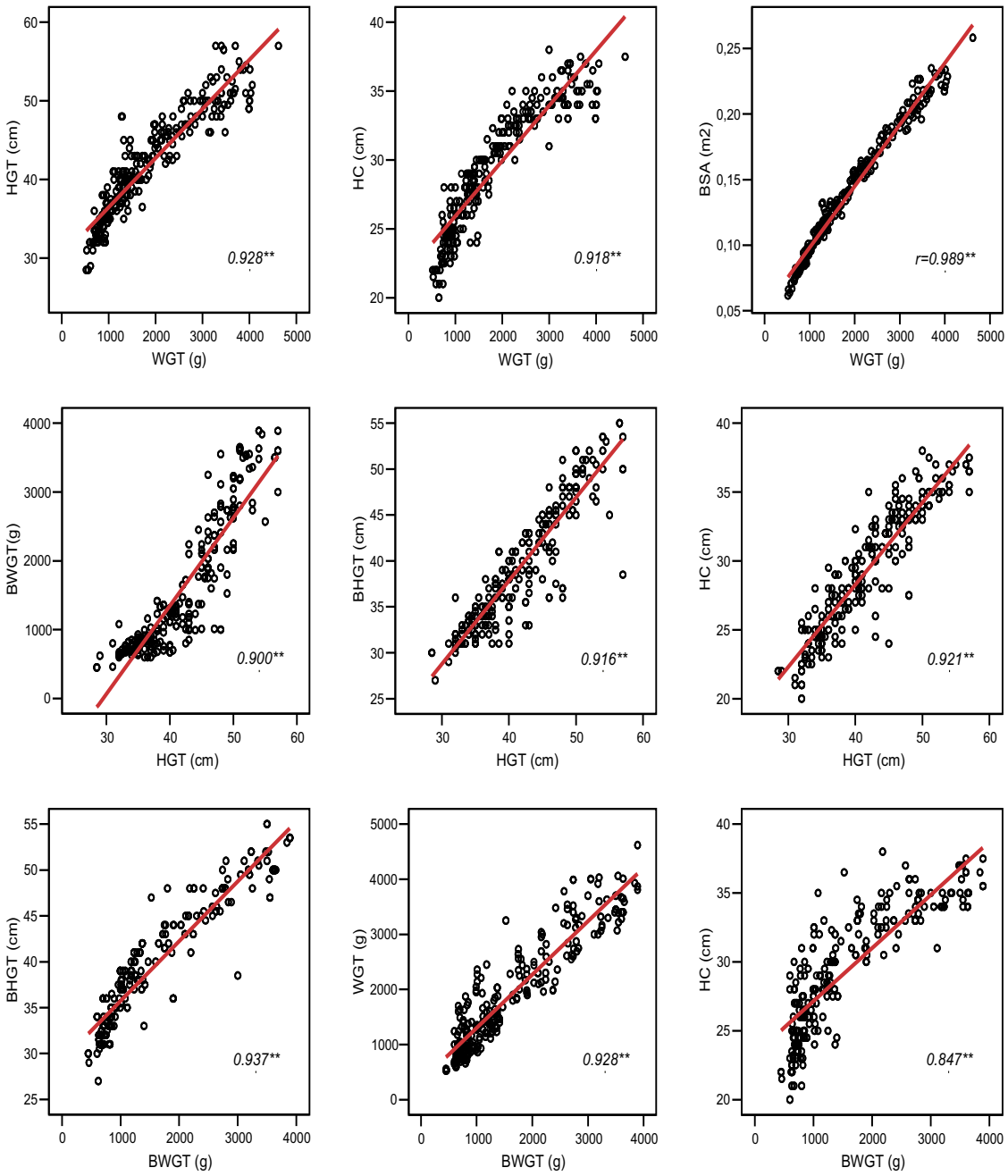


Figure 4.8. Histograms of the distributions of covariates related to body size

The Pearson correlation coefficient was statistically significant ($p<0.01$) among all the covariates related to body size (WGT, BWGT, HGT, BHGT, HC and BSA). A high linear correlation was found in all the cases (Figure 4.9).



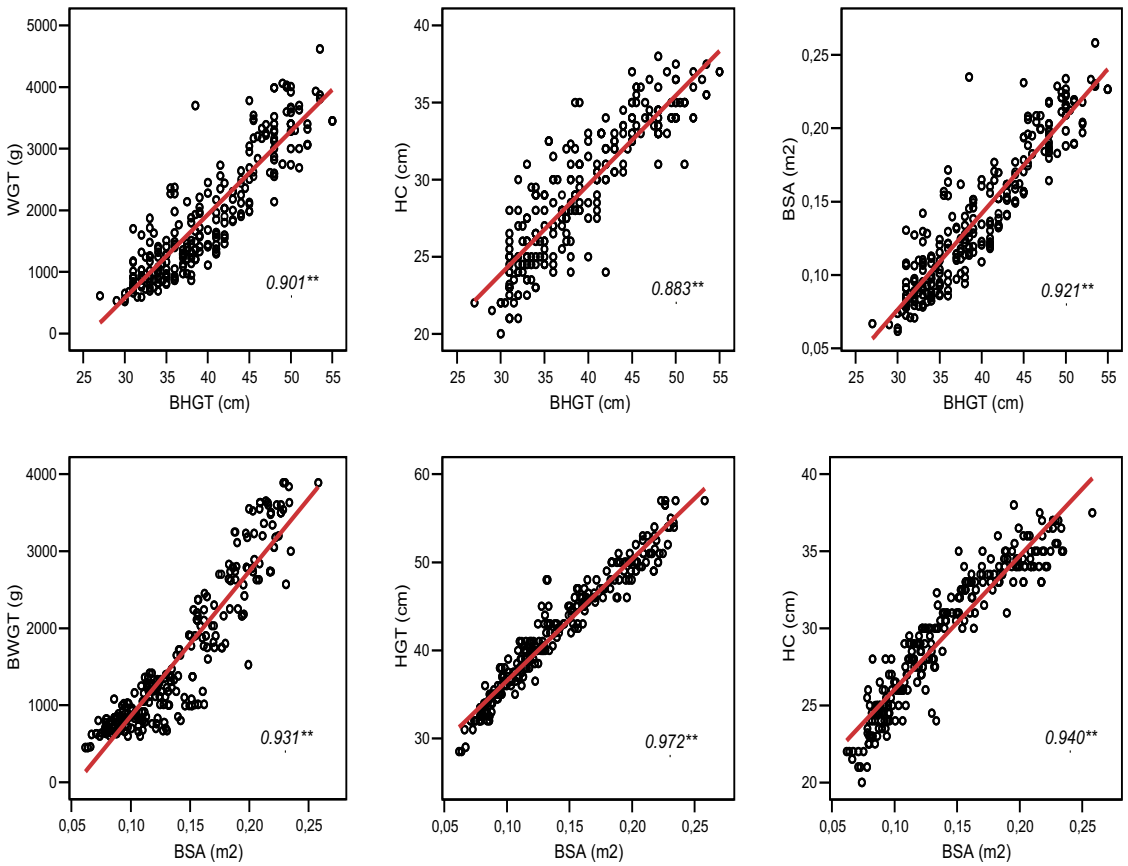


Figure 4.9. Relationships among all the covariates related to body size (*Pearson correlation coefficient at p=0.01 significant level***).

4.3.1.3 Covariates related to renal function

According to the Kolmogorov-Smirnov test, the assumption of normality was rejected for all of these covariates. Figure 4.10 shows the histograms corresponding to the statistical distribution of covariates related to renal function.

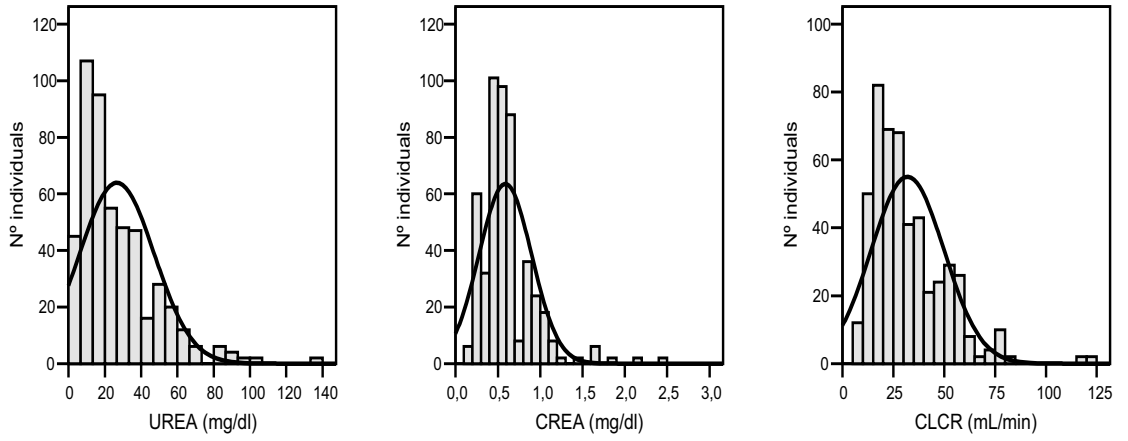


Figure 4.10. Histograms of the distributions of UREA, CREA and CLCR

When the relationships among these covariates (CLCR, CREA and UREA) were investigated, a high negative linear correlation was found between CREA and CLCR (Pearson correlation coefficient = -0.655, p<0.01), being positive between CREA and UREA (Pearson correlation coefficient = 0.529, p<0.01). A low linear correlation was found between CLCR and UREA (Pearson correlation coefficient = -0,165, p<0.01). Figure 4.11 shows the relationships among covariates related to renal function.

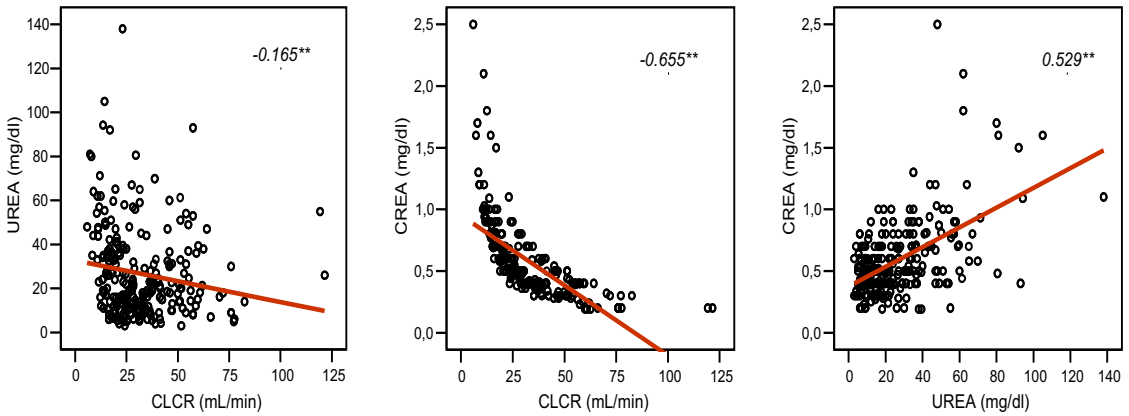


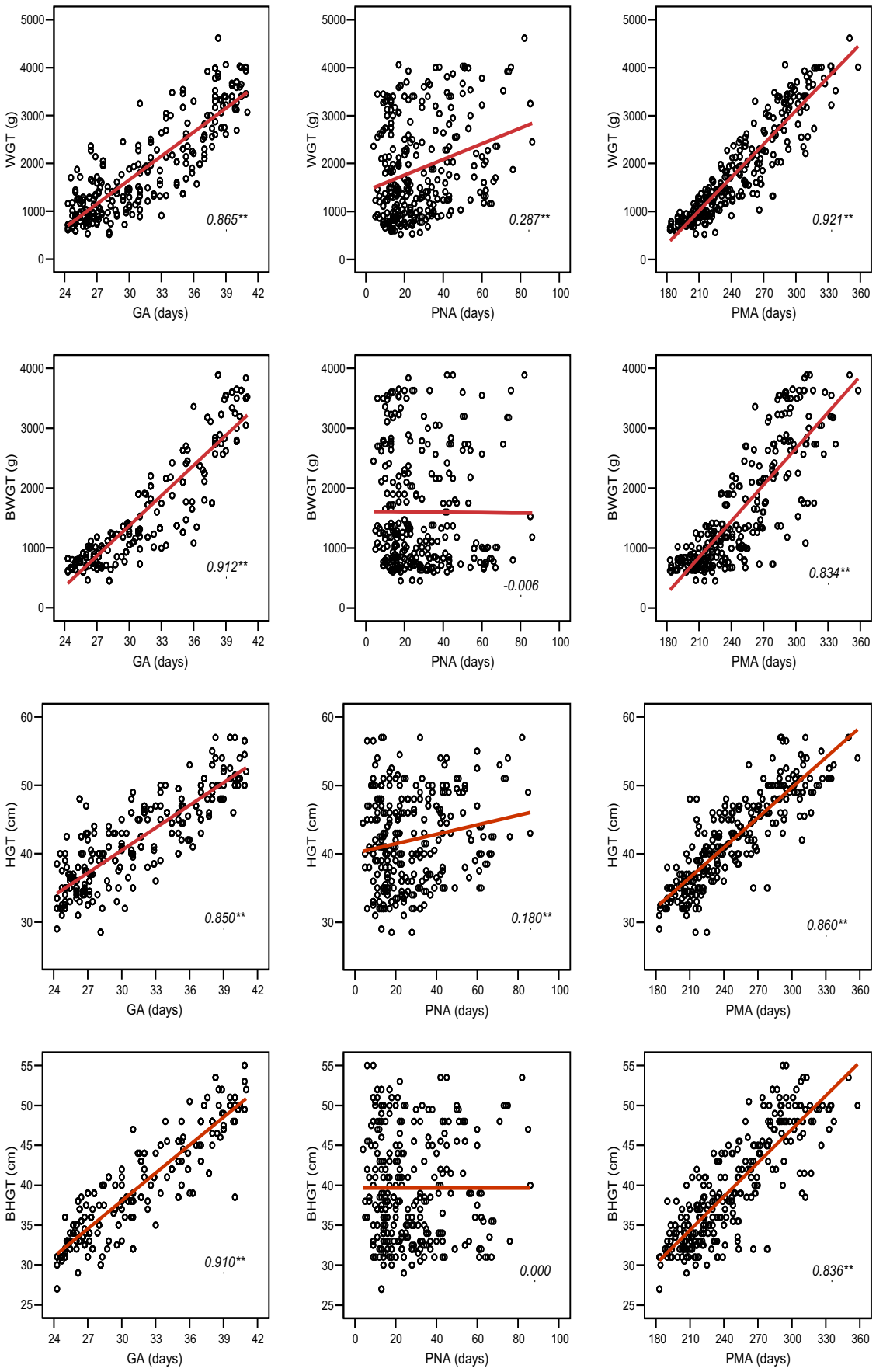
Figure 4.11. Relationships among covariates related to renal function (*Pearson correlation coefficient at p=0.01 significant level***).

4.3.1.4 Relationships among covariates related to renal function, body size and age

The relationships among covariates representing age, body size and renal function were analyzed. Graphics were used for the inspection of potential correlations, and the Pearson correlation coefficient was used to investigate the existence of linear correlation.

Covariates related to body size vs age covariates

Figure 4.12 shows the relationships among covariates related to body size and ages (GA, PMA and PNA). The Pearson correlation coefficient was statistically significant (p<0.01) in most cases, except for the relationship between PNA vs BWGT and PNA vs BHGT. There was a high linear correlation between GA and PMA vs all covariates related to body size (Pearson correlation coefficients between 0.83 and 0.92), while it was low between PNA vs WGT, HGT, HC and BSA (Pearson correlation coefficients between 0.18 and 0.29).


WGT (g)
GA (days)
0.865**
WGT (g)
PNA (days)
0.287**
WGT (g)
PMA (days)
0.921**
BWGT (g)
GA (days)
0.912**
BWGT (g)
PNA (days)
-0.006
BWGT (g)
PMA (days)
0.834**
HGT (cm)
GA (days)
0.850**
HGT (cm)
PNA (days)
0.180**
HGT (cm)
PMA (days)
0.860**
BHGT (cm)
GA (days)
0.910**
BHGT (cm)
PNA (days)
0.000
BHGT (cm)
PMA (days)
0.836**

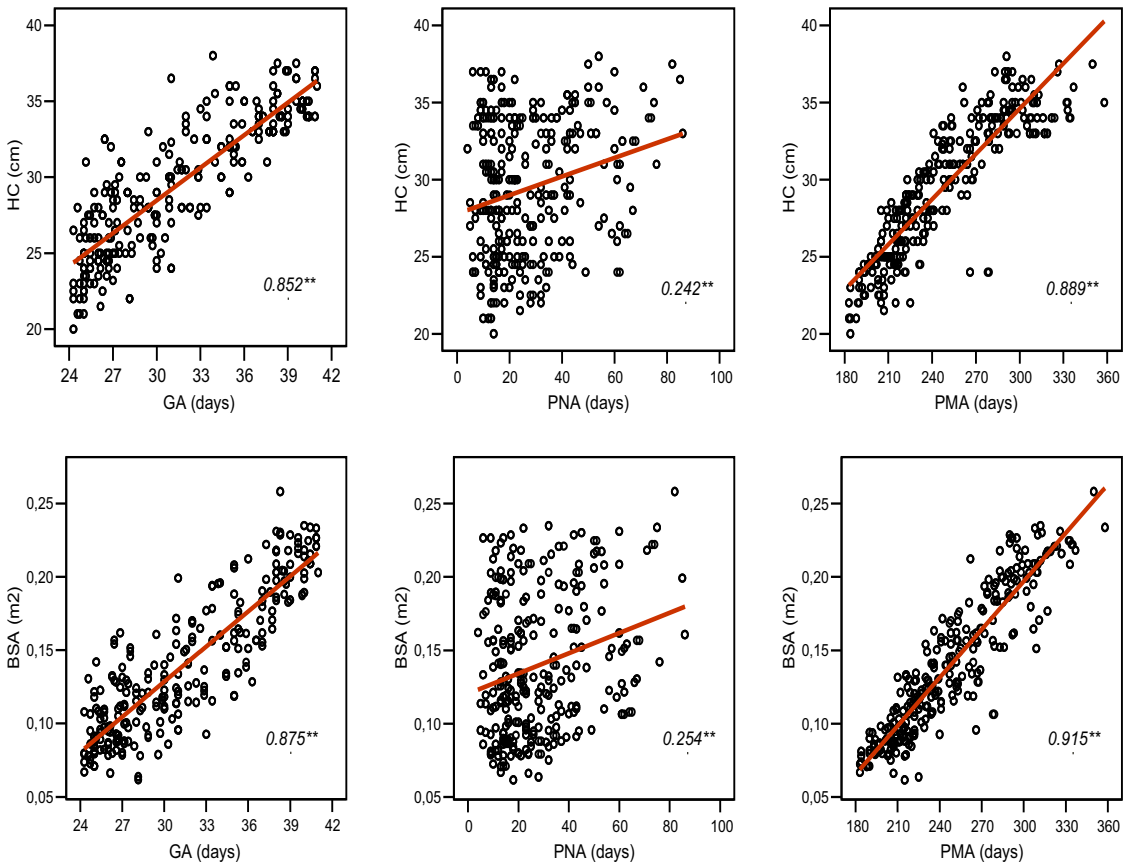


Figure 4.12. Relationships among covariates related to body size and ages (GA, PNA and PMA) (*Pearson correlation coefficient at p=0.01 significant level***).

Covariates related to renal function vs age covariates

Regarding to relationships among age covariates and renal function markers (Figure 4.13), the Pearson correlation coefficient was statistically significant ($p<0.01$) in all the cases with the exception of CREA vs GA relationship. None other kind of relationship was found between CREA and GA. The highest linear correlation was found between CLCR vs PMA (Pearson correlation coefficient = 0.582), followed by CLCR vs GA and PNA, and also between CREA vs PNA relationships, while low correlations were found between UREA vs all age covariates, and between CREA vs PNA.

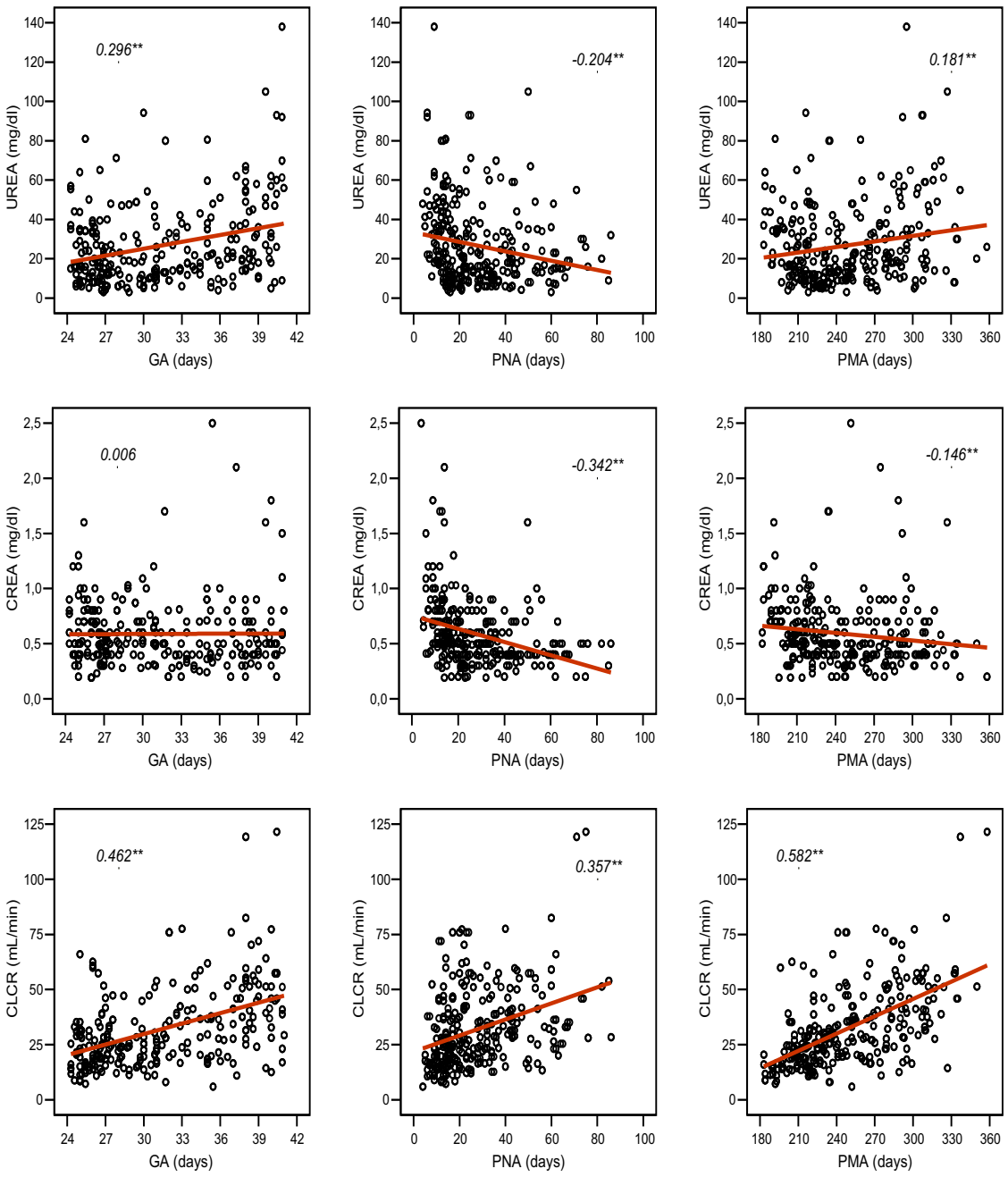


Figure 4.13. Relationships among ages and renal function parameters (*Pearson correlation coefficient at p=0.01 significant level**).*

Because CLCR had a higher linear correlation with age covariates than other renal function parameters, changes of CLCR with time were also investigated. Figure 4.14 shows the evolution of the mean CLCR with GA. According to this plot, CLCR tended to increase gradually with GA, except between 35 and 37 weeks of GA where there was an unexpected decrease of CLCR. Similarly occurred for GA of 41 weeks. Therefore, further inspection of these data would be required to guess the causes of it. Morevoer, high variabilities were observed for any of the GA groups.

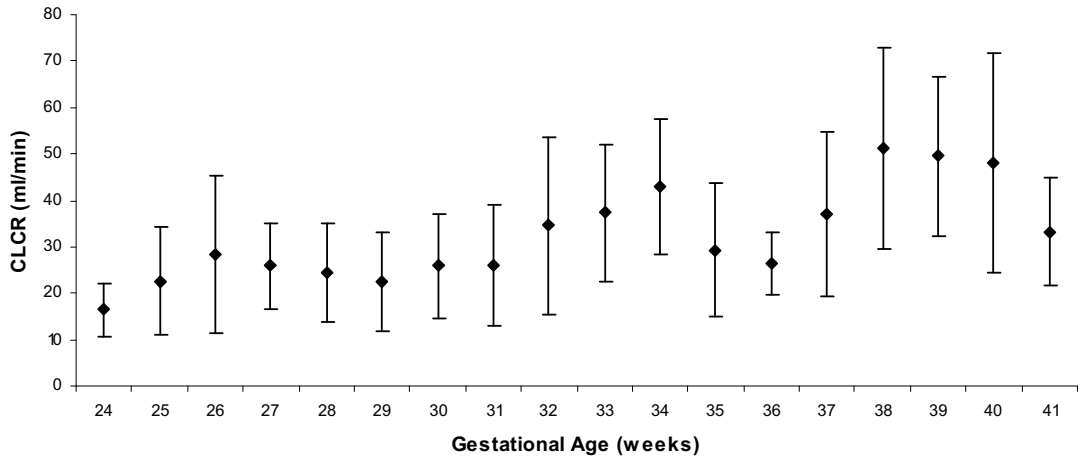


Figure 4.14. Evolution of mean values of CLCR with gestational ages

The evolution of the mean CLCR along PNA within each one of the previous GA groups (≤31 days, 32-36 days and ≥37 days) was also studied (Figure 4.15).

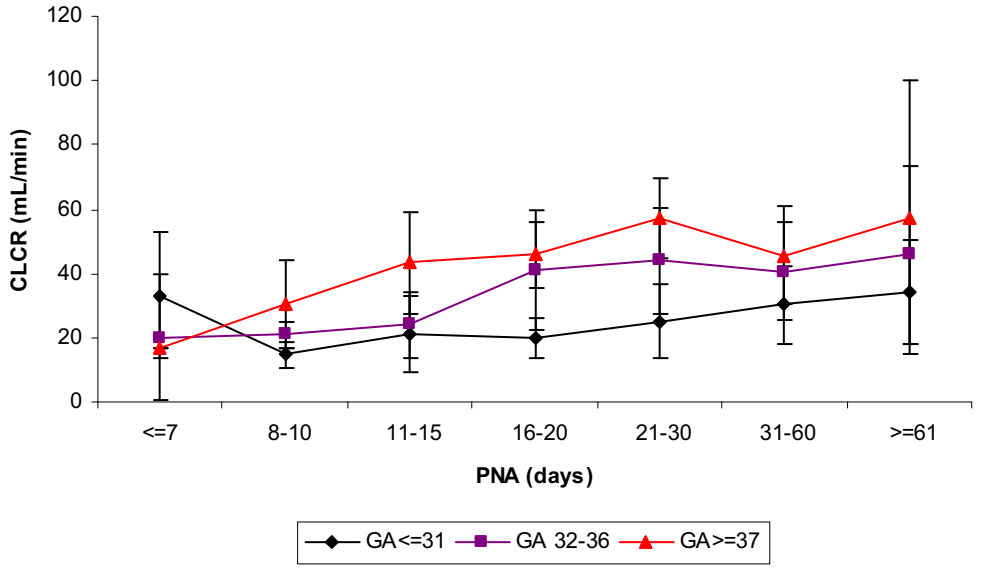


Figure 4.15. Evolution of CLCR (mean) with PNA within the gestational ages groups of ≤31 days, 32-36 days and ≥37 days.

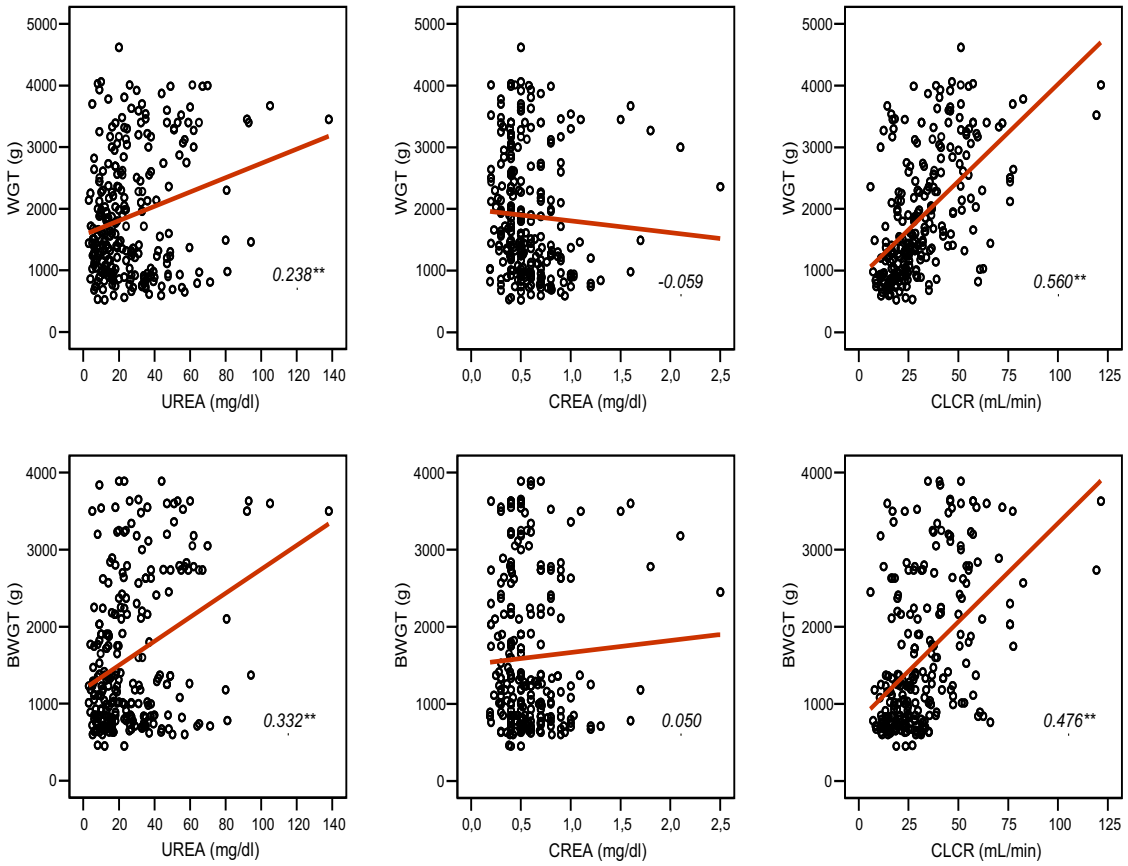
Visual inspection of this plot indicates that CLCR values increased with PNA, regardless of the GA group considered. The group of gestational ages ≤ 31 weeks had the lowest CLCR values independently of PNA. The exception was for PNA ≤ 7 days, in which case the highest values were observed. These results should be taken with caution due to the low number of data available for PNA ≤ 7 days among all GA groups (only 12 CLCR values from 6 individuals with GA ≤ 31 weeks; 6 CLCR values from 3 individuals with GA from 32 to 36 weeks, and 2 values from only 1 individual with GA ≥ 37 weeks).

Regarding to the 32-36 weeks GA group, CLCR increased with PNA with values close to those of the lowest GA group until days 11-15 of life. From this point a greater increase was observed.

CLCR values of the term newborns (GA ≥ 37 weeks) were the highest, excepting for PNA ≤ 7 days, increasing with postnatal age, with values close and higher to those of the other GA groups. The improvement of renal function with both GA and PNA confirmes the renal function maturation with time.

Covariates related to body size vs renal function markers

Relationships among covariates related to body size and renal function parameters are presented in Figure 4.16. The highest correlation with body size covariates was found for CLCR, whereas very low correlations were found for UREA, and no statistically significant correlation was found for CREA. The highest correlation between CLCR and body size parameters could be explained by the fact that body size is taken into account in the calculation of CLCR according to the Schwarz formula.



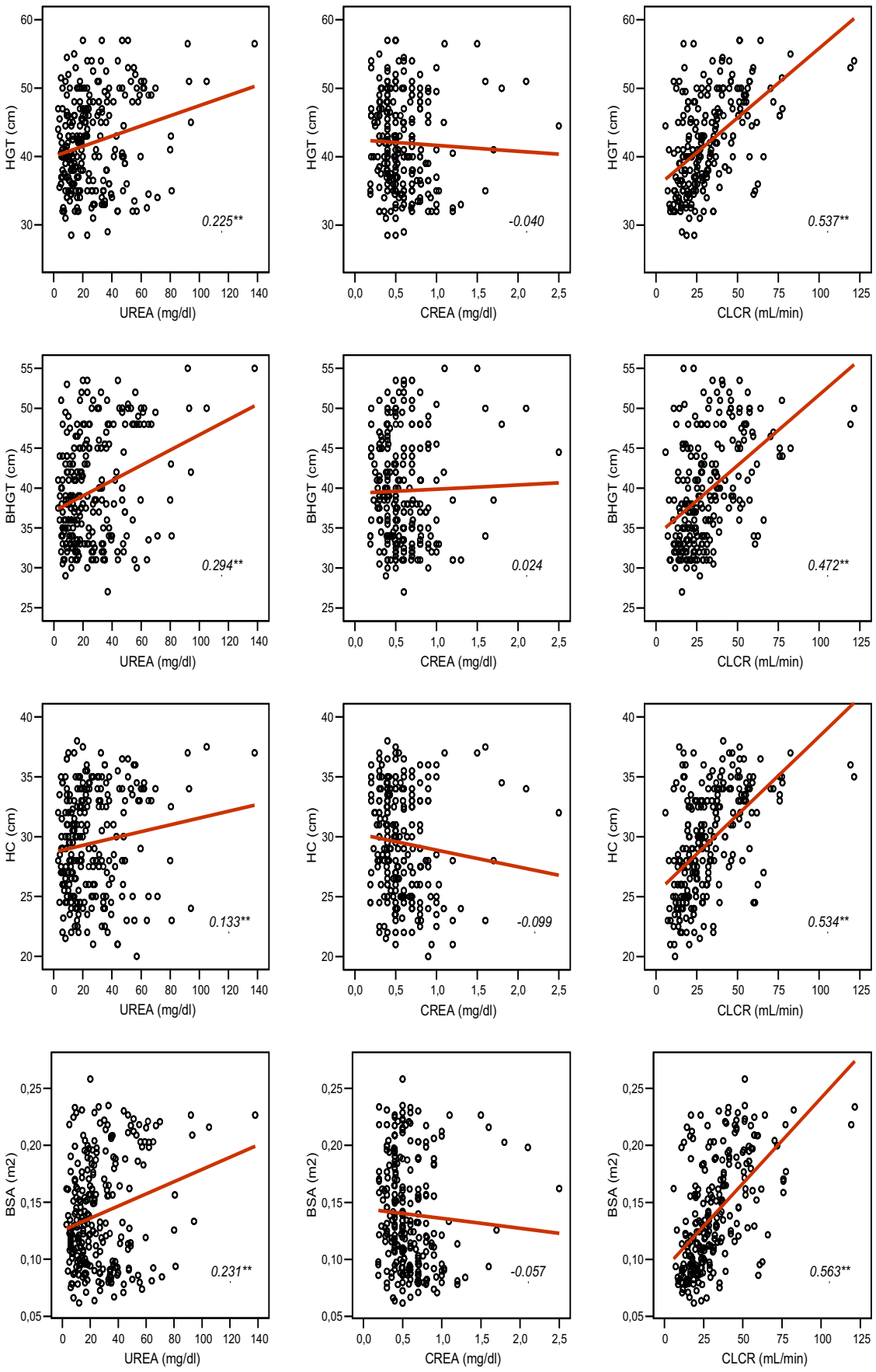


Figure 4.16. Relationships of collinearity among covariates related to body size renal function covariates (*Pearson correlation coefficient at p=0.01 significant level**).*

4.3.2 Model development

4.3.2.1 Base model development

The base model development was performed in several steps, starting out by fitting the most simple pharmacokinetic model (one-compartment model) and then going on through higher complex models. Interoccasion variability modeling was also tested (121). Results corresponding to the three approaches of handling BLQ data before mentioned are described below.

Data analysis including BLQ data, treated as continuous data

Table 4.4 summarizes the most relevant steps of the model building process from simultaneous analysis of concentration vs time data including BLQ values (reporting BLQ values as LLOQ of the analytical method). Number of compartments, parameters to which between-patient variability was associated, type of residual error model and estimation method tested in each step, as well as results and decisions taken from these, are presented in this table.

Table 4.4. Summary of the base model development strategy from concentrations vs time data including BLQ values.

Strategy		Model	Results	ΔOFV	Compared to model	Model selected	Decision
PK Model **IIV** **REM** **EM**	One-compartment CL Additive FOCE	Run 1	**Obj:** 2651.60	-	-	Run 1	Try proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Proportional FOCE	Run 2	**Obj:** 3125.48 • Adjust error	+473.88	Run 1	Run 1	Try additive-proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Additive-proportional FOCE	Run 3	**Obj:** 2442.557 • Significant decrease of Obj with respect to Run1	-209.04	Run 1	Run 3	$EST FOCE I
PK Model **IIV** **REM** **EM**	One-compartment CL Proportional FOCE I	Run 4	**Obj:** 2329.596	-	-	Run 4	Improve of OFV Try additive-proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Additive-proportional FOCE I	Run 5	**Obj:** 2302.867 • Significant decrease of Obj with respect to Run4	-26.73	Run 4	Run 5	IIV on V
PK Model **IIV** **REM** **EM**	One-compartment V Additive-proportional FOCE I	Run 6	**Obj:** 2393.870 • Not significant decrease of Obj with respect to Run5	+91.00	Run 5	Run 5	IIV on CL and V
PK Model **IIV** **REM** **EM**	One-compartment Cl+V Additive-proportional FOCE I	Run 7	**Obj**: 2245.526 • Significant decrease of Obj with respect to Run5	-57.34	Run 5	Run 7	Try two-compartment model
PK Model **IIV** **REM** **EM**	Two-compartment Cl Additive FOCE	Run 8	**Obj:** 2632.98	-	-	Run 8	Try proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment Cl Proportional FOCE	Run 9	**Obj:** 2804.38 • Not significant decrease of Obj with respect to Run8	+171.40	Run 8	Run 8	Try additive-proportional REM

PK Model **IIV** **REM** **EM**	Two-compartment Cl Additive-Proportional FOCE	Run 10	**Obj:** 2391.792 • Significant decrease of Obj with respect to Run8	-241.19	Run 8	Run 10	IIV on CL+V1
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1 Additive-Proportional FOCE	Run 11	**Obj:** 2336.538 • Significant decrease of Obj with respect to Run10	-55.25	Run 10	Run 11	IIV on CL+V1+Q
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1+Q Additive-Proportional FOCE	Run 12	**Obj:** 2317.513 • Significant decrease of Obj with respect to Run11	-19.03	Run 11	Run 12	IIV on CL+V1+Q+V2
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1+Q+V2 Additive-Proportional FOCE	Run 13	**Obj:** 2317.513 • Not significant decrease of Obj with respect to Run12 • RSE of θ3-Q higher than 40%.	+00.00	Run 12	Run 12	$EST FOCE I
PK Model **IIV** **REM** **EM**	Two-compartment CL Proportional FOCE I	Run 14	**Obj:** 2211.346	-	-	Run 14	Try addivitive-proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment CL Additive-Proportional FOCE I	Run 15	**Obj:** 2210.242 • Not significant decrease of Obj with respect to Run14 • Improve precision estimation of parameters.	-1.10	Run 14	Run 15	FOCE I betther than FOCE IIV on CL+V1
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1 Additive-Proportional FOCE I	Run 16	**Obj:** 2169.076 • Significant decrease of Obj with respect to Run15	-41.17	Run 15	Run 16	IIV on CL+V1+Q **FINAL MODEL**
Model PK **IIV** **REM** **EM**	Two-compartment CL+V1+Q Additive-Proportional FOCE I	Run 17	**Obj:** 2132.931 • Significant Decrease of Obj with respect to Run16 • Covariance step aborted.	-36.15	Run 16	Run 16	IIV on CL+V1+V2
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1+V2 Additive-Proportional FOCE I	Run 18	**Obj:** 2166.101 • Not significant decrease of Obj with respect to Run16	+2.98	Run 16	Run 16	-

PK Model: *Pharmacokinetic Model.* ***IIV:*** *Interindividual variability parameters.* ***REM:*** *Residual Error. Model* ***EM:*** *Estimation Method (Method 1: FOCE, First Order Conditional Estimation Method ; Method 1 + Interaction: FOCE1, First Order Conditional Estimation with Interaction)*

After the analysis of all amikacin concentration vs time data, the First Order Conditional Estimation Method with Interaction resulted in a better precision of the pharmacokinetic parameters estimated, so that models that applied this method were considered for comparison and further selection of the best base model. According to the Akaike Criterion, the two-compartment model provided the best fit of the data, so that when models of two compartments were compared to the corresponding one compartment models an statistical reduction of the AIC values was found in all the cases. Specifically, AIC was reduced 76.45 units from model 7 (one-compartment) to 16 (two-compartment). The combined residual error model (additive and proportional) provided a better fit than the proportional error model with a lower OFV achieved (model 9: proportional error OFV=2804.38 vs model 10: combined error OFV=2391.79). Between-patient variability could be associated to plasma clearance (CL) and central compartment distribution volume (V1). Therefore, model 16 was selected as the best base model when BLQ data were included as continuous data values.

Figure 4.17 shows the goodness-of-fit plots corresponding to the base model (#16) developed using concentration vs time data including BLQ values.

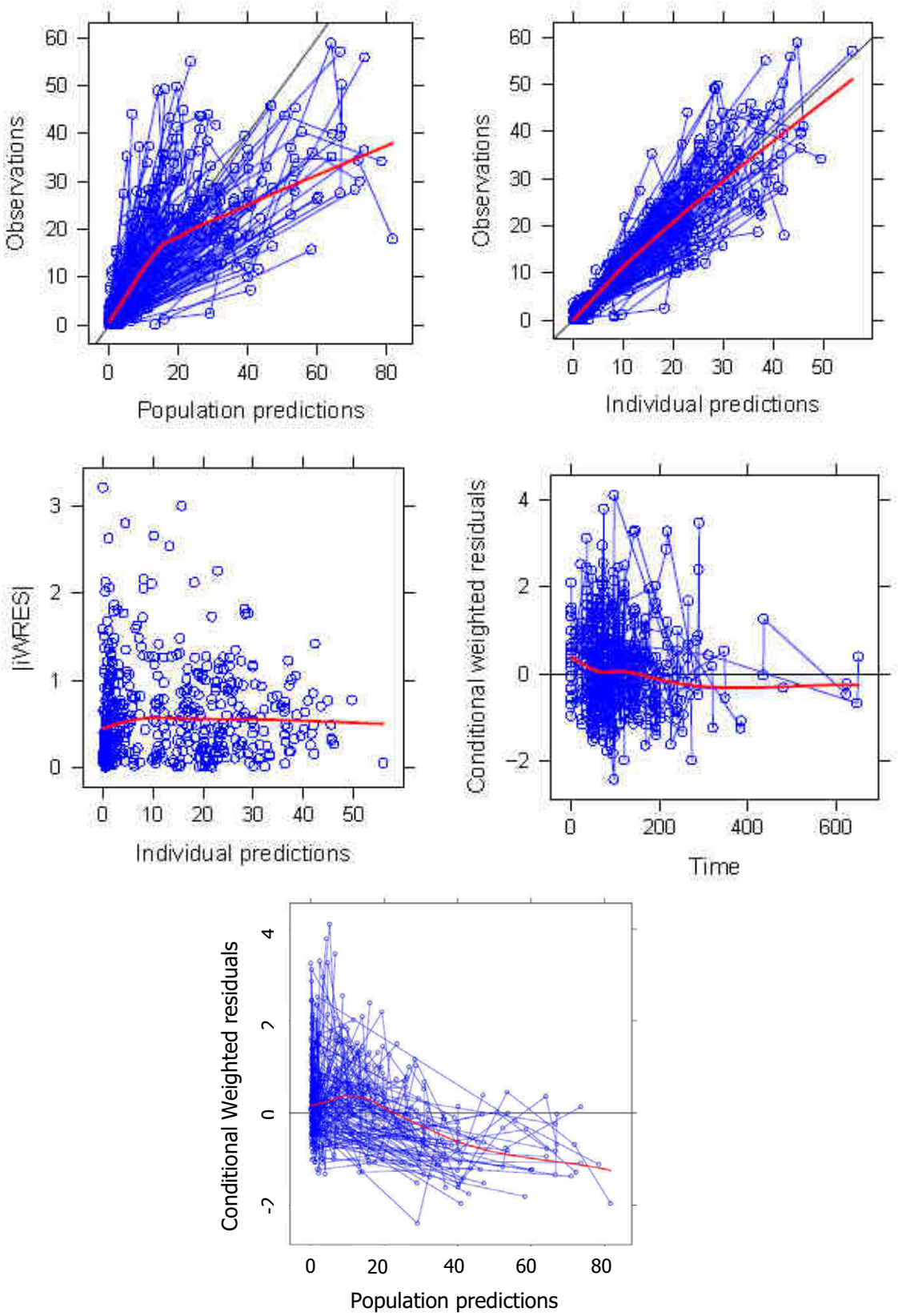


Figure 4.17. GOF plots of the final base PK model developed including BLQ values (#16).Upper panels: DV vs PRED (left) and IPRED (right) concentrations. Middle panels: IWRES vs IPRED (left) and CWRES (calculated as PRED-DV) vs TIME (right). Lower panel: CWRES (calculated as DV-PRED) vs PRED. Concentrations expressed as mg/L. *Black solid line: identity line. Red solid line: smoothed line showing the general trend of the data.*

The evaluation of the structural part of the model was done trough the goodness-of-fit plots of DV vs PRED, CWRES vs TIME and also CWRES vs PRED. All data points of the selected final base model were homogeneously distributed around identity line on DV vs PRED plot, and were fairly well centered on the zero-line on CWRES vs TIME and CWRES vs PRED plots. Otherwise, CWRES vs PRED shows a tendency of the model to overestimate at high concentrations, as can be also observed on DV vs PRED. Goodness-of-fit plots of DV vs IPRED and IWRES vs IPRED have already some variability incorporated, not bringing much information for the evaluation of the structural part of the model.

Data analysis after removing BLQ data

Table 4.5 summarizes the most relevant steps of the model building process from simultaneous analysis of concentration vs time data after removing BLQ values. As before, number of compartments, parameters to which between-patient variability was associated, type of residual error model and estimation method tested in each step, as well as results and decisions taken from these, are presented in this table.

Again, the First Order Conditional Estimation Method with Interaction resulted in a better precision in the pharmacokinetic parameters estimated, so that models that used this method were considered for comparison and further selection of the best base model. According to the Akaike Criterion, the two-compartment model provided the best fit of the data, so that when models of two compartments were compared to the corresponding one compartment models an statistical reduction of the AIC values was found in all the cases. Specifically, AIC was reduced 71.43 units from model 25 (one-compartment) to 32 (two-compartment). The combined residual error model (additive and proportional) provided a better fit than the proportional error model with a lower OFV achieved (model 30: proportional error OFV=2142.475 vs model 32:combined error OFV=2092.812). Between-patient variability could be associated to plasma clearance (CL), central compartment distribution volume (V1) and intercompartmental or distributional clearance (Q). Therefore, model 33 was selected as the best base model when BLQ data were removed from the analysis.

Table 4.5. Summary of the base model development strategy from concentrations vs time data after removing BLQ values.

Strategy		Model	Results	ΔOFV	Compared to model	Model selected	Decision
PK Model **IIV** **REM** **EM**	One-compartment CL Additive FOCE	Run 19	**Obj:** 2477.502	-	-	Run19	Try proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Proportional FOCE	Run 20	**Obj:** 2584.849 • Not significant decrease of Obj with respect to Run19	+107.35	Run19	Run19	Try additive-proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Additive-proportional FOCE	Run 21	**Obj:** 2368.466 • Significant decrease of Obj with respect to Run19	-109.04	Run19	Run21	$EST FOCE I
PK Model **IIV** **REM** **EM**	One-compartment CL Proportional FOCE I	Run22	**Obj:** 2278.247	-	-	Run22	Improve of OFV Try additive-proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Additive-proportional FOCE I	Run23	**Obj:** 2248.981 • Significant decrease of Obj with respect to Run22	-29.27	Run22	Run23	IIV on V
PK Model **IIV** **REM** **EM**	One-compartment V Additive-proportional FOCE I	Run24	**Obj:** 2393.870 • Not significant decrease of Obj with respect to Run23	+144.89	Run23	Run23	IIV on CL+V
PK Model **IIV** **REM** **EM**	One-compartment Cl+V Additive-proportional FOCE I	Run25	**Obj:** 2168.245 • Significant decrease of Obj with respect to Run23	-80.74	Run23	Run25	Try two-compartment model
PK Model **IIV** **REM** **EM**	Two-compartment Cl Additive FOCE	Run26	**Obj:** 2457.019	-	-	Run26	Try proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment Cl Proportional FOCE	Run27	**Obj:** 2519.596 • Not significant decrease of Obj with respect to Run26	+62.58	Run26	Run26	Try additive-proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment Cl Additive-Proportional FOCE	Run28	**Obj:** 2318.367 • Significant decrease of Obj with respect to Run26	-138.65	Run26	Run28	IIV on CL+V1
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1 Additive-Proportional FOCE	Run29	**Obj:** 2336.538 • Not significant decrease of Obj with respect to Run28	+18.17	Run28	Run28	$EST FOCE I
PK Model **IIV** **REM** **EM**	Two-compartment CL Proportional FOCE I	Run30	**Obj:** 2142.475	-	-	Run30	Try additive-proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment CL Additive-Proportional FOCE I	Run31	**Obj:** 2140.493 • Not significant decrease of Obj with respect to Run30 • Improve precision estimation of parameters	-1.98	Run30	Run31	IIV on CL+V1
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1 Additive-Proportional FOCE I	Run32	**Obj:** 2092.812 • Significant decrease of Obj with respect to Run31	-47.68	Run31	Run32	IIV en el CL+V1+Q
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1+Q Additive-Proportional FOCE I	Run33	**Obj:** 2075.969 • Significant decrease of Obj with respect to Run32 • Improve precision estimation of parameters	-16.84	Run32	Run33	**FINAL MODEL**

PK Model: *Pharmacokinetic Model.* ***IIV:*** *Interindividual variability parameters.* ***REM:*** *Residual Error. Model* ***EM:*** *Estimation Method (Method 1: FOCE, First Order Conditional Estimation Method ; Method 1 + Interaction: FOCE1, First Order Conditional Estimation with Interaction)*

Figure 4.18 shows the goodness of-fit-plots of the best base compartment model (#33) developed removing BLQ data from the analysis.

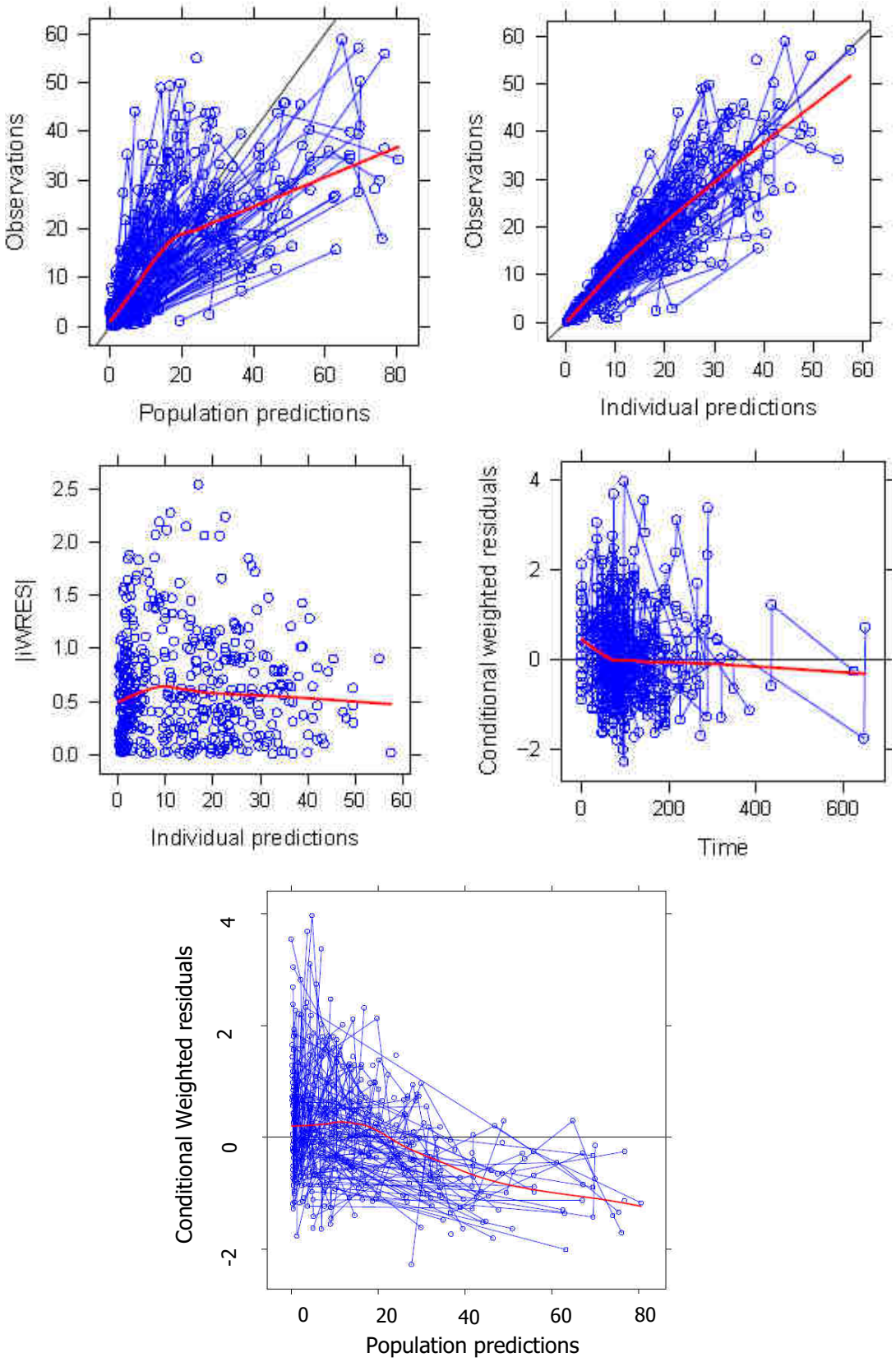


Figure 4.18. GOF plots of the final base PK model developed removing BLQ values (#33).Upper panels: DV vs PRED (left) and IPRED (right) concentrations. Middle panels: IWRES vs IPRED (left) and CWRES (calculated as PRED-DV) vs TIME (right). Lower panel: CWRES (calculated as DV-PRED) vs PRED. Concentrations expressed as mg/L. *Black solid line: identity line. Red solid line: smoothed line showing the general trend of the data.*

All data points of the goodness-of-fit plots that allowed the evaluation of the structural base model were randomly distributed around the indentity line (DV vs PRED) or zero-line (CWRES vs TIME and CWRES vs PRED). As in the model obtained including BLQ data, CWRES vs PRED showed a tendency of the model to overestimate at high concentrations, similarly at DV vs PRED plot.

Data analysis including BLQ data treated as censored data

Table 4.6 summarizes the most relevance steps of the model building process considering BLQ data as described by Method 3. As before, number of compartments, parameters to which between-patient variability was associated, type of residual error model and estimation method tested in each step, as well as results and decisions taken from these, are presented in this table.

Table 4.6 Summary of the base model development strategy from concentrations vs time data including BLQs treated as censored data.

Strategy		Model	Results	ΔOFV	Compared to model	Model selected	Decision
PK Model **IIV** **REM** **EM**	One-compartment CL Additive FOCE I Laplacian	Run 40	**Obj:** 2532.832 • Minimization succesfull. However… covariance aborted	-	-	-	Try proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Proportional FOCE I Laplacian	Run 41	**Obj:** 2927.735 • Minimization succesfull. However… covariance aborted	-	-	-	Try additive-proportional REM
PK Model **IIV** **REM** **EM**	One-compartment CL Additive-proportional FOCE I Laplacian	Run 42	**Obj:** 2418.804 • Minimization terminated due to rounding errors (E=134)	-	-	-	Try two-compartment model
PK Model **IIV** **REM** **EM**	Two-compartment Cl Additive FOCE I Laplacian	Run 43	**Obj:** 2533.548 • Minimization terminated due to rounding errors (E=134)	-	-	-	Try proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment Cl Proportional FOCE I Laplacian	Run 44	**Obj:** 2762.330 • Minimization terminated due to rounding errors (E=134)	-	-	-	IIV on CL+V1
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1 Proportional FOCE I Laplacian	Run 45	**Obj:** 2751.109 • Minimization terminated due to rounding errors (E=134)	-	-	-	IIV en el CL+V1+Q
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1+Q Proportional FOCE I Laplacian	Run 46	**Obj:** 2734.808 • Minimization succesfull. However… covariance aborted	-	-	-	IIV en el CL+V1+V2
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1+V2 Proportional FOCE I Laplacian	Run 47	**Obj:** 2739.139 • Minimization terminated due to rounding errors (E=134)	-	-	-	Try additive-proportional REM
PK Model **IIV** **REM** **EM**	Two-compartment CL Additive-Proportional FOCE I Laplacian	Run 48	**Obj:** 2399.574 • Minimization terminated due to rounding errors (E=134)	-	-	-	IIV on CL+V1
PK Model **IIV** **REM** **EM**	Two-compartment CL+V1 Additive-Proportional FOCE I Laplacian	Run 49	**Obj:** 2352.030 • Minimization succesfull. However… covariance aborted	-	-	-	IIV on CL+Q
PK Model **IIV** **REM** **EM**	Two-compartment CL+Q Additive-Proportional FOCE I Laplacian	Run 50	**Obj:** 2395.683 • Minimization terminated due to rounding errors (E=134)	-	-	-	

PK Model: *Pharmacokinetic Model.* ***IIV:*** *Interindividual variability parameters.* ***REM:*** *Residual Error. Model* ***EM:*** *Estimation Method (Method 1: FOCE, First Order Conditional Estimation Method ; Method 1 + Interaction: FOCE1, First Order Conditional Estimation with Interaction)*

The method that treated BLQ data as censored did not provide good fits in any case, so this method was discarded. For the other two methods, similar values of the most relevant pharmacokinetic parameters were obtained with both (Table 4.7), however the proportional residual error was overestimated when BLQ concentrations were included in the analysis. Due to the fact that the actual concentration values of BLQ data were lacking, the same value (0.09 mg/L) was assigned to all of them. This value may be overestimating the actual concentrations below the limit of quantification resulting in higher proportional residual error. Comparison of the goodness-of-fit-plots of the base population PK model developed either including BLQ data (Figure 4.17) or after removing BLQ data (Figure 4.18), was unsufficient to be used as a unique tool to discriminate and select the best base model.

Table 4.7. Population pharmacokinetic parameters of the base models developed from data either including the BLQ concentrations or not considering them.

		Including BLQ data (#16)	**Removing BLOQ data (#33)**
Pharmacokinetic parameters	CL (L/h)	0.137 (6.92)	0.129 (6.95)
	V (L/h)	-	-
	V1 (L)	0.658 (6.25)	0.595 (6.84)
	Q (L/h)	0.046 (19.89)	0.128 (14.84)
	V2 (L)	0.723 (18.53)	1.15 (11.39)
Between- patient variability	IIV-CL (%)	69.90 (14.62)	74.23 (15.01)
	IIV-V (%)	-	-
	IIV-V1 (%)	57.10 (17.70)	58.48 (19.04)
	IIV-Q (%)	-	80.72 (23.85)
Residual variability	Additive (mg/L)	0.41 (6.72)	0.42 (4.81)
	Proportional (%)	110 (16)	24.00 (32.88)
η_1-shrinkage	%	14.68	10.34
η_2-shrinkage	%	22.60	31.56
η_3-shrinkage	%	-	55.97
ε-shrinkage	%	19.40	19.95

RSE%: Precision given by the relative standard error in parenthesis

According to Bergtrand and Karlsson (115), there is no predictable pattern when BLQ data are replaced by LOQ/2 , sometimes reducing and sometimes inflating the bias in parameter estimates. In our case no relevant differences in the main parameter estimates were observed, but the inflated proportional residual error lead to select the base model developed after omitting BLQ data (#33). According to this:

- Two-compartment kinetic model with first order elimination process, parameterized as clearances and distribution volumes best described the PK of amikacin.
- Between-patient variability modelled exponentially was associated with plasma and distributional clearances and central compartment distribution volume.

- Combined residual error model (additive + proporcional) provided the best fit of data.
- FOCEI was used as estimation method.

The pharmacokinetic parameter estimates corresponding to the base model (model 33) and schrinkage values associated to the between-patient (η) and residual (ε) random effects are listed in Table 4.7. All the parameters were estimated with adequate precision (RSE< 33%). Between-patient variabilities were from 58.48% (V1) to 80.72% (Q). Additive residual error was 0.42 mg/L and proportional error was 24%. η_1-shrinkage value was around 10%, while η_2- and η_3-shrinkage were higher than 20%. ε-shrinkage was around 20%. Interoccasion variabilities on CL and V1 did not reduce significantly the objective function value.

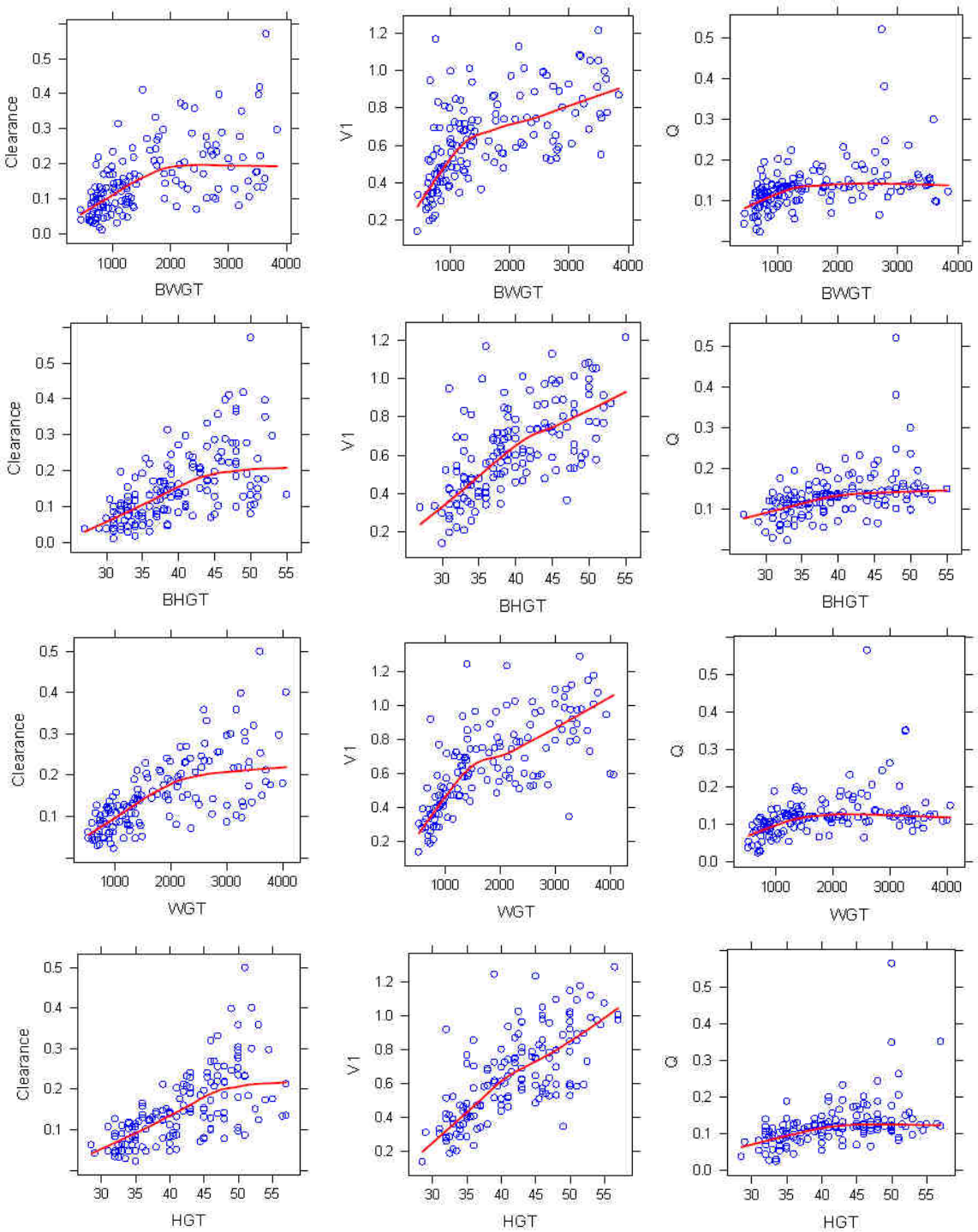
4.3.2.2 Covariate selection and final model development

Once the base model had been obtained, the covariate effects on pharmacokinetic parameters were studied. Among all the variables recorded, CLCR, CREA, UREA, BSA, WGT, HGT, HC, BWGT, HWGT, GA, PMA and PNA were taken as first level covariates, meanwhile BHC, APG1, APG5 were considered as second level covariates. GA and BWGT were also treated as categorical covariates: premature vs term neonates, and low birth weight vs very low birth weight vs extremely low birth weight, respectively. Gender, gestation number and twined pregnancy information was not considered relevant for the amikacin pharmacokinetics, so that their potential effects were not assessed. According with Savic et al (95), who reported that a shrinkage higher than around 20–30%, leads to EBE-based diagnostics lack informativeness, it would be desirable to use alternative diagnostic methods and direct testing of covariates on PK parameters with NONMEM during the covariate model building process. For this reason, .although the steps summarized for the covariate selection, all the covariates physiologically plausible were tested in NONMEM in order to avoid the falsely indicate relationships or even obscure relationships due to the presence of η- and ε-shrinkage, when it existed.

i. **Identification of potential correlations among covariates.** It was studied in order to avoid the possible overparameterization caused by the simultaneous introduction of covariates related to each other. Results corresponding to the most relevant potential correlations among recorded continuous covariates have been presented in section 4.3.1).

ii. **Identification of potential statistically significant covariates.**
   - Figure 4.19, Figure 4.20 and Figure 4.21 show the plots of individual Bayesian estimated from the base model versus the most relevant covariates from a physiological point of view to explain BPV associated with them.

Regarding to covariates related to body size, all covariates (BWGT, BHGT, WGT, HGT, HC, BSA) were correlated with either CL or V1. No marked trend was observed in the relationship between the distributional clearance and any of the covariates related to body size (Figure 4.19).



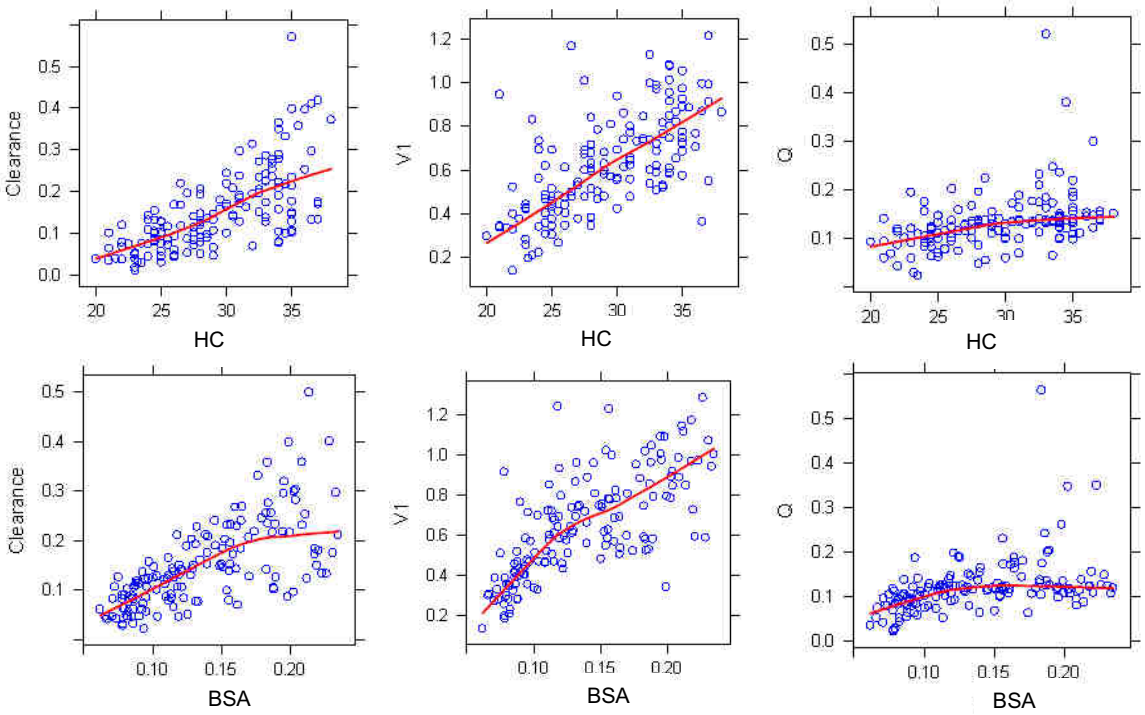
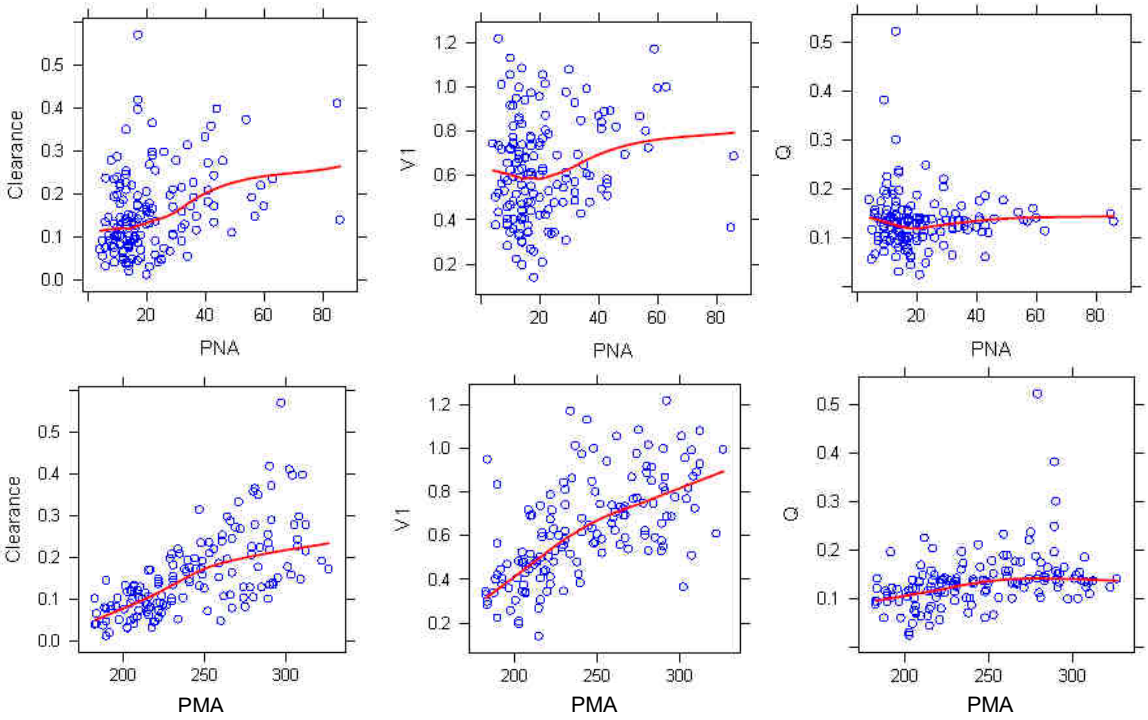


Figure 4.19. Plots of Individual Bayesian values of parameters CL, V1 and Q, vs covariates related to body size. *Solid red line: smoothed line showing the general trend of the data.*

PMA and GA correlated with either CL or V1. A less marked trend existed between PNA and CL, while it did not exist between PNA and CL, but neither between any of the ages nor distributional clearance (Figure 4.20).



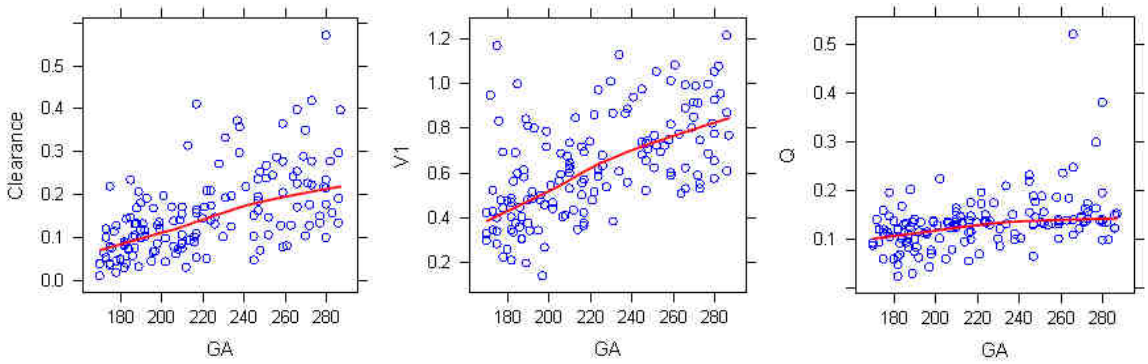
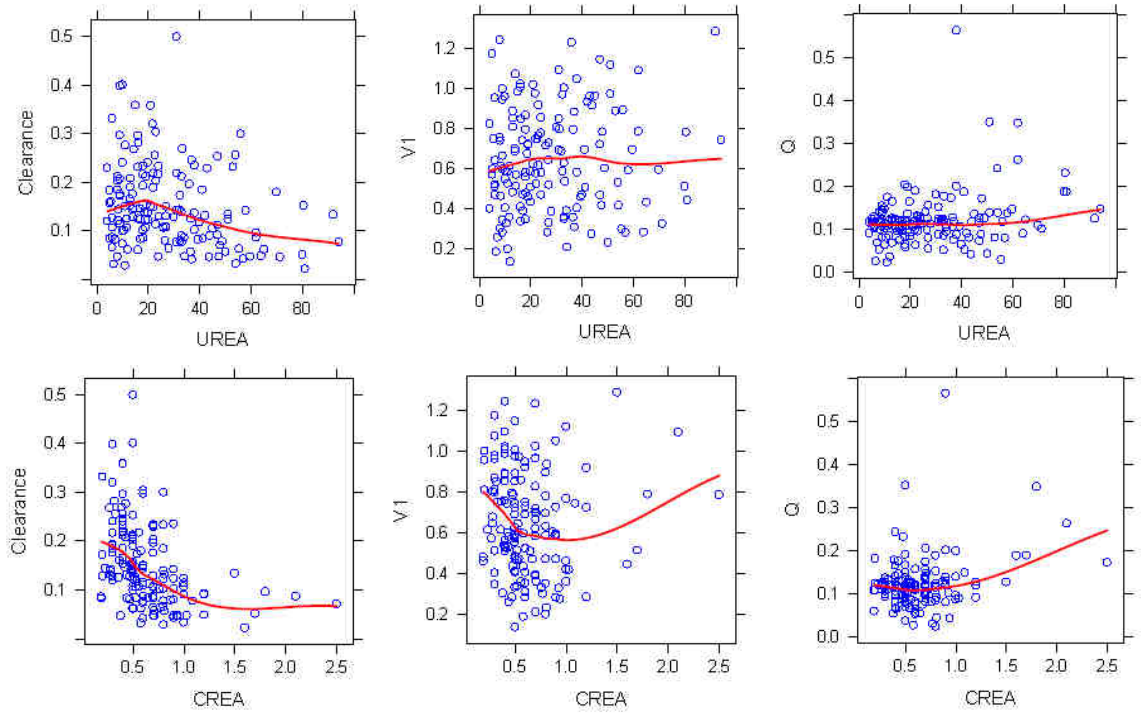


Figure 4.20. Pots of Individual Bayesian values of parameters CL, V1 and Q, vs covariates related to age. *Solid red line: smoothed line showing the general trend of the data.*

Plasma clearance values also correlated with all the covariates related to renal function. Although visual inspection suggested a correlation between V1 and CLCR values, it was not considered further because it did not make sense from a physiological point of view. In fact other factors as body weight could be responsible of this, since CLCR, as described before (section 4.3.1.1), is also highly correlated with bodyweight. No correlations existed between V1 and the other renal covariates (CREA and UREA) neither between any renal function covariate and Q (Figure 4.21).



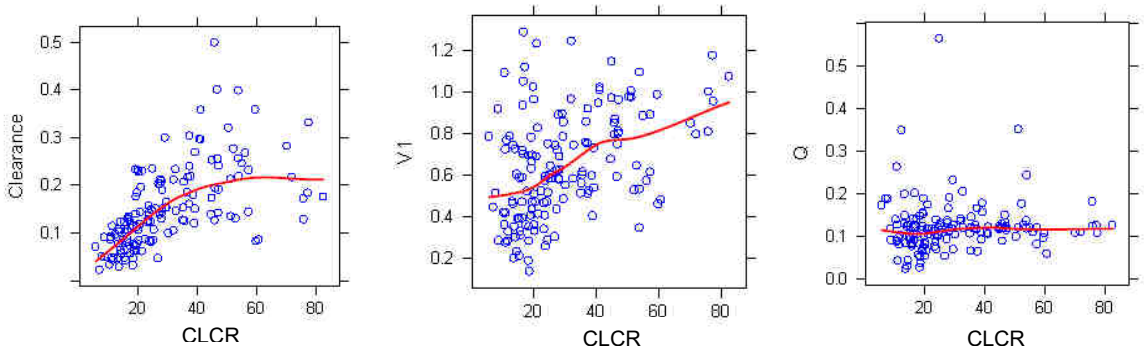
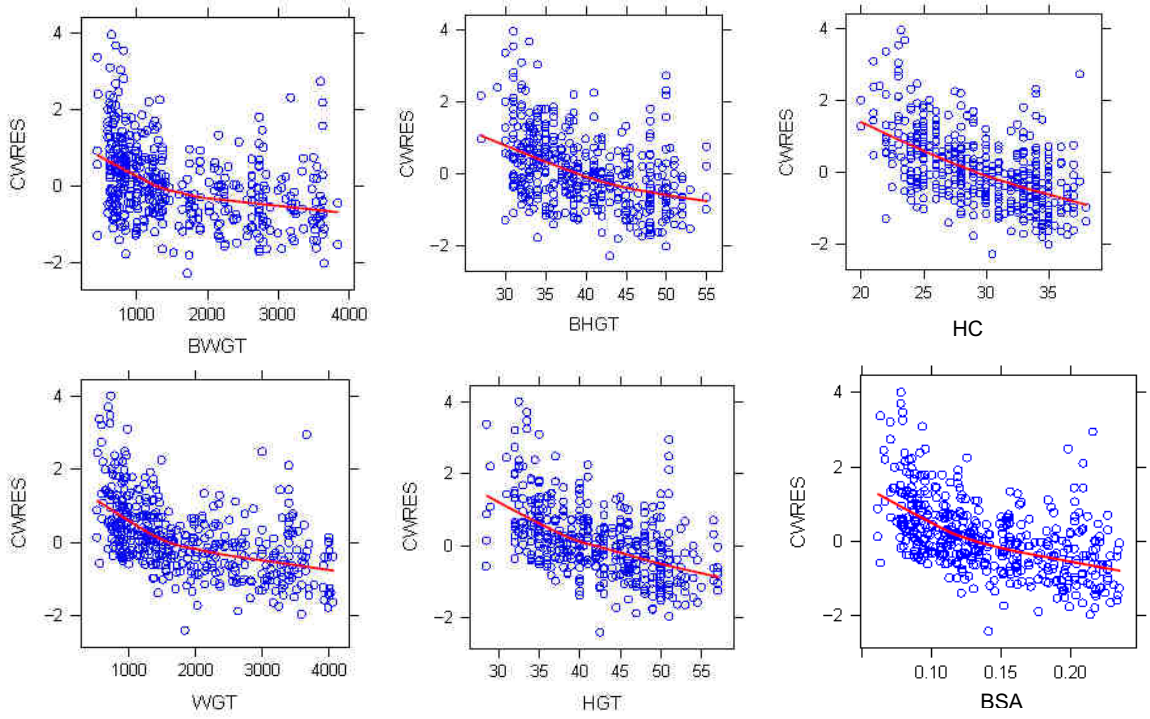


Figure 4.21. Plots of Individual Bayesian values of parameters CL, V1 and Q, vs covariates related to renal function. *Solid red line: smoothed line showing the general trend of the data.*

- Plots of CWRES vs covariates are displayed in Figure 4.22. There was a marked trend in CWRES versus WGT, indicating an underestimation of amikacin concentrations at low weights but an overestimation of them at high weights, and also versus CLCR. Similar trends were observed in CWRES versus HGT, BSA, HC and PMA. There was also a marked but negative trend in CWRES versus CREA, suggesting a more overestimation of amikacin concentrations at more high serum creatinine concentrations. These trends suggest that these covariates could explain some of the variability on the pK parameters of the structural model, being required some adjustment to reduce part of the observed variability. Less trend in CWRES versus BWGT, BHGT, PNA and GA was observed. Finally, no trend was observed in the case of CWRES versus UREA.



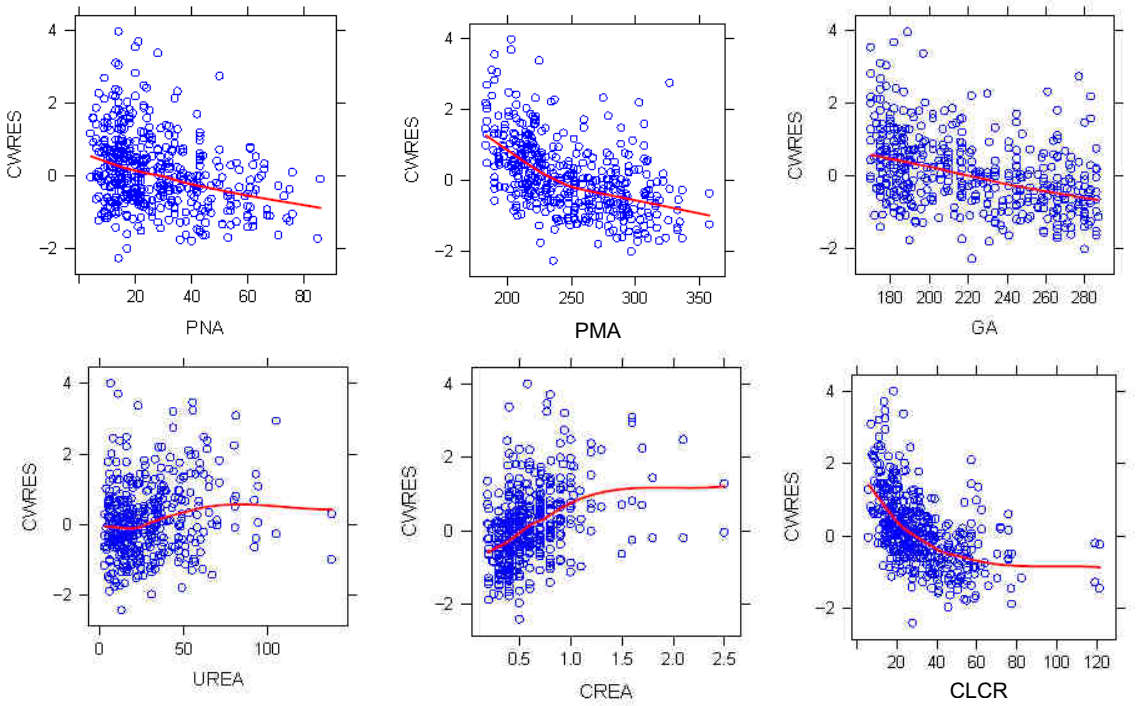


Figure 4.22. Plots of CWRES vs the most relevant covariates (BWGT, BHGT, WGT, HGT, CP, BSA, PNA, PMA, GA, UREA, CREA, CLCR). *Solid red line: smoothed line showing the general trend of the data.*

Although the marked trend observed in some CWRES versus covariates plots (WGT, CLCR) all the covariates physiologically plausible were tested in NONMEM, except gender, gestation number and twined pregnancy, for the above reasons.

- Multivariant analysis using the "Stepwise Generalised Additive Modelling" (GAM) identified as potential pharmacokinetic parameter predictors: WGT, UREA and CREA on CL; BSA on V1; HGT and CREA on Q.

Table 4.8. summarizes the covariate testing strategy during the covariate model development, for the most relevant covariates. The introduction of categorical covariates (sex, gender, prematurity and the classification according to BWGT) did not improve the OFV and neither did precision estimation of the parameters.

Table 4.8. Summary of the univariate covariate testing during the initial steps of the covariate model development.

Pharmacokinetic parameter	Covariate	Model	Results	ΔOFV
FINAL STRUCTURAL MODEL	-	Run33	Obj: 2075.969	-
CL	WGT	Run87	Obj: 1929.819	-146.15
CL	HGT	Run93	Obj: 1955.438	-120.53
CL	BWGT	Run105	Obj: 2008.348	-67.62
CL	BHGT	Run104	Obj: 1999.562	-76.41
CL	HC	Run85	Obj: 1924.644	-151.33
CL	BSA	Run88	Obj: 1930.688	-145.28
CL	PNA	Run102	Obj: 1997.736	-78.23
CL	PMA	Run83	Obj: 1909.177	-166.79
CL	GA	Run101	Obj: 1994.064	-81.91
CL	CLCR	Run80	Obj: 1873.002	-202.97
CL	CREA	Run89	Obj: 1935.717	-140.25
V1	WGT	Run82	Obj: 1905.093	-170.88
V1	HGT	Run84	Obj: 1924.214	-151.76
V1	BWGT	Run97	Obj: 1964.688	-111.28
V1	BHGT	Run95	Obj: 1961.627	-114.34
V1	HC	Run86	Obj: 1927.869	-148.10
V1	BSA	Run81	Obj: 1904.589	-171.38
V1	PNA	Run109	Obj: 2066.497	-9.47
V1	PMA	Run98	Obj: 1965.653	-110.32
V1	GA	Run106	Obj: 2012.050	-63.92
V1	CREA	Run109	Obj: 2080.141	-4.17
V1	CLCR	Run107	Obj: 2036.199	-39.77
Q	WGT	Run90	Obj: 1942.656	-133.31
Q	HGT	Run92	Obj: 1953.954	-122.02
Q	BWGT	Run100	Obj: 1968.139	-107.83
Q	BHGT	Run96	Obj: 1962.439	-113.53
Q	HC	Run94	Obj: 1960.725	-115.24
Q	BSA	Run91	Obj: 1943.927	-132.04
Q	PNA	Run110	Obj: 2072.532	-3.44
Q	PMA	Run99	Obj: 1967.518	-108.45
Q	GA	Run103	Obj: 1998.766	-77.193
Q	CLCR	Run108	Obj: 2052.299	-23.67
Q	CREA	Run111	Obj: 2070.766	-5.20

Because of the existence of η and ε-shrinkage, all of the covariates were tested in NONMEM during the univariate covariate testing. All the covariates introduced univariately in the model resulted in a statistically significant reduction of the OFV, with the exception of CREA on V1 and Q, and PNA on Q. So, although no marked trend was observed when bayesian estimates of distributional clearance were plotted versus almost all the covariates (Figure 4.19, Figure 4.20 and Figure 4.21), the univariate covariate introduction on Q was also tested. All the covariates related to body size produced a statistically significant reduction of the OFV, on CL, V1 and Q. Due to the linear correlations existing among all of them, only those most frequently managed in the clincal practice were considered. Hence, body weight (WGT) was selected, whereas BSA, HGT, BHGT and CH, were not considered further to be introduced in the model. Regarding to covariates related to age, the introduction of GA either on CL, V1 or Q, produced a significant decrease on OFV, but the precision estimation of parameters was incorrect. The most statistically significant covariate related to age was

PMA either on CL, V1 or Q. CLCR was more statistically significant than CREA as renal function covariate on CL. Some other covariates, that produced a significant decrease on the objective function value, resulted in low precision estimates for some parameters, that is HGT and BWGT on V1; and CLCR on Q. After the univariately testing, the most statistically significant covariates were CLCR on CL, followed by two covariates related to body size, BSA and WGT, both on V1, and then PMA, on CL. But BSA was discarded because its less frequent use during the clinical practice.

From this point, sequential forward inclusion of covariates that had showed to be statistically significant was carried out, as summarized in Table 4.9. It should be noted that only the most relevant steps of the process, from a physiological point of view, are displayed in this table.

Table 4.9. Summary of most relevant steps of the sequencial forward covariate inclusion during the covariate model development.

Hypothesis	Model	Results	ΔOFV	Respect to the model	Model selected	Decision
BASE MODEL	Run 33	**Obj: 2075.969**	-	-	-	-
+ CLCR on CL $TVCL=\left[\theta_1\times\left(\frac{CLCR}{31.97}\right)^{\theta_2}\right]$	Run80	**Obj: 1873.002**	-	-	Run80	To introduce the second covariate
+ CLCR on CL **+ WGT on V1** $TVCL=\left[\theta_1\times\left(\frac{CLCR}{31.97}\right)^{\theta_2}\right]$ $TVV1=\left[\theta_3\times\left(\frac{WGT}{1880}\right)^{\theta_4}\right]$	Run121	**Obj:** 1681.002 • Significant decrease of Obj with respect to Run80	-192.00	Run80	Run121	To introduce the third covariate
+ CLCR on CL **+ WGT on V1** **+ PMA on CL** $TVCL=\left[\theta_1\times\left(\frac{CLCR}{31.97}\right)^{\theta_2}\times\left(\frac{PMA}{248}\right)^{\theta_5}\right]$ $TVV1=\left[\theta_3\times\left(\frac{WGT}{1880}\right)^{\theta_4}\right]$	Run122	**Obj:** 1619.904 • Significant decrease of Obj with respect to Run121	-61.10	Run121	Run121	Strong linear correlation PMA vs CLCR No statistically significant reduction of BPV associated to CL was observed To discart covariate
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** $TVCL=\left[\theta_1\times\left(\frac{CLCR}{31.97}\right)^{\theta_2}\times\left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1=\left[\theta_3\times\left(\frac{WGT}{1880}\right)^{\theta_4}\right]$	Run123	**Obj:** 1608.557 • Significant decrease of Obj with respect to Run121	-72.45	Run121	Run123	Reduction of BPV associated to CL To accept the covariate To introduce the fourth covariate **COVARIATE MODEL**
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ WGT on Q** $TVCL=\left[\theta_1\times\left(\frac{CLCR}{31.97}\right)^{\theta_2}\times\left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1=\left[\theta_3\times\left(\frac{WGT}{1880}\right)^{\theta_4}\right]$ $TVQ=\left[\theta_6\times\left(\frac{WGT}{1880}\right)^{\theta_7}\right]$	Run124	**Obj:** 1602.440 • Not significant decrease of Obj with respect to Run125	-6.12	Run123	Run123	To discart covariate

+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ PMA on V1** $TVCL = \left[\theta_1 \times \left(\frac{CLCR}{31.97}\right)^{\theta_2} \times \left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1 = \left[\theta_3 \times \left(\frac{WGT}{1880}\right)^{\theta_4} \times \left(\frac{PMA}{248}\right)^{\theta_6}\right]$	Run125	**Obj:** 1608.628 • Not significant decrease of Obj with respect to Run123	+0.07	Run123	Run123	To discart covariate
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ BWGT on Q** $TVCL = \left[\theta_1 \times \left(\frac{CLCR}{31.97}\right)^{\theta_2} \times \left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1 = \left[\theta_3 \times \left(\frac{WGT}{1880}\right)^{\theta_4}\right]$ $TVQ = \left[\theta_6 \times \left(\frac{BWGT}{11640}\right)^{\theta_7}\right]$	Run126	**Obj:** 1606.529 • Not significant decrease of Obj with respect to Run123	+2.03	Run123	Run123	To discart covariate
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ PMA on Q** $TVCL = \left[\theta_1 \times \left(\frac{CLCR}{31.97}\right)^{\theta_2} \times \left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1 = \left[\theta_3 \times \left(\frac{WGT}{1880}\right)^{\theta_4}\right]$ $TVQ = \left[\theta_6 \times \left(\frac{PMA}{248}\right)^{\theta_7}\right]$	Run127	**Obj:** 1607.531 • Not significant decrease of Obj with respect to Run123	-1.03	Run123	Run123	To discart covariate
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ PNA on CL** $TVCL = \left[\theta_1 \times \left(\frac{CLCR}{31.97}\right)^{\theta_2} \times \left(\frac{WGT}{1880}\right)^{\theta_5} \times \left(\frac{PNA}{28}\right)^{\theta_6}\right]$ $TVV1 = \left[\theta_3 \times \left(\frac{WGT}{1880}\right)^{\theta_4}\right]$	Run128	**Obj:** 1607.269 • Not significant decrease of Obj with respect to Run123	-1.296	Run123	Run123	To discart covariate
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ CLCR on V1** $TVCL = \left[\theta_1 \times \left(\frac{CLCR}{31.97}\right)^{\theta_2} \times \left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1 = \left[\theta_3 \times \left(\frac{WGT}{1880}\right)^{\theta_4} \times \left(\frac{CLCR}{31.97}\right)^{\theta_6}\right]$	Run129	**Obj:** 1608.376 • Not significant decrease of Obj with respect to Run123 • RSE of θ10-V1~CLCR higher than 100%	-0.18	Run123	Run123	To discart covariate
+ CLCR on CL **+ WGT on V1** **+ WGT on CL** **+ PNA on V1** $TVCL = \left[\theta_1 \times \left(\frac{CLCR}{31.97}\right)^{\theta_2} \times \left(\frac{WGT}{1880}\right)^{\theta_5}\right]$ $TVV1 = \left[\theta_3 \times \left(\frac{WGT}{1880}\right)^{\theta_4} \times \left(\frac{PNA}{28}\right)^{\theta_6}\right]$	Run130	**Obj:** 1613.173 • Not significant decrease of Obj with respect to Run123	+4.62	Run123	Run123	Increase of BPV associated to Q respect to Run123 To discart covariate

The covariate model development started out with the creatinine clearance model (#80), given that it had resulted to be the most statistically significant covariate during the univariate analysis. When WGT was added to V1 (#121) from model #80, the objective function value as well as the BPV_V1 associated, decreased 192 units and 41,32%, respectively. WGT was retained in V1, based on phisiological and clinical criteria, and this was considered from here to forwards.Then, from model #121, PMA

was tested on CL, resulting in a statistically significant decrease of the OFV (-61.098). But no clinical reduction of the BPV associated to this parameter was observed, and precision of some estimated parameters worsened. The inclusion of WGT on CL from model #121, (model #123) produced a decrease in the objective function with an additional improvement of BPV_CL (-72,445 units and 30.68%, respectively). Therefore, WGT was keeped into the model (#123). The several body size covariates tested on pharmacokinetic parameters (#124 and #125) did not reduce the OFV in any case and similarly occurred when PMA and PNA were tested on CL, V1 or Q (models #125, #128 and #130). According to these results, model #123 was selected as the best final model. The backward elimination, of each covariate, one by one, from the full model, increased the objective function value in more than 10.8 units in all the cases ($p<0.001$).

4.3.2.3 Final model

The main characteristics of the final population pharmacokinetic model were:

- Two-compartment kinetic model with first order elimination process, parameterized as clearances and distribution volumes.
- Between-patient variability modelled exponentially was associated with plasma and distributional clearances and central compartment distribution volume.
- Combined residual error model (additive + proportional) provided the best fit of data.
- FOCEI was used as estimation method
- WGT and CLCR showed to be the best predictor covariates of CL.
- WGT was the most significant predictor covariate of V1.

Table 4.10 lists the population pharmacokinetic parameter estimates for the base and final models and the corresponding shrinkage values as well as the bootstrap results.

Table 4.10. Population pharmacokinetic parameters of the base and final models and the bootstrap results.

		Base model (#33)	Final model (#123)	Mean value from Bootstrap (95% PI)*
Pharmacokinetic parameters	θ1-CL (L/h)	0.129 (6.95)	0.133 (4.23)	0.133 (0.122 – 0.145)
	θ2-CL~CLCR	-	0.649 (9.78)	0.642 (0.508 – 0.771)
	θ5-CL~WGT	-	0.752 (12.02)	0.758 (0.581 – 0.955)
	θ3-V1 (L)	0.595 (6.84)	0.837 (3.80)	0.834 (0.768 – 0.900)
	θ4-V1~WGT	-	1.09 (4.86)	1.09 (0.978 – 1.201)
	θ7-Q (L/h)	0.128 (14.84)	0.039 (28.13)	0.041 (0.020 – 0.068)
	θ6-V2 (L)	1.15 (11.39)	0.409 (23.94)	0.427 (0.251 – 0.667)
Between-patient variability	BPV-CL (%)	74.23 (15.01)	34.50 (16.39)	33.72 (27.15 – 39.64)
	BPV-V1 (%)	58.48 (19.04)	21.07 (25.23)	20.92 (14.91 – 26.66)
	BPV-Q (%)	80.72 (23.85)	70.29 (40.08)	68.73 (32.27 – 94.24)
Residual variability	Additive (mg/L)	0.42 (4.81)	0.28 (6.97)	0.28 (0.24 – 0.32)
	Proportional (%)	24.00 (32.88)	50.4 (15.34)	51.84 (29.8 – 97.2)
η_1-shrinkage	%	10.34	17.82	-
η_2-shrinkage	%	31.56	43.04	-
η_3-shrinkage	%	55.97	74.18	-
ε-shrinkage	%	19.95	17.64	-

RSE%: Precision given by the relative standard error in parenthesis
** From 1000 resamplings*

*** Final model:*

$$TVCL = \left[0.133\left(CLCR/31.97\right)^{0.649} X \left(WGT/1880\right)^{0.752}\right];\quad TVV1 = \left[0.837\left(WGT/1880\right)^{1.09}\right];\quad TVV2 = 0.409;\quad TVQ = 0.039$$

Inclusion of CLCR on clearance accounted for a reduction of the BPV_CL from the base model of 34.55%. The further inclusion of WGT on V1 reduced BPV_V1 of 63.32%. The final inclusion of WGT on CL, being the final model, accounted for a reduction of the between-patient variability of CL and V1 from the base model of 53.59% and 63.97%, respectively. Figure 4.23 shows the goodness-of fit plots corresponding to the final model.

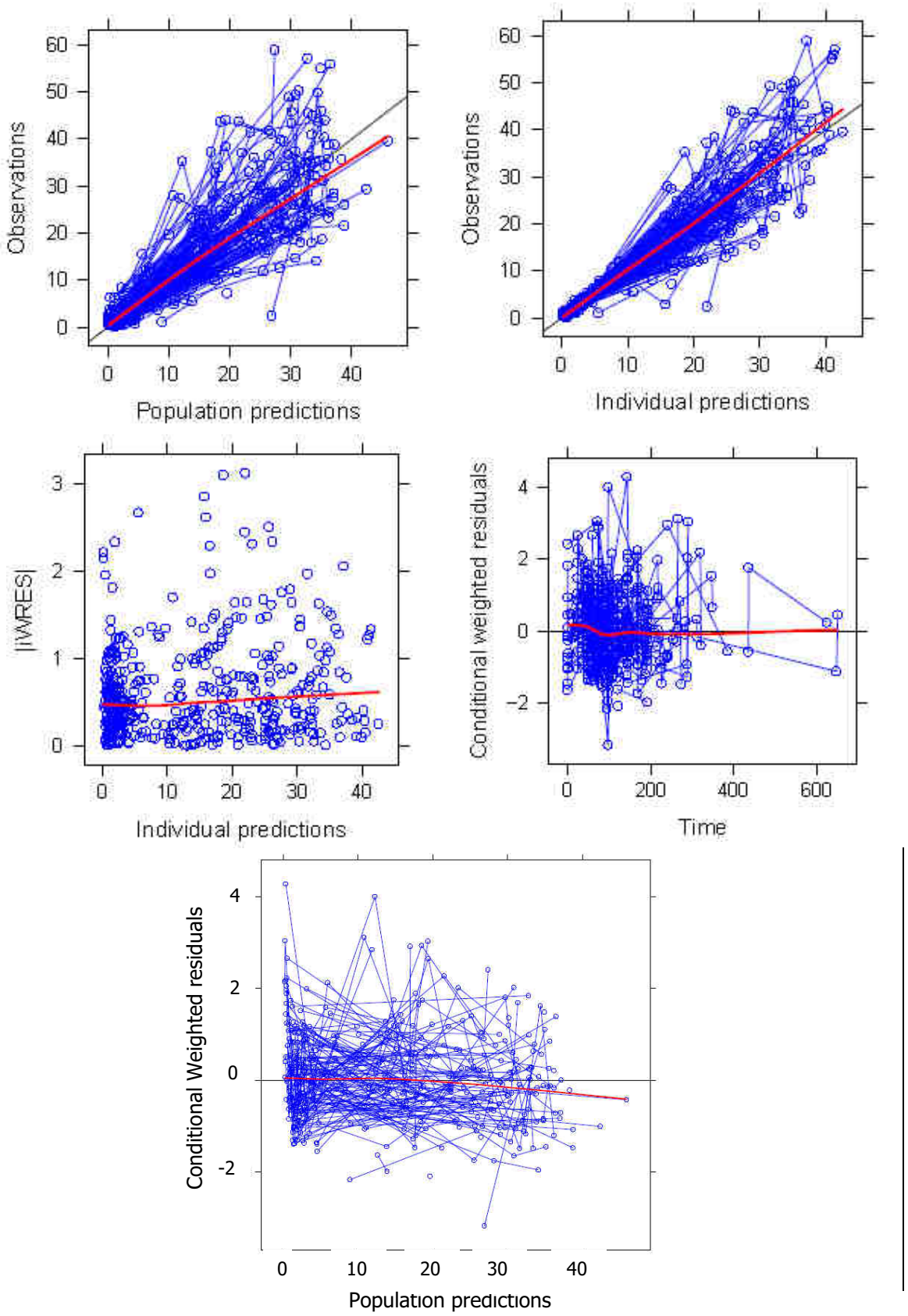


Figure 4.23. GOF plots of the final PK model developed (#123).Upper panels: DV vs PRED (left) and IPRED (right) concentrations. Middle panels: IWRES vs IPRED (left) and CWRES (calculated as PRED-DV) vs TIME (right). Lower panel: CWRES (calculated as DV-PRED) vs PRED. Concentrations expressed as mg/L. *Black solid line: identity line. Red solid line: smoothed line showing the general trend of the data.*

The introduction of covariates on the base model (#33) accounted for an improvement of the structural part of it. Goodness-of-fit plots that allowed the evaluation of the structural population PK model (DV vs PRED, CWRES vs TIME and CWRES vs PRED) showed all data points in the final model closer to the identity line on DV vs PRED plot and to the the zero-line on CWRES vs TIME and CWRES vs PRED plots than in the base model, indicating that the correlation DV/PRED in the final model was better than in the base one. Plots of CWRES vs PRED, presented a clear tendency to overestimation at high concentrations in the base model. After the introduction of covariates, data points were closely and symmetrically distributed around the zero-line, confirming the effect of the introduction of the covariates to achieve a good precision of the model and also the elimination of the trend of the model to overestimation at high concentrations. Moreover, the model was able to predict the individual and population concentration-time profiles of neonates for the whole range of body weights, as illustrated in Figure 4.24.

The comparative plots of CWRES vs WGT and CLCR for the base and the final models (Figure 4.25) confirms the better performance of the final model fitting to data. The marked trend observed in CWRES versus WGT plots for the the base model, suggested an underestimation of amikacin concentrations at low values of WGT but an overestimation of them at high values. This trend disappeared in the final model, indicating an adequate prediction of individual amikacin concentrations for the whole range of WGT. Similar changes in trends of CWRES versus CLCR plots were observed.

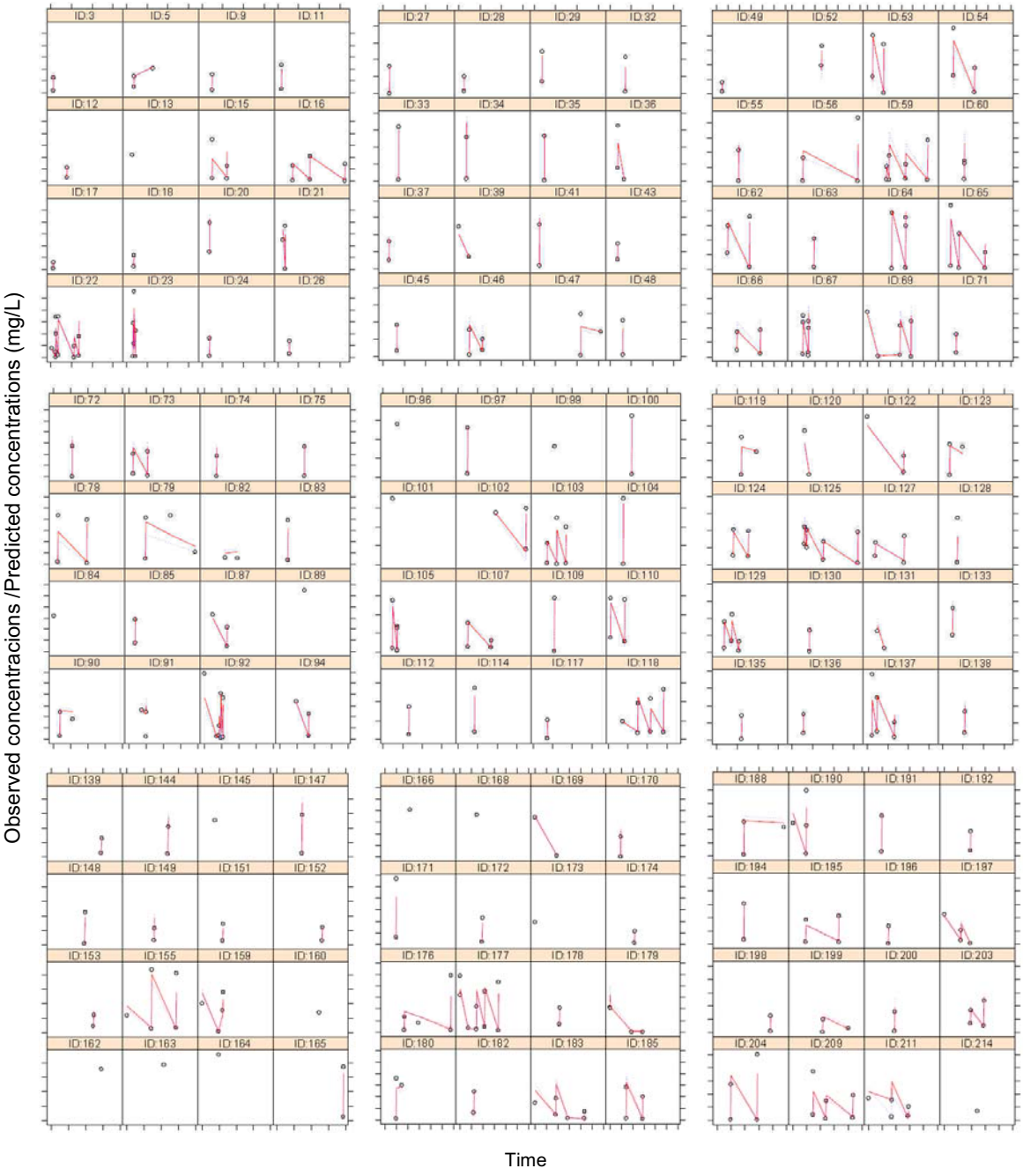


Figure 4.24. Superimposed values of observed (DV), population predicted (PRED) and individual predicted (IPRED) concentrations vs time for each individual of the “Model building dataset”. DV are represented by grey points, IPRED as red continue line and PRED as blue dashed line.

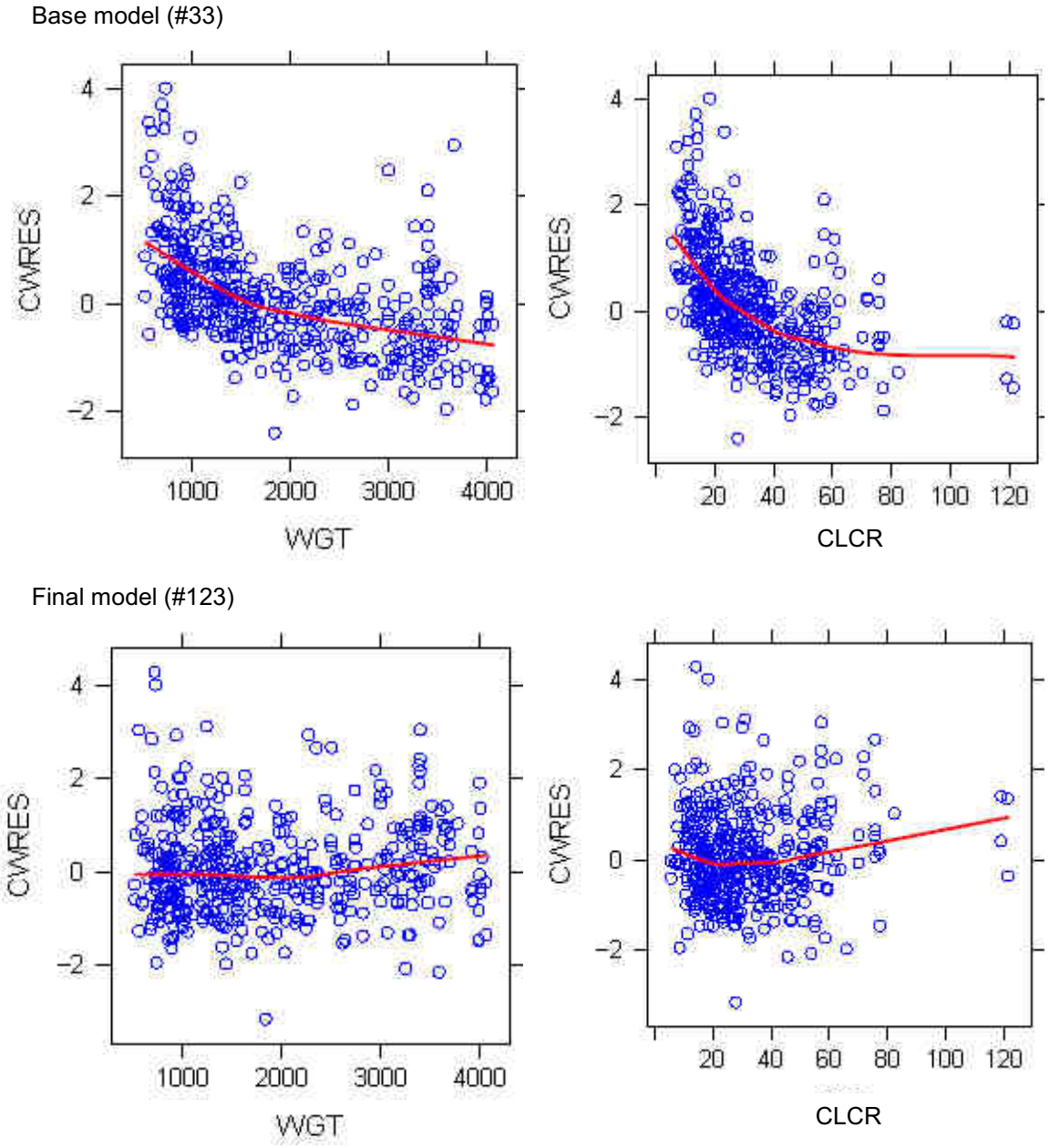


Figure 4.25. CWRES vs WGT and CLCR plots of the base model (upper pannel) and the final model (lower pannel). Concentrations expressed as mg/L. *Solid red line: smoothed line showing the general trend of the data.*

Morevoer, the marked trend observed in the between patient random effects associated with CL (ETA1) and V1 (ETA2) versus covariates WGT and CLCR in the base model disappeared in the final model after adjusting by the corresponding statistically significant covariate (Figure 4.26).

Base model (#33) Final model (#123)

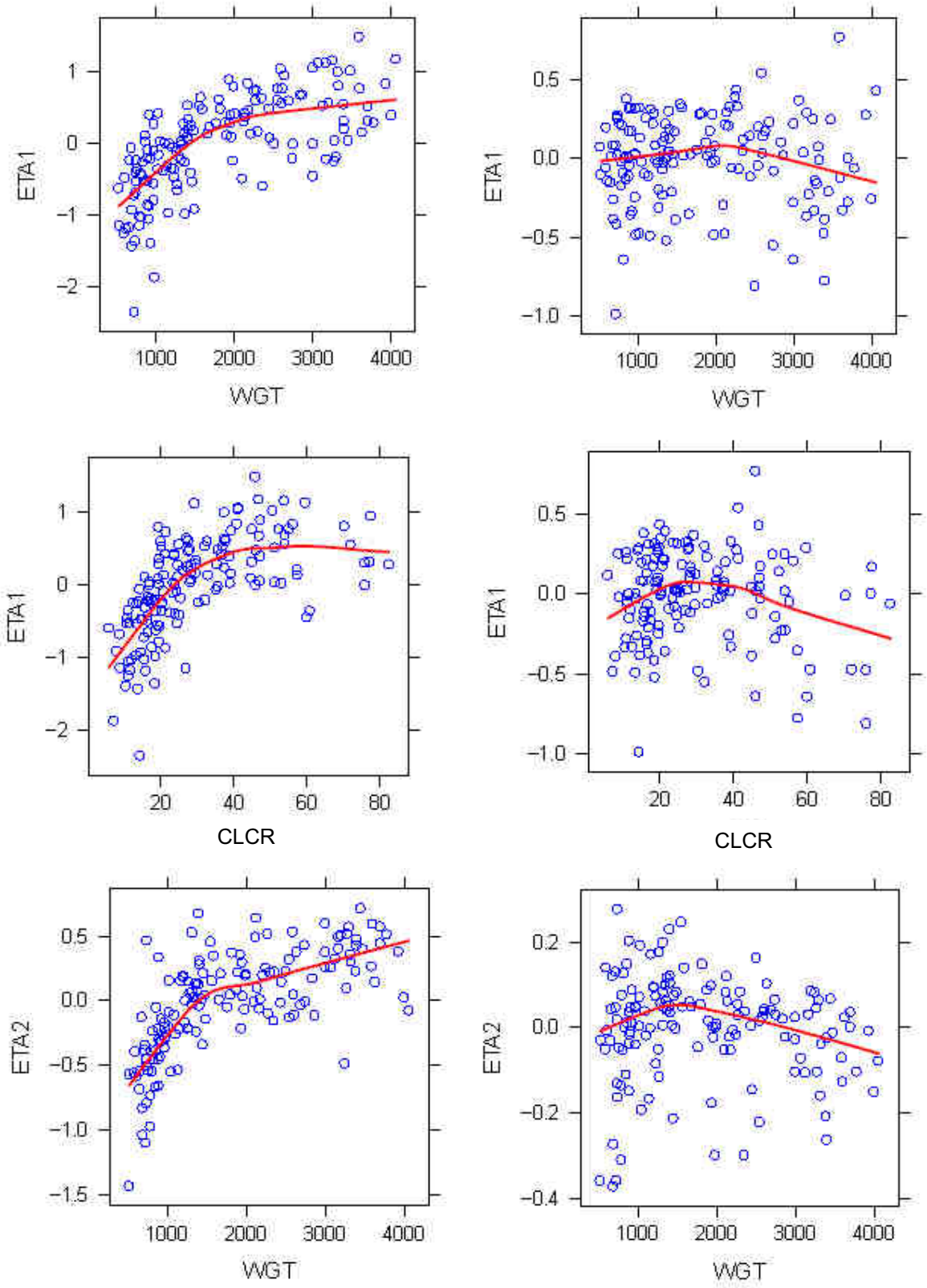


Figure 4.26. Plots of between patient random effects associated to CL and V1 versus covariates on the base (left) and final model (right). *Solid red line: smoothed line showing the general trend of the data.*

Finally, between-patient random effects associated with CL (η1), V1 (η2) and Q(η3) were approximately normally distributed around zero in the final model (Figure 4.27).

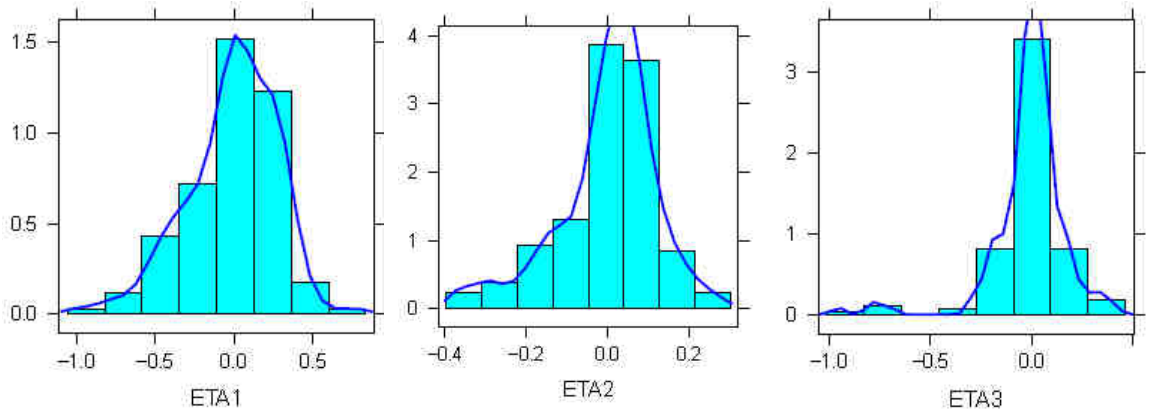


Figure 4.27. Distributions of between-patient random effects associated with CL (η1), V1 (η2) and Q(η3) in the final model.

4.3.3 Final model evaluation and qualification

Results corresponding to the predictive capacity of the model developed are presented in the following sections.

4.3.3.1 External validation techniques

Data set for the external validation comprised 53 newborns, of which 34 were boys (64%) and 19 girls (36%). The main demographic and biochemical characteristics of these patients are summarized in Table 4.1. As Figure 4.28 shows, good agreement between the observed (DV) and either population predicted (PRED) or individual predicted (IPRED) concentrations was found. It suggested that the developed model had a good predictive power. The median values and the 2.5 and 97.5 percentiles of bias and precision are displayed in Table 4.11 for both trough and peak concentrations.

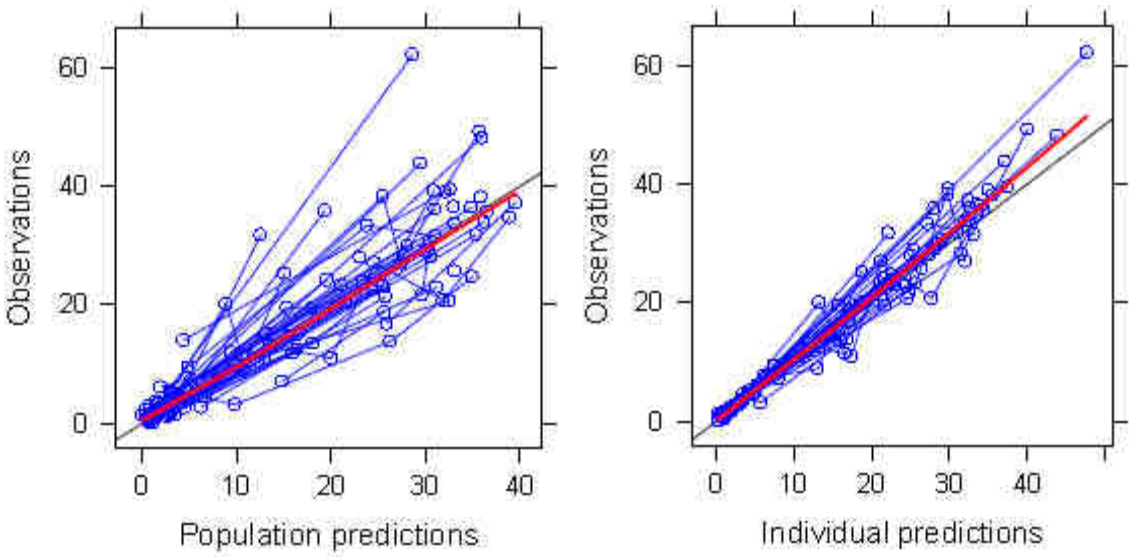


Figure 4.28. Relationship between observed and population/individual model predicted concentrations for the external evaluation dataset using the final model. Concentrations expressed as mg/L. *Red solid line: smoothed line showing the general trend of the data. Black solid line: identity line.*

Totally acceptable median bias and precision values were found, it suggesting a good predictive ability for our model. The 95% confidence intervals of bias associated with both trough and peak concentrations included the zero value suggesting no statistically significant differences from zero. Based on median trough and peak concentrations of 2.21 and 22.12 mg/L, respectively, the median precisions of trough and peak concentrations estimated from individual predictions (0.71 and 1.20, respectively) represented the median errors of 32.12% (for trough concentrations) and 5.42% (for peak concentrations). As expected, accuracy and precision confidence intervals were narrower for individual prediction vs population prediction concentrations.

Table 4.11. Median values and percentiles 2.5 and 97.5 of accuracy and precision for trough and peak concentrations achieved by the external evaluation

	Trough concentrations		**Peak concentrations**	
	Bias (mg/L)	**Precision (mg/L)**	**Bias (mg/L)**	**Precision (mg/L)**
PRED vs OBS	0.09 (-8.92 – 4.50)	0.91 (0.52 – 1.73)	-0.22 (-11.34 – 17.40)	1.26 (0.72 – 2.04)
IPRED vs OBS	0.13 (-1.95 – 1.81)	0.71 (0.45 – 1.24)	1.10 (-5.05 – 9.43)	1.20 (0.71 – 1.75)

4.3.3.2 Internal validation techiques

Bootstrap

Table 4.10 lists the results corresponding to the means and 95% PIs of parameters estimates from 1000 bootstrap samplings. The mean value from bootstrapping were very close to the population means for all the parameters. The percentages of difference between the final model and the bootstrap means were lower than 6% for all the pharmacokinetic structural and random-effect parameters. All the final model parameter estimates lie within the bootstrap 95% prediction intervals. These results indicated that the estimates for the fixed and random effects in the final model were accurate and that the model was stable.

Visual Predictive Check (VPC) and Prediction-corrected Visual Predictive Check (pc-VPC)

The VPC and pcVPC are displayed in Figure 4.29 and Figure 4.30, respectively. In both cases, the simulations of 1000 populations, as the original, were performed from the parameter estimates of the final model. In the case of pcVPC, both the observations and model predictions were normalized by the typical model predictions in each bin of independent variables such as time, dose and covariate values. Hence, the pcVPC allowed to correct for the differences within a bin coming from these variables. Neither VPC nor pcVPC suggested discrepancies between observations and model predictions, and therefore any important model missespecification across post-dosing time or concentrations. The median and the 5% and 95% percentiles of the observed data fell within the 95% confidence intervals of the corresponding model predicted percentiles in all the cases, but a tendency toward underpredicted amikacin concentrations at the predose sample can be observed in these plots from 24 hours post-dosing to upwards. Besides, a slightly overprediction of peak concentrations is also present.

On the other hand, the pcVPC showed slightly narrower blue fields with respect to the VPC. This could be due to the fact that part of the variability could be corrected with normalization by independent variables. Besides, the underprediction of trough concentrations from 24 hours post-dosing to upwards is also present, as well as the overprediction of some peak concentrations.

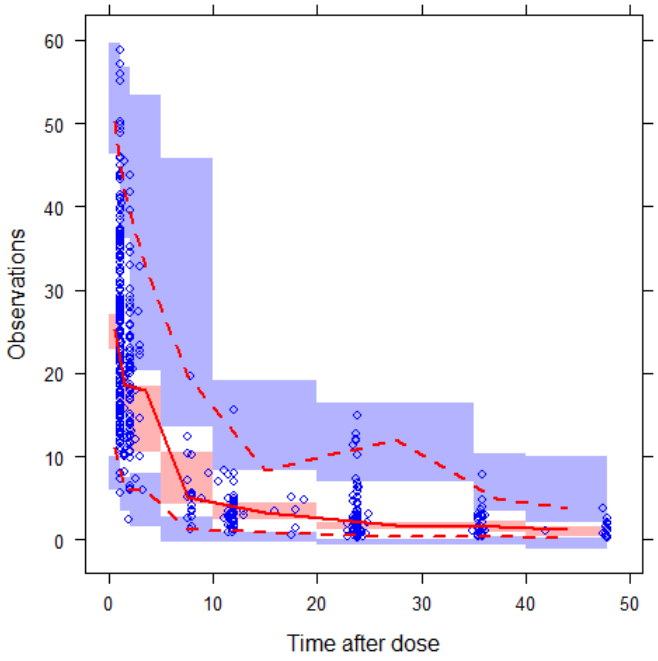


Figure 4.29. Visual Predictive Check for amikacin concentration vs time data for the final model. Median (solid line), 95th and 5 th percentile (dashed lines) of the observations. Pink area cover the 95% CI of the median and light blue areas cover the 95% CI of the 5th and 95th percentiles of the simulated profiles.

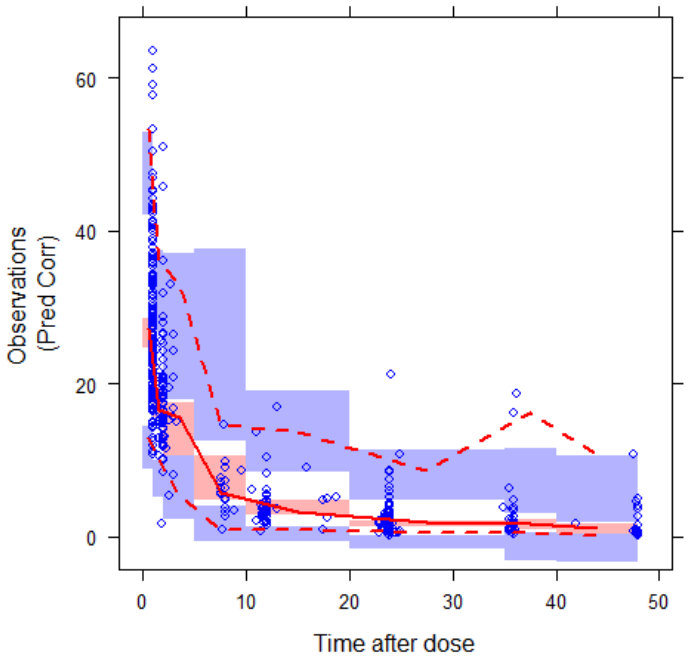


Figure 4.30. Predicted-corrected Visual Predictive Check for amikacin concentration vs time data for the final model. Median (solid line), 95th and 5 th percentile (dashed lines) of the observations. Pink area cover the 95% CI of the median and light blue areas cover the 95% CI of the 5th and 95th percentiles of the simulated profiles.

Posterior Predictive Check (PPC)

Figure 4.31 and Figure 4.32 show the distributions of simulated trough and peak amikacin concentrations (mg/L) respectively, from 1000 populations as the original. The simulated median distributions (middle panel), and the 25% (upper panel) and 75% (lower panel) percentiles distributions were superimposed to the corresponding median values and the 25% and 75% percentiles of observed trough or peak concentrations respectively. The medians of simulated trough and peak concentrations were both adequately within the 25% and 75% percentiles of observed trough and peak concentrations respectively, although the medians of trough concentrations were slightly overpredicted and the medians of peak concentrations were underpredicted some. According to this results, the posterior predictive check (PPC) indicated a reasonable prediction of the original data.

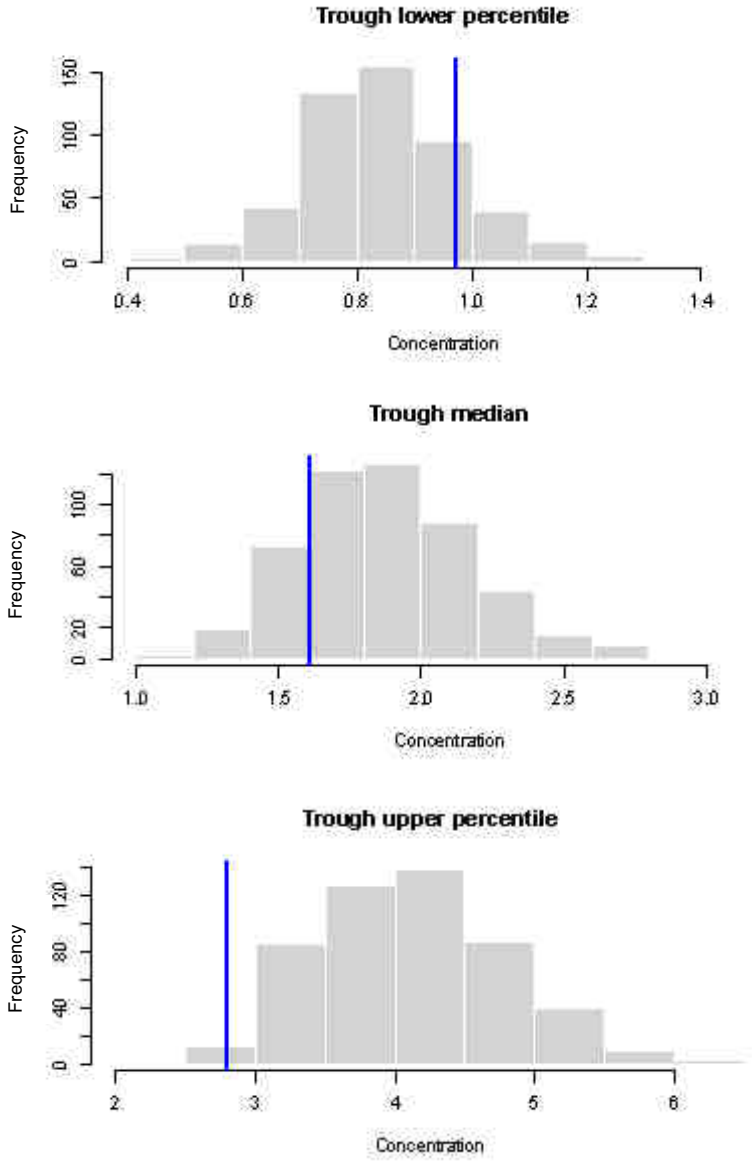


Figure 4.31. Posterior Predictive Check (PPC) for amikacin trough concentration vs time data for the final model.

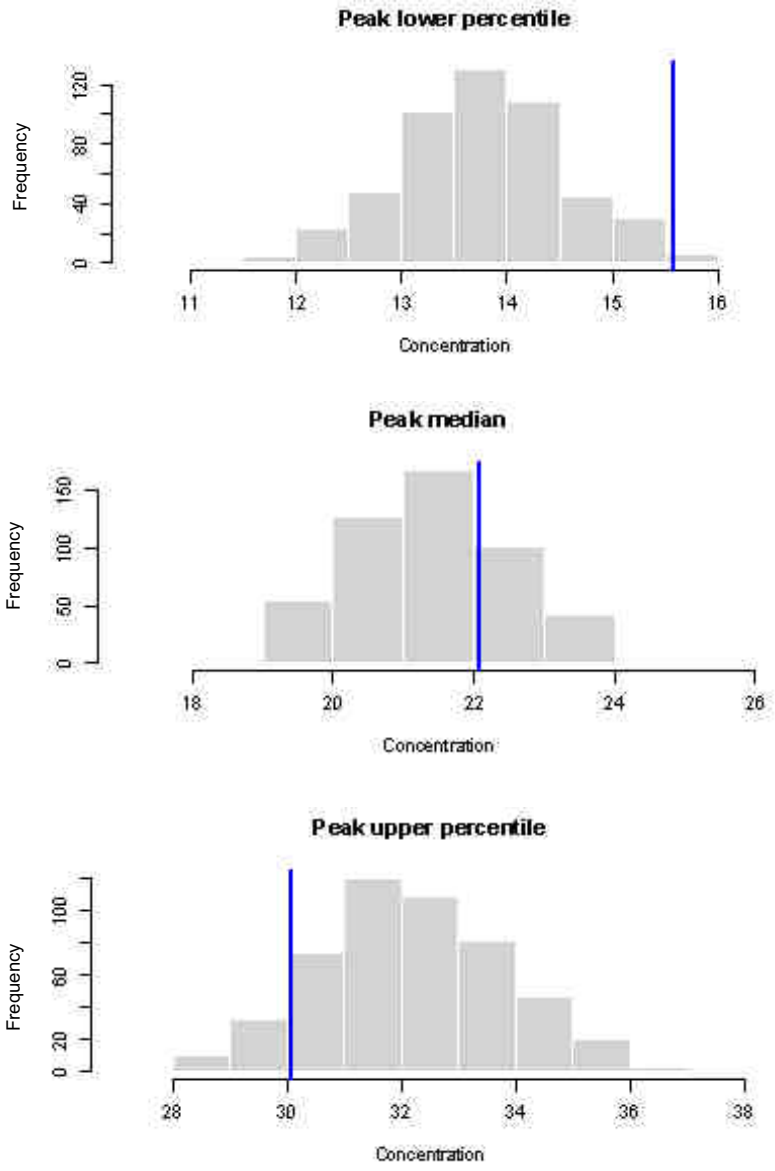


Figure 4.32 Posterior Predictive Check (PPC) for amikacin concentration vs time data for the final model.

Normalised prediction distribution errors (NPDE)

Figure 4.33 shows discrepancies between simulated and observed data for 1000 simulations from the final model graphed as an histogram and a dispersion plot. According to these plots, the density of predictive model discrepancies (npde) went along theorical normal distribution, without extreme valules, indicating that the final model (#123) estimations variance was low. On the other hand, discrepance errors of predicted concentrations had an homogeneous distribution around npde=0, without a specific tendency.

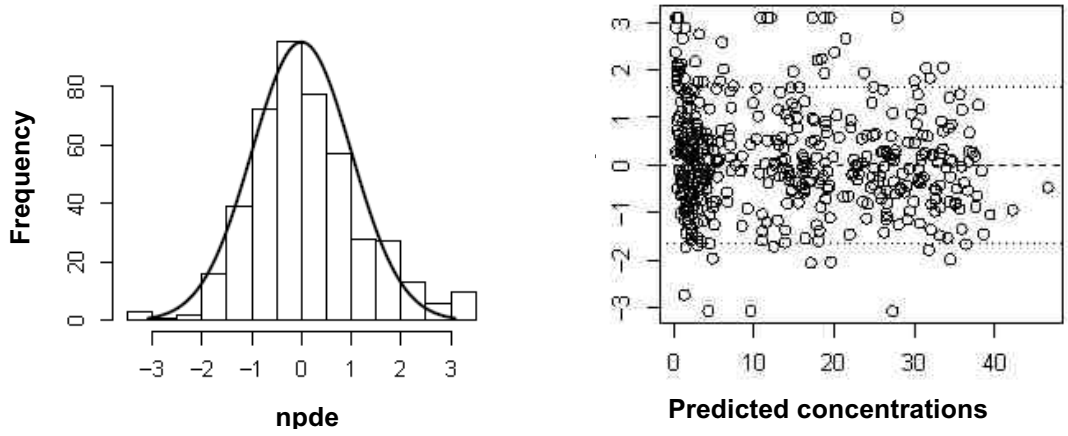


Figure 4.33. Npde obtained from 1000 simulations of the final model

4.3.4 Model based simulations

Results corresponding to the simulations based on the final model parameter estimates are presented in the following sections.

i. Assessment of the influence of the covariates identified as statistically significant on amikacin trough and peak concentrations.

For different cut-offs of WGT and CLCR previously established, simulations of trough and peak concentratios of 1000 virtual patients of the same characteristics and having received the same dosage as those of the original data set, were performed. The different cutoffs of WGT and estimated CLCR values used for the simulations (Table 3.2) were established in an effort to categorize the patients of the entire population into well distinct groups depending on their WGT and CLCR values. Hence, WGT was classified as: low (≤ 1199 g), medium (1200 – 1999 g) and high (≥ 2000 g). Based on CLCR, four groups were considered as representative of the main stages of the renal failure defined by the Chronic Kidney Disease (122).

Table 4.12 summarizes the percentages of patients with trough concentrations below 5 mg/L as well as below the concentration value considered as potentially toxic (10 mg/L). Table 4.13 shows the percentages of individuals with ineffective peak concentrations (< 20 mg/L). The 2.5%, 50% and 97.5% percentiles values of the trough and peak concentrations for each cut-off are also displayed.

Table 4.12. Percentages of patients with amikacin trough concentration < 5 mg/L as well as < 10 mg/L and 2.5%, 50% and 97.5% percentiles of the predicted trough concentrations.

CLCR	WGT	Trough concentrations				
		< 5 mg/L (%)	< 10 mg/L (%)	p2.5%	p50%	p97.5%
<15	≤1199	85.6	96.1	0.03	0.75	8.17
	1200-1999	86.3	96.5	0.02	0.55	8.12
	≥2000	64.2	77.3	0.03	0.58	9.31
15-30.99	≤1999	89.6	97.7	0.04	1.01	9.62
	1200-1999	80.1	91.6	0.04	1.18	8.97
	≥2000	70.7	84.7	0.06	1.90	8.00
31-59.99	≤1999	79.6	90.3	0.02	0.69	8.46
	1200-1999	92.6	97.9	0.03	0.57	6.89
	≥2000	74.7	88.9	0.04	1.04	8.83
≥60	≤1999	98.6	100	0.02	0.46	3.90
	1200-1999	-	-	-	-	-
	≥2000	95.1	98.4	0.02	0.51	7.75

(-) There were no patients with WGT and CLCR values within this range in the original dataset.

Table 4.13 Percentages of patients with amikacin peak concentrations < 20 mg/L and 2.5%, 50% and 97.5% percentiles of the predicted peak concentrations.

CLCR	WGT	Peak concentrations			
		< 20mg/L (%)	p2.5%	p50%	p97.5%
<15	≤1199	46	20.45	31.37	72.41
	1200-1999	36.7	20.53	32.67	80.78
	≥2000	19	20.89	36.93	89.95
15-30.99	≤1199	49.4	5.50	20.21	64.52
	1200-1999	44.6	5.90	21.78	63.57
	≥2000	54.2	20.42	30.87	70.68
31-59.99	≤1999	52	20.38	30.70	65.94
	1200-1999	53	3.99	18.99	59.24
	≥2000	52.3	20.37	29.58	67.26
≥60	≤1999	68.1	20.22	26.64	52.33
	1200-1999	87.3	3.71	11.90	28.98
	≥2000	55.1	4.13	18.58	50.58

According to the results of Table 4.12, the current dosages given provided safe trough concentrations, that is within the therapeutic range, in the 80% or more of the patients, and below the threshold of toxicitiy in the 90% or more of the patients in all the cases, excepting in those patients of WGT higher than 2000 g and CLCR lower than 60 mL/min. In these cases, there was a trend to over-dosing which was greater the smaller was the creatinine clearance. In general, patients with the highest CLCR values were those best dosed in view of the potential toxicity. However, they were also the worst dosed in view of efficacy (Table 4.13), with the highest percentages of peak concentrations below the threshold of 20 mg/L (from 55.1 to 87.3%). In the other groups, there were lower percentages of patients with peak concentrations below 20 mg/L as well as a trend to median peak concentration values closer to 30 mg/L, excepting patients of CLCR ranging from 15 to 30.99 mL/min and WGT below than 1999 g or patients with CLCR values ranging from 31 to 59.99 mL/min and WGT from 1200 to 1999g. In summary, all these results show that the current dosage (dose and dosing interval) provides trough and peak concentrations within the therapeutic range in most cases, but revision would be required for some groups, particularly arranging trough concentrations for the patients of CLCR with CLCR lower than 15 mL/min and WGT ≥ 2000 g, and peak concentrations for the patients with the highest CLCR values except those with WGT ≥ 2000 g.

After stratified the overall resulting simulated concentrations vs time data by the same age groups as those considered by the Neofax guide,
Table 4.14 summarizes, for each group, the percentages of patients with trough concentrations < 5 mg/L and below the concentration value considered as potentially toxic (10 mg/L), as well as the percentages of individuals with ineffective peak concentrations (< 20 mg/L).

Table 4.14 Percentages of through and peak concentrations within the therapeutic range

Neofax age group	**Through concentrations**		**Peak concentrations**
	< 5 mg/L	**< 10 mg/L**	**< 20 mg/L**
Group A	100%	100%	11.4%
Group B	88.7%	97.2%	49.5%
Group C	88.1%	95.2%	51.6%
Group D	75.6%	95.2%	47.7%
Group E	82.1%	92.0%	48.5%
Group F	75.6%	89.0%	49.3%

The percentage of patients with trough concentracions within the therapeutic range (< 5 mg/L) was around 80% in all the groups, from 75.6% (Group D) to 100% (Group A). Besides, the percentage of trough concentrations > 10 mg/L was higher than 88% in all the cases. Regarding peak concentrations, all the groups achieved percentages

around 50%, except for the Group A, with only a 11.4% of trough concentracions < 20 mg/L.

These results show that cutoffs according to our model (CLCR and WGT) are more sensitive to detect an ineffective dosage regimen than stratifying by Neofax in such a way that provides higher percentages of peak concentrations within the therapeutic range for the most immature group (Group A) than when considering CLCR <15 mL/min and WGT ≤1199 g. It suggests that patients of GA ≤29 weeks and PNA 0-7 days do not exactly correspond to patients of CLCR <15 mL/min and WGT ≤1199 g, with probably slightly higher CLCR or WGT values than those of the first cutoff of our distribution. Similarly, a trend to lack of correlation was observed when percentages of target trough concentrations were compared for CLCR ≥60 mL/min and GA ≥35 weeks, in that case higher precentages of trough concentrations within the therapeutic range were observed for CLCR ≥60 mL/min vs GA ≥35 weeks, it suggesting that patients of GA ≥35 weeks tended to show lower CLCR values than 60 mL/min. Therefore no correlation between the type of stratification was found for the most imamature groups when compared peak cocnentrations and for the less immature groups when compared trough concentrations.

ii. To stablish initial dose recommendations, in view of the efficacies and toxicities given by serum amikacin concentrations.

In this case, the dosages to be used for simulations from the final model were chosen by trial and error aiming to achieve mean peak concentrations values of at least 30 mg/L and mean through concentrations below or ranging from 1.5 to 3 mg/L. These doses were selected after testing them in the model with dosing intervals of either 12, 24, 36 or 48 hours and taking into account not only the therapeutic range defined for trough and peak concentrations but also criteria of usefulness in the clinical practice.

Table 4.15 summarizes the initial doses/dosing intervals selected to be given to patients with CLCR values of 10, 20, 30, 50, 60 and 80 mL/min, and WGT values of 500, 1000, 1200, 1500, 2000 and 2500 g, in order to achieve the trough and peak concentrations above mentioned.

Regardless of CLCR values, dose requirements increased with WGT. Similarly, for a given WGT, dosing interval increased with decreasing CLCR. Dosing interval of 12 hours could only be used in patients with normal renal function (≥ 60 mL/min), with the exception of the group with CLCR of 60 mL/min and WGT 500g, that required a 24 hours dosing interval. When the CLCR was 10 mL/min, 48 hours dosing interval was applied, regardless of WGT. For CLCR values of 20 mL/min, the dosing interval was reduced to 36 hours, for the whole range of WGTs. For CLCR values from 30 to 50 mL/min, the dosing interval of 24 hours was the most suitable, regardless of WGT.

Table 4.15. Summary of the first doses (mg) and dosing intervals (hours) estimated from the final developed model to be given in order to achieve the stablished peak and trough concentrations.

CLCR (mL/min)	**WGT (g)**					
	500	**1000**	**1200**	**1500**	**2000**	**2500**
10	9.5 (48h)	16 (48h)	19 (48h)	23 (48h)	30 (48h)	36.5 (48h)
20	10.5 (36h)	18 (36h)	20.5 (36h)	25 (36h)	32.5 (36h)	39.5 (36h)
30	11.5 (24h)	19 (24h)	22 (24h)	26 (24h)	33.5 (24h)	40.5 (24h)
50	14 (24h)	21.5 (24h)	25 (24h)	29.5 (24h)	37 (24h)	44.5 (24h)
60	15 (24h)	22 (12h)	25 (12h)	29.5 (12h)	36.5 (12h)	43.5 (12h)
80	16.5 (12h)	24.5 (12h)	27.5 (12h)	32 (12h)	39.5 (12h)	46.5 (12h)

Table 4.16 show the percentage of patients with trough concentrations below 5 mg/L as the p2.5% and p97.5% percentile values estimated from 1000 simulations from each one of the first dose and dosing interval selected for each CLCR and WGT cutoff. The corresponding percentage of patients with peak concentrations below the threshold defined as effective (20 mg/L), within the therapeutic range (20 – 30 mg/L) and higher than the therapeutic range (30 mg/L), estimated as peak concentrations, and the p2.5% and p97.5% percentile values are shown in Table 4.17.

Table 4.16. Percentages of patients showing amikacin trough concentration < 5mg/L (percentile 2.5 - percentile 97.5) after 1000 simulations of the first dose selected from the final model to be given.

CLCR (mL/min)	WGT (g)					
	500	**1000**	**1200**	**1500**	**2000**	**2500**
10	86.9% (0.167-8.227)	90.7% (0.05-7.65)	90% (0.06-7.71)	89.3% (0.09-8.22)	86.9% (0.11-8.83)	85.5% (0.12-9.17)
20	92.1% (0.05-7.02)	93.9% (0.22-6.53)	94.3% (0.25-6.44)	93.9% (0.30-6.60)	93.2% (0.28-7.21)	92.4% (0.30-7.34)
30	86.8% (0.15-8.52)	89.6% (0.07-7.94)	89.6% (0.10-8.07)	89.7% (0.12-8.31)	86.7% (0.12-9.14)	85.4% (0.11-9.62)
50	94.2% (0.17-6.50)	97.2% (0.38-5.23)	97.2% (0.41-5.26)	97.3% (0.44-5.26)	97.1% (0.46-5.45)	96.5% (0.47-5.74)
60	95.3% (0.27-5.74)	75.5% (0.19-11.82)	75.6% (0.15-12.12)	73.7% (0.17-12.55)	70.5% (0.22-13.04)	65.9% (0.32-13.90)
80	79.5% (0.12-11.50)	83.8% (0.03-10.33)	83.8% (0.05-10.26)	83.3% (0.06-10.50)	81.4% (0.05-11.53)	79.6% (0.01-11.96)

Table 4.17. Percentages of patients showing amikacin peak concentration < 20 mg/L, 20 – 30 mg/L and > 30 mg/L (percentile 2.5 - percentile 97.5) after 1000 simulations of the first dose selected from the final model to be given.

CLCR (mL/min)	WGT (g)																	
	500			1000			1200			1500			2000			2500		
	<20 mg/L	20-30 mg/L	>30 mg/L	<20 mg/L	20-30 mg/L	>30 mg/L	<20 mg/L	20-30 mg/L	>30 mg/L	<20 mg/L	20-30 mg/L	>30 mg/L	<20 mg/L	20-30 mg/L	>30 mg/L	<20 mg/L	20-30 mg/L	>30 mg/L
10	20%	35.5%	44.5%	16.2%	38.3%	45.5%	15.2%	37.1%	47.7%	15.3%	37.2%	47.5%	14.6%	36.9%	48.5%	14.8%	36.6%	48.6%
	(10.70-55.62)			(11.81-54.56)			(12.11-54.95)			(12.18-53.93)			(12.36-54.96)			(12.33-54.58)		
20	23.4%	36.7%	39.9%	16.4%	38.3%	45.3%	16.7%	40.1%	43.2%	15.9%	38.6%	45.5%	14.9%	37.6%	47.5%	15%	36.2%	48.8%
	(10.37-54.41)			(11.88-54.70)			(11.70-52.91)			(11.93-53.73)			(12.20-54.12)			(12.31-54.21)		
30	22.8%	35.9%	41.3%	16.9%	37.8%	45.3%	16.2%	37.5%	46.3%	16.7%	38.6%	44.7%	15.3%	37.9%	46.8%	15.1%	37.4%	47.5%
	(9.88-55.53)			(11.98-54.79)			(11.89-54.49)			(11.79-53.46)			(12.09-53.80)			(12.02-53.71)		
50	23.8%	34.2%	42%	18.9%	39.2%	41.9%	17.5%	38.1%	44.4%	17%	38.6%	44.4%	16.9%	38.3%	44.8%	15.7%	38%	46.3%
	(9.33-57.35)			(10.65-53.84)			(11.34-54.64)			(11.62-53.72)			(11.89-52.97)			(11.88-52.76)		
60	25.4%	33.5%	41.1%	18.6%	38.5%	42.9%	18.2%	39.7%	42.1%	17%	38.3%	44.7%	17%	38.5%	44.5%	15.9%	37.9%	46.2%
	(8.87-58.17)			(10.44-54.30)			(10.88-53.71)			(11.50-54.08)			(11.79-53.13)			(11.84-52.81)		
80	26.4%	33.2%	40.4%	19.5%	36.8%	43.7%	19%	37.7%	43.3%	17.9%	38.2%	43.9%	16.9%	36.9%	46.2%	16.1%	37%	46.9%
	(8.17-59.22)			(10.19-57.32)			(10.88-53.71)			(10.98-56.01)			(11.79-53.13)			(11.78-55.69)		

Percentages of patients with trough concentrations < 5 mg/L were near 80% in all the groups tested. The percentage of peak concentrations between 20 mg/L and 30 mg/L was around 35% and 38% in all the groups. The lowest WGT group (WGT of 500 g) had the highest percentages of ineffective peak concentrations (< 20 mg/L) , from 20% to upwards. The rest of the groups, the percentages of ineffective peak concentrations were lower than 20%. Figure 4.34 and Figure 4.35 show the distribution of trough and peak concentrations for all the groups, respectively. They are in accordance with the results discussed above. So, the initial doses proposed for the different groups based on WGT and CLCR provided safe and effective trough and peak concentrations for a high percentage of patients in all the groups.

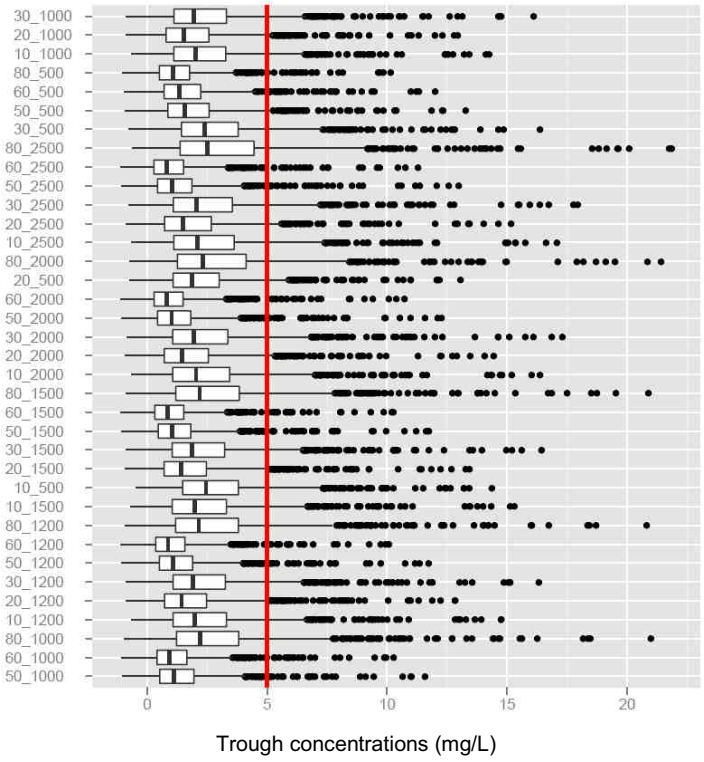


Figure 4.34. Boxplots of amikacin trough concentrations achieved after the first dose administered.

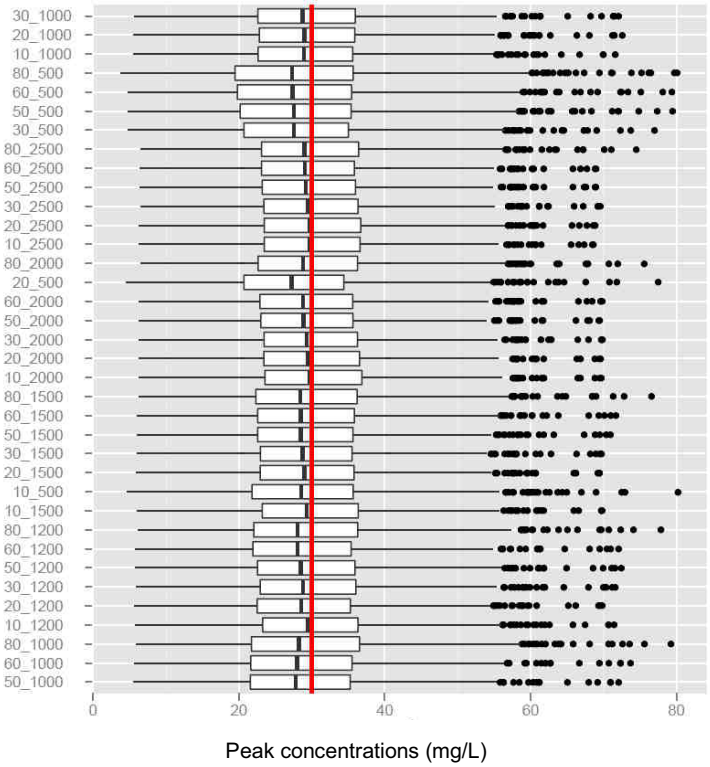


Figure 4.35. Boxplots of amikacin peak concentrations achieved after the first dose administered.

5. DISCUSSION

Understanding the variability associated with pharmacokinetics (PK) and identifying subpopulations with special characteristics can provide clinicians with relevant information for dosage individualization. Uncertainty still exists concerning to the most safe and effective dosing regimen of aminoglycosides in neonates. Target concentrations for amikacin have not been prospectively defined, but clinical convention aims for defined trough and peak concentrations. However, these recommendations have not been based upon studies in neonates. Several studies have demonstrated that conventional paediatric dosing regimens are not well adapted to neonates because of the between-patient variability in pharmacokinetics in this population, resulting in inconsistent serum concentrations, particularly in the most immature individuals (42). In the current study we have developed a population pharmacokinetic model for amikacin in neonates as a first step for establishing the therapeutic range through a dose/exposure-response relationship. The development of a Bayesian estimator from prior population pharmacokinetics information could help to maintain concentrations within a given therapeutic range as well as the dose optimization during the therapeutic drug monitoring (TDM).

Several population PK studies have been conducted to describe the PK of amikacin in neonates (42(123-(125). Major differences among these studies are mainly based on ranges of postnatal (PNA) and gestational (GA) ages, and on the size of the populations studied, apart from the estimation methods applied (parametric vs non parametric). Only analyses of Botha et al (123), Allegaert et al (125) and Sherwin et al (47) were performed according to the parametric approach with NONMEM, while others applied the non-parametric approach. Regarding GAs, some of these studies involved really immature neonates, GA <30 weeks (125). The present study, with a GA median of 31.8 weeks is located between the above and the studies of Bleyzac et al and Botha et al, with GAs medians of 34 and 35 weeks, respectively. Tréluyer et al did not include preterm infants in the studied population. Besides, the present study included a large amount of more extremely premature neonates (GA<28 weeks, 35.6%) and more extremely low-birth-weight infants (birth weight <1000g, 34.2%), similarly than Sherwin et al (47) (57.5% of extremely premature neonates, and 55% of low-birth-weight infants). In relation to PNA, while studies of Bleyzac et al (42) and Allegaert et al (125) were limited to PNAs lower than 2 and 3 days, respectively, median PNA of Tréluyer et al (124) was 69 days, ranging from 1 to 3650 days, and so being a really heterogeneous population. Our population, showed a somewhat higher median PNA (28 days) than that of Sherwin et al (9 days) and with a wider range of variation (4-86 days vs 3-64 days) Only Allegaert et al studied a larger size sample

(n=205) than ours, but their population was restricted to PNAs below 3 days. Therefore, the large size of the sample studied (n=149), the wide range of GAs covered and the relatively high percentage of young premature babies with their associated renal immaturity included, gave relevance to our study compared to the previous.

In the current study, the PK of amikacin was best described by a two-open-compartmental model with zero order input and first order elimination kinetics. All population PK studies of amikacin in neonates above cited reported a one-compartment pharmacokinetic model as the best to describe the respective data, despite the fact that amikacin pharmacokinetics shows a bi-exponential decay. In all these studies, sparse data designs were also applied due to the sampling limitations existing in neonates. As all of these studies, our sampling schedule coincided with the times corresponding to the trough and the so-called peak concentrations, equivalent to an early distributional phase sampling time of 30 minutes after the end of the infusion, as recommended by amikacin dosing protocols (29). However, it should be noted that in our case, due to limitations during the clinical practice, the time of sampling of peak concentrations ranged from 1 to 3.25 hours after the start of the infusion, it allowing a better description of the early distributional phase and hence justifying the better fit of the two-compartment model vs the one-compartment to the data. The PK parameters estimated by omitting data below the limit of quantification (BLQ) were very close to those obtained considering BLQ values reported as 0.09 µg/L, that is a value just lower the LLOQ of the analytical method. However, an inflated proportional residual error was estimated in the later vs the former case. According to Bergstrand and Karlsson (115), there is no predictable pattern when BLQ data are replaced by LOQ/2, sometimes reducing and sometimes inflating the bias in parameter estimates. The inflated proportional residual error found in our case lead to select the base model developed after omitting BLQ data. Handling of BLQ by using “Method 3” recommended by Bergstrand and Karlsson was also tested, but any of the models assayed fit data properly). In fact, the percentage of BLQ data of our study was low (<9%) compared to other studies where this method had been applied successfully.

The population PK model developed in this study allowed the inclusion of between-patient variability in plasma clearance (34.50%), central compartment distribution volume (21.07%) and distributional clearance (70.29%). Inter-occasion variability could not be captured by the model and the fit did not terminate successfully. This could be due to the sparse data design of the study with only two samples per patient at each

occasion and the few number of occasions assayed within each patient (from 2 to 3 occasions). Physiologically feasible estimates of the basic PK parameters of amikacin were found and they did not differ significantly from previous findings of studies in neonates. Unlike adults, differences observed in neonates in growth and maturity, associated with development, can contribute to the variability found in the pharmacokinetic parameters, particularly in clearance. Therefore two scaling approaches can be considered, that is allometric and mechanistic. Allometric scaling is only based on size and says nothing about the maturity of the processes involved in the clearance of the compound, whereas mechanistic scaling considers each process involved in the drug clearance separately and their different maturation rates. These relevant aspects in paediatrics are covered by covariates such as body size covariates, i.e., birth weight (BWGT), current weight (WGT), birth height (BHGT), or current height (HGT) among others; and by age covariates, i.e., gestational age (GA), postnatal age (PNA) and postmenstrual age (PMA), or by renal function covariates, i.e., serum creatinine (CREA) and creatinine clearance (CLCR). The percentage of change observed in these covariates among all the individuals of the population clearly exemplifies their potential contribution to the variability in the pharmacokinetic parameters. For our population, changes of 103.7% and 100% occurred for BHGT and HGT, respectively, and were of approximately the 750% for both BWGT and WGT. Age covariates changed by less than 100% (68.7% for GA and 95.6% for PMA), with the exception of PNA, that increased a 2050% from the lowest to the highest value, reflecting the maturity heterogeneity of our target population. Apart from PNA, the highest percentages of changes were observed for renal function covariates, that is CREA (1215.8%) and CLCR (1971.4%). This lead to the identification of several subpopulations with varying degrees of renal function, either due to immaturity or renal impairment, within the target population. These changes, not being readily apparent in adults, are in line with the high reported between-patient variability (BPV) associated with PK parameters in neonate populations. As expected, correlations were found not only among covariates representing ages, body size or renal function but also between body size parameters and either maturation-related parameters such as ages (as all body size parameters vs GA and PMA) or CLCR (all body size parameters vs CLCR). Correlations between ages and renal function parameters were also found (all age covariates vs CLCR and also between CREA and PNA). Otherwise, as expected, no correlations were observed between PNA and BWGT or BHGT. These correlations resulted to be very informative for the covariate model development. In effect, according to all the above aspects mentioned when clearance in neonates is discussed weight, age and CLCR (provided that the drug is eliminated by renal excretion) should

be considered to explain between patient variability in this parameter and regardless of their relative degree of correlation, they may or may not be mutually exclusive, that is, any one factor may or may not predict between-patient differences in clearance.

Although some authors advocate selection of weight as the first covariate to be entered in the model before investigating the secondary effects of the other covariates related with maturity and renal function, in our case, we firstly tested the inclusion of CLCR on plasma clearance. This deliberate choice was based on known statistical criteria, as CLCR was the covariate that produced the highest decrease of the objective function value (OFV). This was expected for a drug that as amikacin is excreted unchanged by the kidney (5) through glomerular filtration, making renal function given by CLCR (estimated through the Schwarz formula) one of the most determining factors of amikacin disposition. Therefore, CLCR was shown to be the most influential covariate; the estimated CLCR explained 34.55 % of the between-patient variability in CL. Inclusion of CREA on CL resulted in a markedly lower decrease of the OFV than CLCR (-140.25 vs -202.97, respectively). Moreover, although it is also a good marker of renal function in paediatrics, the placental transfer of maternal creatinine, from the first two days of life until several weeks after birth, can contribute to elevated creatinine concentrations inappropriate to age and muscle mass in very preterm neonates. So, it is obvious that the relationship of CREA with amikacin CL during the physiological development of premature infants is complex and CLCR is considered to be a better marker of renal function of the maturation process. Other covariates tested on CL, physiologically and statistically justifiable for the model, were PMA, WGT, BSA, GA, PNA, and BWGT.

From the statistical point of view, postmenstrual age and body weight were the following more influential covariates when tested univariately on amikacin clearance. The cumulative inclusion of PMA in the CLCR-covariate-model did not provide a statistically significant reduction of the BPV associated to CL, which was estimated with very low precision, so that this covariate was removed from the model. Otherwise, body weight tested according to an allometric relationship produced a great reduction of OFV (-72.45) but also of BPV_CL from the base model (53.59%), being the allometric exponent value estimated (0.752) in agreement with biological principles (3/4 or 0.75) and supported by extensive observations from diverse areas in biology. This finding confirmed the expected non linear correlation of renal drug clearances with size factors (as body weight), determining glomerular filtration rate. Thus, total amikacin clearance may be expected to scale with a power of 0.75, even though 0.133 L/h is the typical

clearance for a newborn weighting 1880 g and also a renal function given by a CLCR value of 31.97 mL/min. Allometry has been used to predict either clearance or distribution volumes in infants by scaling the infants weight to that of 70-kg standard adult subject. Of note, a wide range of varying body weights is required to develop such a model and to estimate the scaling exponent. However, in our case, the non-linear allometric scaling power describing the relationship between body size (WGT) and organ function (CL) has been estimated within our neonatal population, this confirming the relative wide range of body weights in this population. However, newborns must grow from an immature form to reach a size that allows reproduction. This maturation factor can not be explained by allometry so that a model describing maturation in required As mentioned before, the inclusion of PMA on the CLCR covariate model was not clinically significant although it has been reported to be a physiological appropriate covariate to explain the time course of changes in clearance. Inclusion of PNA on CLCR covariate model did not provide a statistically significant reduction of the OFV. This was physiologically plausible, since major developing of functional nephrons occurs during the gestational period (126) also discarded PNA as a good descriptor of renal maturation because of the large variability in weight and gestation possible at birth, it also occurring in our study. PNA would have rather been a good predictor if the target population had only included term newborns with larger PNA ranges than that of our population (from 4 to 86 days). In effect at 1-year PNA in neonates born at term the glomerular filtration rate is 90% that of the mature (126). Surprisingly, inclusion of GA in the CLCR-covariate model did not provide a statistically significant significant reduction of the OFV. Therefore, in the current study, unlike other authors (124(125), age was not the best predictor of amikacin renal clearance. This could be due to the high influential effect of CLCR on amikacin clearance it covering differences in renal function associated with factors as maturity, disease and possible drug interactions and hence masking the effect of the own maturity covariates as either PMA or GA. In fact, the studied population has shown a clear increasing of CLCR with either GA or PNA, and hence it may be expected with PMA, with different patterns depending on the degree of prematurity, so that preterm neonates have shown a slower increase in CLCR during the first days of life than full term neonates. This confirms that CLCR can be considered in the current study an accurately measured marker of the maturity degree. On the other hand, a high collinearity has been found between CLCR and both PMA and GA (r^2= 0.921 and 0.865, respectively); so that PMA and GA can be almost predicted by CLCR.

We are aware of results of previous studies reporting age as a good predictor of renal function, i.e., GA (42), PCA, (125) or PMA (47) and PNA, (124). However, we were unable to find among these, any study reporting CLCR as a predictor of amikacin clearance in neonates. This could be justified by the difficulties encountered for determining renal function in children although a high number of formulas have been published. Some of these authors justified this by the fact that CREA is not always routinely measured in neonatal nurseries, converting it into a restricted information, as well as CLCR, and being a reason because of the absence of studies including some marker of renal functionality as a predictor of aminoglycosides CL. In spite of this CLCR has been reported to be a good predictor of plasma clearance of other drugs also excreted by kidneys as vancomycin in neonates (127). The incorporation of CLCR as predictor of amikacin plasma clearance in the clinical setting is a major finding, particularly considering the large variable ranges of CLCR in the neonatal populations confirmed in the current study (from 5.87 to 121.5 mL/min). This maybe due to the presence of more patients with renal impairment, being an enough wide range to quantify some of the differences of amikacin CL independently of body size and also to estimate the covariate effect of CLCR, not included in other studies. On the other hand, some of the population pharmacokinetic studies of amikacin in neonates had reduced ranges of PNAs (125), and hence CLCR/CREA was more a reflex of maternal than neonatal renal function. Then the current model allowed estimating a plasma clearance value of 0.074 L/h/kg for a patient with a renal function given by a CLCR value of 31.97 mL/min. This value resulted to be lower than those reported for adults. On the other hand, this value (0.074 L/h/kg) was slightly higher than that of 0.05 L/h/kg, reported by Sherwin et al. Of note, the target population of Sherwin et al, was that closest to the ours among other studies, but these authors reported a lower CL value probably due to the inclusion of more immaturity.

Regarding other pharmacokinetic parameters estimated by the final model reported herein, as expected BSA, WGT, and the remaining body size parameters were statistically significant on the central compartment distribution volume, as were PMA, GA and PNA due to their correlation with body size parameters. As known, between-patient variability on the central compartment distribution volume, is determining of the peak concentrations achieved after a given dose and secondly the peak concentrations are good markers of efficacy during the amikacin therapeutic drug monitoring. Although BSA provided a more statistically significant reduction of the OFV when tested on V1, vs body weight, only the inclusion of the last, supported by physiological considerations, was considered. For V1, the power function of weight again allowed the

size to be referenced to a 1880 g newborn with allometry using an estimated exponent close to 1 (1.09) as when referenced to a 70 kg-adult. Therefore, the inclusion of WGT on V1 represented a decrease around 63.32% of the BPV_V1, it allowing better individual predictions of the peak concentrations with changes of body weight. A higher central compartment distribution volume was found in the current study (0.465 L/Kg),when compared to adults (0.27 L/Kg) .This could be due to the highest volume of extracellular water reported in neonates (44%) vs the adults (19%), where aminoglycosides use to be distributed because of their polarity.

On the other hand, because of the relatively large extracellular fluid volume of the preterm infants, the volume of distribution is increased and varies greatly from infant to infant, but dramatically decreases during the very first weeks of life. For this reason, some studies have attributed an effect of PCA on volume of distribution of several drugs during the first weeks of life in cohorts of more heterogeneous ages (124,(128(130). In our study, during the univariate covariate testing, all the age covariates decreased significantly the OFV, being PNA which produced the smaller decrease (-9.47). However, the inclusion of either PMA or PNA on the WGT covariate model of V1, not only did not decrease significantly the OFV, but also provided incorrect precision of estimated parameters, revealing a probable overparametrization of the model. Therefore, any of them was finally kept in the model. Although other studies with other drugs have found additional covariates influencing volume of distribution in neonates, such as sepsis and body water (123(131), these were not proved in our dataset. Then, results of the present study predicted that typical CL would range from 0.133 L/h (for a typical patient of 1880 g and CLCR of 31.97 mL/min) to 0.314 L/h (for a typical patient of 1880 g and CLCR of 120 mL/min) or from 0.133 L/h (for a typical patient of 1880 g and CLCR of 31.97 mL/min) to 1.764 L/h (for a typical patient of 4000 g and CLCR of 31.97 mL/min. Moreover the typical V1 would range from 0.837 L (for a typical patient of 1880 g) to 1.906 L (for a typical patient of 4000 g). The therapeutic implications of this are that patients with CLCR/WGT values of 120 mL/min/1880 g would require larger initial doses than patients with CLCR/WGT values of 31.97 mL/min /1880 g to achieve peak concentrations within the therapeutic range, or that patients with CLCR/WGT values of 120 mL/min/1880 g would require also larger doses than patients of 120 mL/min/4000 g to achieve peak concentrations within the therapeutic range.

Regarding to distributional clearance and peripheral compartment distribution volume, typical values of 0.039 L/h and 0.409 L were found, respectively, however given that no

previous models for amikacin in neonates where a two-compartment model best described the pharmacokinetic profile, comparisons were not able to be performed.

Once the model had been developed, its predictive performance assessment was considered necessary before the intended use of this for either initial dose calculations or dose tailoring during the therapeutic drug monitoring. The two widely known approaches (internal and external evaluation methods) were applied and their results compared. Firstly the most methodologically pure approach was considered by measuring the predictive performance of the model in a separate population of 53 neonates. The bias and precision of population and individual predicted concentrations estimated according to Sheiner and Beal (120), were totally acceptable. Although we were not aware of previous developed models for amikacin that were validated externally, our results were in line with those found after an external validation of a previously reported population PK model for vancomycin in neonates (132). Based on the median trough and peak concentrations of 2.21 and 22.12 mg/L, respectively, of the current study, the median precisions of trough and peak concentrations estimated from individual predictions (0.71 and 1.20, respectively) represented the median errors of 32.12% (for trough concentrations) and 5.42% (for peak concentrations).

As far as model validation is concerned, internal validation techniques, more complex and computationally intense were also applied. Hence, the bootstrap, the visual predictive check, or the normalized distribution prediction errors confirmed the good performance of the model. However, the prediction corrected visual predictive check technique was considered more suitable, because of the changing doses and covariate values in the target population). In this case, a slight underprediction of peak concentrations was suggested and also of trough concentrations beyond the 24 hour dosing intervals. Surprisingly, the posterior predictive check proved to be the most sensitive technique to model misspecifications. Results of this also suggested good predictability for the model although a slight under and over prediction of the medians of the trough and peak concentrations, respectively, was observed.

One of the purposes of this study was to use the developed model to evaluate the influence of the statistically significant identified covariates on the amikacin exposure (trough and peak concentrations) with the actual dose regimens being given. Hence, the influence of WGT and CLCR on amikacin trough and peak concentrations was investigated, after establishing several CLCR/WGT cutoffs according to the CLCR/WGT distributions in the target population. Results suggested that for most of the CLCR/WGT cutoffs, the current dosage (dose and dosing interval) provided trough and peak concentrations within the therapeutic range (trough < 5 mg/L) and peak concentrations > 20 mg/L) However, further revision would be required for some

groups, particularly in regard to trough concentrations of patients with CLCR < 15 mL/min and WGT ≥ 2000g (with only 64.2% of trough concentrations <5 mg/L), or peak concentrations of patients with CLCR values ≥60 mL/min. (with more than 68% of peak concentrations < 20 mg/L), except for those with WGT ≥ 2000g. This could be due to the lack of correlation between the stratifying criteria applied by us vs Neofax guide, especially for the most immature groups when compared peak concentrations, and for the less immature groups when compared trough concentrations. That is, probably patients of GA <29 weeks and PNA 0-7 days, showed slightly higher CLCR values or WGT than those of the first cutoff of our distribution, meanwhile, patients of GA ≥35 weeks tended to show lower CLCR values than 60 mL/min. On the other hand, as expected, results of simulated percentages of trough and peak concentrations from the original dataset, once stratified according to the Neofax criteria, strengthened the fact that the current dosage involves a great percentage of underdosed individuals However caution should be taken because as described before the recommendations of Neofax guide were not strictly followed-up in this study.

Secondly, the model was applied to investigate the most recommended dose regimens in order to prevent under or over-exposure based on target trough and peak concentrations. Hence, the initial dosage recommendations were established. According to this, amikacin dosage regimens should be individualised particularly in those patients with more reduced renal function (CLCR< 15 mL/min), either due to immaturity or impairment. These patients would require dose but also dosing interval adjustments to larger values than 24 hours. To avoid toxicity These results are in accordance with the recommendations of Neofax guide, which establishes dosing intervals of 36 hours and 48 hours for the most premature neonates. The base-model simulations also confirmed the impact of changes of CLCR on trough concentration values and hence on the potential toxicity of the drug determining the dosing interval values. Otherwise, changes in body weight are more influential on peak concentration values, efficacy markers determining the amounts of drug to be given.

In summary a validated population pharmacokinetic model has been developed for amikacin in a relatively large paediatric population of young premature babies which are a faithful representation of renal immaturity. Dosing adaptation from the start of the treatment to avoid under or over exposure is feasible in this population treated with repeated doses. Hence, results of this study may assist the optimization of initial dosing regimen aimed at achieving target trough and peak concentrations form the start of the treatment. Moreover the implementation of this model in a Bayesian prediction software can provide feedback dosage adjustments to achieve desired serum concentrations during the therapeutic drug monitoring.

Nevertheless, one of the main limitations associated with the present study is the lack of pharmacodynamic (PD) data, not only because it could allow us to build our own PD model, but also to be taken into account for dosage and monitoring of the antibiotic. In order to solve it in the near future, one possibility could be the use of a published PD model obtained in a population with similar characteristics than our, and validate its use in our population. Secondly, it could be valued the use of cumulative fraction response, based on the distribution of minimum inhibitory effect (MIC) in our hospital area, as another way to improve the PK model developed, and consequently, the dosage and monitoring of amikacin in the neonate population.

One of the main limitations associated with the present study was the lack of pharmacodynamic data (PD) in order to develop a pharmacokinetic-pharmacodynamic model allowing to more precisely establish the therapeutic range in the neonatal population. However alternative approaches can be applied for this purpose as i) to incorporate a previously published PD model , developed in a similar population than ours, and to validate it in our population or ii) to use the cumulative fraction response, based on the distribution of minimum inhibitory concentrations (MIC) of our hospital area.

6. CONCLUSIONS

1. A population pharmacokinetic model for amikacin in newborn patients has been developed.
2. The pharmacokinetics of amikacin in newborn patients has been best described by a two-compartment kinetic model with first order elimination process, parameterized as clearances and distribution volumes.
3. Between-patient variability, modelled exponentially, has been associated with plasma and distributional clearances and central compartment distribution volume. Combined residual error model (additive + proportional) has provided the best fit of data.
4. Current weight and creatinine clearance have showed to be the best predictor covariates of plasma clearance, and current weight the most significant predictor covariate of central compartment distribution volume. Inclusion of current weight on plasma clearance and distribution volume has been performed according to an allometric model.
5. The results of an external validation with a group of patients not included in the development, belonging to the same population as the target one has proved a good predictive ability for the model. Median bias and precision values for trough and peak individual predicted concentrations (IPRED) have been lower than 0.72 mg/L and 1.21 mg/L, respectively.
6. Internal validation techniques as bootstrap, visual predictive checks, Npde and PPC have all confirmed the good predictive power of the model, being in agreement with the results from the external validation.
7. Model-based simulations have allowed to evaluate the impact of changes in WGT and CLCR values on the amikacin exposure.
8. The model developed has allowed to evaluate the current dose regimen and to recommend initial dosing strategies for different cutoffs of the covariates identified as best predictors of the PK behaviour, i.e. WGT and CLCR.
9. This model further implemented in a Bayesian dose optimization software will help in the dose adjustment during the therapeutic drug monitoring of amikacin in the target population.
10. Additionally, the model can be used to further investigate optimal dosing strategies.
11. The implementation of the model in the routine therapeutic drug monitoring practice will allow prospective validation of this model.

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8. APPENDIX

APPENDIX 1

DEMOGRAPHIC DATA:

- Initials/ Medical number:
- Birth date:
- Gender*:

PERINATAL DATA:

- Number of gestation:
- Twin pregnancy: Yes No
- Gestational age:

- APGAR: Apgar1 ../../../../../ Apgar5 ../../../../../
- At birth: Weight:
  Height:
  Head circumference:

* *(1)Male, (0)Female*

TREATMENT:

- Main diagnostic:
- Empirical: Yes No
- Identified germ:

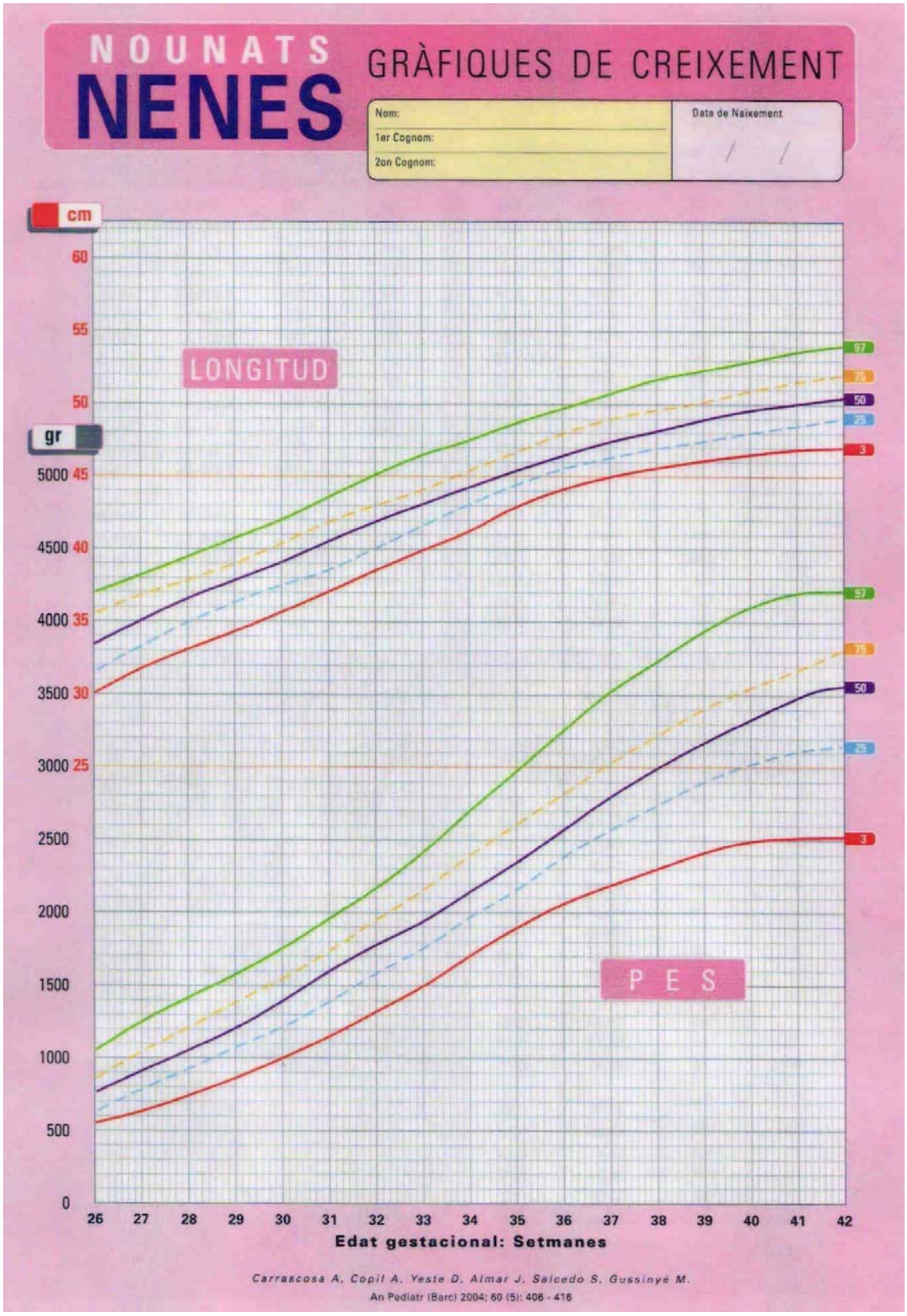
OBSERVATIONS:

AMIKACIN TREATMENT:

Date	Anthropometric data**			Biochemistry data**		Amikacin administration			Blood sampling	
	Weight (Kg)	Height (cm)	Head cir (cm)	Urea (mg/dl)	Creatinine (mg/dl)	Hour	Dose (mg)	Interval	Hour Ctrough	Hora Cpeak

** Registrat el mateix dia de l'extracció de sang o alternativament entre els ± 2 dies consecutius.*

APPENDIX 2.1 GROWTH GRAPHIC (GIRLS)



APPENDIX 2.2 GROWTH GRAPHIC (BOYS)