

Determination of the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr George Mukhari Academic Hospital: Gauteng Province

Dissertation submitted by

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in partial fulfilment of the requirements for the degree of

Master of Science (Medical) in Pharmacy

of the

University of Limpopo, South Africa

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January 2013

DECLARATION

I, Deirdré Engler, hereby declare that the work on which this dissertation is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for any other degree at this or any other university.

Signature

Date

DEDICATION

To my husband, *Kevin* and our two sons,
Reinhardt and *Rüdiger*

I dedicate this dissertation to you as you all had to make a lot of sacrifices during these times. Thank you for your love, support and encouragement.

ACKNOWLEDGEMENTS

I would like to extend my sincere thanks and appreciation to the following people, for without their help, none of this would have been possible:

- ∞ **Dr. Natalie Schellack and Prof. Andries Gous** for all their advice, assistance, patience and perseverance

- ∞ **Mrs. Alida Naude** for co-supervising the study and interpretation of audiology results

- ∞ **Ms. Celeste Botha** for her input in the initial protocol

- ∞ **Mrs. Liepollo Ntlhakana and Mr. Melvin Mogano** for their assistance with data collection

- ∞ **Neonates and their parents** for their participation in the study

- ∞ **Doctors and nurses** in the NICU for their cooperation

- ∞ **Mrs Ingrid Bornman** of Tshwane NHLS for all her help and co-operation to obtain the amikacin peak and trough levels

- ∞ **The interns (Amma Yeboah, Heleen Esterhuizen, Zanli de Jager and Christinah Mokou) and Department of Pharmacy** for logistical support

- ∞ **Prof. Hermann Schoeman, Mrs. Salomie Nel and Dr. Hannelie Meyer** for their assistance with the analysis of the data

DISSEMINATION OF FINDINGS

PODIUM PRESENTATIONS

NATIONAL CONFERENCES

1. **Engler D**, Schellack N, Naude A, Gous AGS. Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr George Mukhari Academic Hospital - Gauteng Province. South African Society of Clinical Pharmacists Conference. Grahamstown. 25 - 27 June 2012.
2. **Engler D**, Schellack N, Naude A, Gous AGS. Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr George Mukhari Academic Hospital - Gauteng Province. Neonatal Nurses Association of South Africa. Bloemfontein. 4 - 5 October 2012.

POSTER PRESENTATIONS

LOCAL CONFERENCES

1. **Engler D**, Schellack N, Naude A, Gous AGS. Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr George Mukhari Academic Hospital - Gauteng Province. Academic Day. University of Limpopo. 22 - 23 August 2012.

PUBLICATION

Paediatrics: Use of amikacin in neonates and related ototoxicity

Use of amikacin in neonates and related ototoxicity

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Keywords: amikacin, neonates, related ototoxicity

Abstract

Neonates frequently receive aminoglycosides as empiric therapy for severe infections caused by suspected Gram-negative bacteria. Amikacin is classified as an aminoglycoside. Optimum dosing of aminoglycosides is required because of the inter-individual variability in the pharmacokinetics of aminoglycosides in the neonatal population. Aminoglycosides have the ability to produce nephrotoxicity and ototoxicity. This is a major limitation and concern regarding the use of this class of antibiotics. The ototoxic effects of AG treatment are dose-dependent. Preterm infants are especially susceptible to the ototoxic effects of aminoglycoside drugs, because of the anatomical and functional maturation development of the inner-ear system. Identification of AG ototoxicity is important to minimise long-term damage.

Introduction

Neonates, particularly when premature, are prone to more infections because they are relatively immunocompromised.¹ Sixty per cent of preterm neonates receive at least one antibiotic and 43% of the antibiotics that are administered to these neonates are aminoglycosides.² This class of antibiotics is concentration-dependent.³ Therefore, achieving a therapeutic maximum concentration of amikacin in plasma is associated with a significant decrease in the rate of mortality because of infection in critically ill patients.⁴ Amikacin has a very narrow therapeutic range and can cause serious side-effects, such as nephrotoxicity and ototoxicity.⁵ Neonates are a high-risk population for hearing loss,⁶ and when ototoxicity occurs, it places a burden of disability on the affected individual.⁷

Amikacin: mechanism of action

The aminoglycosides primarily act by binding to the amino-acyl site of 16S ribosomal RNA within the 30S ribosomal subunit, leading to misreading of the genetic code and inhibition of translocation. The initial steps required for peptide synthesis are uninterrupted, such as binding of mRNA and the association of the 50S ribosomal subunit, but elongation fails to occur because of disruption of the mechanisms to ensure translational accuracy. Usually, the ensuing antimicrobial activity is

bactericidal against susceptible aerobic Gram-negative bacilli.⁸ Amikacin has the widest antimicrobial spectrum⁹ and is resistant to inactivating enzymes. This allows it to be more active against pathogens resisted by other aminoglycosides.¹⁰ The aminoglycosides as a class that demonstrate concentration-dependent killing and produce prolonged post-antibiotic effects.¹¹

Pharmacokinetics of amikacin

"Pharmacokinetics" is defined as the study of the time course of drug absorption, distribution, metabolism and excretion. Less than 1% of aminoglycosides is absorbed from the gastrointestinal tract and has to be administered parentally.¹¹ Amikacin is a highly hydrophilic drug and distributes primarily into extracellular fluid (ECF).¹² The ECF compartment is larger in a newborn baby (40% of bodyweight, compared to 25% of bodyweight in adults), comprising 70-75% of the total bodyweight.

These characteristics affect the volume of distribution of aminoglycosides, so a higher dose per kilogram of bodyweight needs to be administered in order to achieve adequate peak blood concentrations.¹³ The volume of distribution of amikacin is stated to be 0.25 l/kg with an elimination half-life in low birthweight neonates (1-3 days) as 7-9 hours, and in term neonates (< 7 days) as 4-5 hours.¹² Studies have shown that the volume of distribution is larger, and elimination half-lives are longer, the earlier

the gestational age.¹⁴ Amikacin is almost exclusively eliminated by glomerular filtration in neonates. In general, renal clearance of drugs in preterm and term neonates is lower than that in infants and children, and increases with postnatal and postmenstrual age. Neonatal renal clearance is dependent on the glomerular filtration rate.¹⁵ Nephrogenesis is completed by end of the thirty-fourth week of gestation.¹⁶ The results of a study carried out on 275 infants confirmed a median glomerular filtration rate reference value (ml/minute per 1.73 m²) in infants aged 27-31 weeks' gestation ranged from 7.9-30.3 on day 7, 10.7-33.1 on day 14, 12.5-34.9 on day 21, and 15.5-37.9 on day 28.¹⁷

The main determinants of amikacin clearance are current weight and postmenstrual age.¹⁸ The primary determinant of volume of distribution is current weight, and the foremost predictor of treatment failure is peak/minimum inhibitory concentration (MIC) ratio < 8 µg/ml. Differences in body composition and the renal immaturity associated with neonates contribute to the variability of the pharmacokinetic parameters, particularly clearance, gestational age, post-natal age, haematocrit, fever and lean body weight.¹⁸

Dose and plasma concentration of amikacin

The recommended dose for amikacin in neonates is 15 mg/kg once daily.^{19,20} The target plasma levels are a peak of > 30 µg/ml and a trough of < 1 µg/ml.¹⁹ Neofax 2009 Online states a peak of 20-30 µg/ml and a trough of 2-5 µg/ml. Conventional paediatric dosing regimens are not well adapted to neonates and result in inconsistent serum concentrations, particularly in extremely low birthweight neonates. Peak concentrations are often too low to achieve an optimal bactericidal effect, and trough concentrations too high, with increased risk of toxicity.¹⁸ Higher peak concentrations may result in more rapid killing of susceptible organisms. According to the schedule by Young and Mangum,²⁰ peak concentrations should be > 40 µg/ml.² Optimal antibacterial activity of amikacin is achieved when the peak is 8-10 times greater than the MIC. The clinical MIC breakpoint for problematic pathogens, e.g. Enterobacteriaceae and *Pseudomonas aeruginosa*, in intensive care unit patients is 8 µg/ml.²¹ The post-antibiotic effects of amikacin refer to amikacin retaining its antibacterial effect while the concentration falls below the MIC. Although the post-antibiotic effects of aminoglycosides is usually in the order of 2-4 hours,¹⁴ the post-antibiotic effects of amikacin last from 13.5-27.6 hours.²² The bactericidal efficacy of amikacin mainly relates to intermittent, discontinuous peak concentrations as a result of the post-antibiotic effects.¹⁵

Uncertainty about the safest and most effective dosing regimen of any AG in neonates prevails. Dosing is based on a balance of maximal efficacy and minimal toxicity, while still avoiding the induction of bacterial resistance.¹⁵

Toxic side-effects of amikacin

Although the use of aminoglycosides is limited because of its toxic effects, they are cost-effective and therefore widely used, particularly in developing countries.²³ All aminoglycosides cause renal toxicity, which is often reversible, as well as irreversible ototoxicity.²⁴ Various studies have shown that both the therapeutic response and toxic effects of aminoglycosides depend on plasma concentrations.^{4,13} Renal side-effects and ototoxicity relate to the average plasma concentration based on the saturation of the renal and cochlear cell-binding sites.¹⁵ aminoglycosides accumulate in the kidney and can account for 40% of the total drug in the body.¹¹

Accumulation of aminoglycosides occurs within the proximal tubular epithelial cell in the lysosomal phospholipid complexes which rupture and initiate cell death.¹⁴ Although aminoglycosides are hydrophilic in nature and do not readily cross cell membranes, it is proposed that both endocytosis and transport through ion channels mediate the uptake of aminoglycosides into sensory hair cells.²⁵ Free radicals are generated within the inner ear by aminoglycosides, with subsequent permanent damage to sensory cells and neurons.²⁶ Other reasons for AG ototoxicity have been indicated by studies as abnormal iron transport, dysfunction of mitochondria, gene mutations and drug-drug interactions.²⁷

Ototoxic hearing loss is secondary to the destruction of the outer-hair cells of the inner ear and will initially produce a high-frequency, sensory-neural hearing loss. Latency exists to the ototoxic effects of aminoglycosides because of the slower clearance from inner-ear fluids, compared to the clearance from serum. This latency can result in progression of hearing loss²⁸ because of prolonged exposure of the cochlear cells to aminoglycosides.²⁷ aminoglycosides progressively accumulate in the endolymph and perilymph of the inner ear. The half-life in these fluids is 5-6 times greater than that of plasma half-life. Ototoxicity is more likely to occur in patients with persistently elevated concentrations in plasma because of the dependence of back-diffusion on concentration of the drug in plasma.²⁹ Amikacin is mainly cochleotoxic.²⁵ Symptoms include tinnitus and high frequency hearing loss. The latter is especially crucial for speech recognition. Damage to the cochlea, referred to as sensorineural hearing loss, can produce permanent hearing loss because the hair cells in the cochlea do not regenerate.²⁶

Therapy with aminoglycosides that exceeds 10 days, prior or concurrent treatment with ototoxic drugs and pre-existing renal impairment, increases the risk for ototoxicity.¹¹

Monitoring the neonate

The use of ototoxic drugs in the neonatal population

requires careful monitoring by healthcare professionals, because of the characteristics and special needs of this population.³⁰ Prevention of AG ototoxicity will require effective therapeutic drug monitoring, as well as hearing evaluation prior to, during, and after, the drug treatment.³¹

Therapeutic drug monitoring refers to the individualisation of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range or window. Its goal is to individualise therapeutic regimens for optimal patient benefit.³² The bactericidal efficacy of amikacin mainly relates to intermittent, discontinuous peak concentrations because of the post-antibiotic effect, while renal side-effects and ototoxicity relate to the average plasma concentration, based on the saturation of renal and cochlear cell-binding sites.¹⁵ Drug concentrations may be used as surrogates for drug effects so therapeutic drug monitoring can optimise patient management and improve clinical outcomes.³³ Optimising AG therapy should be achieved by tight-serum concentration monitoring because of the wide interindividual variability of pharmacokinetic abnormalities.²¹ Regular monitoring of levels is recommended to assure the adequacy of the dosing regimen and to monitor for drug accumulation and possible toxicity. A reduction in toxicity caused by aminoglycosides was observed when the trough concentration was < 2 µg/ml.¹³

The primary objective of ototoxicity monitoring is to identify cochlear changes, even before they become apparent during behavioural audiometric testing.³⁴ Transient-evoked otoacoustic emissions (OAEs) and distortion-product OAEs are evoked OAEs that are clinically applied in various settings, such as neonatal hearing screening and ototoxicity monitoring.³⁴ OAEs can be applied during ototoxic monitoring, as they are site-specific for cochlear outer-hair cell damage. Ototoxic drugs, such as amikacin, exert their effect on outer-hair cell functioning.²⁵ Aminoglycosides affect high-frequency hearing earlier than low-frequency hearing. High-frequency hearing losses are audiometrically more detectable before they become severe enough to involve the speech frequency range.¹³ Measurement of OAEs provide an objective evaluation of the cochlear outer-hair cell system.³⁵ Distortion-product OAEs are especially valuable when monitoring ototoxicity in very young children who may not consistently provide reliable, complete, ear-specific, pure-tone threshold responses. The measurement of distortion-product OAEs does not require the child's active participation.³⁵ An advantage of distortion-product OAEs over transient-evoked OAEs is that the former test at higher frequencies (500-8000 Hz) vs. 500-5000 Hz in the case of transient-evoked OAEs, making them more sensitive to the frequency area that is affected first.³⁶

Conclusion

Hearing impairment can be viewed as the silent epidemic of developing countries, owing to its invisible, nonlife-

threatening, but highly prevalent nature.³⁷ Infants who are admitted to the neonatal intensive care unit are a population who will benefit from early hearing detection and intervention services. It has long been known that these infants are a high-risk population.⁶ Factors such as prematurity, low birthweight and the provision of ototoxic drugs, e.g. aminoglycoside drug treatment, increase these infants' risk for hearing loss.

The use of ototoxic drugs in the neonatal population requires careful monitoring by healthcare professionals because of the characteristics and special needs of this population.³⁰ Regular monitoring of levels is recommended to assure the adequacy of the dosing regimen and to monitor for drug accumulation and possible toxicity.

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ABBREVIATIONS AND ACRONYMS

AABR	Automated Auditory Brainstem Response
AG	Aminoglycoside
ASHA	American Speech – Language – Hearing Association
dB	Decibel
DGMH	Dr George Mukhari Hospital
DPOAE(s)	Distortion Product Otoacoustic Emission(s)
ECF	Extracellular fluid
EHDI	Early hearing detection and intervention
ELBW	Extremely low birth weight
GA	Gestational age
GFR	Glomerular filtration rate
HCU	High Care Unit
HFA	High frequency audiometry
HPCSA	Health Professions Council of South Africa
Hz	Hertz
ICU	Intensive Care Unit
IHC	Inner hair cell
IQR	Inter-quartile range
JCIH	Joint Committee of Infant Hearing
kHz	Kilohertz
KMC	Kangaroo mother care
LBW	Low birth weight
MD	Maintenance dose
MIC	Minimum inhibitory concentration
NBW	Normal birth weight
NEC	Necrotizing enterocolitis
NHS	Newborn hearing screening
NICU	Neonatal Intensive Care Unit
OAE	Otoacoustic Emission
OHC	Outer hair cell

PAE	Post-antibiotic effect
PD	Pharmacodynamic
PK	Pharmacokinetic
PMA	Post-menstrual age
PNA	Postnatal age
SOAE	Spontaneous Otoacoustic Emission
SPL	Sound pressure level
TDM	Therapeutic Drug Monitoring
TEOAE	Transient Evoked Otoacoustic Emission
U&E	Urea-and-Electrolyte
Vd	Volume of distribution
VLBW	Very low birth weight

ABSTRACT

Neonates, particularly when premature, are prone to more infections due to being immunocompromised. Sixty percent of preterm neonates receive at least one antibiotic and 43% of the antibiotics administered to these neonates are aminoglycosides (AGs). This class of antibiotics is concentration dependent thus achieving a therapeutic maximum concentration of amikacin in plasma is associated with a significant decrease in the rate of mortality due to infection in critical ill patients. Amikacin has a very narrow therapeutic range and can cause side effects such as nephrotoxicity and ototoxicity. Neonates are a high risk population and the internal and external risk factors necessitate close monitoring in this population. The main aim of the study was to determine a possible correlation between amikacin serum concentrations and ototoxicity in neonates by using otoacoustic emissions (OAEs).

The study was done at the Neonatal Intensive Care Unit of Dr George Mukhari Hospital (DGMH), a public sector academic hospital in Ga-Rankuwa, west of Pretoria in the Gauteng Province. A descriptive quantitative study with a correlation research design was used for the study. The correlation design was between the amikacin peak and trough levels and otoacoustic emission amplitudes measured at four different frequencies. Although the clinical therapeutic range for amikacin trough level is less than 10 mcg/mL, for the purpose of the study trough levels higher than 2 mcg/mL were used as a referral end point for OAEs.

Over the period of five months, 83 neonates receiving amikacin therapy were recruited. Data were obtained from 55 (66%) of these recruited neonates. Kinetic-only data were obtained from 33 neonates and kinetic-and-audiology data from 22 neonates.

The total population group had a mean gestational age (GA) of approximately 33 weeks with a mean weight of 1.91kg. This group received a mean maintenance dose of 19.59 mg/kg per day. Their glomerular filtration rate (GFR) was within limits and the mean was 19.58 mL/min per 1.73m². Pharmacokinetic (PK) calculations revealed

a mean true peak value of 47.45 mcg/mL which was higher than the recommended range of 30 to 40 mcg/mL. The reference half-life is between 4 and 8 hours, but was much longer at 10.93 hours. With regards to the volume of distribution (V_d), it was towards the upper limit, calculated at 0.665 L/kg. The interquartile range (IQR) for the whole population ($n = 55$) showed that 50% of the true trough levels ranged between 2.07 and 6.15 mcg/mL with true peak levels between 36.14 and 53.43 mcg/mL.

The kinetics-only group had a mean GA of 33 weeks with a mean weight of 1.84 kg. This group received a mean maintenance dose of 19.84 mg/kg and had a GFR of 19.51 mL/min per $1.73m^2$. A mean peak of 45.67 mcg/mL and a mean trough of 7.07 mcg/L, both much higher than desired. The mean half-life was much higher than the reference range at 12.88 hours and the volume of distribution within range but towards the upper limit.

The kinetic-and-audiology group had a mean GA of 33 weeks with a mean weight of 2 kg. This group received a mean maintenance dose of 20.12 mg/kg and had a GFR of 139.71 mL/min per $1.73m^2$. A mean peak of 50.13 mcg/mL and a mean trough of 5 mcg/L, both much higher than desired. The half-life was toward the upper limit and the volume of distribution within range.

During the ototoxic monitoring, a DPOAE assessment was completed at baseline for the sub-population of 22 patients. These baselines were determined within 24 hours from when the patient was identified and consent obtained. A follow-up DPOAE was performed on the day of the third MD and therapeutic drug monitoring (TDM).

Distortion product otoacoustic emissions (DPOAEs) were obtained at four different frequencies for each ear, namely at 2, 4, 6 and 8 kHz. Trough levels above 2 mcg/mL, but below the accepted therapeutic level of 10 mcg/mL, affected seven neonates ears (left and/or right), thus 32% of the sub-population. Three neonates ($n = 22$; 14%) had trough levels above 10 mcg/mL and reflected a change in outer hair cell function at some to all the frequency levels. Peak levels above 50 mcg/mL affected eight neonates' ears (left and/or right), accounting for 36% of the sub-

population. A total of six patients had both ears affected with peak and trough levels, thus 27.3% of the sub-population.

This study indicated that outer hair cell (OHC) function is affected from baseline to follow-up audiology in neonatal patients treated with amikacin. A multidisciplinary approach between pharmacists, audiologists and doctors is imperative to reduce the morbidity in vulnerable population groups. Diagnostic OAE and PKs for ototoxic medications should be further investigated in a larger study population.

Keywords: neonates, aminoglycoside, amikacin, ototoxicity, outer hair cell, otoacoustic emission

CHAPTER 1

INTRODUCTION

This introductory chapter describes the background and rationale for the study. The aim and objective of the study are provided and this chapter ends with an outline of the dissertation.

1.1 BACKGROUND TO THE STUDY

Neonates, particularly when premature, are prone to more infections due to being relatively immunocompromised (Yeung and Davies 2005) and receive aminoglycosides (AGs) as empiric therapy for severe infections caused by suspected Gram-negative bacteria. According to Pacifici (2008), 60% of preterm neonates receive at least one antibiotic and 43% of the antibiotics administered to these neonates are AGs. Aminoglycosides are concentration-dependent, thus high peak blood concentrations of AGs are associated with increased survival and a better therapeutic response in Gram-negative infections (Touw, Westerman and Sprij 2009). The optimum dosing of AGs is required due to the inter-individual variability in the pharmacokinetics of AGs in the neonatal population (Pacifici 2008; Siddiqi, Khan, D.A., Khan, F.A. and Razzaq 2009). The extracellular-fluid (ECF) compartment differs in neonates, infants and adults, with premature neonates having the largest ECF compartment. These changes will influence the pharmacokinetic properties of hydrophilic drugs like amikacin. Various studies have shown that both the therapeutic response and toxic effects of AGs depend on plasma concentrations (Touw *et al.* 2009; Tréluyer, Merlé, Tonnelier, Rey and Pons 2002). Aminoglycosides have the ability to produce nephro- and ototoxicity, rendering it a concern surrounding the use of this class of antibiotics. Maudonnet *et al.* (2008) stated that the ototoxic effects of AG treatment are dose dependent. A reduction in toxicity caused by AGs was observed when the trough concentration was < 2 mcg/mL (Touw *et al.* 2009). Aminoglycosides also induce a post-antibiotic effect (PAE), thus a period of delayed regrowth (Turnidge 2009) and the PAE of amikacin lasts from 13.5 to 27.6 hours (Tsui, Yew, Li, *et al.* 1993). Regular monitoring of levels is recommended to assure the adequacy of the dosing regimen and to monitor for drug accumulation and possible toxicity.

The incidence of hearing loss in neonates is two to four cases in every 1000 live births (Zamani, Daneshjou, Ameni and Takand 2004). Preterm infants are especially susceptible to the ototoxic effects of AG drugs, due to the anatomic and functional maturation development of the inner ear system (Naeimi, Maamouti, Baskabadi *et al.* 2009). Ototoxicity, in relation to this drug, is defined as cochlear injury which can manifest as hearing loss and affects up to 33% of patients receiving AGs (Deng 2012). Amikacin is mainly cochleotoxic and hearing loss is secondary to the destruction of the outer hair cells (OHC) of the inner ear. Symptoms include tinnitus and high frequency hearing loss, the latter being especially crucial for speech recognition (Huth, Ricci and Cheng 2011) and optimal language and speech development (Swanepoel, Störbeck and Friedland 2009). Identification of AG ototoxicity is important to minimise long-term damage to the cochlea of the inner ear. Cochlear damage can be monitored via electrophysiological measurements, such as otoacoustic emissions (OAE) and automated auditory brainstem response (AABR). The Joint Committee of Infant Hearing (JCIH 2007) states that these tests should be used as a standard protocol to screen infants and children for hearing loss before three months of age.

An OAE is a sound which is generated from within the inner ear. Transient evoked OAEs (TEOAE) and DPOAE require a stimulus to generate this sound. Otoacoustic emissions are site-specific for cochlear outer hair cell damage which makes them ideal to monitor ototoxic drugs such as amikacin exerts their effect on the OHC functioning (Huth *et al.* 2011). Measurement of OAEs provides an objective evaluation of the cochlear OHC system (Knight *et al.* 2007). Distortion Product OAEs do not require the child's active participation (Knight *et al.* 2007) and are especially valuable for monitoring ototoxicity in very young children who may not consistently provide reliable, complete, ear-specific pure-tone threshold responses. Aminoglycosides affect high-frequency hearing earlier than low-frequency hearing. During high frequency audiometry hearing losses are audiometrically more detectable before they become severe enough to involve the speech frequency range (Touw *et al.* 2009).

The use of ototoxic drugs in the neonatal population requires careful monitoring by health care professionals, due to the characteristics and special needs of this population (Schellack 2010). Prevention of AG ototoxicity requires effective TDM as well as hearing evaluation prior to, during and after the drug treatment (Naeimi *et al.* 2009).

1.2 RATIONALE FOR THE STUDY

Neonates are a high risk population for hearing loss (JCIH 2007) and when ototoxicity occurs, it places a burden of disability on the affected individual (Roland and Rutka 2004).

The recommended dose for amikacin in neonates according to the South African Medicines Formulary is 15 mg/kg once daily. The target plasma levels are a peak of > 30 mcg/mL and a trough of < 1 mcg/mL (Rossiter 2012). Conventional paediatric dosing regimens are not well adapted to neonates, resulting in inconsistent serum concentrations, particularly in extremely low-birth-weight neonates (ELBW). Peak concentrations are often too low for optimal bactericidal effect and trough concentrations too high with increased risk of toxicity (Sherwin, Svahn, Van der Linden *et al.* 2009). Currently a loading dose of 25mg/kg/dose is used as the dosage regimen in the NICU at DGMH. A maintenance dose is administered irrespective of gestational age, post-natal age or renal functioning (Schellack 2010). A study conducted in this unit by Ntlhane (2004), concluded that the once-daily dose of 20mg/kg/dose is appropriate, but that peak and trough serum levels should still be monitored in neonates with conditions that could interfere with the amikacin pharmacokinetics, e.g. birth asphyxia, meconium aspiration and congenital pneumonia (Schellack 2010).

Serum creatinine levels are routinely requested and the glomerular filtration rate (GFR) calculated to monitor the kidney functions of neonates admitted to the NICU of DGMH. Currently there is no hearing screening or ototoxic monitoring being provided within the NICU, at DGMH, in spite of the advantages such a programme provides.

This study was motivated by the possibility of establishing a multidisciplinary treatment approach for NICU neonates. The aim was to determine a possible correlation between peak and trough levels (as obtained with the plasma concentration) and audiological test results via OAE, for neonates receiving amikacin in the NICU at DGMH. With a multidisciplinary approach, attempts could be made to optimise treatment while preventing or at least minimizing the risk of damage to both the cochlear and renal functioning of the neonate.

1.3 AIM OF THE STUDY

The main aim of the study was to determine a possible correlation between amikacin serum concentrations and ototoxicity in neonates by measuring outer hair cell functioning with otoacoustic emission assessments.

1.4 OBJECTIVES OF THE STUDY

The following objectives were formulated:

- ∞ To determine peak and trough levels for neonates treated with amikacin.
- ∞ To compare the DPOAE amplitude of infants with amikacin kinetic levels.
- ∞ To determine if there is a correlation between amikacin induced toxicity and cochlear OHC functioning measured with DPOAEs.

1.5 OUTLINE OF THE DISSERTATION

Chapter One introduces the reader to the study and includes the background and rationale for the study. The aim and objectives of the study is thereafter laid out.

An extensive review of the literature is covered in Chapter Two and is discussed under the following headings: Therapeutic drug monitoring, which includes a short explanation and background to TDM; Rationale of TDM and The role of the pharmacist in TDM. The literature review thereafter covers the chemical properties of amikacin, its mechanism of action, the dosing schedule, PKs and PDs pertaining to amikacin ending off with the toxic effects of this drug. Under the section with regards to Audiology and Ototoxic monitoring a background to audiology is given, following with a section on ototoxic monitoring, explaining the types of tests and their use in the study.

Chapter Three follows hereafter and covers the methodology of the study. The research design is discussed including a workflow table of the research design for clear and easy understanding thereof. In this chapter the study site, period and population is given as well as the sampling method with some information of the control patients. Instruments used for the data collection is laid out as well as a complete explanation on the processes followed for data collection. This chapter on methodology ends with an explanation on the data analysis and ethical considerations.

The results obtained in the study and a discussion follows in Chapter Four. Firstly the study population is described with a full description on the patient demographics. Wherever applicable, the total study population of 55 neonates are discussed followed by the sub-population of 33 and 22 respectively. Under patient demographics, the gender, gestational ages and birth weight are described and discussed. A section follows on the diagnoses made and when amikacin therapy was initiated. The dosages of amikacin, PK data and serum concentration results are presented. The section on serum concentrations includes two figures to illustrate peak and trough levels measured respectively for the study population of 55 neonates. A discussion on inter quartile ranges for all three study populations follow. Thereafter a complete discussion on pharmacokinetic values per gestational age group follow and individual patients identified as outliers are highlighted and discussed. Again, the discussion first covers the total study population and thereafter the two sub-populations. A section on the conclusion for the study patients is presented. Finally chapter four ends with the results and a discussion on pharmacokinetic data and audiology data with comparisons for the sub-population of 22 neonates.

Chapter Five is the final Chapter and includes a description on the limitations of the study with recommendations. A conclusion to the study ends off the dissertation.

CHAPTER 2

LITERATURE REVIEW

In this chapter the literature review will focus on previous studies of authors which are relevant to the area of study. Section 2.1 is an overview of research conducted on therapeutic drug monitoring. The involvement of the pharmacist is discussed in Section 2.2. An overview of amikacin is presented in Section 2.3. The audiological component with ototoxic monitoring is discussed in Section 2.4.

2.1 THERAPEUTIC DRUG MONITORING

Therapeutic drug monitoring (TDM) refers to the individualization of drug dosage by maintaining plasma or blood drug concentrations within a targeted therapeutic range or window. The goal of TDM is to individualize therapeutic regimens for optimal patient benefit (Kang and Lee 2009). Two major sources of variability exist between individual patients in drug response. These variations in the relationship between dose and effect can be separated into pharmacokinetic (dose-concentration) and pharmacodynamic (concentration-effect) components. Concentration provides the link between pharmacokinetics (PK) and pharmacodynamics (PD) and is the focus of the target concentration approach of rational dosing (Birkett 1997).

Pharmacokinetics deals with the dose-concentration part and is determined by the PK processes of absorption, distribution and elimination (Holford 2009). Major sources of pharmacokinetic variability includes compliance, age, gender, disease states, drug inter actions, environmental influences and genetic polymorphisms (Birkett 1997). Pharmacodynamics describes the concentration- and time-dependent interaction of antibiotics against pathogens in the host (Holford 2009).

During a second meeting of the subcommittee of the Expert Committee on the Selection and Use of Essential Medicines, held in Geneva during 2008, monitoring of drug levels and for toxicity was recommended.

2.1.1 Rationale for TDM

The bactericidal efficacy of amikacin mainly relates to intermittent, dis-continuous peak concentrations due to the post-antibiotic effect, while renal side-effects and ototoxicity relate to the average plasma concentration, based on saturation of renal and cochlear cell binding sites (Allegaert *et al.* 2006). Amikacin has a very narrow therapeutic range (Siddiqi *et al.* 2009) while the efficacy and toxicity of AGs show a strong positive relationship with blood drug concentrations (Touw *et al.* 2009). Due to developmental differences early in life, neonates display large inter-individual differences in the PKs of AGs (Touw *et al.* 2009). The desired or adverse effects may correlate better with plasma or blood concentrations than they do with dose. Drug concentrations may be used as surrogates for drug effects so TDM can optimise patient management and improve clinical outcomes (Ghiculescu RA 2008). Optimizing AG therapy should be achieved by tight-serum concentration monitoring because of the wide inter-individual variability of PK abnormalities (Taccone *et al.* 2010).

2.1.2 The role of a pharmacist in therapeutic drug monitoring

Antibiotic therapy should be started as soon as infection is suspected due to the morbidity and mortality caused by bacterial infections in neonates (Pacifici 2008). In 1993 Langhendries *et al.* recommended a once daily dosing of amikacin. Administration of relative larger doses of amikacin with extended dosing intervals to obtain efficacy and reduce toxicity (Allegaert *et al.* 2006) has since been applied, but the remarkable inter-individual variability in the PKs of AGs requires that their optimum dosing be defined (Pacifici 2008). A multidisciplinary approach is required to perform TDM. Accurate and clinically meaningful drug concentrations are attainable only by complete collaboration with a TDM team, typically comprised of scientists, clinicians, nurses and pharmacists (Kang and Lee 2009). Clinical pharmacists have a vital role to play in TDM, offering advice to medical and nursing staff about the use of TDM, dose calculations and interpretation of the results obtained (Jones 2009). The American Society of Health-System Pharmacists (ASHP 1998) stated that the pharmacists' clinical functions include appropriate and cost-conscious TDM and provision of clinical PK assessments. Clinical PK monitoring is necessary when the range between minimal effectiveness and toxicity is narrow and

the results of the drug assay provide significant information for clinical decision-making (ASHP 1998).

2.2 AMIKACIN

2.2.1 Chemical properties

Susceptible gram-negative organisms allow AGs to diffuse through porin channels in their outer membranes. An oxygen-dependent system in the organisms transports the drug across the cytoplasmic membrane. The drug then binds to the 30S ribosomal subunit prior to ribosome formation. It interferes with assembly of the functional ribosomal apparatus and/or can cause the 30S subunit of the completed ribosome to misread the genetic code. Aminoglycosides interrupt the process of polysome disaggregation and assembly and causes the polysomes to become depleted (Harvey and Champe 2009).

The AG class of compounds consists of an aminocyclitol moiety with two or more sugar rings. Aminoglycosides are highly polar and polycationic due to a characteristic quaternary ammonium group (Huth *et al.* 2011). The chemical structure of amikacin is shown in Figure 2.1.

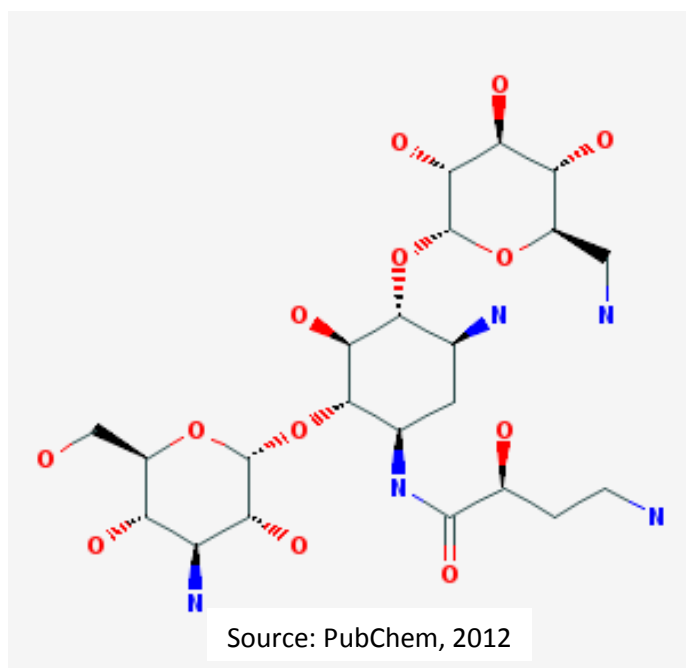


Figure 2.1: The chemical structure of amikacin

2.2.2 Mechanism of action

Aminoglycosides act at various sites of the bacterial cell. As a first step, cationic AGs bind to the anionic outer membrane of the gram-negative organisms, thereby disrupting the integrity of the cell wall's normal permeability function. Their uptake into the bacterial cell is increased in an alkaline environment producing greater intracellular concentrations, thus enhancing the antibacterial effect of the drug. Second and most important, aminoglycosides impair bacterial protein synthesis by binding to the 30S ribosomal subunit leading to misreading of the genetic code and inhibition of translocation. Elongation of the amino acid chain fails to occur, resulting in bacterial death (Roland and Rutka 2004). Amikacin has the widest antimicrobial spectrum (Rang *et al.* 2003) and is resistant to inactivating enzymes, allowing it to be more active against pathogens resisted by other AGs (Frymark *et al.* 2010). Aminoglycosides have good activity against many Gram-negative bacteria, particularly the Enterobacteriaceae and *Pseudomonas spp.* (Inglis 2003). Amikacin is indicated for serious infections caused by strains of *Klebsiella pneumoniae*, *Serratia sp.* and specifically in the neonatal population, late-onset sepsis caused by coagulase-negative *Staphylococcus* species (Sherwin *et al.* 2009).

The AGs as a class demonstrate concentration-dependent killing and produce prolonged post-antibiotic effects (Craig 2010). Higher peak concentrations may result in more rapid killing of susceptible organisms. According to the schedule by Young and Mangum (2007), peak concentrations should be > 40 mcg/mL (Pacifici 2009). Optimal antibacterial activity of amikacin is achieved when the peak is 8 to 10 times greater than the MIC. The clinical MIC breakpoint for problematic pathogens e.g. Enterobacteriaceae and *Pseudomonas aeruginosa* in ICU patients is 8 mcg/mL (Taccone *et al.* 2010). The post-antibiotic effect (PAE) of amikacin refers to amikacin retaining its antibacterial effect while the concentration falls below the MIC. Although the PAE of AGs, according to Turnidge (2003), is usually in the order of two to four hours, the PAE of amikacin lasts from 13.5 to 27.6 hours (Tsui *et al.* 1993). The bactericidal efficacy of amikacin relates to intermittent, discontinuous peak concentrations due to the PAE (Allegaert *et al.* 2006).

2.2.3 Dosing schedule

Although once-daily dosing is widely used for standard therapeutic situations, there is no consensus on doses. The dose for amikacin varied from 11 to 20 mg/kg in prospective once-daily comparative studies (Turnidge 2003). For AGs it has been demonstrated that therapeutic success greatly depends on the C_{max}/MIC ratio to the pathogen involved (Touw *et al.* 2009). As confirmed in numerous studies, larger intermittent doses of AGs have equal or greater efficacy and equal or lesser toxicity compared to multiple daily doses (Peloquin *et al.* 2004).

Allegaert *et al.* (2006) stated that uncertainty on the safest and most effective dosing regimen of any AG in neonates prevails and that dosing is based on a balance of maximal efficacy and minimal toxicity, while still avoiding the induction of bacterial resistance.

2.2.4 Pharmacokinetics and Pharmacodynamics

Pharmacokinetics is defined as the study of the time course of drug absorption, distribution, metabolism and excretion whereas pharmacodynamics refers to the dynamics of drug action. It is the relationship between the fluctuating concentrations seen with dosing and killing of bacteria and is referred to as pharmacokinetics-pharmacodynamics (Turnidge 2003).

Less than 1% of AGs is absorbed from the gastro-intestinal tract and has to be administered parentally (Craig 2010). Amikacin is a highly hydrophilic drug and distributes primarily into extracellular fluid (ECF) (Porter 2012). In a newborn the extracellular fluid compartment is larger (40% of bodyweight compared to 25% of bodyweight in adults) and makes up 70 – 75% of the bodyweight. These characteristics affect the volume of distribution (V_d) of AGs and for adequate peak blood concentrations, a higher dose per kilogram of bodyweight needs to be administered (Touw *et al.* 2009). Porter (2012) states the V_d to be 0.25 L/kg with an elimination half-life in LBW neonates (1 – 3 days) as 7 to 9 hours and term neonates (< 7 days) as 4 to 5 hours. According to Turnidge (2003), studies have shown that V_d are larger and elimination half-lives are longer the earlier the GA. Amikacin is almost exclusively eliminated by glomerular filtration in neonates. Renal clearance of

drugs in preterm and term neonates is in general lower than that in infants and children and increases with postnatal and postmenstrual age (PMA). Neonatal renal clearance is dependent on the glomerular filtration rate (GFR) (Allegaert *et al.* 2006). Nephrogenesis is completed by the end of the 34th week of gestation (European Medicines Agency 2004) and the results of a study done on 275 infants confirmed a median GFR reference values (mL/min per 1.73 m²) in infants aged 27 to 31 weeks' gestation ranged from 7.9 to 30.3 on day 7, 10.7 to 33.1 on day 14, 12.5 to 34.9 on day 21, and 15.5 to 37.9 on day 28 (Vieux *et al.* 2010).

In a study conducted by Sherwin *et al.* (2009), the principle findings were that the main determinants of amikacin clearance were current weight and PMA. The main determinant of Vd was current weight and the main predictor of treatment failure was peak/MIC ration < 8 mcg/mL. They further concluded that differences in body composition and the renal immaturity associated with neonates contribute to the variability of the PK parameters, particularly clearance, GA, post-natal age (PNA), haematocrit, fever and lean body weight (Sherwin *et al.* 2009).

2.2.5 Toxic effects

Aminoglycosides are extremely efficacious antibiotics and have been widely used in the past, despite their toxicities (Deng 2012). Patients in developed countries can afford to make a change to newer antibiotics with fewer side effects, while patients in developing countries continue to depend on the only economically affordable antibiotic – AGs (Deng 2012). All AGs cause renal toxicity (often reversible) and irreversible ototoxicity (Levison 2009). The efficacy and toxicity of AGs show a strong direct positive relationship with blood drug concentrations (Touw *et al.* 2009; Tréluyer *et al.* 2002). Renal side-effects and ototoxicity relate to the average plasma concentration, based on saturation of renal and cochlear cell binding sites (Allegaert *et al.* 2006). Aminoglycosides accumulate in the kidney and can account for 40% of the total drug in the body (Craig 2010). Accumulation of AGs occurs within the proximal tubular epithelial cell in lysosomal phospholipid complexes which rupture and initiate cell death (Turnidge *et al.* 2003).

Reasons for AG ototoxicity have been indicated by studies as abnormal iron transport, dysfunction of mitochondria, gene mutations and drug-drug interactions (Yu *et al.* 2011). Although AGs are hydrophilic in nature and do not readily cross cell membranes, it is proposed that both endocytosis and transport through ion channels mediate the uptake of AGs into sensory hair cells (Huth *et al.* 2011). The ion channels are also known as mechanotransducer (MET) channels and our sense of hearing and balance relies on the very rapid gating of these channels known to be located close to the top of the hair cell stereocilia within the stereociliary bundle (Furness, Hackney and Evans 2010). Free radicals are generated within the inner ear by AGs, with subsequent permanent damage to sensory cells and neurons (Selimoglu 2007). Another hypothesis of AG ototoxicity has been based on the over-activation of glutamate receptors on cochlear synapses (Roland and Rutka 2004). There appears to be a genetic predisposition linked to two or more mutations in the mitochondrial chromosome and these genetic factors may also contribute to the susceptibility for ototoxicity (Roland and Rutka 2004).

Latency exists to the ototoxic effects of AGs due to the slower clearance from inner ear fluids compared to the clearance from serum. This latency can result in progression of hearing loss (Mudd 2012) due to prolonged exposure of the cochlear cells to AGs (Yu *et al.* 2011). Aminoglycosides persist in inner ear fluids for months after treatment, which may account for the delayed hair cell death (Roland and Rutka 2004). Ototoxicity is more likely to occur in patients with persistently elevated concentrations in plasma due to the dependence of back-diffusion on concentration of the drug in plasma (Geneva 2008).

Therapy with AGs exceeding ten days, prior or concurrent treatment with ototoxic drugs and pre-existing renal impairment increases the risk for ototoxicity (Craig 2010). The relationship between AG PK-PD parameters and auditory toxicity is unclear (Craig 2011; Turnidge 2003). Different aminoglycosides have variable cochleotoxicity and vestibulotoxicity properties: streptomycin and gentamycin are primarily vestibulotoxic, whereas kanamycin, streptomycin and amikacin are primarily cochleotoxic (Huth *et al.* 2011). Hearing loss and/or tinnitus are symptoms of cochleotoxicity while those of vestibulotoxicity affects balance and cause dizziness

(Huth *et al.* 2011). Damage to the cochlea, referred to as sensorineural hearing loss, can produce permanent hearing loss because the hair cells in the cochlea do not regenerate (Selimoglu 2007). Inner hair cells (IHCs) show less vulnerability to AGs, and susceptibility of OHCs shows a marked cochlear base-to-apex gradient. Hair cells are the most susceptible to apoptosis and those at the basal coil of the cochlea exhibit greater degeneration than at the apical coil (Leitner, Halaszovich and Oliver 2010). These structural alterations are accompanied by a reduced auditory sensitivity beginning at high frequencies and progressing to lower frequencies (Durrant *et al.* 2009; Leitner *et al.* 2009). High-frequency hearing losses are audiometrically more detectable before they become severe enough to involve the speech frequency range (Touw *et al.* 2009).

Serum creatinine is routinely requested in a ward setting to monitor GFR and kidney function, but in contrast, ototoxicity cannot practically be monitored for early detection before the onset of symptoms. Therefore the frequency of clinically reported ototoxicity is much lower than nephrotoxicity (Turnidge 2003).

2.3 AUDIOLOGY AND OTOTOXIC MONITORING

2.3.1 Background to audiology

Hearing impairment can be viewed as the silent epidemic of developing countries, due to its invisible, non-life-threatening, but highly prevalent nature (Theunissen and Swanepoel 2008). Critical developmental periods for optimal language acquisition are forfeited in children with undetected hearing loss (Swanepoel 2009). Patients with acquired hearing loss often withdraw from social situations in an attempt to avoid the difficulties of trying to converse. Studies show that hearing loss is consistently associated with increased levels of depression and reduced quality of life (Deng 2012).

It is estimated that in South Africa, approximately 6 116 babies a year are born with permanent bilateral hearing loss or acquire it in the first weeks of life. This means that every day 17 babies are born with or will develop hearing loss, 15.5 of them in the public health sector (Swanepoel 2009). Despite these figures, a recent study by Theunissen and Swanepoel (2008) revealed that an estimate of only 7.5% of public

sector hospitals in South Africa provide some form of newborn hearing screening (NHS) and less than 1% of these institutions provide universal NHS. These figures are in stark contrast to those of developed countries such as the United States of America (USA) and the United Kingdom, where 90% or more of all live births are being screened for hearing loss (Theunissen and Swanepoel 2008).

As mentioned previously, AGs have been popular in the developing world because of their low cost and potent antibacterial activities. As a result, the incidence of ototoxicity in developing countries, due to the use of AGs, may increase in comparison to the industrialized world (Huth *et al.* 2011).

The 1994 position statement of the Joint Committee on Infant Hearing (JCIH) endorses the goal of universal detection of infants with hearing loss as early as possible. All infants with hearing loss should be identified before three months of age and receive intervention by six months of age (JCIH 1994). Early hearing detection and intervention (EHDI) programmes, as proposed in the position statement of The Health Professions Council of South Africa (HPCSA) (2007), are recommended to identify, diagnose and treat newborns and infants with hearing loss. The early identification and consequent early intervention of infants with hearing loss, is essential to facilitate the development of speech, language, cognitive skills, as well as social-emotional development and scholastic achievement (Luterman 1999). According to Yoshinaga–Itano (2003) the language abilities of hearing-impaired children, identified before the age of six months are significantly better compared to those identified later than six months of age. These infants (identified prior to six months of age and depending on the type of intervention) have the opportunity to develop and maintain language skills within the normal developmental range, proportionate to their cognitive development during early childhood. These benefits associated with the early identification of hearing loss, outweigh the expenditure of financial, technological and human resources involved in the implementation of early hearing screening programmes (Swanepoel, Hugo and Louw 2005).

A population that will benefit from these EHDI services are those infants admitted to the Neonatal Intensive Care Unit (NICU), as it has long been known that these

infants are a high-risk population (JCIH 2007). Factors such as prematurity, low birth weight and the provision of ototoxic drugs e.g. AG drug treatment, increase these infants' risk for hearing loss (Bielecki, Horbulewicz and Wolan 2011). Jiang, Brosi and Wilkinson (2001) concluded that about one in four preterm VLBW babies has peripheral and/or central hearing impairment at full-term. Due to the toxic effect of AGs on the auditory system (ototoxic effect) a multidisciplinary approach between audiologists and pharmacists would be of an advantage to the overall health care of these neonates.

2.3.2 Ototoxic monitoring

The JCIH (2007) states that electrophysiological measurements, such as otoacoustic emissions (OAE) and automated auditory brainstem response (AABR) testing should be used to screen infants and children for hearing loss. These technologies are both non-invasive objective testing procedures of the physiological activities which underlie normal auditory functioning (JCIH 2007).

Otoacoustic emissions measure the cochlear response to acoustic stimuli in the external auditory canal and are dependent on outer hair cell integrity (JCIH 2007). The cochlea is the auditory component of the inner ear. The sensory cells of the auditory system lie along the turns of the cochlea on a flexible membrane, called the basilar membrane. Inner hair cells (IHC) and OHC are two types of sensory cells that lie on the basilar membrane. These sensory cells have different roles in hearing e.g. while the OHCs modify the incoming signal by altering the fluid motion at the top of the IHCs, the IHCs are responsible for transferring the fluid motion at their surface into a neural signal that can be passed to the brain to be perceived as hearing (Roland and Rutka 2004). Otoacoustic emissions are waves generated by movement of the basilar membrane, measured in the external auditory canal and can be directly related to the OHC function (Cunningham 2011). According to a study done by Abdala and Dhar in 2011, the compressive nature of the basilar membrane motion is mature at birth and they have concluded that newborn ears produce strong reflection.

When these two measures, AABR testing and OAEs, are used in combination with one another a comprehensive view of auditory functioning can be obtained, as the OAE reflects the functioning of the peripheral auditory system up to the OHCs and the AABR extends through towards the functioning of the cranial nerve VIII and the brainstem auditory pathway (JCIH 2007).

Transient evoked OAE (TEOAE) and DPOAE, are evoked OAEs clinically applied in various settings, such as NHS and ototoxicity monitoring (Hall 2000). Otoacoustic emissions can be applied during ototoxic monitoring, as they are site-specific for cochlear OHC damage, and ototoxic drugs such as amikacin exert their effect on the OHC functioning. Otoacoustic emissions are normally very stable with time and are valuable as a sensitive monitor of changes in cochlear status over time, e.g. in relation to sudden hearing loss (Knight *et al.* 2007). As mentioned in the paragraph on the toxic effects of amikacin, AGs affect high-frequency hearing earlier than low-frequency hearing (Leitner *et al.* 2010). Otoacoustic emissions are sensitive to initial ototoxic damage and may detect changes in hearing or auditory function before ototoxicity affects hearing at frequencies important for speech recognition. An advantage of DPOAEs over TEOAEs is that the former test at higher frequencies (500 – 8000 Hz) than the latter (500 – 5000 Hz), making them more sensitive to the frequency area affected first (Cunningham 2011). Distortion Product OAEs are especially valuable for monitoring ototoxicity in very young children who may not consistently provide reliable, complete, ear-specific responses. The measurement of DPOAEs does not require the child's active participation (Knight *et al.* 2007). Measurement of OAEs provides an objective evaluation of the cochlear OHC system (Knight *et al.* 2007).

The clinical applicability of DPOAE amplitude to assess cochlear functioning has been studied by several investigators. When middle-ear function is normal, the amplitude of recorded energy (OAE) reflects the functional status of the cochlea (Knight *et al.* 2007). The healthy cochlea creates internal vibrations whenever it processes sound (Kemp 1978). With ototoxicity, OAEs have been shown to decrease simultaneously with changes in High Frequency Audiometry (HFA) thresholds (Durrant *et al.* 2009).

Initial studies investigating drug-induced hearing loss focused on a decrease in OAE amplitude to indicate ototoxic effects (Beahan *et al.* 2006). Knight *et al.* (2007) saw changes in OHC function as decreases in DPOAE amplitudes, decreases in dynamic range of response, and/or loss of DPOAEs, specific to regions of OHC damage. They stated that DPOAEs and hearing thresholds are negatively correlated – a decrease in DPOAE level is correlated with an increase in hearing level. For the purpose of their study, decreases in DPOAE greater than 8 dB sound pressure level (SPL) were considered a significant clinical change. They have based this on the work of Beattie *et al.* (2003) who reported that differences in DPOAE amplitude must exceed 7 dB SPL at one to four kHz to be statistically significant at the 0.05 level of confidence (Knight *et al.* 2007). Although reports vary, there is no agreed upon universal dB SPL amount that indicates a significant change from baseline to follow-up tests. In 2002 Stavroulaki *et al.* reported a change of 2.4 dB SPL as a significant decrease (Cunningham 2011). Clinical experience suggests that changes of 3-6 dB SPL are generally accepted as significant and indicate a change in cochlear function (Cunningham 2011).

As explained in the section on the mechanism of action of amikacin, this drug is concentration-dependent and exhibits a PAE. During 1998, Kakigi *et al.* found that changes in DPOAE amplitude were observed even after the treatment period had finished. This implies that clinically, a period of monitoring is needed both during and after the treatment of AG to determine the full and final ototoxic effect of these drugs. The American Speech-Language-Hearing Association (ASHA 1994) states that patients that receive AG treatment should be monitored every three days during the treatment period as monitoring is based on drug therapy schedules. Thereafter monitoring tests are performed at approximately three and six months post-treatment to determine any long-term residual effects of these drugs on the auditory system.

Preterm infants are especially susceptible to the ototoxic effects of AG drugs, due to the anatomic and functional maturation development of the inner ear system (Naeimi *et al.* 2009). The use of ototoxic drugs in the neonatal population requires careful monitoring by health care professionals, due to the characteristics and special needs of this vulnerable population (Schellack 2010). Prevention of AG ototoxicity will

require effective TDM as well as hearing evaluation prior to, during and after the drug treatment (Naeimi *et al.* 2009). An effective ototoxicity monitoring programme should consist of various aspects, including specific criteria to identify ototoxicity, timely identification of the at-risk population, pre-treatment counselling regarding the potential ototoxic effect of the drug treatment, valid baseline measurements, on-going monitoring during and after the completion of the drug treatment (ASHA 1994).

According to Hall (2000), the primary objective of ototoxic monitoring is to identify cochlear changes even before they become apparent during behavioural audiometric testing. For the purpose of this study the results obtained were correlated with the amikacin serum concentrations to allow early intervention when necessary.

CHAPTER 3

METHODOLOGY

The overall aim of the study was to determine a possible correlation between amikacin serum concentrations and ototoxicity in neonates by using otoacoustic emissions.

A lay-out of the research design is given in this chapter including a description of the study site and study period followed by the population used and the sampling thereof. This chapter further describes the data collection process, which includes the data collection instruments and the methodology used to obtain serum concentration levels for amikacin. The methodology to obtain otoacoustic emissions is explained thereafter. A brief explanation of the data analysis follows and the chapter ends with a description of the ethical considerations.

3.1 RESEARCH DESIGN

A descriptive quantitative study, with a correlation research design was used during the study. A descriptive study, presents a picture with specific details of a given situation, social setting or relationship focusing on *how* and *why* questions (Neuman 2003). This enabled the researcher to assess the nature of the existing conditions within the given research context, by examining the situation as it is (McMillan and Schumacher 2010). The correlation design enabled the researcher to determine the degree of the relationship between the two phenomena investigated during this research study (McMillan and Schumacher 2010). The correlation design was between the amikacin peak and trough levels and DPOAEs measured at four different frequencies. To allow amikacin to reach steady state, peak and trough levels were taken around the third maintenance dose. As a measure to confirm this relationship, audiology baseline testing via DPOAE was conducted on the study patients before or within 24 hours of initiation with amikacin therapy. Follow-up DPOAEs were repeated before the trough blood sample for amikacin was taken. Distortion-product otoacoustic emissions elicited with stimulus frequencies less than or equal to 8 kHz have been used in hearing clinics to assess whether the inner ear and cochlea are normal, but high-frequency hearing (>4 kHz) is most vulnerable to

cochlear pathology (Dreisbach and Siegel 2001). Figure 3.1 provides an outline of the research design.

RESEARCH DESIGN

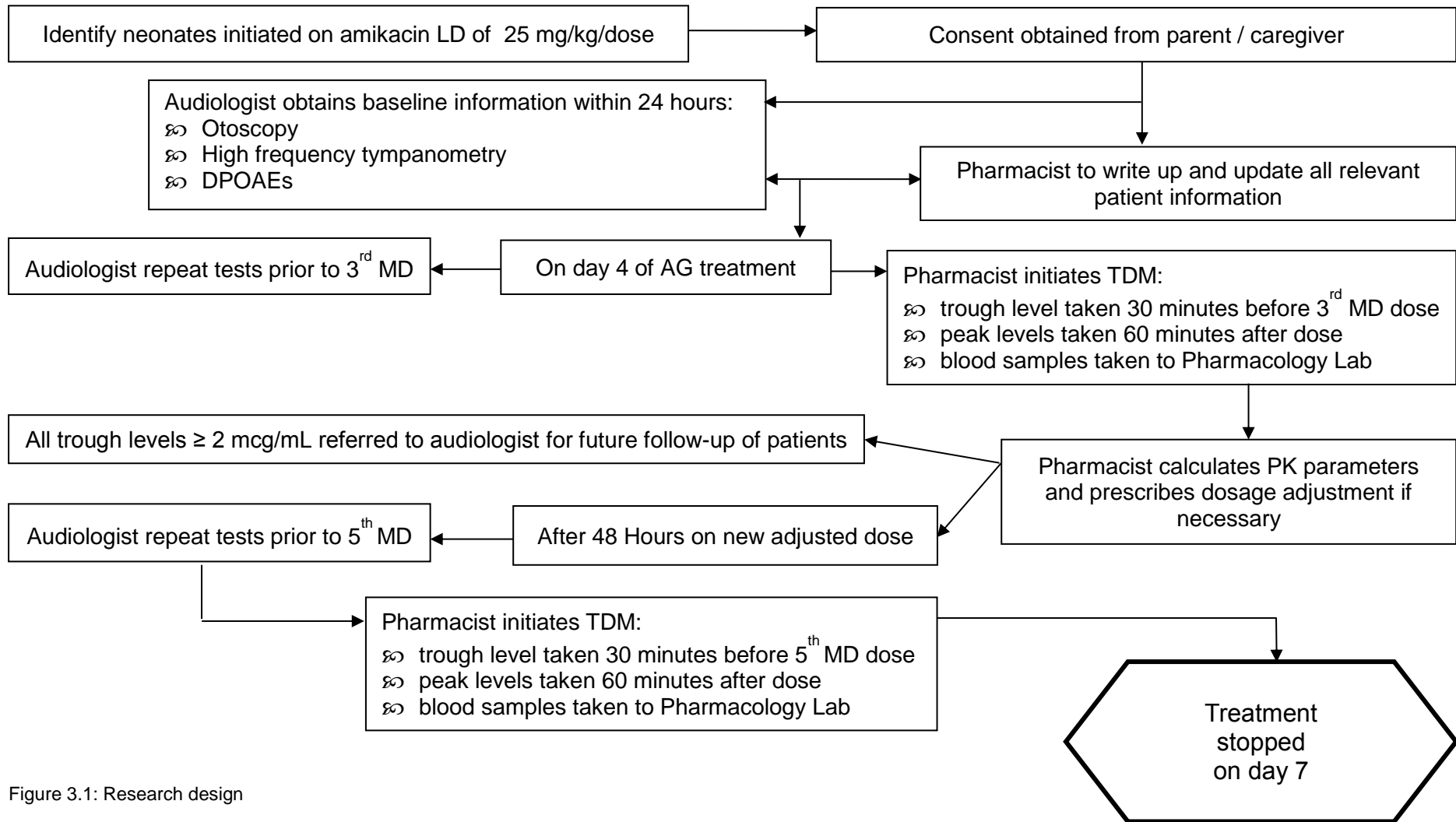


Figure 3.1: Research design

3.2 STUDY SITE

The study was conducted in the Neonatal Intensive Care Unit (NICU) at DGMH which is a public sector academic hospital, situated in Ga-Rankuwa, North-West of Pretoria, South-Africa.

The NICU at DGMH is a 55 bed unit, divided into an ICU, High Care Unit (HCU) as data were collected in all of the sub-units.

3.3 STUDY PERIOD

A pilot study was done during November 2011 to test the feasibility of the study and to test the data collection instruments. The actual data collection took place from January to May 2012.

3.4 STUDY POPULATION

The study population consisted of neonates admitted to the NICU and HCU (ward 24) at DGMH. All newborns initiated on amikacin were considered for the study, but excluded if their regimen included previous or concurrent ototoxic medication. The patients were also followed for follow-up audiology and TDM should they have been moved to the KMC unit.

This site was chosen due to logistical constraints pertaining to the collection of the data as well as convenience.

3.5 SAMPLING METHOD

Purposive sampling method is a form of non-probability sampling in which decisions concerning the individuals to be included in the sample are taken by the researcher, based upon a variety of criteria which may include specialist knowledge of the research issue, or capacity and willingness to participate in the research. Some types of research design necessitate researchers taking a decision about the individual participants who would be most likely to contribute appropriate data, both in terms of relevance and depth (Oliver 2006). In this study purposive sampling was used to identify patients initiated on amikacin.

During the study period of five months, 83 informed consents were obtained. Of the 83 patients, therapeutic drug monitoring was done on 55 of these patients. The

remaining 28 (drop-out rate of 33.7%) patients not included were due to factors such as a change in therapy, an IV line that was out or the patient had passed away.

Out of the 55 kinetic patients, audiology data was obtained from a sub-population of 22 patients. Unfortunately the 33 (drop-out rate of 60%) patients that had no audiometric results were due to other factors such as e.g. high noise level of the setting or audiologic equipment errors.

The participants have been enrolled in the following way, refer to Figure 3.2:

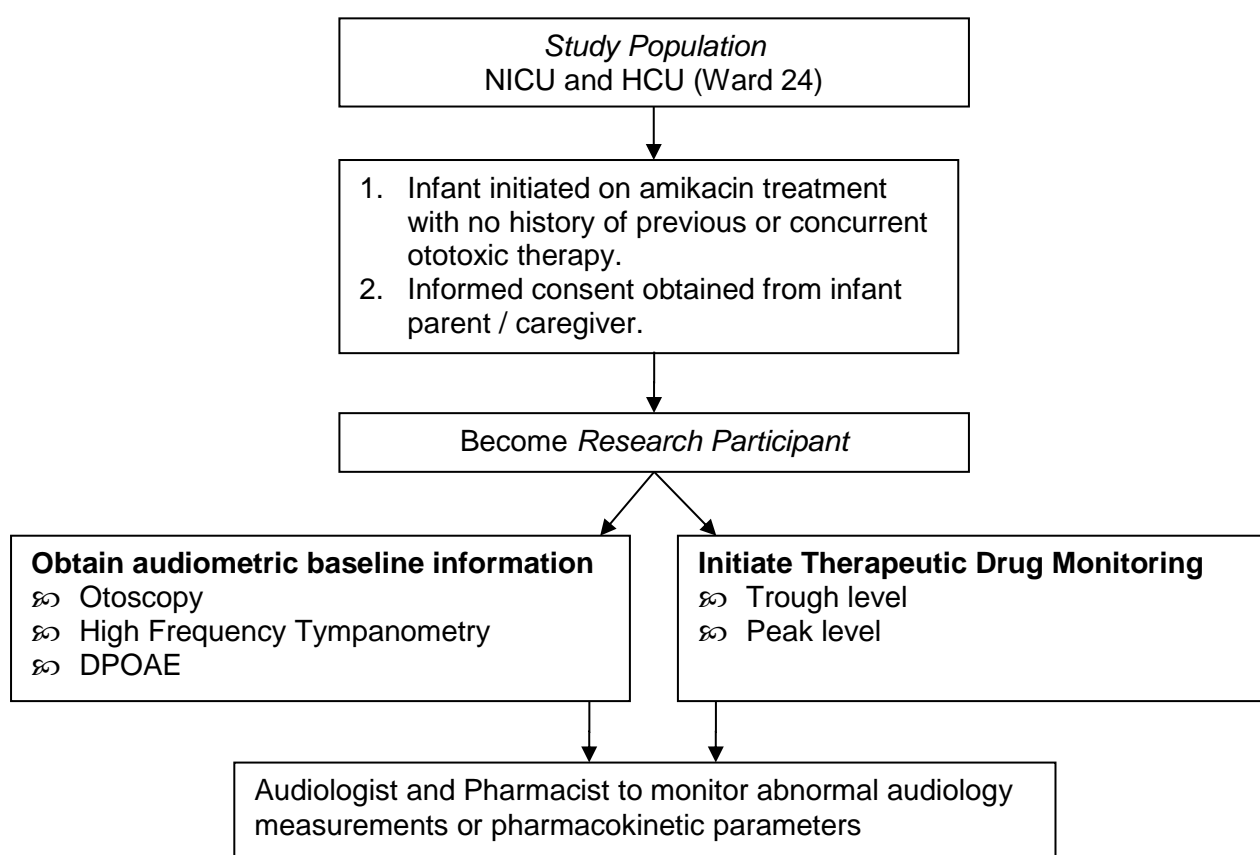


Figure 3.2: Sampling of study patients

3.6 DATA COLLECTION INSTRUMENTS

The following materials and instruments were used during the research project:

3.6.1 Consent forms

The consent form provided the study patient's parent or caregiver with the relevant information relating to the study and enabled the researcher to obtain informed consent from parents or caregivers of the potential study patient. The consent forms included a patient information leaflet.

(Appendix 2 – English; Appendix 3 – Setswana)

3.6.2 Audiology-Pharmacy-Referral sheet

The form was used to document the study patient's demographic information, co-morbid conditions, results obtained during TDM, relevant Urea-and-Electrolyte (U&E) results and a calculation to determine glomerular filtration rate. The researcher was able to view the study patient's information on one sheet and to keep track of their TDM and ototoxic monitoring. (Appendix 4)

3.6.3 Tympanometer MAICO MI-34 with 1000 Hz probe tone

The instrument was used to conduct high frequency tympanometry, defined as the measure of acoustic admittance in the ear canal as a function of changes in the air pressure within the ear canal (Katz *et al.* 2009). The procedure was conducted before DPOAE testing as compromised middle ear functioning due to middle ear effusion or mesenchyme in middle ear cavities has a negative effect on OAE results (Keefe *et al.* 2003).

3.6.4 Madsen Capella Diagnostic OAE with OAE system module version 2.12 – ran on XP software

This instrument was used to obtain OAE results prior to and during the treatment to determine changes in OAE amplitude related to the treatment with amikacin.

3.6.5 Pharmacy Feedback forms

During the study a Pharmacy Feedback form (Appendix 5) was developed to provide feedback to the treating physician.

3.7 DATA COLLECTION

3.7.1 Therapeutic drug monitoring

3.7.1.1 Administration of medication

As mentioned previously (Section 1.2; page 3): a loading dose was administered as an intravenous bolus at 25 mg/kg initially, thereafter 20 mg/kg/day as maintenance dose for the rest of the treatment period. The amikacin dosages were prepared and injected as a bolus into a running IV solution by the nursing staff over an assumed period of five minutes.

The researcher was responsible for the amikacin TDM and calculating the PK parameters. The researcher recorded all the information e.g. dose and exact times of administration onto the audiology - pharmacy referral sheet (Appendix 4).

3.7.1.2 Drawing and handling of specimens

Blood specimens were taken on the third day of maintenance dosing to ensure steady state has been reached. Specimens for the trough level were drawn half an hour before the next dose and the peak levels were drawn one hour after administration of the third maintenance dose. A volume of 0.5 mL of venous blood was drawn from the study patients by the treating physician, respectively for trough and peak levels.

The calculations of the PK parameters were cross-checked by her supervisors before feedback was provided to the ward. The researcher recorded all the information e.g. dose and exact times of administration onto the audiology - pharmacy referral sheet (Appendix 4).

The blood specimens and the laboratory request forms were taken to DGMHs National Health Laboratory Service (NHLS). The necessary data was captured on site were after the samples were couriered to the NHLS of Tshwane. The following information was noted on the pharmacology request form by the researcher:

- ∞ Patient name
- ∞ Date of birth
- ∞ Gender
- ∞ Weight

- ∞ Hospital file number
- ∞ Date when the sample was requested
- ∞ Determination required
- ∞ Current and recent therapy (drug name(s), dosage, duration of therapy)
- ∞ Nature of sample, namely blood sample
- ∞ Date and time when blood sample was drawn
- ∞ Date and time when last dose of amikacin was administered
- ∞ Route of administration, namely intravenous
- ∞ Indication being treated
- ∞ Signs of toxicity e.g. reduced GFR

3.7.1.3 Analytical techniques

The specimens were analysed by using a QMS[®] Amikacin Immunoassay reagents kit on the Beckman Coulter DXC 880i[®] system. Due to the reagents kit not being a product of the latter, the QMS[®] Amikacin Immunoassay reagents kit was used as “user defined reagents”. The laboratory technicians had to set up the reagents for the Beckman Coulter DXC 880i[®] system and calibrate it according to the suppliers recommendations.

3.7.1.4 Assay methodology

The QMS[®] Amikacin assay is intended for the quantitative determination of amikacin in human serum or plasma on automated clinical chemistry analysers. It is a homogenous particle-enhanced turbidimetric immunoassay.

The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody binding sites of the amikacin antibody reagent. The amikacin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-amikacin antibody reagent and in the absence of any competing drug in the sample.

The rate of absorbance change is measured photometrically. When a sample containing amikacin is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. A concentration-dependent classic

agglutination inhibition curve can be obtained with maximum rate of agglutination at the lowest amikacin concentration and the lowest agglutination rate at the highest amikacin concentration.

3.7.1.5 Calculation of pharmacokinetic parameters

The AG (amikacin) peak and trough levels were obtained from the Tshwane NHLS laboratory where it was analysed by using a one compartment open pharmacokinetic model (Herfindal *et al.* 1992) and using the Sawchuk-Zaske method (Sawchuk and Zaske 1976) to calculate the different pharmacokinetic parameters.

The pharmacist used the following procedure:

- ∞ Recorded the time the trough was taken, the doses administered to the patient and the time the peak was taken
- ∞ Recorded the day of therapy and the amount of drug given
- ∞ By using a pharmacokinetic graph (see Figure 3.3), recorded the amount and time of doses given to the patient and the values and times of measured drug concentrations
- ∞ Calculated the elimination rate constant (k_e) and half-life ($t_{1/2}$) from peak to trough concentrations
- ∞ Determined the C_{max} (concentration at the time the drug administration is completed) – this is the *true* C_{max}
- ∞ Determined C_{min} (concentration at the end of the dosing interval) – this is the *true* C_{min}
- ∞ Calculated the volume of distribution (V_d)
- ∞ Decided the desired peak and trough levels for each individual patient
- ∞ Calculated an alternative dose and/or dosing interval when necessary.

Figure 3.3 depicts how the amount and times of doses given to the patient, and the values and times of the measured serum drug concentrations (peak and trough) were used to calculate individual patient pharmacokinetic parameters: k_e , $t_{1/2}$, C_{max} , C_{min} , V_d , CL.

The PK parameters were calculated by using the following equations:

The elimination constant (k_e) was calculated as follows:

$$k_e = \frac{\ln C1/C2}{\Delta t} \quad [3.1]$$

An estimation of the half-life ($t_{1/2}$) is allowed through the measured serum drug concentrations, using peak and trough levels. Therefore, the following equation has been used to calculate the $t_{1/2}$:

$$t_{1/2} = \ln 2/k_e = \underline{0.693} \quad [3.2]$$

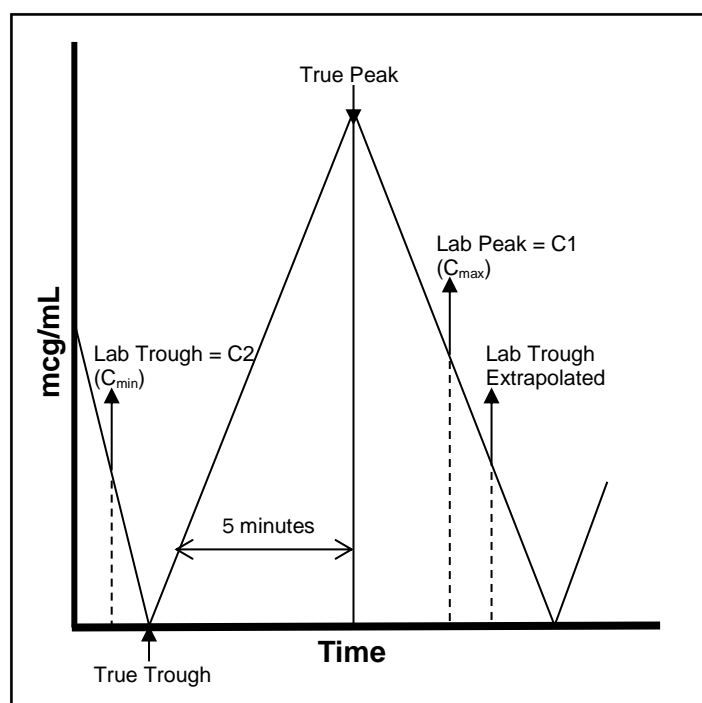


Figure 3.3: Therapeutic drug monitoring graph

$$\text{True } C_{\max} = \frac{C1}{e^{-k_e t}} \quad [3.3]$$

$$\text{True } C_{\min} = C2 \times e^{-k_e t} \quad [3.4]$$

$$V_d = \frac{D (1-e^{-keT})}{k_e \times T [C_{max} - (C_{min} \times e^{-keT})]} \quad [3.5]$$

$$\text{Dose (D)} = V_d \times k_e \times T \times C_{max} [(1-e^{-ke\tau}) / (1-e^{-keT})] \quad [3.6]$$

$$CL = k_e \times V_d \quad [3.7]$$

Key to the pharmacokinetic abbreviations 3.1 to 3.7:

C1 = peak concentration; the concentration measured at time 2 (t2)

C2 = trough concentration, the concentration measured at time 1 (t1)

Δt = elapsed time between the two concentrations, C1 and C2, extrapolated to the same dosing interval

k_e = first order elimination rate constant

t_{1/2} = elimination half-life

C_{max} = theoretical maximum serum concentration; i.e. the concentration at the end of the infusion

C_{min} = theoretical minimum serum concentration; i.e. the concentration at the end of the dosing interval

t = the time difference between the two concentrations

τ = dosage interval

V_d = the apparent volume of distribution

T = infusion time

CL = clearance

It is possible to evaluate the standard regimen through a comparison between the calculated peak and trough serum concentrations and the recommended literature concentrations. The t_{1/2}, V_d and CL were calculated to compare the study population with previously studied populations. An adjusted dose was calculated when the peak level was below or the trough level was above the recommended ranges for a particular patient.

Quantitative data was collected. According to McMillan and Schumacher (2010), quantitative data collection use some form of instrumentation to obtain numerical indices that correspond with the characteristics of the research participants.

3.7.2 Audiology

3.7.2.1 Middle ear status

The MAICO MI-34 tympanometer was used and in accordance with the manufacturer recommendations, the equipment was switched on ten minutes prior to the test to ensure reliable measurements. Probe calibration was conducted prior to

testing of the study patients. The probe tip (without an ear tip) was placed in the whole of the test cavity, labelled 0.5 ml. Calibration was done automatically. When prompted to, the same procedure has been repeated in the 2 ml cavity. After calibration, the MI-34 tympanometer was automatically switched to tympanometry mode. An appropriate ear-tip size was selected and placed in the external ear canal and once a good seal had been obtained, the testing began automatically.

A 1000 Hz frequency probe tone was presented to the ear canal, by means of a hand held probe. This tone measured the change in the compliance of the middle ear system, while the air pressure automatically varied between positive +200 daPa and negative -400 daPa. Maximum compliance was recorded on the tympanometry chart. Measurement took place over an average of two to four seconds.

The tympanogram was interpreted by looking at the ear canal volume, compliance and pressure. The measurement was repeated for the opposite ear and the results stored on the computer.

3.7.2.2 Outer Hair Cell Functioning

The MADSEN Capella Cochlear Emissions Analyser was used. As soon as a baseline measurement has been obtained the new study patient information was captured in the database. If a follow up measurement was conducted, the study patient's name / research number was captured onto the database.

The test ear was selected on the programme. After the appropriate ear tip size had been selected, it was mounted onto the probe tip. The ear tip was then placed into the study patient's external ear canal. The probe fit was checked by clicking on the *Probe Fit* icon. When needed, adjustments were made until a good seal was obtained (indicated by a green flashing light). DPOAE measurement was selected by clicking on the **DP-Gram** icon.

The given test frequencies were selected by clicking on each frequency on the programme, namely 2, 4, 6 and 8 kHz.

The DPOAE measurement began automatically once the researcher pressed the *Start* icon.

As soon as the stop criteria for one frequency had been met, the software automatically resumed with the measurement of the next frequency.

Once all frequencies had been tested, the measurement was repeated in the same ear. The whole process was done and repeated in the opposite ear. All results were stored on the database in PDF format.

3.8 DATA ANALYSIS

The data was entered and analysed using a Microsoft Excel™ spread sheet and the expertise of a statistician utilized. The statistician made use of SAS Release 9.2 running under Microsoft Windows.

Pharmacokinetic parameters for each of the 55 patients have been calculated and used to evaluate the patient's dose. When necessary, dosage adjustments were recommended based on these pharmacokinetic parameters. Patient demographic data and the different pharmacokinetic parameters were analysed and described.

For the sub-population group of 22 patients, a descriptive correlation technique was employed to determine the correlation between:

- i. The amikacin peak and trough levels, and
- ii. Otoacoustic emissions at four different frequencies

3.9 ETHICAL CONSIDERATION

Approval for the study was obtained from the Medunsa Campus Research and Ethics Committee (Appendix 1). Permission to conduct the study was obtained from the CEO of DGMH and the head of the NICU.

Each study patient was allocated a specific study number to maintain strict patient confidentiality. Participant's privacy was maintained throughout the study and all information handled confidentially. Only the researcher had access to the participants' information during the study and the research data was stored appropriately, to ensure the participant's privacy.

The researcher has an ethical obligation to protect subjects against any form of physical and/or emotional harm (Leedy and Ormrod 2001). Informed consent was obtained by providing a full explanation of the study either verbally in English or in the participants' native language. The information was also presented in a written format in English or Setswana (Appendix 2 and 3). This was important to ensure that the participants comprehend the nature of the study and could consequently make an informed decision regarding their participation. Participants were given the opportunity to terminate their participation at any time, without penalty. It is important to note, that since the participants used in this study were too young to give assent to participate in the study, informed consent was obtained from the infant's parents or caregivers (McMillan and Schumacher 2010).

Balancing the benefits and risks during medical care is a moral and frequent challenge for clinicians and researchers (Olusanya, Luxon and Wirz 2006). Full disclosure of the risks involved in the study was provided to the parents or caregivers of the research participants, so as to prevent non-maleficence. Screening for hearing loss is not without some risks for the child and the parents, as screening test are not perfect. It is possible for a child to fail the screening in the absence of hearing loss, causing unnecessary anxiety and stress until the final diagnosis is made. In contrast it is possible to obtain a false-negative result, which assures the parents, that all is well, even when the child has a hearing loss. Screening devices for hearing loss have improved with technology advances, and sensitivity and specificity scores are now close to 100% (Swanepoel 2009).

With regards to the TDM, the parents or caregivers were informed about the possibility of amikacin affecting the ear and the importance of acquiring the drug levels in the neonate. With the information at hand, the researcher was able to suggest dosage recommendations or a change in regimen. All discrepancies and suggested recommendations were discussed with the treating physician where after the physician decided upon the best intervention for the particular study patient.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter describes the analysis of data followed by a discussion of the research findings. The findings relate to the research questions that guided the study.

A total of 83 consents were obtained. Due to various reasons e.g. a change in therapy, an IV-line that was out or the unavailability of physicians to assist with taking samples, TDM could be performed only on a total of 55 neonates. This group was divided into two sub-populations:

- ☞ 33 neonates (n = 55; 60%) that did not receive any audiology and only PK data could have been obtained
- ☞ 22 neonates (n = 55; 40%) from which both audiology and PK data could have been obtained

Refer to Figure 4.1 for an illustration of the study population.

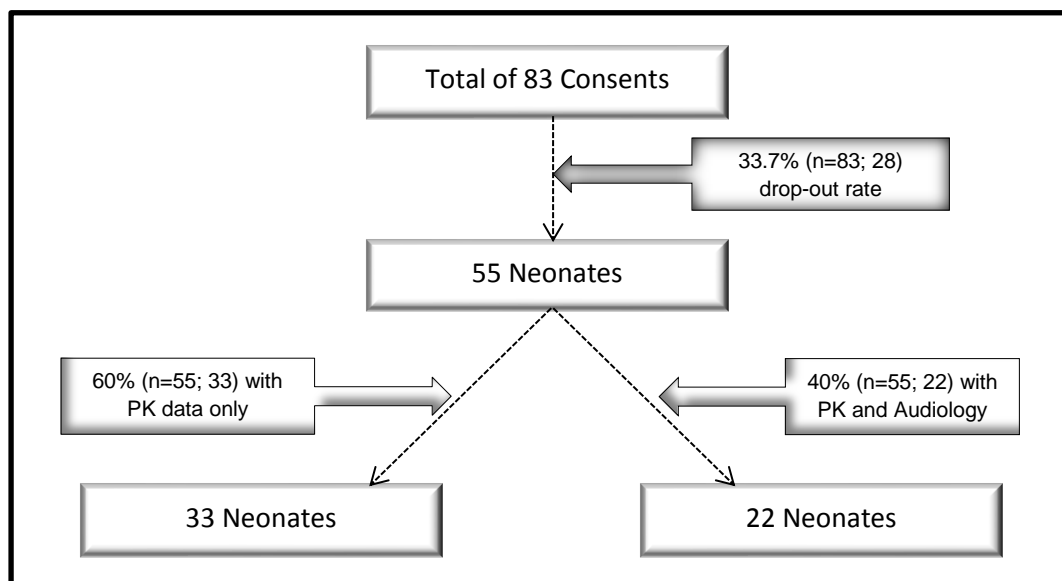


Figure 4.1: Illustration of the study population

The data will be presented in this chapter for the whole study population followed by the data for the two sub-populations in the following order:

- ☞ Section 4.1 Patient Demographics
- ☞ Section 4.2 Pharmacokinetic Data with discussions on serum concentrations
- ☞ Section 4.3: Pharmacokinetic and Audiological data with comparisons

4.1 PATIENT DEMOGRAPHICS

Demographic variables of the samples are described and the possible influence on the research findings assessed. By discussing the two sub-populations separately, it enabled the researcher to confirm whether the groups are representative of each other. The demographic data consisted of the gestational ages, body weight at birth and gender.

Table 4.1: Patient demographics

Parameters		All patients (n = 55)	PK only (n = 33)	PK and Audio (n = 22)
Gender	Male	30	15	15
	Female	25	18	7
Gestational age (weeks)	Mean (± SD)	33.1 (± 3.96)	33.09 (± 3.79)	33.14 (± 4.30)
	Median (range)	33 (25 – 40)	33 (25 – 40)	32 (27 – 40)
Birth weight (kg)	Mean (± SD)	1.91 (± 0.89)	1.84 (± 0.85)	2.0 (± 0.97)
	Median (range)	1.55 (0.9 – 4.6)	1.6 (0.95 – 4.2)	1.50 (0.9 – 4.6)

The two groups (n = 33) and (n = 22) did not differ in terms of demographic and clinical findings according to two statistical findings, namely the Wilcoxon Test and the Two Sample t-Test.

The Wilcoxon test was developed to analyse data from studies with repeated-measures and matched-subjects designs. The purpose of matched-subjects designs is to evaluate whether the pairs of participants differ significantly under the two conditions.

The results for the two groups were as follows:

1. For variable gestational age: $p = 0.8425$
2. For variable weight: $p = 0.6363$

In the Two-Sample Test for Variances task, testing can be done for two variables to determine if there is any different variances or if a single variable exists that contains values for the two groups. This can be used to determine if there is any variance between the groups.

The results for the two groups were as follows:

1. For variable gestational age: $p = 0.9457$
2. For variable weight: $p = 0.5226$

4.1.1 Gender

While no influence of gender was found on amikacin clearance (Schreuder *et al.* 2009), some subtle sex differences in the auditory system are evident at birth. Studies have found that female neonates produce significantly greater amplitude and more numerous OAEs compared to boys (Sax 2010). For these reasons information on gender is included.

The latest census statistics of South Africa became available during October 2012 and reflected a sex ratio at birth of 1.02 male(s)/female.

In the population group of 55 study patients, 30 were male and 25 were female. The gender ratio of the study patients reflected a slightly higher male-to-female ratio namely 1.2:1.

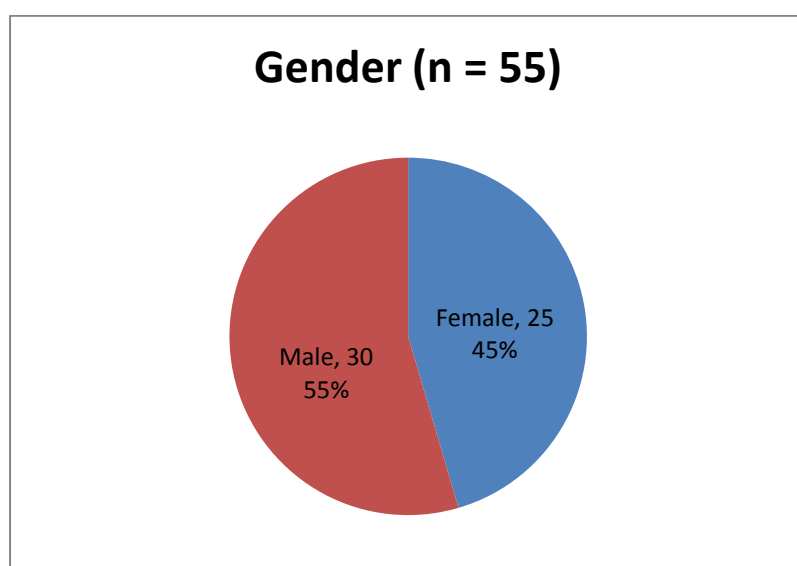


Figure 4.2: Gender ratio of the total study population

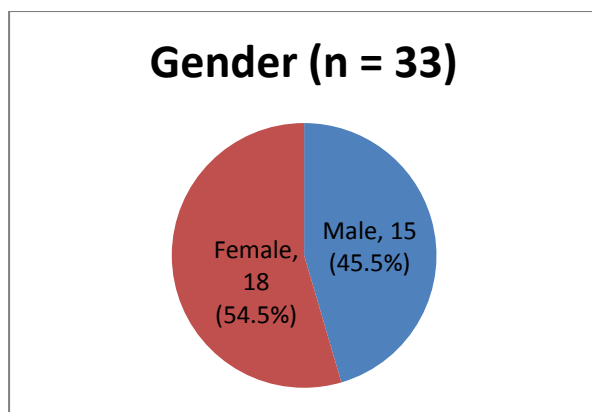


Figure 4.3: Gender ratio of sub-population n = 33

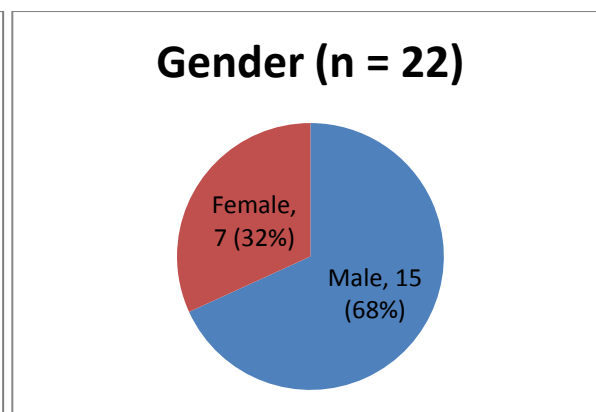


Figure 4.4: Gender ratio of sub-population n = 22

In the sub-population group of 33 study patients, 15 were male and 18 were female, thus a lower male-to-female representation of 1:1.2. The male-to-female ratio was much higher in the sub-population group of 22 study patients which had 15 males versus 7 females. The ratio was 2.1 male(s)/female.

4.1.2 Gestational age

The gestational age of a population is an important factor when it comes to the pharmacokinetics of a specific drug. In Chapter 2 (Section 2.2.4) it has been explained that amikacin is a hydrophilic drug which distributes mainly into the ECF compartment and is excreted renally. It was further explained how the ECF compartment changes in relation to different gestational ages. Prematurity, for example, is associated with a low nephron endowment and it can be expected that neonates who are born prematurely have a lower GFR (Schreuder *et al.* 2009). These factors mentioned can ultimately influence the distribution and excretion of amikacin and it is therefore important to analyse the gestational age distribution/composition of the study population. The prevalence of neonatal hearing disorders has also been reported to be increased 10- to 50-fold in infants at risk, which includes preterm infants (Behrman 2007).

In order to report on the different stages, the classification according to the WHO (2012) was used, namely:

- ∞ extremely preterm (<28 weeks)
- ∞ very preterm (28 to <32 weeks)

so moderate to late preterm (32 to <37 weeks)

Babies born at 38 to 41 completed weeks are classified as full-term.

For the population group of 55 neonates the mean gestational age was approximately 33.11 ± 3.96 weeks (range 25 to 40 weeks). These results were evaluated and it became apparent that for the 25 females, the mean gestational age was 33.84 ± 4.09 weeks (with a range of 27 to 40 weeks). For the 30 males, the mean gestational age was 32.5 ± 3.81 weeks (with a range of 25 to 39 weeks).

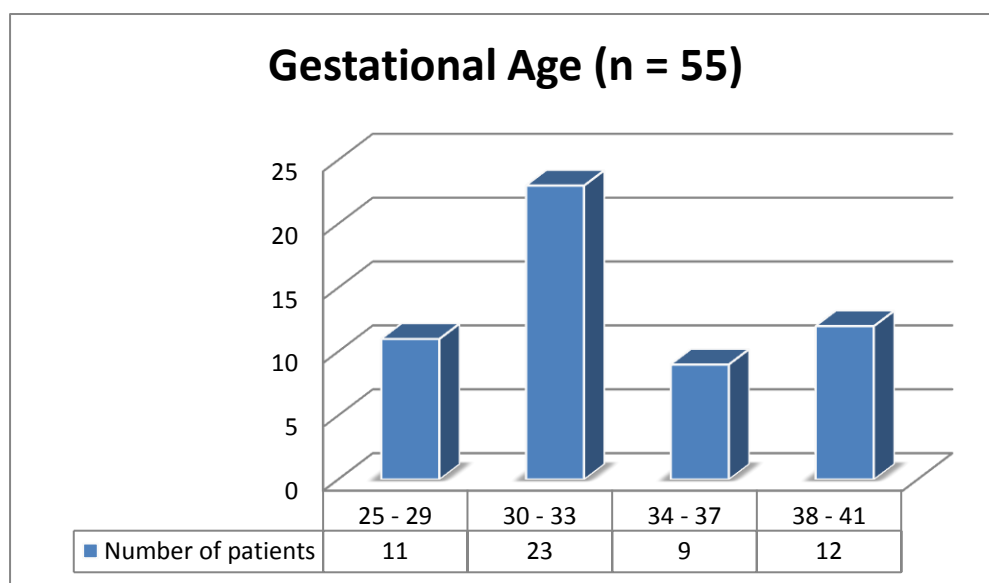


Figure 4.5: Number of patients grouped according gestational age

In Figure 4.5 it is shown that 43 study patients were born at or before 37 weeks gestational age, thus classified as preterm. Study patients born prematurely accounted for 78.18% of this population.

The mean gestational age of the sub-population of 33 neonates was 33.09 ± 3.79 weeks with a median of 33 (range 25 to 40 weeks). The different GA groups are reflected in Figure 4.6.

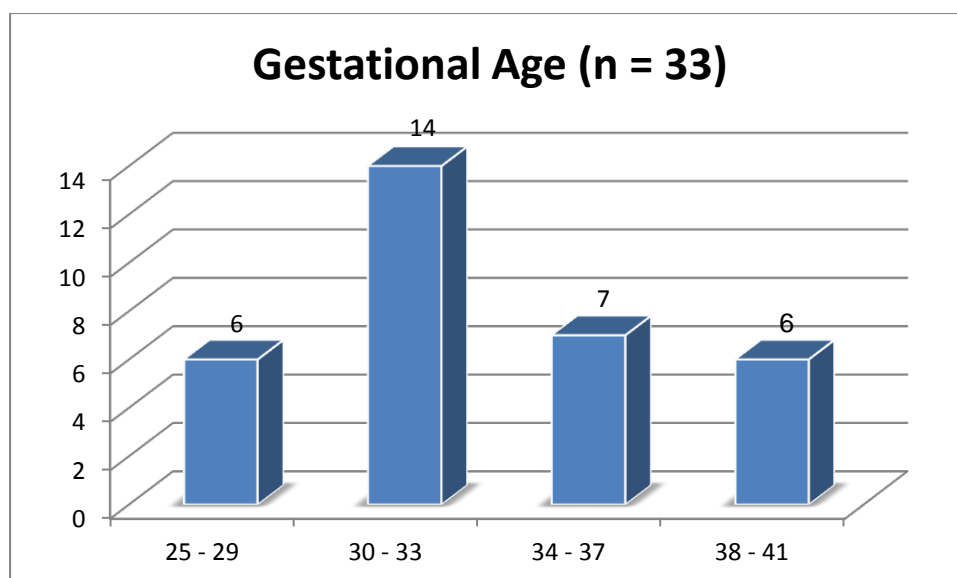


Figure 4.6: Number of patients in different gestational age groups

The majority of neonates (42%; $n = 14$), in this group were born between 30 – 33 weeks. In this sub-population 81.82% ($n = 27$) were born prematurely. Of the six neonates born term (>37 weeks), five of them were of female gender. Table 4.2 summarises the gestational ages of the patients by gender groups.

Table 4.2: Gestational ages of patients in the subgroup ($n = 33$)

Gestational age (weeks)	Female	Male	Total
25 – 29	2	4	6
30 – 33	8	6	14
34 – 37	3	4	7
38 – 41	5	1	6
Total	18	15	33

The mean gestational age of this sub-population of 22 neonates was 33.14 ± 4.30 weeks with a median of 32 (range 25 to 40 weeks).

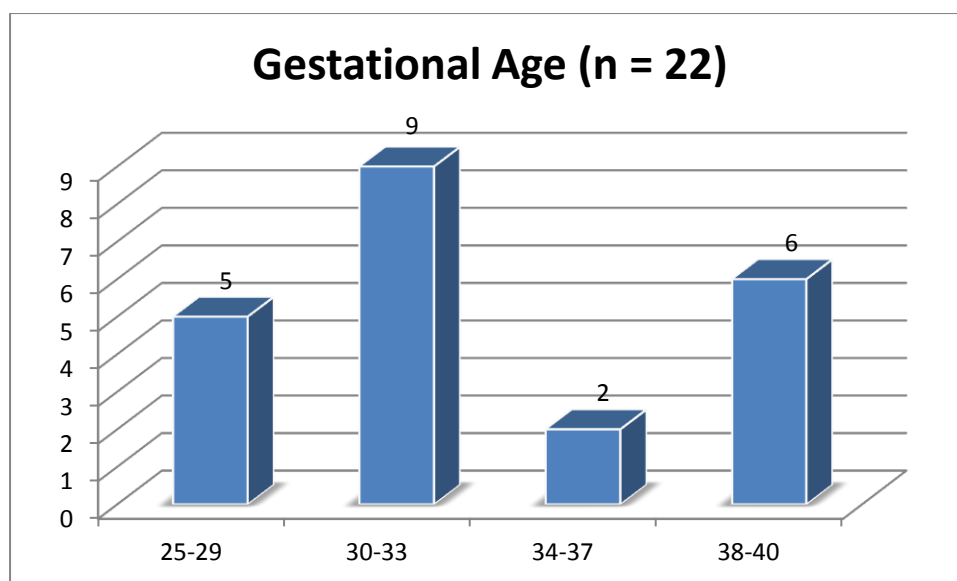


Figure 4.7: Number of patients grouped according gestational age

Figure 4.7 shows that the majority (40.9%; $n = 9$) were born between 30 and 33 weeks gestational age, the same as with the sub-population of 33. Study patients born prematurely accounted for 72.7% ($n = 16$) of this population.

Of the six neonates born term (>37 weeks), four of them were of male gender. Table 4.3 summarises the gestational ages of the patients by gender groups.

Table 4.3: Gestational ages of patients in the subgroup ($n = 22$)

Gestational age (weeks)	Female	Male	Total
25 – 29	3	2	5
30 – 33	2	7	9
34 – 37	0	2	2
38 – 41	2	4	6
Total	7	15	22

Specific statistics pertaining to South Africa is not available but in the Born Too Soon report (WHO 2012) it was estimated that 11.1% of the world's live births, 60% of premature births occur in Africa and South Asia.

4.1.3 Birth weight

According to the WHO report of 2012 a newborn baby weighing less than 2.5 kg at birth is classified as a low birth weight (LBW) neonate. Low birth weight neonates are further classified as very low birth weight (VLBW < 1550 g) and extremely low birth weight (ELBW < 1000 g) neonates (Gupta 2008).

The distribution of birth weights in the four categories is illustrated in Figure 4.8.

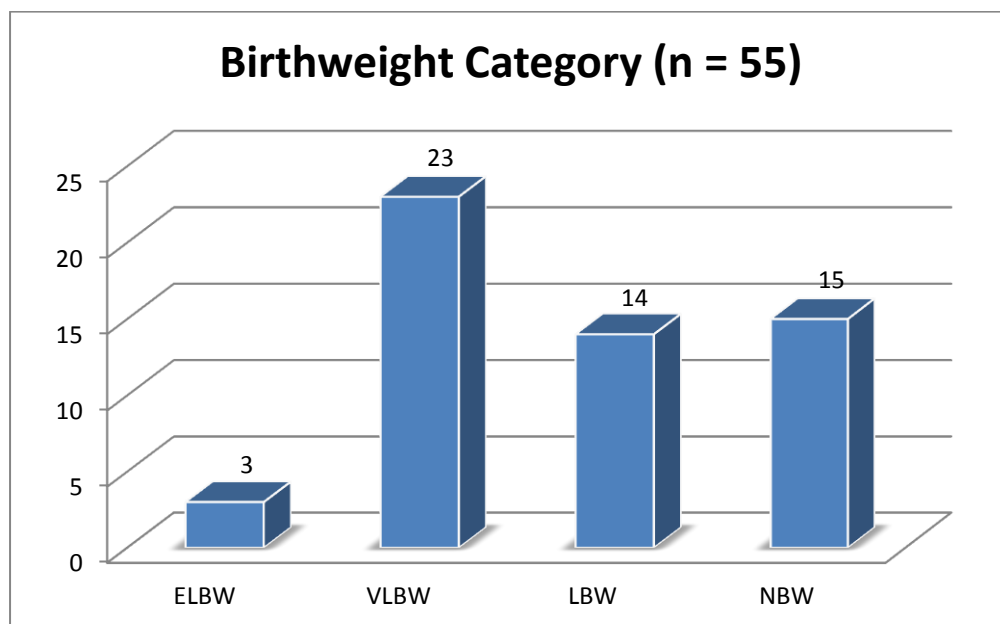


Figure 4.8: Distribution of birth weights

The mean weight for the pharmacokinetic population of 55 neonates was 1.91 kg \pm 0.89 with a median of 1.55 kg (ranges 0.9 kg to 4.6 kg).

Mean birth weights for the different genders are summarised in Table 4.4.

Table 4.4: Birth weight of study population according to gender

	Female	Male	All
n =	25	30	55
Mean birth weight (kg)	1.97	1.85	1.91
Standard deviation	± 0.85	± 0.94	0.89
Median	1.65	1.46	1.55
Range	0.90 – 3.90	1.00 – 4.60	0.90 – 4.60

Birth weight has an influence on pharmacokinetics as concluded by Myers, Roberts and Mirhij (1997). In their study a prolonged serum half-life was associated with the related variables of birth at an early gestational age, low birth weight, and hypoxemia.

A total of 72.7% of the study population weighed less than the accepted normal birth weight of above 2.5 kg. This was expected as only 21.8% of the study population was between 38 and 41 weeks GA.

It is also evident that the male neonates weighed more than the female neonates and this correlates with a study done by Malik, Vaqar and Razaq (2008) confirming that male neonates are heavier when compared to their female counterparts.

The distribution of birth weights in the four categories for the two sub-populations is presented in Tables 4.5 and 4.6.

Table 4.5: Birth weight categories according to gender (n = 33)

Birth weight category	Female	Male	Total
< 1 kg (ELBW)	2	-	2
1 – 1.49 kg (VLBW)	4	9	13
1.5 – 2.5 kg (LBW)	7	5	12
> 2.5 kg (NBW)	5	1	6
Total	18	15	33

The majority of neonates weighed between (and including) 1 – 2.5 kg (75.8%, n = 25) and only 18% (n = 6) were of NBW. The latter correlate with their GA as all six these neonates had a GA of above 37 weeks.

Table 4.6: Birth weight categories according to gender (n = 22)

Birth weight category	Female	Male	Total
< 1 kg (ELBW)	1	-	1
1 – 1.49 kg (VLBW)	3	7	10
1.5 – 2.5 kg (LBW)	-	3	3
> 2.5 kg (NBW)	3	5	8
Total	7	15	22

In the sub-population group of 22 neonates the majority of neonates (45.5%; n = 10) weighed between 1-1.49 kg, while 36.4% (n = 8) were of NBW. Six of the eight neonates in the NBW category had a GA of above 37 weeks.

Overall, the weight range of 0.9 kg to 4.6 kg was similar to that found in previous studies conducted in the same unit. Ntlhane (2004) found the range in weight as 0.9 – 3.98 kg, while Schellack (2010) found the range as 0.85 – 3.05 kg.

4.2 DIAGNOSES

Very low birth weight and respiratory distress syndrome (RDS) were the two diagnosis most often made in New South Wales, Australia (Ford *et al.* 2007). In a paediatric hospital in Mexico City four conditions (congenital cardiopathy, prematurity, specific congenital syndromes and respiratory distress syndrome) accounted for more than two-thirds of the diagnoses (Feria-Kaiser 2002).

In this study the diagnoses were obtained from the patients' file as noted by the treating physicians. In total, 23 different diagnoses were made. Different combinations of diagnoses were made, accounting to a total of 195 diagnoses for the 55 study patients. A minimum of 2 diagnoses and a maximum of 6 diagnoses per patient were made. The mean was 3.55 diagnoses with a median of 3 diagnoses per patient for this population. The most frequent combination of diagnoses was VLBW, prematurity and respiratory distress syndrome (RDS).

The 23 diagnoses with the number of neonates diagnosed with the specific condition/s are listed in Table 4.7 below.

Table 4.7: Different diagnoses made for 55 study patients

	Diagnoses	Number of neonates
1	ELBW: \leq 1000g	4
2	VLBW: 1001 – 1500g	22
3	LBW: 1501 – 2500g	16
4	NBW: >2500g	13
5	Prematurity	43
6	Respiratory Distress Syndrome (RDS)	35
7	Birth asphyxia	1
8	Congenital Pneumonia	4
9	Meconium Aspiration	2
10	Septicaemia	8
11	Jaundice	8
12	Retroviral Disease (RVD) Exposed	17
13	Infant to Diabetic Mother (IDM)	2
14	Thrombocytopenia	2
15	Anaemia	4
16	Omphalocele	1
17	Respiratory acidosis	2
18	Collodion	1
19	Chorioamnionitis	1
20	Hypothermia	3
21	Hypoglycaemia	3
22	Necrotizing enterocolitis (NEC)	2
23	Metabolic acidosis	1

In the pharmacokinetic population, 20 diagnoses were made for the 33 neonates and in the pharmacokinetic-and-audio population, 18 diagnoses were made for the 22 neonates. In both these population groups VLBW, prematurity and RDS were the diagnosis most made. This correlates well with the results obtained in the studies done by Ford *et al.* (2007) and Feria-Kaiser (2002).

4.3 INITIATION OF AMIKACIN THERAPY

Amikacin is a commonly prescribed drug used for the empirical treatment of suspected Gram-negative bacterial infections (Siddiqi *et al.* 2009). The NICU of DGMH has amikacin included as first-line therapy in the ward protocol (Antibiotic Policy DGMH 2005). Sixty percent of preterm neonates receive at least one antibiotic, and 43% of the antibiotics administered to these neonates are AGs (Pacifci 2009). Bacterial sepsis is a major problem in the newborn unit and according to Kuschel (2007) 10% of all neonates admitted to the NICU are treated with antibiotics for suspected sepsis. Prematurity is a risk factor for sepsis and antibiotic therapy should be considered in any baby with signs of sepsis (e.g. RDS), particularly in the presence of risk factors (Kuschel 2007).

Early initiation of antibiotic therapy can be life-saving in the NICU (Dellinger *et al.* 2008) - for this reason, day until antibiotic therapy was initiated was analysed and a short discussion follows next.

The duration from birth, taken as day one, until initiation of a loading dose of 25 mg/kg amikacin, was 2.47 days (mean value), with a minimum of 1 day and a maximum of 7 days. Of this population, 40% (n = 22) was started on day two with amikacin therapy. In Figure 4.9, this information is plotted on a graph.

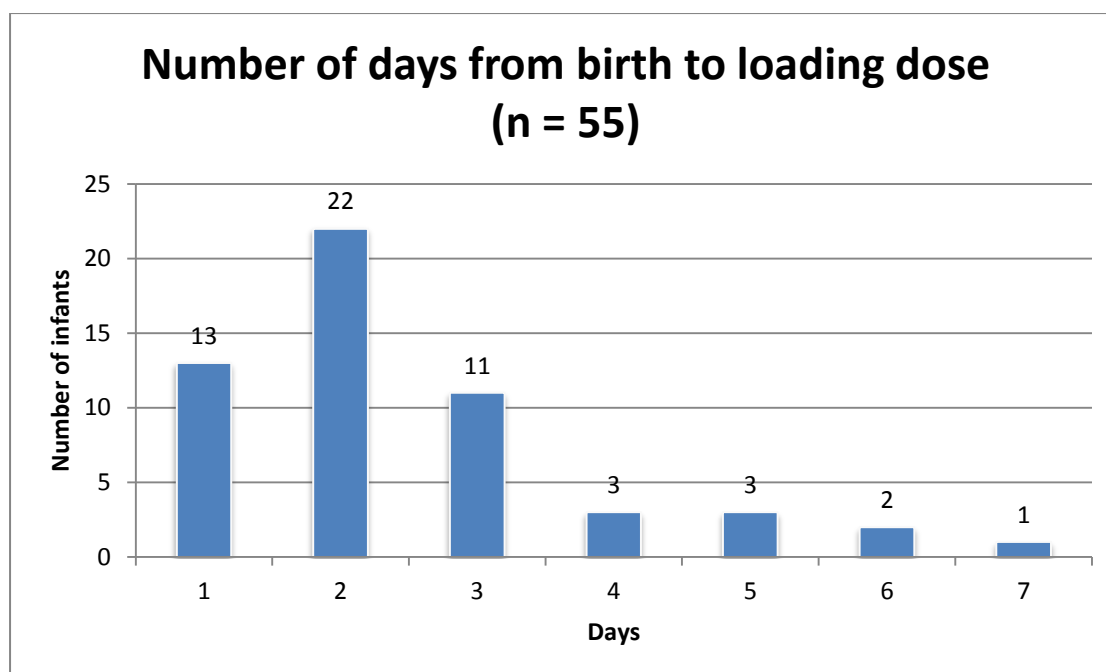


Figure 4.9: Duration in days until initiation with amikacin therapy

The study population of 33 neonates reflected a 2.18 days duration from birth until initiation of amikacin therapy with a minimum of 1 day and a maximum of 6 days. Similar to this the study population of 22 neonates reflected a duration of 2.91 days from birth until initiation of amikacin therapy with a minimum of 1 day and a maximum of 7 days.

The early initiation of amikacin therapy correlates well with the 78.18% (43; n = 55) premature neonates in the study population.

4.4 DOSAGES OF AMIKACIN

The antibiotic policy of the NICU in DGMH states as first line therapy an amikacin loading dose of 25 mg/kg and a maintenance dose of 20 mg/kg.. The dosage used was measured and analysed using the antibiotic policy.

It is important that amikacin is dosed per kilogram of body weight. Table 4.10 shows calculated dosages of amikacin grouped into the four weight categories, as stipulated under Section 4.1.3, at the time of administration.

Table 4.8: Dosages of amikacin per kilogram body weight

Weight at time of administration	Number of patients	Minimum dose (mg/kg)	Maximum dose (mg/kg)
ELBW (< 1 kg)	3	21.05	22.22
VLBW (1.0 – 1.49 kg)	23	17.39	23.81
LBW (1.50 – 2.5 kg)	14	17.39	21.21
NBW (> 2.5 kg)	15	17.39	21.43
Total	55		

The mean MD for the study population of 55 neonates was 19.96 mg/kg with a standard deviation of 1.139. The median MD was 20 mg/kg with ranges between 17.39 and 23.81 mg/kg.

The dosages of amikacin per kilogram body weight for the study population of 33 neonates reflected a MD of 19.84 mg/kg with a standard deviation of 1.207. For the study population of 22 neonates the MD was 20.12 mg/kg with a standard deviation of 1.033. The median MD for both the n = 33 and n = 22 study groups was 20 mg/kg with ranges of 17.39 and 23.81 mg/kg (n = 33) and 17.39 and 22.22 mg/kg (n = 22). The calculated MDs were appropriate. When the dose varied significantly e.g. in those neonates who had received 17.39 mg/kg, the physician was notified and dosage adjustments made to obtain a dose of 20 mg/kg daily.

4.5 PHARMACOKINETIC DATA OF ALL THE STUDY PATIENTS

Pharmacokinetic (PK) parameters have been calculated for all 55 study patients and are presented in Table 4.11. The individual values for the patients in the different categories of gestational age are shown in Tables 4.13 to 4.16 and discussed thereafter.

Table 4.9: Summary of pharmacokinetic data

n = 55	True peak (mcg/mL)	True trough (mcg/mL)	K value	t_{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m²) n = 33
Mean (± SD)	47.45 (± 14.97)	6.24 (± 9.59)	0.12 (± 0.10)	10.93 (± 22.89)	0.665 (± 0.685)	19.58 (±8.93)
Median (Range)	49.89 (6.87-81.58)	3.86 (0.01-65.99)	0.103 (0.004-0.658)	7.00 (3.77-174.45)	0.456 (0.244-3.71)	18.25 (4.23-49.23)
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾		4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)

(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

PK calculations revealed a true peak value of 47.45 mcg/mL which is higher than the reference ranges provided by literature (Adams *et al.* 2010; Segar, Patel and Tierney 2012; ed. Rossiter 2012). The median value was much higher than the accepted values and had a very wide range.

The accepted clinical range for amikacin trough level is less than 10 mcg/mL (Adams *et al.* 2010). Touw *et al.* (2009) have stated, a reduction in toxicity was observed when the trough concentrations were less than 2 mcg/mL. For the purpose of the study, trough levels higher than 2 mcg/mL were used as a referral point to the audiologist. The mean trough level was 6.24 mcg/mL (±9.59) and the median was 3.86 mcg/mL. Both the mean and the median were below the clinical reference range (Adams *et al.* 2010) but above the desired 2 mcg/mL (Touw *et al.* 2009).

The Vd of AGs shows a strong relationship with bodyweight and tends to be larger in more premature infants (Touw *et al.* 2009). They further explain that the Vd is increased in premature infants due to the larger ECF compartment. The larger Vd means that a higher dose per kilogram of bodyweight is needed to obtain adequate peak blood concentrations.

Amikacin is a water-soluble drug and mainly eliminated by the kidneys. Preterm and term neonates have immature renal function (EMEA 2004) which leads to the reduced elimination of amikacin (Pacifci 2008). Serum creatinine levels were requested by the treating physician in 34 of the 55 (61.8%) study patients. This resulted that the glomerular filtration could be calculated in 61.8% (34; n = 55) of the study population. Kidney function could subsequently not be monitored in the remaining 38.18% (21; n = 55) of the participants.

The study population group of 33 neonates reflected a true peak value of 45.67 mcg/mL (SD \pm 15.56) and a true trough value of 7.07 mcg/mL (SD \pm 11.90). The median for these two parameters were 47.9 mcg/mL (ranges 6.87 – 81.58) and 3.86 mcg/mL (ranges 0.74 – 65.99) respectively. The k-value had a mean of 0.117 (SD \pm 0.105) with a median of 0.099 and ranges of 0.004 – 0.658. The half-life of amikacin in this study population was 12.88 hours with a standard deviation of \pm 29.22. The mean half-life was 7.13 hours within a range of 3.77 – 174.45 hours. These neonates had a volume of distribution of 0.712 L/kg (SD \pm 0.72) with a median of 0.515 L/kg (ranges 0.244 – 3.714). Their mean GFR was 19.51 mL/min per 1.73m² (SD \pm 7.22) with a median of 19.22 mL/min per 1.73m² ranging between 4.23 – 33.19 mL/min per 1.73m².

The study population group of 22 neonates reflected a true peak value of 50.13 mcg/mL (SD \pm 13.96) and a true trough value of 5.00 mcg/mL (SD \pm 4.26). The median for these two parameters were 52.11 mcg/mL (ranges 15.42 - 77.47) and 3.55 mcg/mL (ranges 0.01-14.3) respectively. The k-value had a mean of 0.135 (SD \pm 0.105) with a median of 0.113 and ranges of 0.023-0.567. The half-life of amikacin in this study population was 8.01 hours with a standard deviation of \pm 5.72. The mean half-life was 6.59 hours within a range of 3.88 – 30.26 hours. These neonates had a volume of distribution of 0.594 L/kg (SD \pm 0.643) with a median of 0.428 L/kg (ranges 0.254 – 3.4). Their mean GFR was 19.71 mL/min per 1.73m² (SD \pm 11.5) with a median of 15.7 mL/min per 1.73m² ranging between 9.82 – 49.23 mL/min per 1.73m².

Both the two study populations had peak levels higher than the accepted reference ranges as stipulate above. The trough levels were also above the desired 2 mcg/mL (used for referral purposes in the study) for both these population groups. The mean half-life was slightly longer in the n = 33 group compared to the n = 22 group, 12.88 hours vs 7.13 hours. This correlated with the volume of distribution as well which was longer in the n = 33 group (0.712 L/kg) compared to the n = 22 group (0.515 L/kg). The GFR was similar in both study population groups and within the reference range of 10 – 40 mL/min per 1.73m². The n = 33 group had a mean GFR of 19.51 mL/min per 1.73m² and the n = 22 group had a mean GFR of 19.22 mL/min per 1.73m².

4.6 SERUM CONCENTRATIONS

Amikacin peak and trough levels were measured for 55 neonates and compared to the desired therapeutic range for this population. Uncertainty still remains on the most safe and effective dosing regimen of any AG in neonates (Allegaert *et al.* 2006). Variability exists within the reference ranges of desired peak and trough levels for amikacin. The most important microorganisms involved in neonatal infections have a MIC of around 1 mcg/mL and an AG peak concentration of at least 10 mcg/mL should be aimed for (Levison 2009; Touw *et al.* 2009). According to Allegaert *et al.* (2006) an initial amikacin target concentration range of 15-30 mcg/mL and trough concentration of less than 5 mcg/mL to an average steady state concentration of about 10 mcg/mL, are adequate targets in a once-daily approach.

Figure 4.10 and 4.11 illustrates the measured peak and trough levels respectively for the study population according to the GA groups. In Section 4.1.8 a more detailed discussion follow on pharmacokinetic results found in the different groups.

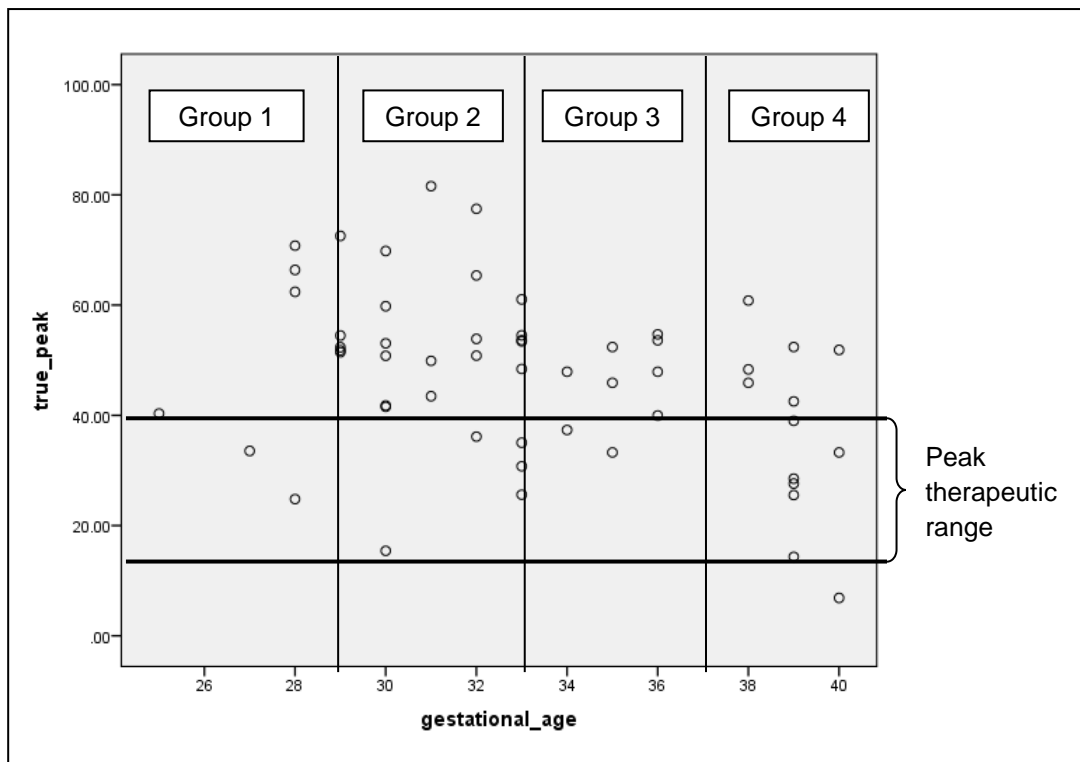


Figure 4.10: Amikacin serum peak concentrations measured for 55 neonates

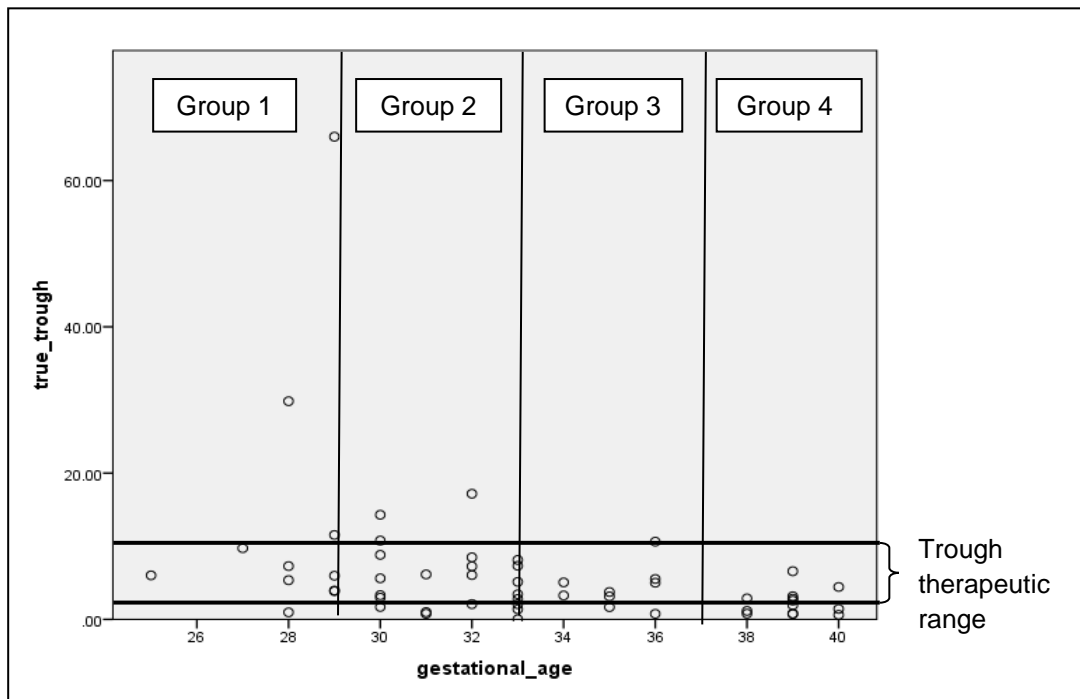


Figure 4.11: Amikacin serum trough concentrations measured for 55 neonates

4.7 INTERQUARTILE RANGE

Another measure of dispersion is known as the interquartile range (IQR). The use of an IQR is applied to illustrate 50% of the particular population that falls within the minimum and the maximum. Refer to Table 4.10 below.

Table 4.10: Interquartile range for all the study patients

	n = 55	
	True peak (mcg/mL)	True trough (mcg/mL)
Minimum	6.87	0.01
Lower quartile (25%)	36.14	2.07
Mean (\pm SD)	47.45 (\pm 14.97)	6.24 (\pm 9.59)
Median	49.89	3.86
Upper quartile (75%)	53.43	6.15
Maximum	81.58	65.99

For the purpose of this study, a reference range of 20 – 40 mcg/mL has been used as guideline for peak levels and < 10 mcg/mL for trough levels for toxicity; and trough levels \geq 2 mcg/ml were used for referral purposes (to the audiologist) in this study.

Fifty percent of the study population had a true peak level for amikacin between 34.16 and 53.43 mcg/mL. This falls within the ranges as stipulated above, but higher than the guideline used for the study purpose.

The IQR for the study population showed a true trough level for amikacin between 2.07 and 6.15 mcg/mL, the latter being higher than the reference range of < 5 mcg/mL used in many references, but lower than the 10 mcg/mL used for the study purpose.

The study done by Sherwin *et al.* (2009) confirmed that the existing amikacin dosing regimen recommended target peak concentrations between 20 and 30 mcg/mL. The target concentrations were revised and adjusted to 24-35 mcg/mL, which was considered to be an acceptable upper limit with regards to toxicity.

The Nebraska Medical Centre accepts a peak for amikacin of 40 – 60 mcg/mL for once daily dosing (Gross, 2012), while Adams *et al.* (2010) use optimal serum amikacin levels of 15 – 40 mcg/mL as a guideline. As mentioned above Allegaert *et al.* (2006) stated that an initial amikacin target concentration range of 15-30 mcg/mL and trough concentration of less than 5 mcg/mL to an average steady state concentration of about 10 mcg/mL, are adequate targets in a once-daily approach. While the recommended target concentrations for amikacin trough levels are < 5 mcg/mL (Sherwin *et al.* 2009), the Nebraska Medical Centre recommends an undetectable trough level (Gross, 2012).

Table 4.11: Interquartile range for the study population of 33 neonates

	n = 33	
	True peak (mcg/mL)	True trough (mcg/mL)
Minimum	6.87	0.74
Lower quartile (25%)	44.0775	1.4675
Mean (\pm SD)	45.67 (\pm 15.55)	7.07 (\pm 11.90)
Median	47.90	3.86
Upper quartile (75%)	54.645	8.4275
Maximum	81.58	65.99

Table 4.12: Interquartile range for the study population of 22 neonates

	n = 22	
	True peak (mcg/mL)	True trough (mcg/mL)
Minimum	15.42	0.01
Lower quartile (25%)	38.17	1.82
Mean (\pm SD)	50.13 (\pm 13.95)	5.00 (\pm 4.25)
Median	52.11	3.55
Upper quartile (75%)	54.18	6.92
Maximum	77.47	14.3

In both the study populations as set out in Table 4.11 and Table 4.12 the mean peak levels were above the reference range of 20 – 40 mcg/mL (45.67 mcg/mL \pm 15.55 and 50.13 mcg/mL \pm 13.95 respectively). The mean trough levels were below 10 mcg/mL, but above the 2 mcg/mL that was used as a referral point to the audiologist (7.07 mcg/mL \pm 11.90 and 5.00 mcg/mL \pm 4.25 respectively).

4.8 PHARMACOKINETIC DATA BY GESTATIONAL AGE

The study population has been divided into four gestational age groups (refer to section 4.1.2). The PK parameters will be shown and discussed in the following four tables. Out of range values are shaded in grey.

4.8.1 Neonates born at 25 to 29 weeks gestational age

Table 4.13: Pharmacokinetic values for Group 1: Gestational age 25–29 weeks (11; n = 55)

ID	Gender	GA (weeks)	Weight (kg)	Peak (mcg/mL)	Trough (mcg/mL)	t _{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m ²)
2	F	29	0.95	72.56	65.99	174.45	3.032	8.61
7	F	28	0.95	70.78	7.28	7.29	0.326	12.86
18	M	29	1.1	51.45	3.86	6.37	0.379	17.08
23	M	28	1.25	66.38	29.83	20.75	0.551	22.36
25	M	28	1.1	24.81	0.96	5.10	0.758	27.10
42	M	29	1.2	51.79	3.95	6.43	0.421	22.08
49	F	29	1.1	52.35	11.54	10.98	0.485	19.50
72	M	25	1.05	40.33	6.02	8.71	0.690	22.67
78	F	27	1.20	33.54	9.73	13.35	0.860	-
82	F	29	0.90	54.48	5.97	7.49	0.477	10.47
83	M	28	1.60	62.40	5.36	6.75	0.329	18.17
Mean (± SD)		28.09 (±1.22)	1.13 (±0.19)	52.81 (±15.19)	13.68 (±18.97)	24.33 (±49.99)	0.755 (±0.775)	18.02 (±5.91)
Median		28	1.10	52.35	6.02	7.49	0.485	18.84
Minimum		25	0.9	24.81	0.96	5.10	0.326	8.61
Maximum		29	1.60	72.56	65.99	174.45	3.032	27.10
Reference range				15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47- 0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

The majority of patients (n=8) in Group 1 had peak values exceeding the reference range (>40 mcg/mL). Three patients had higher than the reference range trough levels (< 10 mcg/mL) and all but one neonate (id 25) had trough levels > 2 mcg/mL.

The half-life of amikacin was longer than the norm for four of the patients. Three patients had a volume of distribution exceeding 0.7 L/kg. According to Pacifici (2009) and Turnidge (2003) there is a correlation between the volume of distribution and the half-life; when the volume of distribution increases, the half-life will increase. This occurred in study patient 2 and 78. Although study patient 78 had a Vd of 0.86 L/kg and a half-life of 13.35 hours, the patient had peak and trough levels within the reference range. The serum creatinine was not requested for this patient and the GFR could not be calculated.

Study patient 2 had the largest Vd (3.032 L/kg) with the longest half-life. The half-life of amikacin for this individual was exceptionally high, calculated as 174 hours. This patient presented with very high peak and trough levels, 72.56 mcg/mL and 65.99 mcg/mL respectively. The participant was of female gender, born at 29 week's gestational age and weighed a mere 0.95 kg. She received 21.05 mg/kg amikacin as a maintenance dose, which was higher than the desired 20 mg/kg. The kidney function was impaired and reflected with a GFR of 8.61 mL/min per 1.73m² – the only neonate with a value below the reference range compared to the other study patients in this group. Pacifici (2009) reviewed published data on the PKs and PDs of AGs and concluded that the clearance and half-life of AGs are influenced by the maturation of the kidney. The patient was Retroviral Disease (RVD) exposed and also diagnosed with Hyaline Membrane Disease (also known as RDS) sepsis and jaundice. These conditions could have influenced the PKs of amikacin, e.g. a condition like sepsis results in a larger volume of distribution (Taccone *et al.* 2010). Frequent changes in pH can occur in critically ill patients as a result of e.g. respiratory failure, shock states and renal failure. The ionized state, and therefore the subsequent distribution of amikacin are affected by the pH of the environment (Boucher *et al.* 2006).

These mentioned factors might have caused the limited drug excretion in these patients and a resultant accumulation with high peak and trough levels.

Study patient 25 was born at 28 weeks gestational age and weighed 1.1 kg. He presented with a slightly larger volume of distribution (0.758 L/kg) which might have resulted in peak and trough level towards the lower reference range, namely 24.81

and 0.96 mcg/mL respectively. The half-life and GFR were within normal reference ranges.

Except for the peak levels that were increased in patients 7, 18, 42, 82 and 83 all the other PK values were within normal reference range. The trough levels were all above 2 mcg/mL and dosage adjustments were recommended in all of these patients. Study patient 83 for example, received a MD of 32 mg (20 mg/kg/dose) per day and had a laboratory peak and trough of 54.6 mcg/mL and 5.5 mcg/mL respectively. Calculations were done and reflected a true peak of 62.4 mcg/mL and a true trough of 5.36 mcg/mL. The patient's V_d was 0.329 L/kg with a longer half-life of 6.75 hours. It was recommended that the dose be reduced to 20 mg (12.5 mg/kg/dose) per day to achieve a peak of 39 mcg/mL and trough of 3.3 mcg/mL (Appendix 5).

Of these patients, patient 7 had the highest peak level (70.78 mcg/mL) and a trough level of 7.28 mcg/mL. She was born at 28 weeks, weighed 0.95 kg and was diagnosed with RDS. Amikacin was administered at a dose of 21.05 mg/kg/day as maintenance therapy. A dose of 11 mg (11.58 mg/kg) was recommended. With this dose a peak level of 44 mcg/mL and a trough level of 4.54 mcg/mL could have been expected. The intervention was not accepted as it was close to day 7 of therapy. The therapy was subsequently changed to tazobactam/piperacillin on 1/12/2011.

Study patient 72 was the only patient in this gestational age-group that had a normal GFR and within range PK values. He was born at a gestational age of 25 weeks and weighed 0.75 kg. This patient was treated with amikacin at a MD of 19.05 mg/kg for septicaemia and necrotising enterocolitis (NEC). A diagnosis of anaemia had also been made. No dosage adjustment was required in this patient.

The two tables below contain the information of the GA groups 25 – 29 weeks of the two sub-populations groups separately.

Table 4.14: Pharmacokinetic values for neonates born at gestational age 25–29 weeks (6; n = 33)

n = 6	Peak (mcg/mL)	Trough (mcg/mL)	t_½ (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	54.39	18.99	37.11	0.956	18.45
SD (±)	19.099	25.242	67.521	1.031	6.897
Min	24.81	0.96	5.1	0.326	8.61
Max	72.56	65.99	174.45	3.032	27.10
Median	58.92	6.65	8.00	0.621	19.72
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(6) Adams *et al.* (2010)(7) Segar *et al.* (2012)

(8) ed. Rossiter (2012)

(9) Pacifici (2008)

(10) Vieux *et al.* (2010)**Table 4.15: Pharmacokinetic values for neonates born at gestational age 25–29 weeks (5; n = 22)**

n = 5	Peak (mcg/mL)	Trough (mcg/mL)	t_½ (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	50.91	7.31	9.00	0.514	17.56
SD (±)	10.599	3.187	3.033	0.203	4.994
Min	33.54	3.95	6.43	0.329	10.47
Max	62.40	11.54	13.35	0.860	22.08
Median	52.35	5.97	7.49	0.477	18.84
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(11) Adams *et al.* (2010)(12) Segar *et al.* (2012)

(13) ed. Rossiter (2012)

(14) Pacifici (2008)

(15) Vieux *et al.* (2010)

The mean peak levels for both the above study population groups were above 50 mcg/mL. The mean trough level was much higher in the n = 33 group than the n = 22 group, 18.99 vs 7.31 mcg/mL but this was due the very wide range in the n = 33 group, (e.g. study patient id 2, discussed above). The half-life was much longer in the n = 33 group, also due to individual patients with exceptionally long half-lives. The median for the half-lives in the n = 33 and the n = 22 group was similar, 8.00 vs. 7.49 hours. The volume of distribution as well as the GFR was within limits in both these groups.

4.8.2 Neonates born at 30 to 33 weeks gestational age

Table 4.16: Pharmacokinetic values for Group 2: Gestational age 30-33 weeks

(23; n = 55)

ID	Gender	GA (weeks)	Weight (kg)	Peak (mcg/mL)	Trough (mcg/mL)	t _{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m ²)
8	F	32	2.1	36.14	8.48	11.43	0.686	14.24
14	F	33	2.7	54.51	0.01	3.95	0.337	49.23
16	M	30	1	41.55	2.95	6.26	0.515	14.17
24	M	33	1.1	30.76	2.07	5.89	0.669	16.71
27	M	30	1.35	59.79	5.61	7.00	0.341	19.50
34	M	30	1.15	53.07	3.31	5.97	0.417	16.15
36	M	33	2.3	48.41	5.13	7.38	0.463	-
37	F	33	1.8	25.61	8.12	14.41	1.111	-
39	F	31	1.25	81.58	0.99	3.77	0.244	19.22
40	M	31	1.35	43.47	0.77	4.10	0.467	13.52
41	F	32	1.4	65.38	7.25	7.55	0.361	9.82
52	F	32	1.45	53.88	17.18	14.50	0.548	-
55	F	33	1.65	53.64	7.33	8.35	0.465	-
59	F	33	1.60	35.04	3.43	7.13	0.590	21.96
66	F	33	1.55	53.43	2.77	5.59	0.410	-
67	F	31	1.15	49.89	6.15	7.97	0.388	-
68	M	32	1.45	50.83	6.06	7.80	0.423	-
69	M	32	1.35	77.47	2.08	4.59	0.254	-
73	M	30	1.46	50.81	1.70	4.88	0.426	-
74	M	30	1.35	69.82	14.30	10.50	0.365	13.69
75	M	30	1.10	41.78	10.77	12.22	0.685	12.39
79	M	33	2.20	61.03	1.39	4.39	0.340	15.70
80	M	30	1.40	15.42	8.82	30.26	3.400	12.67
Mean (± SD)		31.61 (±1.27)	1.53 (±0.43)	50.14 (±15.71)	5.51 (±4.40)	8.52 (±5.69)	0.605 (±0.637)	17.78 (±9.60)
Median		32	1.40	50.83	5.13	7.13	0.426	14.97
Minimum		30	1.0	15.42	0.01	3.77	0.244	9.82
Maximum		33	2.70	81.58	17.18	30.26	3.400	49.23
Reference range				15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

In group 2 (Table 4.16), eighteen of the patients (78%; n = 23) had peak levels higher than the reference range. Three patients had a trough level above 10 mcg/mL (13%; n = 23) and five patients (22%; n = 23) had a trough level less than 2 mcg/mL. Only 13% (3; n=23) had trough levels more than the recommended reference range of 10 mcg/mL and 78% (18; n = 23) had trough levels more than the desired 2 mcg/mL for the study. With regards to the half-life, eight patients (35%; n = 23) had a half-life outside the reference range of 4 to 8 hours – only one having a half-life less than 4 hours. The volume of distribution was normal in this group, except for study patients 37 and 80. Both these patients also presented with much longer half-life's. This correlates with what was mentioned in the previous gestational age group with regards to a longer half-life and increased Vd (Turnidge 2003; Pacifici 2009). The mean GFR for group 2 was within range - only study patient 14 had an above normal GFR while study patient 41 had a reduced GFR.

Compared to the other 22 study patients in this gestational age group (n = 23), the highest GFR resulting in the lowest trough level have been seen in study patient 14. She had a peak level of 54.51 mcg/mL and a trough level of 0.01 mcg/mL. Although the patient was born prematurely at 33 weeks, the birth weight was normal at 2.7 kg. The patient was diagnosed with RDS and received a MD of 18.52 mg/kg. The volume of distribution was within the reference range at 0.337 L/kg and the half-life was 3.95 hours. The shorter half-life and low trough level could have been due to normal kidney function, calculated as a GFR of 49.23 mL/min per 1.73m². As renal function increases, the elimination rate constant increases and half-life decreases (Munar n.d.). Another reason for a short half-life can be because the patient has received a lower MD than the accepted 20 mg/kg. The dose prescribed was 50 mg, resulting in a dose per kilogram for this patient of 18.52 mg. The dose was recalculated to lower the peak level, but still keeping the trough level below 2 mcg/mL. A dose of 36 mg per day would have delivered an expected peak and trough level of 39.68 and 0.6 mcg/mL respectively. It was however close to day seven of therapy and the intervention was not accepted with an acceptable trough of less than 2 mcg/mL.

Study patient 39 had the highest peak level in this gestational age group (n = 23) as well as overall (n = 55) - 81.58 mcg/mL. This participant was born prematurely at 31 weeks with a VLBW of 1.25 kg. The gender was female and she was diagnosed with RDS and had been RVD exposed. She received an amikacin MD of 20 mg/kg and had a trough level of 0.99 mcg/mL, well within the desired level of < 2 mcg/mL for the study purpose. Although kidney function is underdeveloped in premature neonates (Sherwin *et al.* 2009), the GFR of 19.22 mL/min per 1.73m² was low but still within limits. The half-life of amikacin in this individual was slightly shorter than the accepted 4 – 8 hours, at 3.77 hours. The patient's volume of distribution was small, 0.2 L/kg and might have played a significant role in causing such a high peak level. In the two Tables (Tables 4.17 and 4.18) below, the two study populations of n = 33 and n = 22 can be compared to one another for the GA group 30 – 33 weeks.

Table 4.17: Pharmacokinetic values for neonates born at gestational age 30-33 weeks (14; n = 33)

n = 14	Peak (mcg/mL)	Trough (mcg/mL)	t _{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m ²)
Mean	48.12	5.68	8.10	0.519	17.42
SD (±)	14.001	4.029	3.193	0.209	2.914
Min	25.61	0.99	3.77	0.244	14.17
Max	81.58	17.18	14.50	1.111	21.96
Median	50.36	5.37	7.26	0.464	16.71
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(6) Adams *et al.* (2010)

(7) Segar *et al.* (2012)

(8) ed. Rossiter (2012)

(9) Pacifici (2008)

(10) Vieux *et al.* (2010)

Table 4.18: Pharmacokinetic values for neonates born at gestational age 30-33 weeks (9; n = 22)

n = 9	Peak (mcg/mL)	Trough (mcg/mL)	t_½ (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	53.29	5.23	9.16	0.737	18.15
SD (±)	18.489	5.174	8.468	1.006	13.820
Min	15.42	0.01	3.95	0.254	9.82
Max	77.47	14.3	30.26	3.4	49.23
Median	54.51	2.08	4.88	0.365	13.52
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

Again, the mean peak levels for both the study population groups were above the reference range and the mean trough levels were below 10 mcg/mL but above the referral point of 2 mcg/mL for the audiologist. The mean half-lives only slightly differed between the two groups, 8.10 hours (14; n = 33) and 9.16 hours (9; n = 22), which were both longer than the reference range. The volume of distribution as well as the GFR was within the accepted values for both these groups.

4.8.3 Neonates born at 34 to 37 weeks gestational age

Table 4.19: Pharmacokinetic values for Group 3: Gestational age 34-37 weeks (9; n = 55)

ID	Gender	GA (weeks)	Weight (kg)	Peak (mcg/mL)	Trough (mcg/mL)	t _{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m ²)
6	M	36	2.8	53.61	10.62	10.24	0.464	21.18
17	M	34	2	47.91	5.06	7.37	0.465	-
21	M	36	1.55	54.69	0.76	3.88	0.365	-
28	F	34	1.4	37.35	3.27	6.74	0.584	-
30	M	35	1.95	33.26	3.14	7.02	0.644	4.23
33	M	35	1.8	52.39	1.68	4.82	0.385	13.20
47	M	35	1.7	45.91	3.74	6.61	0.491	18.33
51	F	36	2.3	47.90	5.53	7.67	0.410	-
53	F	36	2.50	39.99	5.01	7.98	0.572	25.64
Mean (± SD)		35.22 (±0.83)	2.0 (±0.46)	45.89 (±7.54)	4.31 (±2.84)	6.93 (±1.83)	0.487 (±0.096)	16.52 (±8.22)
Median		35	1.95	47.90	3.74	7.02	0.465	18.33
Minimum		34	1.40	33.26	0.76	3.88	0.365	4.23
Maximum		36	2.80	54.69	10.62	10.24	0.644	25.64
Reference range				15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 - 40 ⁽⁵⁾

(1) Adams *et al.* (2010)

(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

Similarly to group 1 and 2, most of the patients in group 3 (66.7%, n = 9) had peak levels exceeding the reference range. The trough level was above 10 mcg/mL only in study patient id 6. Six of the study patients in this gestational age group (66.7%, n = 9) had a trough level below 10 mcg/mL but above 2 mcg/mL. The volume of distribution was normal for all these patients and one patient (id 6) had a longer than normal half-life. Serum creatinine levels were requested for five patients (55.6%, n =

9), which enabled the researcher to calculate the GFR. The GFR was within range except for study patient 30. He had a reduced GFR although all other parameters were within range.

Study patient 6 was of male gender, born at 36 weeks gestational age and although classified as premature his weight was of normal birth weight. He was also diagnosed with RDS and congenital pneumonia. A prolonged serum amikacin half-life in association with hypoxemia was apparent in a study done by Myers *et al.* (1997). This correlates well with the longer than normal half-life that resulted in increased peak and trough levels in this study patient. He received a dose of 56 mg per day (20 mg/kg/day) as MD and this resulted in a peak of 53.61 mcg/mL, a trough of 10.62 mcg/mL and a half-life of 10.25 hours. After PK calculations a MD of 42 mg/day could have been suggested with expected peak and trough levels of 42 mcg/mL and 8.26 mcg/mL, but the patient had been discharged.

Data of the neonates born between 30 and 33 weeks GA in the two study population groups of 33 and 22 neonates are displayed in Table 4.20 and 4.21 below.

Table 4.20: Pharmacokinetic values for neonates born at gestational age 34-37 weeks (7; n = 33)

n = 7	Peak (mcg/mL)	Trough (mcg/mL)	t_½ (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	43.53	3.92	6.89	0.507	15.35
SD (±)	6.815	1.364	1.035	0.096	9.00
Min	33.26	1.68	4.82	0.385	4.23
Max	52.39	5.53	7.98	0.644	25.64
Median	45.91	3.74	7.02	0.491	15.77
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)**Table 4.21: Pharmacokinetic values for neonates born at gestational age 34-37 weeks (2; n = 22)**

n = 2	Peak (mcg/mL)	Trough (mcg/mL)	t_½ (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	54.15	5.69	7.06	0.415	21.18
SD (±)	0.764	6.972	4.49	0.070	-
Min	53.61	0.76	3.88	0.365	21.18
Max	54.69	10.62	10.24	0.464	21.18
Median	54.15	5.69	7.06	0.415	21.18
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

For both the study population groups the mean peak levels were above the reference range e.g. 43.53 and 54.15 mcg/mL. The mean trough level for the n = 33 group was 3.93 mcg/mL and for the n = 22 group 5.69 mcg/mL. The mean half-lives only slightly differed between the two groups, 6.89 hours (7; n = 33) and 7.06 hours (2; n = 22), which were both within the reference range. The volume of distribution as well as the GFR was within range for both these groups.

4.8.4 Neonates born at 38 to 41 weeks gestational age

Table 4.22: Pharmacokinetic values for Group 4: Gestational age 38-41 weeks (12; n = 55)

ID	Gender	GA (weeks)	Weight (kg)	Peak (mcg/mL)	Trough (mcg/mL)	t _{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m ²)
5	M	38	2.85	48.34	2.87	5.87	0.421	37.86
12	F	40	3	51.87	4.43	6.74	0.419	-
22	F	40	2.7	33.26	0.62	4.31	0.610	-
26	F	40	3.2	6.87	1.43	10.54	3.714	24.55
29	F	39	2.9	42.54	1.94	5.37	0.523	31.61
31	M	39	4.2	27.62	0.74	4.58	0.792	-
38	M	39	2.8	52.40	2.83	5.68	0.430	-
44	F	39	2.8	38.99	0.78	4.25	0.503	26.32
45	F	39	3.9	25.53	6.58	12.22	1.079	-
54	F	38	2.90	60.82	0.78	3.80	0.330	33.19
76	M	38	4.60	45.90	1.16	4.50	0.444	-
81	M	39	3.45	28.51	3.15	7.52	0.717	-
Mean (± SD)		39 (±0.74)	3.28 (±0.63)	38.55 (±14.93)	2.28 (±1.82)	6.28 (±2.64)	0.832 (±0.931)	30.71 (±5.37)
Median		39	2.95	40.77	1.69	5.53	0.513	31.61
Minimum		38	2.70	6.87	0.62	3.80	0.330	24.55
Maximum		40	4.60	60.82	6.58	12.22	3.714	37.86
Reference range				15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47- 0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

Although the median peak level was within range for group 4, six patients (50%; n = 12) had levels above 40 mcg/mL. All the patients in this group had trough levels

within range, with seven patients (58.3%; n = 12) having a trough level below 2 mcg/mL. The GFR was calculated for five patients and all were within the reference range. It is evident how the GFR improves towards the older gestational age groups – the lower the GA at birth the higher is the serum creatinine (EMA 2004).

Study patient 26 had a desirable trough level of 1.43 mcg/mL but a true peak of 6.87 mcg/mL. This participant was of female gender, born at 40 weeks gestational age with a normal body weight of 3.2 kg. A diagnosis of anaemia was made and she received an amikacin MD of 20.31 mg. Although her GFR was 24.55 mL/min per 1.73m^2 , thus within the normal reference range, the calculated half-life of amikacin was longer at 10.54 hours. The volume of distribution was 3.7 L/kg, thus an extremely large Vd. The latter could have been the reason for the low peak and trough values obtained for this patient. The pharmacokinetics of amikacin has been investigated in a small study done by Kaojarem, Maoleekoompairoj and Atichartakam (1989) and the results revealed that there was a marked increase in volume of distribution of amikacin in patients with haematologic malignancies compared with normal.

Two patients had very large volumes of distribution with extended half-lives – one was study patient 26, already discussed above, the other was study patient 45. Study patient 45 was a female patient born at 39 weeks GA to a diabetic mother. She weighed 3.9 kg and was diagnosed with septicaemia. As Taccone *et al.* (2010) explains, increased cardiac index and interstitial fluid shifts in sepsis result in a larger Vd. This study patient's Vd was 1.079 L/kg and had a peak amikacin serum level of 25.53 mcg/mL and trough of 6.58 mcg/mL. The half-life was above normal at 12.22 hours. All these PK parameters correlate with each other e.g. increased Vd, decreased peak levels and extended half-life. The trough level was below the reference range of 10 mcg/mL but much higher than the desired 2 mcg/mL. The patient was not evidently oedematous and no serum creatinine levels were requested. The treating physician was subsequently notified to request serum creatinine levels to enable calculation of the GFR.

Data of the neonates born between 38 and 41 weeks GA in the two study population groups of 33 and 22 neonates are displayed in Tables 4.23 and 4.24 below.

Table 4.23: Pharmacokinetic values for neonates born at gestational age 38-41 weeks (6; n = 33)

n = 6	Peak (mcg/mL)	Trough (mcg/mL)	t_{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	33.73	2.04	6.79	1.16	28.92
SD (±)	18.249	2.274	3.629	1.279	4.136
Min	6.87	0.74	3.80	0.33	24.55
Max	60.82	6.58	12.22	3.714	33.19
Median	33.31	1.11	4.98	0.66	28.965
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)

(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

Table 4.24: Pharmacokinetic values for neonates born at gestational age 38-41 weeks (6; n = 22)

n = 6	Peak (mcg/mL)	Trough (mcg/mL)	t_{1/2} (h)	Vd (L/kg)	GFR (mL/min per 1.73m²)
Mean	43.38	2.51	5.77	0.507	37.86
SD (±)	10.079	1.394	1.25	0.126	-
Min	28.51	0.62	4.31	0.419	37.86
Max	52.4	4.43	7.52	0.717	37.86
Median	47.12	2.85	5.77	0.437	37.86
Reference range	15-40 ⁽¹⁾ 20-30 ⁽²⁾ > 30 ⁽³⁾	< 10 ⁽¹⁾ 2-5 ⁽²⁾ < 1 ⁽³⁾	4-8 ⁽⁴⁾	0.47-0.85 ⁽⁴⁾	10 – 40 ⁽⁵⁾

(1) Adams *et al.* (2010)

(2) Segar *et al.* (2012)

(3) ed. Rossiter (2012)

(4) Pacifici (2008)

(5) Vieux *et al.* (2010)

The mean peak level for the n = 33 population group was within reference range but slightly higher for the n = 22 group e.g. 33.73 mcg/mL and 43.38 mcg/mL respectively. The mean trough levels were slightly higher in both the population groups; with n = 33 reflecting a level of 2.04 mcg/mL and the n = 22 group reflecting a level of 2.51 mcg/mL. The half-lives of the two groups were within range although the volume of distribution for the n = 33 group was much higher than the reference range. The latter was due to the very large volume of distribution in study patients 26 and 45 as discussed above. The glomerular filtration rate for both population groups was within limits.

4.9 CONCLUSION ON PHARMACOKINETICS

When comparing the different tables according to gestational ages, it is evident that the highest mean peak amikacin levels occurred in the neonates born the earliest. The mean peak amikacin levels decreased towards neonates born at full term. Between the different gestational age groups, the neonates classified as extremely preterm had a mean peak amikacin level of 52.81 mcg/ml, the very preterm neonates a mean peak of 50.14 mcg/mL, the moderate to late preterm neonates 45.89 mcg/mL and the full term neonates 38.55 mcg/mL.

The same is apparent with the mean trough amikacin level, where the trough level was the highest in the extremely preterm category at 13.68 mcg/mL. The mean trough levels reduced to 5.51 mcg/mL in the very preterm neonates, 4.31 mcg/mL in the moderate to late preterm neonates and 2.28 mcg/mL in the full term neonates. Only in the extremely preterm neonates the mean amikacin trough level exceeded the recommended 10 mcg/mL. For the other three categories the mean amikacin trough level was below 10 mcg/mL but all above the desired 2 mcg/mL.

As the volume of distribution became smaller from the extremely preterm to the full term born neonates, so the half-life became shorter. This is evident in the first three categories, but not in the full term category and was due to individual study patients (id 26 and 45 discussed under section 4.8.4). This correlates well with the statement that premature neonates have larger ECF compartments and changes within the different gestational ages.

Although the GFR was all within the reference range for the four GA groups, it was clear that the full term neonates had the fastest GFR at a mean of 30.71 mL/min per 1.73m². The other three GA groups all had very similar GFRs and differed between 18.02 and 16.52 mL/min per 1.73m².

4.10 PHARMACOKINETIC AND AUDIOLOGY DATA WITH COMPARISONS

Pharmacokinetics and audiologic tests were performed on the study population of 22 neonates. The PKs have been discussed in the previous sections. This section concentrates on the audiology results obtained with a discussion around the correlation between PKs and audiology test results.

A Maico MI34 H middle-ear analyzer was used to record middle ear functioning in the form of tympanograms. A high frequency probe tone of 1000 Hz was utilized to measure Y-admittance tympanograms, with a positive to negative pressure sweep of 200 daPa at a pump speed of 200 daPa/s - recommended for young infants (Holte, Margolis and Cavanaugh 1991). The maximum point on a recorded tympanogram was marked to obtain the uncompensated peak admittance value with the corresponding pressure value at this point. All the neonates admitted to the study presented with peaked high frequency tympanograms as well as admittance values and tympanic peak pressure within suggested normative data (Swanepoel *et al.* 2007). This is important as compromised middle ear functioning due to middle ear effusion or mesenchyme in middle ear cavities has a negative effect on OAE results (Keefe *et al.* 2003).

During the ototoxic monitoring, a DPOAE assessment was completed at baseline for the sub-population of 22 patients. These baselines were determined within 24 hours from when the patient was identified and consent obtained. A follow-up DPOAE was performed on the day of the third MD and therapeutic drug monitoring (TDM).

DPOAEs were obtained at four different frequencies for each ear, namely at 2, 4, 6 and 8 kHz. Distortion Product OAEs and hearing thresholds are negatively correlated and for the purpose of the study a change of 2.4 dB SPL from baseline to follow-up was considered as significant.

Table 4.25 contains a summary of all the results. The four GA groups are grouped together and highlighted in different colours. The audiology results appearing in red are the outliers and discussed thereafter.

Table 4.25: Pharmacokinetic-and-DPOAE results (n = 22)

ID	GA (weeks)	Gender	Weight (kg)	Dose (mg/kg)	Peak (mcg/mL)	Trough (mcg/mL)	t _{1/2} (hours)	Vd (L/kg)	DP_Dif_2kHz	DP_Dif_4kHz	DP_Dif_6kHz	DP_Dif_8kHz
78	27	F	1.20	20.83	33.54	9.73	13.35	0.860	-0.45	0.70	0	1.15
83	28	M	1.60	20.00	62.40	5.36	6.75	0.329	5.7	7.20	4.05	13.05
42	29	M	1.20	20.00	51.79	3.95	6.43	0.421	12	10.7	4.7	5.7
49	29	F	1.10	20.00	52.35	11.54	10.98	0.485	21.3	19.9	16.8	19.6
82	29	F	0.90	22.22	54.48	5.97	7.49	0.477	2.1	3.55	-2.6	22.05
73	30	M	1.46	20.55	50.81	1.70	4.88	0.426	-0.1	-1.25	-4.45	-1.5
74	30	M	1.35	22.22	69.82	14.30	10.50	0.365	-4.85	-3.95	-15.45	20.6
75	30	M	1.10	20.00	41.78	10.77	12.22	0.685	2.65	-2.05	-1.45	-2.55
80	30	M	1.40	21.43	15.42	8.82	30.26	3.400	1.3	1.2	-12.2	21.05
40	31	M	1.35	20.00	43.47	0.77	4.10	0.467	-2.4	-3.2	-3.85	2.35
41	32	F	1.40	20.00	65.38	7.25	7.55	0.361	13	24.7	18	19.3
69	32	M	1.35	20.00	77.47	2.08	4.59	0.254	-9.2	-11.5	-5.55	8.85
14	33	F	2.70	18.52	54.51	0.01	3.95	0.337	-3.6	9.4	7.1	2.4
79	33	M	2.20	20.45	61.03	1.39	4.39	0.340	-11.15	-12.85	-7.6	-7.2
6	36	M	2.80	20.00	53.61	10.62	10.24	0.464	-3.7	-5.8	0.3	-2
21	36	M	1.55	20.00	54.69	0.76	3.88	0.365	1.65	1.2	2.95	0.35
5	38	M	2.85	19.30	48.34	2.87	5.85	0.421	5.85	0.8	-1.3	-2
76	38	M	4.60	20.00	45.90	1.16	4.50	0.444	0.35	-0.2	-4.7	-0.45
38	39	M	2.80	19.64	52.40	2.83	5.68	0.430	-6	-9	-8.6	-9.2
81	39	M	3.45	17.39	28.51	3.15	7.52	0.717	-0.3	-1.75	-4.7	-6.65
12	40	F	3.00	20.00	51.87	4.43	6.74	0.419	-0.5	-0.15	-0.5	-0.85
22	40	F	2.70	20.00	33.26	0.62	4.31	0.610	4.65	5.2	5.95	0.2

Four of the five neonates in the study population of 22 neonates and born at a GA of 25 to 29 weeks, reflected a change in DPOAE baseline and follow-up of more than 2.4 dB SPL. The PK values for study patients 83, 42 and 82 were all within normal reference ranges except for high peak amikacin values of more than 40 mcg/mL. Their trough levels were below the accepted 10 mcg/mL but above the 2 mcg/mL used as referral point to the audiologist. Study patient 82 had a change of more than 2.4 dB SPL at frequencies 4 kHz and 8 kHz, while the other two neonates reflected such change at all four the frequency levels. Study patient 49 had out-of-range PK values for peak, trough and half-life and a change of more than 2.4 dB SPL at all four frequency levels.

In the GA group of 30 to 33 weeks, six of the nine neonates had a DPOAE difference of more than 2.4 dB SPL from baseline to follow-up. Study patient 74, 80, 69 reflected this change only at the highest frequency level of 8 kHz. These results correlate well with the statement by Touw *et al.* (2009) that high-frequency hearing is affected earlier than low-frequency hearing and high-frequency hearing losses are audiometrically more detectable. Peak amikacin levels of above 40 mcg/mL appeared in study patients 74 and 69. Study patient 74 also had a trough amikacin level of more than 10 mcg/mL, while study patients 80 and 69 had a trough level within reference range but above the 2 mcg/mL for study purposes.

The PK values for study patient 41 were above reference range only for the peak level but an insult to the cochlea was evident at all four the frequency levels.

Study patient 14 reflected an above reference range peak level, but a trough level below 2 mcg/mL and changes of more the 2.4 dB SPL at 4, 6 and 8 kHz.

One neonate born at 36 weeks GA had a slightly higher than 2.4 dB SPL change in DPOAE at a frequency level of 6 kHz. This was with study patient 21 whom only had a peak amikacin level higher than 40 mcg/mL and a trough level lower than 2 mcg/mL. All the other PK values were within reference range.

Of the six full-term neonates only two reflected a change in DPOAE baseline and follow-up of more than 2.4 dB SPL, being study patients 5 and 22. Study patient 5

had a peak amikacin level above the reference range and a trough level below 10 mcg/mL but only slightly higher than the desired 2 mcg/mL.

Study patient 22 reflected above 2.4 dB SPL DPOAE difference in readings at 2, 4 and 6 kHz although all the PK parameters were within reference range values. This patient was diagnosed with septicaemia and jaundice. Zamani *et al.* (2003) stated that hyperbilirubinemia causes hearing loss and referred to a study performed by Sheykholeslami and Kaga whom found that in severe hyperbilirubinemia at least some defects exist in the cochlea and especially in the OHCs and even moderate forms of hyperbilirubinemia would be able to cause sensory-neural hearing loss.

For study patient 81, whom also had all the PK parameters within reference range, differences in DPOAE results indicated no change in cochlear function.

In the study population of 22 neonates, 13 neonates (59%) had a change in DPOAE readings between baseline and follow-up of more than 2.4 dB SPL at one or more frequency levels.

From these results it is evident that the younger GA groups are affected the most and this is also the group that had the highest peak-and-trough levels as revealed in the PK results and concluded in section 4.9. These findings implicate that the target population to be monitored by the pharmacist and the audiologist should be extreme premature and VLBW neonates.

CHAPTER 5

LIMITATIONS, RECOMMENDATIONS AND CONCLUSION

During the study certain limitations were encountered and are presented in this chapter. Recommendations are made where possible. This chapter ends with a conclusion to the study.

5.1 LIMITATIONS AND RECOMMENDATIONS

5.1.1 Obtaining blood samples on time: when performing TDM it is important to take the required blood samples on time. Pharmacokinetic studies on a specific drug e.g. amikacin, requires a trough blood sample taken half an hour before the administration of the next dose. Peak blood samples need to be taken an hour after administering a dose. The study was conducted in an academic teaching hospital and the researcher was dependent on the registrars to take the blood samples. The registrars were not always available at the required times. Although this was a frustration to the researcher the formulas used in the calculations allowed for adjustment of time. As long as the blood sample was taken, calculations could still be performed when the required times differ, although it is not desirable or preferable.

5.1.2 On time audiology monitoring of patients: when monitoring neonates for possible ototoxicity it is important to obtain a baseline reading before commencing treatment with a possible ototoxic drug. Monitoring thereafter is based on drug dosing schedules. For the purpose of this study follow-up readings had to be obtained shortly before the third MD. The audiologists helping with this study were either full time students or had commitments at their work and were not always available when audiology monitoring was required. This was one of the main reasons why the study population involving audiology was small.

Recommendation: each person involved in a study should realize the importance to adhere to time limits etc. and be committed. A good understanding of the process is always important as well as a good working relationship between colleagues and staff from different disciplines.

5.1.3 Noise level of the setting: the daily operation in a NICU makes it a rather noisy surrounding and being that of an academic teaching hospital, even more so. Numerous DPOAE readings obtained by the audiologist could not be used for the study due to a too high noise level which interfered with the results. Distortion Product OAEs are sensitive to surrounding noise.

5.1.4 Reliable audiology hard-and-software: as explained in the beginning of chapter four, 83 neonates were recruited initially for the study but only 22 of these neonates received audiology monitoring at the end. One of the reasons for this, including above mentioned limitations, were problems experienced with the audiology hard-and-software.

Recommendation: it is important that the staff and students are informed that the tests performed by an audiologist is noise sensitive and that the surrounding noise level should be reduced when and if possible. This is not always possible and therefore a large number of patients should be enrolled in similar studies where there is a high percentage drop-out rate.

With regards to the hard-and-software of audiology, it is important that audiologist involved in the study are experienced and equipped to use the equipment. This equipment should be reliable to reduce the number of study patients lost.

5.1.5 Administration of drugs: during the study some lower than expected peak levels were obtained. Although there might be numerous reasons for this, retrograde flushing could be one. Timely administration of the drug was also important in this study as trough levels were taken shortly before and peak levels taken an hour after administration of amikacin. Although all staff was well informed about the study, due to staff rotation etc. not all staff was at all times aware of the importance of the administration requirements.

Recommendation: the researcher should ensure that the nursing staff helping with the study is well aware of the importance of the time of administration as well as the technique involved as this might influence the peak levels obtained.

5.1.6 Laboratory away from site: the laboratory at DGMH could not perform peak-and-trough levels for amikacin during the study period and services of another

laboratory were used. The blood samples had to be couriered and were time consuming. This would cause that results were not always obtained on time to make appropriate interventions.

Recommendation: to have results of peak-and-trough levels available timeously and on site will enable the researcher to calculate dosage adjustments sooner and suggest interventions quicker for the benefit of the patient.

5.1.7 Small sample size: due to too few neonates receiving audiology monitoring statistical significant outcome could not be stated.

Recommendation: a future study should be conducted with higher recruitment numbers and an attempt should be made to reduce the reasons for the high drop-out rate as mentioned above.

5.1.8 Control patients: neonates qualify as control patients for audiology should they not receive any previous or current ototoxic drugs.

Recommendation: control patients should be enrolled to enable the researcher to compare audiology results of neonates receiving ototoxic drugs to those not receiving any drugs that could affect the readings.

5.2 CONCLUSION TO THE STUDY

The main aim of the study was to determine a possible correlation between amikacin serum concentrations and ototoxicity in neonates by using otoacoustic emissions.

Therapeutic drug monitoring was performed on all the study patients and it was evident that the neonatal population exhibits inter-individual variability in the PKs of AGs. The two sub-population groups were representative of each other as the study patients did not differ in terms of demographic and clinical findings according to statistical findings.

The peak levels measured for the study population was above the recommended reference range at all the different GAs, the highest mean peak level being in the VLBW neonates. The trough levels of amikacin were also the highest in the neonates classified as extremely preterm and were the only GA group where the

amikacin trough level exceeded the recommended 10 mcg/mL. This population group was also affected the most with regards to DPOAE results.

The mean trough level for the other three GA groups was within the clinical therapeutic range (0-10 mcg/mL) but above the (>2 mcg/mL) referral level. The total neonates affected in the left and/or right ear was 24% (n = 17; 4), indicating that although the trough level is within the therapeutic range, changes in hearing can already be detected.

In the sub-population of 22 neonates, 13 neonates (59%) had a change in DPOAE readings between baseline and follow-up of more than 2.4 dB SPL at one or more frequency level.

The therapeutic response and toxic effects of AGs depend on plasma concentrations. Optimum dosing of AGs is therefore required and necessitates TDM, especially in the extreme premature and VLBW neonates.

A multidisciplinary approach between pharmacists, audiologists and doctors are important for the well-being of patients. Diagnostic OAEs and pharmacokinetics for ototoxic medications in vulnerable populations should be further investigated in a larger study population.

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Appendix 1: MREC approval certificate

UNIVERSITY OF LIMPOPO

Medunsa Campus



MEDUNSA RESEARCH & ETHICS COMMITTEE

CLEARANCE CERTIFICATE

P O Medunsa
Medunsa
0204
SOUTH AFRICA

MEETING: 07/2011

PROJECT NUMBER: MREC/H/151/2011: PG

Tel: 012 - 521 4000
Fax: 012 - 560 0086

PROJECT :

Title: Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach in Dr George Mukhari Academic Hospital: Gauteng Province.

Researcher: Mrs. D Engler
Co-worker Ms C Botha (Speech-Language Pathology & Audiology)
Supervisor: Dr N Schellack
Dr A Naude (Speech-Language Pathology and Audiology)
Hospital Superintendent: Dr J Fisher (Dr George Mukhari Hospital)
Other Involved HODs: Prof PMB Mawela (Paediatrics)
Prof AGS Gous (Pharmacy)
Prof S Du Plessis (Speech-Language Pathology and Audiology)
Department: Pharmacy
School: Health Care Sciences
Degree: MSc (Med)

DECISION OF THE COMMITTEE:

MREC approved the project.

DATE: 13 September 2011


PROF GA OGUNBANJO
CHAIRPERSON MREC

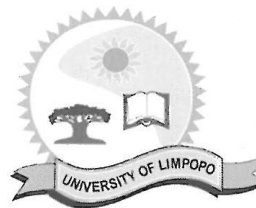
Note:

- i) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
- ii) The budget for the research will be considered separately from the protocol. PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

African Excellence - Global Leadership

Appendix 2: English consent letter

UNIVERSITY OF LIMPOPO
Medunsa Campus
Department of Pharmacy



P O Box 218

Medunsa

0204

Tel: (012) 521 5866

Fax: (012) 521 3992

Email: nschellack@ul.ac.za

TO: Parent / Caregiver of possible participant in research study

Please read this information and feel free to ask any questions about the study, before deciding if you are willing to allow your baby to take part in this study.

The name of the research study is: "Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr George Mukhari Academic Hospital - Gauteng Province."

This research study will be done in the NICU (Ward 24) of Dr George Mukhari Academic Hospital. While your baby is in ward 24, he/she will receive specific medication as part of this unit's treatment protocol. This treatment is used to help your baby get better. To determine the amount of medicine in your baby's body, the medical team will have to take 2 blood samples from your baby, while he/she is in ward 24. If the amount of medicine in your baby's body is very high the medical team will have to take one more sample. The blood samples are done as part of usual monitoring in the unit when they are administering the medicine.

To see what effect this medication has on your baby's hearing; two different tests have to be done on your baby while he/she is in ward 24. These two tests look at different parts of your baby's hearing system. During this time your baby will remain in ward 24 and the medical treatment he/she receives in ward 24 will be changed if needed.

The hearing tests will be done while your baby is sleeping or just lying still. These tests will not hurt your baby and will not be harmful to his/her health.

If there are any problems with your baby's hearing, your baby will receive follow up services at the speech therapy and audiology department at Dr George Mukhari Hospital, even though this is not part of the research study.

At any time during this study, you are free to let us know if you no longer want your baby to take part in the research study. If you decide not to take part in the study anymore, it will not change the medical treatment that your baby gets while he /she stay in ward 24.

All the personal information about your baby will be kept private during and after the research study. Personal information will not be discussed with any person not involved in the research study. If this is needed, we will ask for you permission before this is done.

We will be very thankful if you will let your baby take part in this research study.

Kind Regards

Melvin
Audiologist (Co-Worker)

Mrs D Engler
Pharmacist (Researcher)

Mrs. A. Naude
Audiologist (Supervisor)

Dr. N. Schellack
Pharmacist (Supervisor)

Informed Consent from parent / caregiver

- I have read the information and heard the aims of the proposed study and was given the opportunity to ask questions and given time to rethink the issue.
- The reason for the study is clear to me.
- I have not been pressurised to participate in any way.
- I understand that the participation in this study is completely voluntary and that I may withdraw from it at any time and without supplying reasons. This will have no influence on the regular treatment that holds for the condition of my infant neither will it influence the care that my infant will receive from his / her regular doctor.
- I know that this study has been approved by the Research, Ethics and Publications committee of Medunsa and the management of Dr George Mukhari Hospital.
- I am fully aware that the results of this study will be used for scientific purposes and may be published. I agree to this, provided that my infant's privacy is guaranteed.
- I hereby give consent that my infant may participate in this study.

Name of caregiver / parent

Signature of caregiver / parent

Place

Date

Witness

STATEMENT BY RESEARCHER

I have provided verbal and / or written information regarding this study.
I agree to answer any future questions concerning the study as best as I am able to.
I will adhere to the approved protocol.

Name of Researcher

Signature of researcher

Place

Date

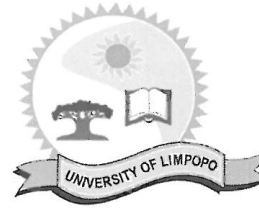
Witness

Appendix 3: Setswana consent letter

UNIVERSITY OF LIMPOPO

Medunsa Campus

Department of Pharmacy



P O Box 218

Medunsa

0204

Tel: (012) 521 5866

Fax: (012) 521 3992

Email: nschellack@ul.ac.za

Go: Motsadi / Motlhokomedi wa motsayakorolo mo patlisisong

Ka kopo buisa ka ga kitsiso e, mme o nne le bolokologi go ka botsa dipotsa mabapi le patlisiso e, pele o ka dira tshwetso fa o batla gone ngwana wag ago a tseye karolo mo patlisisong e.

Leina la kgetho e, ke: "Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr. George Mukhari Academic Hospital, Gauteng Province."

Ke tla leboga thata fa o ka retlella ngwana wag ago go tsoya karalo mo patlisisong e.

Ka ditebogo

Ms L Ntlhakana
Audiologist (Co-Worker)

Mrs D Engler
Pharmacist (Researcher)

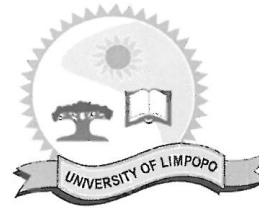
Mrs. A. Naude
(Supervisor)

Dr. N. Schellack
(Supervisor)

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Go: Motsadi / Motlhokomedi wa motsayakorolo mo patlisisong

Ka kopo buisa ka ga kitsiso e, mme o nne le bolokologi go ka botsa dipotsa mabapi le patlisiso e, pele o ka dira tshwetso fa o batla gone ngwana wag ago a tseye karolo mo patlisisong e.

Leina la kgetho e, ke: "Determining the correlation between amikacin serum concentrations and ototoxicity in neonates using otoacoustic emissions: A multidisciplinary approach at Dr. George Mukhari Academic Hospital, Gauteng Province."

Ke tla leboga thata fa o ka retlella ngwana wag ago go tsoya karalo mo patlisisong e.

Ka ditebogo

Ms L Ntlhakana
Audiologist (Co-Worker)

Mrs D Engler
Pharmacist (Researcher)

Mrs. A. Naude
(Supervisor)

Dr. N. Schellack
(Supervisor)

Appendix 4: Audiology-Pharmacy-Referral form

Date Initial TDM: _____		Date Follow Up TDM: _____	
Time trough was taken	Reason for follow-up TDM		
Time the drug was administered	Time the trough was taken		
Time the peak was taken	Time the drug was administered		
Route of administration of drug	IVI	Time the peak was taken	
Results	Trough	Peak	Results
		Peak	Peak

Laboratory requirements for Amikacin

Indication	Staph aureus	Pseudomonas	Empirical	Other
Signs of Toxicity	Absent	Nephrotoxicity	Ototoxicity	Other
Renal Function	Normal	Mild Impairment	Gross Impairment	Albumin [27 – 43]
Potassium [3.5 – 5.0]		Serum Creatinine [10 – 56]	Urea [1.4 – 4.3]	GFR [40 – 65 ml/min/1.73m²]
Hyperkalaemia		GFR Calculation		
Hypokalaemia		$GFR = \frac{\text{Urea} \times \text{Creatinine}}{\text{Creatinine} \times \text{Time}} \times 1.73$ <p style="text-align: center;"> $\mu\text{mol/L (SCr)}$ $\text{cm} \times 40$ ml/min/1.73m^2 </p> <div style="border: 1px solid black; padding: 5px; width: fit-content; margin-left: auto; margin-right: auto;"> Newborn – 4 weeks: 40-65 ml/min/1.73m² Neonates, K = 40 LBW, K = 30 </div>		

Additional Lab Values - ABNORMAL

Appendix 5: Department of Pharmacy Feedback form – Example

DEPARTMENT OF PHARMACY Feedback	
	Date of assessment: 20120312
Patient: ██████████ Hospital Number: ██████████ Weight: 1.65 kg Current Dose: 35 mg	
<u>PHARMACOKINETIC PARAMETERS</u>	
○ Lab Peak: 43.5 mg/L	True Peak: 53.64 mg/L
○ Lab Trough: 7.4 mg/L	True Trough: 7.33 mg/L
○ Volume of Distribution (Vd): 0.465 L/kg [0.4 – 0.6 L/kg]	
○ Half-life: 8.35 hours [4 – 8 hours]	
○ Calculated dose to reach a peak of 40µg/ml: 35 mg	
○ GFR for LBW: No U&E with SCr done	
<u>INTERPRETATION</u>	
<p>Although the peak level is at the desired concentration, the trough is too high and might increase the risk of toxic effects. The volume of distribution is within normal range. The GFR could not be calculated. The higher than normal half-life might indicate that the patient is not excreting the drug fast enough and is accumulating – this being the reason for the high trough level. To maintain a serum peak level of around 40 mg/L but reducing the trough level to below 2 mg/L, the dose has to be adjusted to 35 mg every 48 hours. A peak level of 47.12 mg/L and a trough level of 0.88 mg/L can be expected with this dosing regimen.</p>	
Recommendation: Please request U&E including SCr. Amikacin therapy was initiated on 8/3/2012 and should be given for a maximum of 7 days. Today, 15/3/2012, will be the 8 th day of therapy. Please stop amikacin therapy and depending on patients' status, consider a change in therapy.	
Pharmacist: Deirdré Engler	Signature: _____ Date: 15/03/2012