AMIKACIN TREATMENT WITH OR WITHOUT AMINOPHYLLINE IN NEONATES WITH SUSPECTED SEPSIS AT KORLE-BU TEACHING HOSPITAL: A PHARMACOKINETIC AND PHARMACODYNAMIC STUDY

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DECLARATION

I hereby declare that this thesis is entirely the work of Seth Kwabena Amponsah under the supervision of the undersigned senior members.

Except for references to other people's work, which have been duly acknowledged, this thesis is the result of my own research. This work has never been presented anywhere in part or in full for the award of a degree. I therefore accept full responsibility for any errors it may contain.

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DEDICATION

This work is dedicated to my wife, children and family.

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ABSTRACT

Background: Sepsis is a major cause of death among neonates. In neonates, symptoms of sepsis are often non-specific and diagnosis requires a high index of suspicion. Thus, the role of additional diagnostic markers is important. Amikacin is used as first-line treatment of neonatal sepsis and it is usually co-administered with aminophylline in preterm neonates in Ghana. Amikacin dosing is well established, however, optimal effect requires specific dose titration and maintenance therapy should be individualized.

Aim: To describe the PK and evaluate selected aspects of the PD of amikacin in neonates with suspected sepsis.

Methods: Neonates with suspected sepsis (n=322) and requiring amikacin or amikacin with aminophylline treatment were recruited at the Neonatal Intensive Care Unit, Korle-Bu Teaching Hospital, Ghana. Admission clinical and demographic information was collected, using case record forms. Blood culture and sensitivity, selected hematological (FBC), and biochemical [urea, creatinine, total bilirubin, C-reactive protein (CRP) and procalcitonin (PCT)] parameters were measured before treatment. A standard dose of amikacin was administered as per local guidelines. Brainstem auditory evoked potential was done before treatment commencement and seven days later in a randomly selected group of neonates. Serum amikacin concentration was measured at specified times after treatment initiation and amikacin concentration data was analyzed, using population PK modeling.

Results: A total of 163 (50.6%) of admitted neonates were born preterm, of which 14 (4.3%) were extreme preterm. A total of 79 (24.5%) had signs/symptoms consistent with birth asphyxia. There was 13.6% (41/302) culture positivity. Among neonates

categorized as having “highly probable”, “probable” or “less probable” sepsis, mean PCT was significantly different (P<0.001). The sensitivity, PPV, NPV and AUC was higher compared with CRP. The proportion of neonates with elevated PCT on admission was significantly higher in the “highly probable” group compared with the “probable group” [91% (20/22 versus 31.6% (6/19), p<0.001]. Overall mortality was 12%, with case fatality being highest among extreme preterms or those with birth weight less than 1 kg, or with elevated PCT. A total of 419 plasma concentration profile data was available for 247 neonates for population pharmacokinetic modeling. A one-compartment model best fitted amikacin disposition. The mean peak amikacin serum concentration was 20.56±8.7 µg/mL, and trough 6.68±3.86 µg/mL. Neonates administered amikacin with or without aminophylline showed varying CL and V, but with a high BSV, suggesting possible lack of effect of aminophylline co-administration on amikacin disposition. The population clearance (CL), and volume of distribution (V) of amikacin were related as: CL = 0.153 (birth weight/2.5)$^{1.31}$, V = 2.94 (birth weight/2.5)$^{1.18}$, with 58.9 and 50.7% between-subject variability in clearance and volume, respectively. Mean half-life ($t_{1/2}$) of amikacin was 13.6 hours. There was no difference in the baseline and follow-up BAEP of neonates who received amikacin and those who did not.

Conclusion: Birth weight was an important predictor of amikacin CL and V. Co-administration of aminophylline with amikacin did not influence the pharmacokinetics of amikacin. There was a relatively large V and long $t_{1/2}$ of amikacin in recruited neonates. No difference existed in baseline and follow-up BAEP results of neonates treated with amikacin, although one subject showed a high BAEP threshold post-amikacin dose. PCT was a more sensitive marker than CRP in the diagnosis of early onset neonatal sepsis.

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−2LL	–	-2log likelihood
ACCP	–	American College of Chest Physicians
AMG	–	Aminoglycosides
ANC	–	Absolute neutrophil count
ANOVA	–	Analysis of variance
AUC	–	Area under curve
APG5	–	Apgar score at 5 minutes
BAEP	–	Brainstem auditory evoked potential
bpm	–	Beats per minute
BSV	–	Between subject variability
BW	–	Birth weight
CI	–	Confidence interval
CL	–	Clearance
Cpeak	–	Peak concentration
Ctrough	–	Trough concentration
CO_2	–	Carbon dioxide
CoNS	–	Coagulase negative staphylococcus
cpm	–	Cycles per minute
CRCL	–	Creatinine clearance
CRP	–	C-reactive protein
CWRES	–	Conditional weighted residuals
DNA	–	Deoxyribonucleic acid
DPD	–	3,5-dichlorophenyldiazonium tetrafluoroborate
DV	–	Observations
EDTA	–	Ethylenediaminetetraacetic acid
EOS	–	Early onset sepsis
FBC	–	Full blood count
FOCEI	–	First-order conditional estimation + interaction

GA	–	Gestational age
GBS	–	Group B streptococcus
GFR	–	Glomerular filtration rate
GOF	–	Goodness-of-fit
HABA	–	Dihydroxyaminobutyric acid
HB	–	Hemoglobin
IL-1ra	–	Interlukin-1 receptor antagonist
IL-6	–	Interlukin-6
IL-8	–	Interlukin-8
IL-10	–	Interlukin-10
IM	–	Intramuscular
IPRED	–	Individual model predictions
IV	–	Intravenous
IZD	–	Inhibitory zone diameter
KBTH	–	Korle-Bu Teaching Hospital
LCD	–	Liquid crystal display
LOS	–	Late onset sepsis
MIC	–	Minimum inhibitory concentration
nCD64	–	Neutrophil cluster of differentiation 64
NICU	–	Neonatal intensive care unit
NMDA	–	N-methyl-D-aspartate
NPV	–	Negative predictive value
NONMEM	–	Non-linear mixed-effects modeling
NSAIDs	–	Non-steroidal anti-inflammatory drugs
O_2	–	Oxygen
OAE	–	Otoacoustic emissions
OFV	–	Objective function value
p	–	p value
PCT	–	Procalcitonin
PCR	–	Polymerase chain reaction
PD	–	Pharmacodynamic

PK	–	Pharmacokinetic
PLT	–	Platelet
PNA	–	Postnatal age
Pop PK	–	Population pharmacokinetics
PPV	–	Positive predictive value
PRED	–	Population model predictions
RES	–	Residuals
RCT	–	Randomized controlled trials
RNA	–	Ribonucleic acid
ROC	–	Receiver operator characteristics
SCCM	–	Society of Critical Care Medicine
SCR	–	Serum creatinine
SD	–	Standard deviation
SE	–	Standard error
SIRS	–	Systemic inflammatory response syndrome
TNF-α	–	Tumor necrosis factor alpha
V	–	Volume of distribution
VPC	–	Visual predictive check
WBC	–	White blood cell
WHO	–	World Health Organization
WRES	–	Weighted residuals

CHAPTER ONE

1.0 INTRODUCTION

This chapter summarizes the epidemiology, clinical manifestation and diagnosis of neonatal sepsis. The chapter also gives a brief background to the treatment of neonatal sepsis with aminoglycosides, pharmacokinetics and pharmacodynamics of aminoglycosides and relevant drug interactions with common drugs used in neonatal care. Additionally, the problem statement, justification, aim and specific objectives of this study are outlined.

1.1 BACKGROUND

1.11 Epidemiology of neonatal sepsis

Sepsis is a clinical syndrome that complicates severe infection.[1] It is characterized by signs of inflammation such as vasodilation, leukocyte accumulation, and increased microvascular permeability occurring in tissues that are remote from the infection. Suspected sepsis is any clinical concern for infection to warrant the start of antibiotics before microbiological or laboratory evidence of infection. Neonates, especially those born prematurely (also called preterm) are highly susceptible to sepsis, which is partly ascribed to immaturity of neonatal immune system.[2] In 2013, of the 6.3 million children who died before age 5 years, 51.8% died of infectious causes and 44% died in the neonatal period.[3] In Ghana, it was estimated that 21.9 neonates died per 1000 live births within the first 6 days of life in the year 2013.[4] Despite the fact that globally child mortality is on a decline, neonatal mortality has remained relatively stagnant in Africa in the last decade.[5]

Neonatal sepsis can be classified as early or late onset. Early onset sepsis (EOS) manifests within the first few days of life, likely resulting from vertical transmission of bacteria from mother or during the intrapartum period, whereas late onset sepsis (LOS) originates from community or nosocomial source.[6,7]

There are geographical and temporal differences in the etiology of neonatal sepsis.[8] Also, differences exist between bacteria causing sepsis in term and pre-term neonates. While Gram-positive cocci still prevail among term and near-term infants,[9] the predominance of Gram-negative rods among very low birth weight neonates has also been reported.[10] The most frequent bacterial isolates in EOS in developing countries include *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp* and *Streptococcus pyogenes*. [6,11-13] Associated trends in the incidence of EOS caused by other microorganisms have varied between countries and study centers. In developing countries Gram-negative bacilli account for 52–62% of LOS cases, with *Klebsiella spp* being the most frequently isolated microorganism.[14,15]

1.12 Diagnosis of neonatal sepsis

The clinical manifestation of neonatal sepsis are often non-specific, and demand a high index of suspicion for early diagnosis and treatment. The clinical signs and symptoms of neonatal sepsis include fever or hypothermia, respiratory distress including cyanosis and apnea, tachycardia, feeding difficulties, lethargy or irritability, hypotonia, and poor perfusion.[16,17] In addition, the clinical picture may vary significantly depending on the population studied (term or preterm neonates), time of infection (early or late onset), or

underlying bacterial etiology (Gram-positive, Gram-negative or fungi). Although there is some diagnostic uncertainty with sepsis, the gold standard is isolation of causative organisms from blood cultures.[18] Presently, a combination of perinatal risk factors, clinical signs and hematological markers (absolute neutrophil count, immature leukocyte count, etc) are used for early identification of newborns with possible sepsis.[19] However, the utility of these markers in accurate diagnosis of sepsis remain uncertain.

Lack of reliable clinical signs and symptoms and commonly used laboratory indicators has prompted a widespread search for additional laboratory markers for the diagnosis of neonatal sepsis. Example of such markers include C-reactive protein (CRP) and procalcitonin (PCT). Some studies show CRP to be a valuable screening tool for the diagnosis of neonatal sepsis, [20,21] while other studies do not support the use of CRP because of low sensitivity and/or positive predictive values.[22,23] It has also been reported that PCT, increases markedly in septic conditions.[24] Some studies have reported the usefulness of PCT for early diagnosis of neonatal sepsis.[25,26,27] However, others have reported a lack of accuracy of PCT in detecting neonatal sepsis.[28,29] The role of these two widely used markers in the diagnosis of neonatal sepsis remain controversial.

1.13 Treatment of neonatal sepsis

The cornerstone of treatment of sepsis in the newborn period is antibiotic therapy. Broad spectrum antibiotics are usually administered empirically pending definite diagnostic test results. In most neonatal intensive care units (NICUs), a combination of an aminoglycoside and a β-lactam is the choice of treatment for suspected and proven

neonatal sepsis. Indeed, approximately 43% of antibiotics administered for treating infections in the neonatal period are aminoglycosides.[30]

1.14 Aminoglycosides

The aminoglycoside antibiotics comprise a large group of naturally occurring or semi-synthetic polycationic compounds. Streptomycin was the first aminoglycoside identified as a natural product of a soil bacterium, *Streptomyces griseus*. Aminoglycosides are indicated primarily for the treatment of infections caused by aerobic and facultative Gram-negative bacilli. Aminoglycosides such as gentamicin are (in combination with other antibiotics such as β-lactams), among the most frequently used antibiotics for neonatal sepsis treatment. However, near-ubiquitous resistance of common pathogenic organisms responsible for neonatal sepsis has necessitated introduction of other aminoglycosides, like amikacin.[31]

1.15 Pharmacokinetics of aminoglycosides

Aminoglycosides are polar drugs which are poorly absorbed from the gastrointestinal tract. They are usually administered by parenteral routes; intravenous (IV) or intramuscular (IM). Intracellular distribution of aminoglycosides is limited by their polar nature and thus their distribution space is to extracellular fluid compartment. Aminoglycosides are primarily eliminated by glomerular filtration and accumulate if renal function is impaired. The plasma half-life of aminoglycosides is estimated to be 2-3 hours.[32] Substantial variability is reported in volume of distribution, clearance and half-

life of aminoglycosides in neonates, especially the preterm. This variability is as a result of dynamic changes in renal function and extracellular fluid, in neonates.[33]

1.16 Pharmacodynamics of aminoglycosides

The therapeutic effect of aminoglycosides are as a result of their concentration dependent bactericidal activity and post-antibiotic effect.[34] Post-antibiotic effect, residual bactericidal activity after drug concentrations have reached values below the minimum inhibitory concentration (MIC), persists up to 0.5–7.5 hours for aminoglycosides.[35] The combination of concentration-dependent bactericidal activity and post-antibiotic effect provides the theoretical basis supporting the use of aminoglycosides at high doses and wide dosage intervals (once daily dose administration).[36]

1.17 Adverse effects of aminoglycosides

Aminoglycosides exhibit a relatively high potential risk of ototoxicity and renal toxicity. Aminoglycoside-induced renal toxicity can be attributed to its uptake by proximal tubular cells and to its long-term retention in the renal cortex.[37] Reports suggest high trough concentrations of aminoglycosides are generally related to renal toxicity.[38] Ototoxicity can occur after exposure to aminoglycosides.[39] Aminoglycoside-associated ototoxicity usually manifests as cochleo- or vestibulotoxicity.[40] While gentamicin is predominantly vestibulotoxic, amikacin is cochleotoxic.[41] The cochleotoxicity of amikacin can produce permanent hearing loss due to hair cell destruction.[42] A number of studies have reported aminoglycoside-induced ototoxicity in neonates. [43,44]

1.18 Methylxanthines and their potential interaction with antibiotics

Methylyxanthines such as aminophylline and caffeine have been found to be effective in the prophylaxis and treatment of apnea of prematurity. A proposed mechanism for this effect is through competitive antagonism of central adenosine receptors by methylxanthines, leading to an increase in the responsiveness of respiratory center to carbon dioxide.[45]

Aminoglycosides are distributed in extracellular fluid and primarily eliminated by renal excretion. Reports suggest that some methylxanthines increase urine output and glomerular filtration fraction.[46,47] Methylxanthine-induced changes in glomerular filtration rate and extracellular fluid volume may have an influence on the disposition of aminoglycosides. Additionally, i*n vitro* studies report potentiating and inhibitory effects of antibiotics combined with methylxanthines.[48,49] Although aminophylline and caffeine had no antimicrobial effect, they potentiated the antimicrobial action of carbenicillin, ceftizoxime and gentamicin against *Staphylococcus aureus* and *Pseudomonas aeruginosa in vitro*.[49] This implies that co-administration of certain antibiotics with methylxanthines may influence the pharmacodynamics of the co-administered antibiotic either directly, or via a possible pharmacokinetic effect. There have been no previous reports on the potential drug-drug interaction between aminophylline and amikacin.

1.2 PROBLEM STATEMENT

Infectious diseases remain one of the leading causes of neonatal deaths in developing countries.[3] While reports of the incidence of neonatal sepsis ranges from 1 to 5 cases per

1000 live births, estimates of neonatal sepsis in developing countries range between 49 – 170 per 1000 live births.[50] In resource-poor settings, the high risk of acquisition of infections by neonates may be due to poorly implemented infection control measures. There is also a high-dependency on clinical signs and symptoms for diagnosing neonatal infection, as basic laboratory investigations may be unavailable.[51]

Aminoglycosides are widely used, in spite of their potential ototoxicy and nephrotoxicity,[52-55] among others from their cost-effectiveness.[56] Although aminoglycosides are widely used among neonates in low income countries, there is a paucity of data on their pharmacokinetics (PK) and pharmacodynamics (PD).

1.3 JUSTIFICATION

The diagnosis of neonatal infection in low income settings is challenging because of non-specific presentation of the clinical condition and often inadequate clinical skills and laboratory services. As a result, antimicrobial therapy is commenced on the slightest clinical suspicion of infection. For the purpose of this work it is important to accurately diagnose neonatal infections; as the PK and PD of drugs may vary between those with proven neonatal sepsis and its associated inflammatory and hemodynamic effects versus suspected cases without proven infection.[57,58] No known previous published data assesses the potential influence of inflammatory biomarkers (acute phase reactants) on the disposition of aminoglycosides in neonates with suspected or proven sepsis.

Amikacin and cloxacillin were introduced as first line empirical treatment for suspected neonatal sepsis at the Neonatal Intensive Care Unit (NICU), Korle-Bu Teaching Hospital (KBTH) in 2009. The use of this combination (amikacin and cloxacillin) was due to high level resistance to World Health Organization (WHO) recommended ampicillin and gentamicin and patterns of microorganisms causing nosocomial infections at the Unit. At the NICU, KBTH, neonates admitted display large inter-individual differences in gestational age, weight and comorbidities. The PK of amikacin has been found to differ in various local populations on account of gestational age and weight differences.[59-61] Thus, amikacin is a drug for which therapeutic drug monitoring, PK and PD studies has a well-established role.

Additionally, at the Neonatal Intensive Care Unit (NICU), Korle-Bu Teaching Hospital (KBTH), preterm neonates with sepsis are co-administered amikacin with aminophylline. Aminophylline is used as prophylaxis and treatment of apnea of prematurity. There has been no known previous published report on the potential interaction between aminophylline, which has the tendency to increase urine output and filtration fraction, and amikacin *in vivo*.

1.4 AIM

The aim of this study was to describe the pharmacokinetics and evaluate selected aspects of the pharmacodynamics of amikacin with or without aminophylline in neonates with sepsis at a teaching hospital in Accra, Ghana.

1.5 SPECIFIC OBJECTIVES

To achieve our aim, the following specific objectives were defined:

1. To determine for a population of neonates with suspected sepsis treated with amikacin ± aminophylline, the PK parameters of amikacin, and explore the influence of selected covariates on amikacin disposition

2. To determine brainstem auditory evoked potential (BAEP) in neonates as a surrogate marker for potential ototoxicity after treatment with amikacin

3. To determine the diagnostic utility of selected acute phase reactants in neonates with suspected sepsis and evaluate the effect of these reactants on amikacin pharmacokinetics

4. To determine bacteria etiological agents of neonatal infection and their antibiogram, and evaluate the presence of positive bacterial isolate on amikacin pharmacokinetics

CHAPTER TWO

2.0 LITERATURE REVIEW

This section of the thesis reviews literature pertinent to various aspects of the study. In brief, literature is reviewed on what neonatal sepsis is, etiological agents of neonatal infection, principles of diagnosis of neonatal sepsis and its empirical treatment. This chapter also addresses what aminoglycosides are, their pharmacokinetics and pharmacodynamics. The pharmacokinetics of drugs, absorption, distribution and elimination, in the neonate is also explored in this section. Additionally, literature on population pharmacokinetic modeling of aminoglycosides in neonates is reviewed.

2.1 SEPSIS

2.1.1 TERMINOLOGY/ DEFINITION

The word "sepsis" has its origin from a Greek word which means decomposition or putrefaction.[62,63] Bone *et al*, proposed the term ''sepsis syndrome'', defining it as hypothermia (temperature < 35.5°C) or hyperthermia (temperature > 38.3°C), tachycardia (heart rate > 90 beats/min), tachypnea (respiratory rate > 20 breaths/min), clinical evidence of an infection, and at least one end-organ demonstrating inadequate perfusion or dysfunction.[64] This terminology was somewhat superfluous as sepsis was already a known syndrome.

The American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) held a conference to create a set of definitions for patients with

sepsis.[65] It was realized that the lack of a single definition for sepsis created difficulties in identifying patients. The same systemic response seen in patients with severe infections could occur in other patients without infection but with other inflammatory processes such as pancreatitis, multiple trauma, etc. A key aspect of the consensus conference definitions was the introduction of the term Systemic Inflammatory Response Syndrome (SIRS), to define this phenomenon. SIRS was defined as being the presence of more than one of four clinical criteria: body temperature > 38°C or < 36°C, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or hyperventilation with a $PaCO_2$ less than 32 mmHg, and white blood cell count >12000/mm^3, <4000/mm^3, or with >10% immature neutrophils. A combination of SIRS with a confirmed infectious process was then called sepsis. Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion abnormality, or sepsis-induced hypotension. This SIRS approach was rapidly adopted by many and has been widely used to define population of patients for inclusion in clinical trials. However, not all have considered the SIRS criteria useful, arguing that they are too sensitive for use in clinical diagnosis.[66]

2.1.2 NEONATAL SEPSIS

Neonatal sepsis is normally defined as the presence of bacteria in the blood, bacteremia, in combination with SIRS within the first month of life.[67] Since blood culture has a low sensitivity in neonates with sepsis,[68] many studies include infants with clinical signs of sepsis but a negative blood culture. This is normally termed clinical, probable or suspected sepsis,[69-71] but this has not been sufficiently defined. In addition, the clinical signs that are used vary greatly and are poorly evaluated.

2.1.3 CLASSIFICATION OF NEONATAL SEPSIS

Neonatal sepsis may be classified according to the time of onset into: early onset (EOS) and late onset (LOS).[72] This distinction has clinical relevance, as EOS is due to bacteria acquired before and during delivery. LOS on the other hand, is due to bacteria acquired after delivery (nosocomial or community sources). In literature, however, there is little consensus, with EOS ranging from 48 hours to 6 days after delivery. This makes it difficult to compare studies where cases are grouped into EOS and LOS without further details.[73] Few reports distinguish between very early onset (within 24 hours), EOS (24 hours to six days), and LOS (more than six days).[74,75]

The most common route of EOS is via an ascending amniotic infection. Members of the maternal genital flora, such as Group B streptococcus (GBS) and *Escherichia coli* (*E. coli*), may ascend through the birth canal to the amniotic fluid either through intact amniotic membranes or, more commonly, after rupture of membranes.[76] Amniotic fluid can be aspirated or swallowed by fetus, resulting in pathogens penetrating immature mucosal barriers, resulting in pneumonia or bacteremia.

LOS most commonly occurs via horizontal or nosocomial transmission. Skin or mucosal colonization with potential pathogens may be acquired from hands of health care workers, water used in incubator or ventilator humidification systems, or from contaminated fomites such as stethoscopes.[77] Colonizing organisms may enter the bloodstream through breaks in the skin or introduced through invasive devices such as vascular catheters, endotracheal tubes, or feeding tubes. Alternately, nosocomial infection

may result from infusion of contaminated intravenous solutions (especially lipid-based or high-glucose solutions) or from contaminated formula or breast milk.

2.1.4 MICROORGANISMS CAUSING NEONATAL SEPSIS

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Overall, Gram negative organisms are more common and include *Klebsiella spp*, *E. coli*, *Pseudomonas* and *Salmonella spp*.[75] Of the Gram-positive organisms, *Staphylococcus aureus*,[78] *Streptococcus pneumoniae*,[79] and *Streptococcus pyogenes*,[80] are the most common isolates.

There appears to be a wide variety of bacteria causing EOS and LOS in developing countries. GBS is generally rare,[81] although maternal rectovaginal carriage rates of GBS may be similar to those recorded in developed countries.[82] Neonatal infection surveillance in developed countries generally identifies GBS and *E. coli* as the dominant EOS pathogens and coagulase negative staphylococcus (CoNS) the dominant LOS pathogen.[83,84] Thus, to reduce EOS among neonates in developed countries, vaginal-rectal screening for GBS colonization and intrapartum antibiotic prophylaxis is recommended. However, this is not standard protocol in most developing countries, including Ghana.

2.1.4.1 Gram-positive bacteria

Group B streptococcus (GBS): GBS or *Streptococcus agalactiae* causes invasive disease primarily in infants, pregnant or postpartum women, and older adults, with the highest

incidence among young infants. The pathogenicity of GBS has been attributed to a number of virulence factors, including lipoteichoic acid, a thick polysaccharide capsule and capsular sialic acid, which inhibits neutrophil accumulation at the site of infection.[76]

Staphylococcus aureus: It is a less common cause of neonatal sepsis, however, it can be a highly virulent pathogen in immunocompromised patients such as premature neonates.[76] *S. aureus* is perhaps the pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life threatening infections, and its capacity to adapt to different environmental conditions. Mortality as a result of *S. aureus* bacteremia remains approximately 20–40%.[85] Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs.

Coagulase negative Staphylococcus: CoNS are the etiologic agents of the majority of nosocomial infections in premature neonates. CoNS are common commensals with little pathogenicity, however, premature neonates are particularly susceptible to invasive infection. The first step in the pathogenicity of CoNS involves adherence of the bacteria to skin, mucosal surfaces, or indwelling artificial devices, such as intravascular catheters, which are commonly used in preterm infants. Adherence of CoNS is facilitated by a capsular polysaccharide adhesin consisting of poly- N-succinyl glucosamine.[76] Of the 31 species of CoNS and the 13 known to colonize human skin, those reported to cause disease in infants include *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. warneri*, *S. saprophyticus*, *S. cohnii*, and *S. capitis*. The major species involved in neonatal infection is *S. epidermidis*, which accounts for approximately 50 to 80% of CoNS colonization.[86]

2.1.4.2 Gram-negative bacteria

Escherichia coli: *E. coli* is the most common gram-negative bacteria that causes sepsis among neonates. A number of *E. coli* virulence factors have been identified and linked to neonatal sepsis, including the K1 capsule, fimbriae, hemolysin, and cytotoxic necrotizing factor 1. A pathogenicity island, or cluster of genes present in pathogenic but not in avirulent strains, was found in *E. coli* C5, a strain commonly implicated in neonatal meningitis. Mutant strains lacking this pathogenicity island were less able to induce high-level bacteremia in a neonatal-rat model.[87]

Enterobacter, *Klebsiella*, and *Serratia* species: Gram-negative enteric organisms of the *Enterobacteriaceae* family, notably *Enterobacter*, *Klebsiella*, and *Serratia* species, are common inhabitants of the neonatal intestine which may cause nosocomial sepsis. Like the other well-known member of the family, *E. coli*, these organisms are surrounded by a capsule and fimbriae that contribute to their virulence in neonates. This capsular polysaccharide prevents activation of the alternative complement system protecting the bacteria from opsonization, phagocytosis, and bacteriolysis.[76]

Pseudomonas aeruginosa (*P. aeruginosa*): A non-fermentative, gram-negative rod, is responsible for a wide variety of clinical syndromes in NICU patients, including sepsis, pneumonia, meningitis, and conjunctivitis. Nevertheless, if compared to other gram-negative bacteria, outbreaks by *P. aeruginosa* in NICU settings have been less reported.[88] Due to its ubiquitous nature, high affinity for moist environments, and ability to survive at various conditions, it remains a common pathogenic agent at NICUs. Immunodeficient

newborns, invasive diagnostic procedures and indiscriminate use of antimicrobials are predisposing factors to *P. aeruginosa* septicemia.[89]

2.1.5 DIAGNOSIS OF NEONATAL SEPSIS

The quest for optimal diagnostic tools for neonatal sepsis has been ongoing, but despite all efforts the basic problem still prevails; many infants, both full term and preterm, present with vague and unspecific symptoms. In clinical practice, the recommended approach is to liberally start intravenous antibiotics and then perform a "rule-out" procedure that normally lasts for several days. If all tests are negative and the infant has recovered, the antibiotics can be discontinued and the patient can be discharged from the NICU. This rule-out procedure normally includes cultures (blood, cerebrospinal fluid, urine and possibly skin cultures), x-rays, and a combination of laboratory tests. While these are performed, the patient is closely monitored for additional signs of sepsis.

2.1.5.1 Blood culture

Blood culture is the gold standard in diagnosing neonatal sepsis. Blood from arterial or venous puncture can be used, as well as blood from newly inserted umbilical catheters. It is recommended that a minimum blood volume of 1 mL be taken, and this should be used for aerobic cultures, since anaerobic bacteria are rare at NICUs.[90,91] Despite this, for practical reasons (to minimize skin punctures, blood loss, and pain) smaller volumes are often used, which could lead to a suboptimal sensitivity.[92] Even if optimal blood volumes are used, blood culture has obvious limitations in sensitivity.

Negative blood culture alone cannot support withdrawal of antibiotic therapy, especially if patient's clinical condition indicates ongoing sepsis.[92] In addition to the limited sensitivity of blood culture, the method is time consuming. Most microbiology laboratories take 5−7 days before delivering a full report even though a majority of clinically important bacteria can be detected within 48 hours.[93] Cultures from superficial sites like the axilla, umbilical stump, and ear correlate very poorly with blood culture results.[94] As such, they should not be used either to diagnose neonatal sepsis or as guidance for optimal antibiotic treatment.

2.1.5.2 Hematological parameters

Hematological parameters, white blood cell count, absolute neutrophil count, and immature/total neutrophil ratio, have been used as diagnostic tests for neonatal sepsis.[95-97] However, interpretation of these is complicated, due to the fact that values are affected by post-gestational age, asphyxia, and maternal factors such as fever and hypertension.[98,99] This could be one reason why hematological tests show fairly poor results in large clinical surveys.[100] Results are better when several tests are merged together into a scoring system, but the sensitivity and specificity may still not be high enough to be recommended for routine clinical use.[97,101] In contrast to these, two studies using granulocyte colony-stimulating factor in neonates with suspected sepsis showed high sensitivity and moderate specificity.[102,103]

2.1.5.3 Cytokines and acute phase proteins

Cytokines are endogenous chemical mediators that carry information between different cells and are important factors in the human inflammatory response. In case of infection, both pro-inflammatory and anti-inflammatory cytokines are upregulated according to a specific time schedule. Therefore, by studying the process of upregulation in blood samples, a conclusion can be made on whether systemic inflammation is present or not. Inflammation may be due to sepsis, or triggered by trauma, tissue damage, or even the normal birth process.[104] To achieve optimal specificity, a cytokine that is specific to sepsis-related inflammation needs to be defined. Similarly, acute phase proteins, functionally unrelated proteins whose plasma concentration increase or decrease in response to tissue injury or acute infections, can be studied.

C-reactive protein

The most thoroughly studied acute phase protein is C-reactive protein (CRP). CRP is induced by interleukin-6 (IL-6), and it is not the earliest marker to rise in the event of an infection. Rather, it rises within 6-8 hours after the onset of infection and peaks 24−48 hours later. It has a half-life of 19 hours, and has the capacity for a 1000 fold increase.[105] This means that CRP is not a good screening test for sepsis at an early stage.[106] Furthermore, IL-6, IL-8, tumor necrosis factor α (TNF-α), and procalcitonin (PCT) have been suggested as routine tests to diagnose sepsis, which seems logical as they are precursors for CRP release.

It has been reported that both IL-6 and TNF-α appear to be more sensitive than CRP in detecting sepsis at an early stage.[107] Kuster *et al*, found increased levels of IL-6 and IL-1 receptor antagonist (IL-1ra) 1−2 days before clinical diagnosis of preterm neonatal sepsis was made.[108] IL-8 has similar kinetics to IL-6, and also performs well as a diagnostic marker. A multicenter randomized controlled trial showed that a combination of IL-8 and CRP tests can significantly reduce the number of infants with suspected nosocomial bacterial infection who receive antibiotic therapy unnnecessarily.[109]

Procalcitonin:

Procalcitonin, another acute phase protein, produced in the liver and macrophages responds faster than CRP in neonatal sepsis. Serum PCT concentrations begin to rise 4 hours after exposure to bacterial endotoxin, peak at 6−8 hours, and remain raised for at least 24 hours.[110] PCT diagnostic profile in neonatal sepsis has been claimed to be superior to that of other acute phase reactants, with sensitivity and specificity ranging from 80–100% in term neonates.[104,110,111] Vazzalwar and colleagues also reported that PCT was more sensitive than CRP in predicting LOS in very low birth weight infants.[112] Studies in preterm neonates have yielded more conflicting results with suggested cut-off limits ranging from 0.5 to 2.3 ng/mL, and the corresponding sensitivity and specificity varying from below 50 to 92% and 63 to 97%, respectively.[113-115] However, false negative cases,[115,116] and elevated serum PCT levels due to perinatal conditions, like birth asphyxia and chorioamnionitis, have been reported.[112,117] The added value of PCT when combined with other markers of sepsis like IL-6 or IL-10 has been suggested.[111,118]

2.1.5.4 Clinical scoring system for neonatal sepsis

Often, the first clinical symptoms of sepsis in a neonate may be minimal and similar to those observed in non-infected neonates. The most prominent signs of EOS are usually respiratory distress, lethargy, irritability, fever or hypothermia, jaundice, hepatomegaly, vomiting, abdominal distension and diarrhea. There have been attempts to evaluate the early clinical signs of sepsis by constructing an algorithm or a score that can separate signs of sepsis from all the other signs that newborns display. Such scores have been reported by Gitto *et al*,[119] and Ali *et al*.[120] An example of a table showing the criteria for a septic score is shown in Table 2.1.

Table 2.1: Criteria employed for defining sepsis score

High probable sepsis	At least 3 sepsis-related clinical signs* CRP > 5 mg/ml At least 2 other altered serum parameters† Blood culture: positive or negative
Probable sepsis	Less than 3 sepsis-related clinical signs* CRP > 5 mg/ml At least 2 other altered serum parameters† Blood culture: negative
Possible sepsis	Less than 3 sepsis-related clinical signs* CRP < 5 mg/ml Less than 2 other altered serum parameters† Blood culture: negative
No sepsis	No sepsis-related clinical signs* CRP < 5 mg/ml No altered serum parameters† Blood culture: negative

Adopted from Gitto *et al*.[119]

** Sepsis-related clinical signs: temperature instability; apneic spells; need for supplemented oxygen; need for ventilation; tachycardia/bradycardia; hypotension; feeding intolerance; abdominal distension; necrotizing enterocolitis*

† Serum parameters other than CRP: white blood cells (WBC) count; absolute neutrophil count (ANC); platelet (PLT) count

These scores might be of help to the clinician, but sensitivities and specificities reported are currently not high enough to justify changing the current practice of liberal use of antibiotics.[121,122]

2.1.5.5 Molecular methods for diagnosis of neonatal sepsis

Polymerase chain reaction (PCR) has been found to be a useful method for identification of bacteria in clinical samples. Amplification targeting 16S ribosomal ribonucleic acid (RNA) gene is a potentially valuable clinical tool in samples with low bacterial DNA. The first attempt to use molecular approach to diagnose neonatal sepsis was made by Laforgia *et al*.[123] They successfully detected DNA encoding for the 16S subunit of bacterial ribosome in four out of four blood culture positive infants with early onset sepsis. Another study showed that PCR is useful and superior to blood culture for early diagnosis of sepsis in neonates, having 100% sensitivity and 95.6% specificity.[124] A few others have also reported the utility of molecular methods in neonatal sepsis diagnosis.[125,126,127]

2.1.6 TREATMENT OF NEONATAL SEPSIS

The standard antimicrobial therapy of neonatal sepsis consists of a combination of two or more antibiotics administered parenterally usually for 7 days.[128] However, duration of treatment can be longer if the neonate has certain infections example meningitis. Usually, a combination of a penicillin and an AMG is used as initial therapy. This combination of antimicrobial agents has synergistic activity against most bacteria.[129,130] However, third-

generation cephalosporins, example cefotaxime, represent a reasonable alternative to an AMG, and also constitute standard therapy in most NICUs.

Antibacterial therapy for neonatal sepsis can be empiric, when antibiotic is administered to patients based on the presence of certain risk-factors; or definitive treatment, when infection has been confirmed with a culture. Most infants admitted to NICUs receive empirical antibiotics when in fact the incidence of culture-proven EOS is only between 1 and 4.6 cases per 1000 live births.[131,132] The clinical manifestations of neonatal sepsis are nonspecific, and the fear of missing the diagnosis is high because of the increased morbidity and mortality associated with sepsis. Early and prompt empirical antibiotic therapy, based on risk-factor-driven decision in EOS,[133] and clinical symptoms in LOS,[134] have been shown to reduce mortality in neonates.

2.1.6.1 Empirical antimicrobial regimen for EOS

A meta-analysis[135] of 2 randomized control trials comparing gentamicin plus benzylpenicillin with ceftazidime, and comparing ticarcillin plus clavulanic acid (Timentin) with piperacillin plus gentamicin, failed to show that one regime was superior to the other.[136,137] There was no significant difference in mortality, treatment failure, or bacteriological resistance.

Based on the common antibiotic susceptibilities of the predominant organism causing EOS, the recommended initial empiric therapy for a neonate with suspected bacterial sepsis and/or meningitis includes ampicillin and an AMG.[138] This combination expands

the antimicrobial spectrum and also offers synergistic bacterial killing. The other advantages are low cost and low rates of emergence of bacterial resistance.[139] However, in developing countries where the causative organisms of EOS are different from developed countries, the above combination of ampicillin and gentamicin may not be the best empirical antibiotic of choice.

Lubell *et al*,[140] reviewed literature regarding the antibiotic susceptibility patterns of community-acquired pathogens causing neonatal sepsis in sub-Saharan Africa and Asia. The two common pathogens, *S. aureus* and *Klebsiella spp*., exhibited high rates of resistance to almost all commonly used antibiotics (ampicillin, ceftriaxone, chloramphenicol, cotrimoxazole, macrolides, and gentamicin). Only *Streptococcus pneumonia* exhibited good susceptibility to all drugs other than cotrimoxazole. However, the use of other AMGs, like amikacin, has reduced resistance of pathogenic organisms responsible for neonatal sepsis,[31] and finds use in empiric treatment of sepsis in most NICUs in developing and developed countries.

2.2 AMINOGLYCOSIDES (AMGs)

2.2.1 DISCOVERY OF AMGs

AMG antibiotics were the first drugs discovered by systematic screening of natural product sources for antibacterial activity. Streptomycin was the first AMG to be identified and characterized by Waksman's group in 1944. In contrast to penicillin which was isolated from fungi, streptomycin was the first antimicrobial to be isolated from a bacterial source. The discovery of streptomycin was a landmark in the history of

antimicrobials, since it was the first effective treatment for tuberculosis, a disease that had caused tremendous human suffering for centuries.[141] This was followed by the discovery of neomycin by the same group, in 1949. Neomycin, isolated from *Streptomyces fradiae*, had better activity than streptomycin against aerobic gram-negative bacilli.[142]

Gentamicin, isolated from *Micromonospora* in the year 1963, was a breakthrough in the treatment of gram-negative bacillary infections, including those caused by *P. aeruginosa*.[142] Netilmicin and sisomicin were isolated from different species of *Micromonospora*. Thereafter, research was focused on chemical modification of known compounds in order to increase antibacterial activity and reduce associated toxicity.[143] Amikacin and dibekacin are derivative compounds of kanamycin through chemical modifications, while netilmicin is a semi-synthetic derivative of sisomicin.

2.2.2 CHEMISTRY OF AMGs

AMGs are a complex family of compounds and their classification can be based on chemical structure. There are different structural classes of AMGs, characterized by having an aminocyclitol nucleus (streptamine, 2-deoxystreptamine or streptidine) linked to amino sugars through glycosidic bonds.[144]

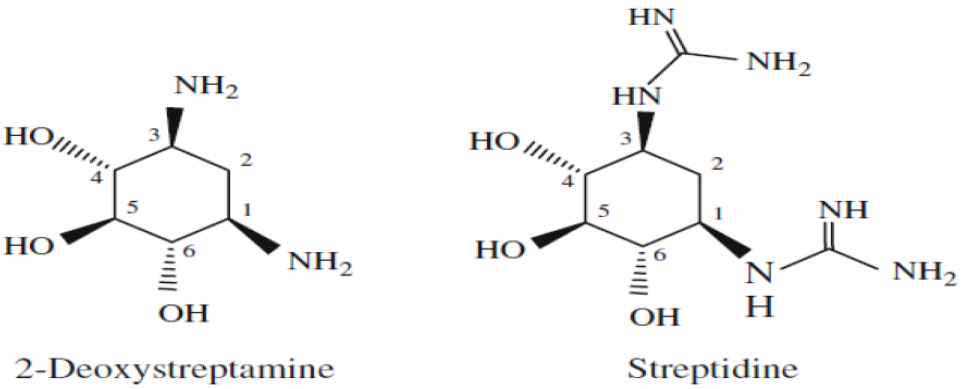


Fig. 2.1: Backbone structure of AMGs (Adopted from Jana and Deb [145])

Streptomyces, *Micromonospora*, *Bacillus*, and other bacterial genera have been shown to produce AMG-aminocyclitol antibiotics.[141] Compounds derived from *Streptomyces* are named with the suffix "-mycin", example tobramycin, while compounds derived from *Micromonospora* are named with the suffix "-micin", example gentamicin. Gentamicin is special among the AMGs because it is made up of three major and several minor components.[146]

Amikacin differs in its chemical structure from kanamycin by the acylation with dihydroxyaminobutyric acid (HABA) at the C-1 amino group of the 2-deoxystreptamine nucleus. The addition of this HABA moiety confers upon amikacin its unique properties, which include a broadening of its antibacterial spectrum over that of kanamycin, and resistance to most plasmid mediated inactivating enzymes.[147] Physicochemically, amikacin is a white, crystalline, basic and water soluble antibiotic with a molecular weight of 585.60 g/mol, a melting point of 201º–204ºC, and a specific optical rotation from +97º to 105º.[148]

2.2.3 PHARMACOKINETICS OF AMINOGLYCOSIDES

2.2.3.1 Absorption

Due to the fact that AMGs are highly polar, they are poorly absorbed from the gastrointestinal tract so systemic infections cannot be treated by this route of administration. However, bioavailability is better when AMGs are given intramuscularly. AMGs are rapidly absorbed with maximal concentrations occurring about 1 hour after injection. In critically ill patients, example those in shock, absorption of aminoglycosides from intramuscular sites may be erratic.[149]

2.2.3.2 Distribution and elimination

Aminoglycosides do not penetrate into most cells and the central nervous system. Volume of distribution of AMGs is similar to extracellular fluid content of the body, and fluid balance is an important factor when estimating AMG volume of distribution for a patient. Febrile patients may be significantly dehydrated and have lower volume of distribution until rehydrated. Due to the fact that AMGs are eliminated primarily by glomerular filtration, renal dysfunction is the most important disease state that affects AMG PK. [150,151] Elimination rate constant decreases in proportion to creatinine clearance because of decline in drug clearance.[152,153]

Premature infants (gestational age ≤37 completed weeks) have a larger amount of body water compared to adults.[154-156] Thus, AMG volume of distribution is larger, 0.5–0.6 L/kg, because of this physiologic difference. Additionally, their kidney functions are immature, and this results in decreased glomerular filtration and decreased AMG

clearance. A large volume of distribution and low clearance rate results in a prolonged average half-life in preterms, often between 6–10 hours. Full-term neonates also have a volume of distribution averaging 0.4–0.5 L/kg and half-life of 4–5 hours.

The volume of distribution of amikacin is estimated to be 0.25 L/kg, with a half-life of 7 – 9 hours in low birth weight neonates. In term neonates, however, the half-life is estimated to be between 4–5 hours.[157] Studies have shown that the volume of distribution is larger, and elimination half-lives longer, in lower gestational age neonates.[158]

2.2.4 PHARMACODYNAMICS OF AMINOGLYCOSIDES

2.2.4.1 Mechanism of action of aminoglycosides

Aminoglycosides are potent bactericidal antibiotics, mostly used against gram negative bacteria. They penetrate bacteria by binding to positively charged moieties of the cell membrane; this is believed to result in the displacement of Mg^{2+} and Ca^{2+} ions that link adjacent lipopolysaccharide molecules. The process damages the membrane and enhances its permeability.[159] Inside the cell, they inhibit bacterial protein synthesis by binding to ribosomes. The precise mechanism of their antimicrobial activity is still a subject of study but all AMGs bind to the 16-S-ribosomal DNA portion of the 30S ribosomal sub-particle, impairing the proofreading function of the ribosome. This leads to misreading of ribonucleic acid (RNA) templates and results in the formation of "non-sense proteins". The most relevant of these unnatural protein syntheses is the effect on bacterial membrane and its function. The defective membrane allows additional

quantities of AMGs to enter the cell. With increased concentration of AMGs within the cell, protein biosynthesis ceases all together. The effect brings about its bactericidal action.[160]

2.2.4.2 Spectrum of activity of AMGs

AMGs exhibit *in vitro* activity against a broad-spectrum of clinically important Gram-negative bacteria such as *E. coli*, *Klebsiella spp*., *Pseudomonas spp*., *Shigella spp*., *Salmonella spp*., *Enterobacter spp*., *Citrobacter spp*., *Acinetobacter spp*., *Proteus spp*., *Serratia spp*., and *Morganella spp*. They also have activity against Gram-positive bacteria such as *S. aureus* and some streptococci.[159] Therapeutic steady-state peak concentrations for gentamicin, tobramycin, and netilmicin are generally 5–10 μg/mL for gram-negative infections. Infection sites with more susceptible bacteria, such as intra-abdominal infections usually can be treated with steady-state peak concentrations at the lower end of this range (typically 5–7 μg/mL). Bacteria that have high MIC values, such as *P. aeruginosa* usually require steady-state peak concentrations in the higher end of the range (typically 8–10 μg/mL). Optimal antibacterial activity of amikacin is achieved when peak concentration is 8-10 times greater than MIC. The MIC breakpoint for a pathogen such as *Pseudomonas aeruginosa,* in intensive care unit patients is 8 μg/mL.[161]

2.2.4.3 Adverse effects of AMGs

Aminoglycoside ototoxicity:

Aminoglycosides can accumulate in the lymphatic fluid of the inner ear and are potentially cochlea- and vestibulotoxic. Outer hair cells, inner hair cells and spiral

ganglional neurons can be damaged in a process of excitotoxic cell death due to enhancement of glutamate N-methyl-D-aspartate (NMDA) receptor activity. Ototoxicity occurs both in a dose-dependent and idiosyncratic fashion.[162] The idiosyncratic pathway is presumably due to genetic predispositions. The genetic predisposition is known to be maternally inherited.[162] Two mutations in the mitochondrial 12S ribosomal RNA gene have been previously reported to predispose carriers to aminoglycoside-induced ototoxicity. The first is A1555G mutation, and a second is the deletion of the T nucleotide at position 961, combined with a heteroplasmic increase in the number of C nucleotides surrounding position 961.[163]

One postulate, linking mutations in the mitochondrial 12S ribosomal RNA gene and aminoglycoside-induced ototoxicity is that aminoglycosides cause misreading in mitochondrial protein synthesis such that tissues rich in mitochondria are affected.[162] A decrease in mitochondrial ATP synthesis results in a compromised ion pump activity, which will progressively lead to a decrease in endocochlear potential.[163]

Electroacoustic and electrophysiologic tests, in particular otoacoustic emissions (OAE) and the brainstem auditory evoked potential (BAEP), are recommended for defining the behavioral audiogram in children aged below 6 months. All sensory-neural structures submitted to stimulus will normally emit bioelectrical potentials as response. Thus, a stimulation of the human auditory receptor triggers a number of electrical responses or evoked potentials, which result in the successive activation of the eighth nerve, cochlea and neurons which make up the auditory pathway with a sequence of five major

waves.[164] Waves I and II arise from the distal and rostral portions of the eighth nerve, respectively; wave III from the pons and waves IV and V are from the midbrain.

It is important to note that, in many normal individuals, all BAEP waves are not always present. However, waves III and V should be detectable in all healthy individuals. Abnormalities in BAEP waves and likely lesions along the brainstem-auditory pathway are summarized in Table 2.2.

Table 2.2: Patterns of Brainstem Auditory Evoked Potential (BAEP) abnormalities

BAEP finding	**Lesion**
Prolonged wave latency	Distal cranial nerve VIII
Prolonged I-III interpeak latency	Between proximal cranial nerve VIII and pons
Prolonged III-V interpeak latency	Between caudal pons and midbrain
Prolonged I-III and III-V latency	Midbrain and acoustic nerve or caudal pons
Absent wave I with normal III and V	Mild to moderate peripheral hearing loss
Absent wave III with normal I and V	Normal variant
Absent wave V with normal I and III	Above the caudal pons
Absence of all waves	Severe hearing loss

Adopted from Blum and Rutkove,[165]

Aminoglycoside nephrotoxicity:

Following glomerular filtration, a small portion of AMGs accumulate in the epithelial cells of mainly the proximal, but also distal and collecting tubules for an extended period and results in intracellular alterations, causing damage that can range from loss of the brush border to complete tubular necrosis.[166,167] Furthermore, AMGs cause significant

functional damage to the mechanisms involved in water and solute transport, causing tubular luminal obstruction.[168] Nephrotoxicity due to AMG therapy is unlikely to occur before 3–5 days of therapy with proper dosing of the antibiotic. Due to the fact that many patients receiving AMGs are critically ill, other sources of nephrotoxicity, such as hypotension or other nephrotoxic drug therapy, should be ruled out before a diagnosis of AMG renal damage is made in a patient. Unlike ototoxicity, AMG-induced nephrotoxicity is usually reversible with little, if any, residual damage if the antibiotic is withdrawn soon after renal function tests change.

2.2.4.4 Therapeutic drug monitoring of AMGs

Conventionally, AMGs are monitored during therapy to reduce the risk of toxicity, particularly useful when treatment is extended more than 48 hours. Often the process requires two blood samples. One, taken 60 minutes after the start of IV dose, is known as maximum or peak concentration (Cpeak). The other, taken just before the administration of the next dose, corresponds to the minimum or trough concentration (Ctrough). The therapeutic range for peak and trough amikacin concentrations have been reported to be 20 – 30 μg/mL and 2 – 5 μg/mL respectively.[169] Data on therapeutic drug monitoring in pediatrics is scanty, and often adult threshold is used.

2.2.4.5 Drug interactions of AMGs

Most important drug interactions of AMGs are PD, and not PK, in nature. Vancomycin,[190] amphotericin B,[191] and cyclosporin[192] enhance the nephrotoxic potential of AMGs. Each of these agents can cause nephrotoxicity when administered alone. When these drugs are administered concurrently with an AMG, serum creatinine concentrations should be monitored on a daily basis. Additionally, serum concentrations of vancomycin or cyclosporin, as well as AMGs, should be measured. Methylxanthines have been revealed to function with antibiotics synergistically and antagonistically *in vitro*. One study evaluated the interaction of some penicillin antibiotics and caffeine against *S. aureus*. Caffeine was seen to raise the antimicrobial effect of amoxicillin, as shown in Table 2.3. Also, caffeine reduced the effects of benzylpenicillin, but had no influence on ampicillin.[173]

Table 2.3: Combined activity (Inhibitory Zone Diameter [mm] ± SEM) of amoxicillin and caffeine against *Staphylococcus aureus*.

Concentration (mg/ml)	Amoxicillin alone	Amoxicillin + 5 mg/ml caffeine	Amoxicillin + 10 mg/ml
7.81	13.5 ± 0.5	18.8 ± 0.5 (39.3)	19 ± 0.5 (40.7)
15.63	14.7 ± 0.6	19.7 ± 0.3 (34.0)	20 ± 0.0 (36.1)
31.25	16.3 ± 0.6	21.0 ± 0.0 (28.8)	21.0 ± 0.0 (28.8)
62.5	18.2 ± 0.3	22.07 ± 0.7 (21.3)	22.2 ± 0.3 (22.0)
125.0	20.5 ±0.0	23.0 ± 0.0 (12.2)	23.5 ± 0.5 (14.6)
250	22.0 ± 0.0	24.0 ± 0.5 (9.1)	24.5 ± 0.5 (11.4)
500	24.0 ± 0.6	24.7 ± 0.7 (2.9)	25.0 ± 0.0 (4.2)
1000	26.5 ± 0.5	27.4 ± 0.5 (3.4)	27.4 ± 0.5 (3.4)

Values in parenthesis represent percent increase in IZD.

Adopted from Esimone *et al*,[173]

It is also documented that methyl-substituted xanthines, caffeine and theophylline potentiate the antimicrobial action of another AMG, neomycin, on *S. aureus* and *P. aeruginosa in vitro*.[48] The mechanism for the potentiating effect of neomycin by the methyl-substituted xanthines *in vitro* is unclear.

Methylxanthines

Methylxanthines, such as theophylline and aminophylline, administered orally are rapidly and completed absorbed from the gastrointestinal tract. They often distribute into all body compartments; crossing the placenta and passing into breast milk.[149] Methylxanthines are primarily metabolized in the liver, however, about 10% of administered drug is excreted unchanged in urine. Methylxanthines are known to possess diuretic properties,[46] and have a greater effect on glomerular filtration rate than on renal blood flow.[47] There is marked between subject variation in the rate of elimination of theophylline owing to both genetic and environmental factors. The drug often follows first-order elimination kinetics but at higher concentrations, zero-order kinetics becomes evident.

2.3 PHARMACOKINETICS OF DRUGS IN THE NEONATE

Although infants and toddlers are understood to be very different from adults in their disposition of and response to drugs, information relevant to this group is almost always collected after a particular drug has been tested in adults. Moreover, the accumulation of these data in infants is often slow and incomplete. Ethical considerations hinder rigorous studies, and in many cases the lack of micro methods for drug analysis in small volumes hampers detailed drug trials in infants.

Compared with older children and adults, neonates differ in their physiology, and this affects drug PK: absorption, distribution, metabolism, and excretion. Disease, critical illness, specialized therapies, and developmental changes in the expression of organ-specific drug transporters may further contribute to these differences.[174,175] Table 2.4 summarizes some of the most important PK differences between the preterm, term neonates and adult, and the derived PK consequences.

Table 2.4: PK differences between neonates and adults

PK parameter	Age group		PK consequence	Examples
	Preterm	Term		
Absorption	↓	↔	↓ AUC	Sulfonamides
Distribution				
Body water	↑↑↑	↑↑	Cmax of water-soluble drugs	Gentamicin, digoxin
Body fat	↓↓	↓	Minimal clinical affect	–
Metabolism				
Hydroxylation	↓↓↓	↓↓	↓Clearance	Diazepam
N-demethylation	↓↓↓	↓		Theophylline
Acetylation	↓	↓		Sulfonamides
Glucuronidation	↓↓	↓		Chloramphenicol
Renal excretion				
Glomerular excretion	↓↓	↓	↑ AUC	Gentamicin
			↑ $t_{1/2}$	
Tubular secretion	↓↓	↓	↑ AUC	Gentamicin
			↑ $t_{1/2}$	

Adopted from Rylance[176]

2.3.1 Drug absorption in neonates

Characteristics of the neonatal gastrointestinal tract that affect absorption of orally administered drugs include increased gastric pH, decreased intestinal motility, delayed gastric emptying time, and a reduction in bile acid synthesis.[177,178] Additionally those that affect intramuscular absorption in neonates include decreased muscle mass, reduced overall muscular perfusion, and decreased contractility.[179,180] Reduction in muscle perfusion due to hypotension, sepsis, or decreased cardiac output can lead to reduced absorption and unpredictable PK of drugs administered intramuscularly.[177] Water soluble drugs tend to have greater intramuscular absorption in neonates than children or adults due to higher muscular water content and increased density of skeletal muscle capillaries in neonates.[179,181]

Rectal absorption of drugs is generally increased in the neonate compared with children and adults.[179,180] However, variability in the depth of insertion or retention of drug in the rectal vault can lead to variability in absorption.[182] Drugs absorbed deep inside the rectum undergo first-pass metabolism by accessing the liver through the superior rectal veins, whereas drugs inserted in a shallow manner will enter systemic circulation directly through the inferior and middle rectal veins.[183]

2.3.2 Drug distribution in neonates

Compared with children and adults, neonates have higher volumes of extracellular fluid and total body water, and lower proportions of adipose tissue.[184,185] Premature neonates have lower fat and higher water content than term neonates.[177,185] Neonates have a

decreased drug protein-binding affinity relative to children and adults. Only unbound drug travels across membranes, exerts biological effect, and is eliminated from the body. Neonates have decreased plasma concentrations of albumin and α1-acid glycoprotein, resulting in increased plasma concentrations of unbound drug.[186] At time of birth, neonates have low concentrations of α1-acid glycoprotein and albumin, which gradually increase to adult levels by 1 year of age.[187] Additionally, some drugs compete with bilirubin for albumin binding. This can render the neonate susceptible to kernicterus (if the unbound bilirubin fraction is increased) or drug toxicity (if the fraction of free drug is increased). Drugs which can compete with bilirubin include phenytoin.[187]

Gentamicin and amikacin have been shown to have greater distribution in neonates, and this decreases gradually during childhood.[188] These changes are explained mainly by changes in the percentage of body water, given that the AMGs bind minimally to plasma proteins. The large volume of distribution in infants and small children means that, at equal doses (per body weight), peak concentrations produced in the blood will be lower than in adults.

2.3.3 Drug elimination in neonates

Drug metabolism by the neonatal liver is affected by the ontogeny of many drug-metabolizing enzymes. Rates of hepatic drug metabolism generally correspond with the expression of these enzymes, which is typically low at birth and gradually increases over time.[189,190] The ontogeny of drug metabolizing enzymes involved in Phase I drug biotransformation reactions is summarized in Table 2.5. Glucuronidation systems also

mature more slowly than other Phase II biotransformation systems. In human fetuses, at 20 weeks gestation, there is often no evidence of any expression of glucuronyl transferase enzyme.[191] This explains the high incidence of jaundice in newborns (high bilirubin levels). Despite lower enzyme expression, reduced protein binding in neonates can sometimes lead to unexpectedly higher metabolic clearance of certain drugs.[192]

Table 2.5: Ontogeny of human hepatic Phase I drug metabolizing enzymes

Gene	Prenatal Trimester			Neonate	1 Month to 1 Year	1 to 10 Years	Adult
	1	2	3				
CYP1A1	+	+	?	−	−	−	−
CYP1B1	?	±?	?	?	−	−	−
CYP1A2	−	−	−	−	+	+	+
CYP2A	−	−	−	?	+	+	+
CYP2B6	−	−	?	?	?	+	+
CYP2C	−	−	−	+	+	+	+
CYP2D6	−	±	±	+	+	+	+
CYP2E1	?	+?	+?	+	+	+	+
CYP2J	?	+	?	?	?	+	+
CYP3A7	+	+	+	+	−	−	−
CYP3A4/3A5	−	−	−	+	+	+	+
FMO1	+	+	+	−	−	−	−
FMO3	±	−	−	±	+	+	+
ADH1	+	+	+	+	−	−	−
ADH2	−	+	+	+	+	+	+
ADH3	−	−	+	+	+	+	+

Adopted from Hine and McCarver,[190]

** *ADH: alcohol dehydrogenase; CYP: cytochrome P450; FMO: flavin-containing monooxygenase.*
(+): activity or protein detectable; (−): no detectable activity or protein; (?): not determined;
(±): activity or protein detectable but in only a fraction of the samples examined; (+?): presence or absence is controversial.

Renal clearance of drugs increases with increasing gestational age, postnatal age, and body weight.[174,185] Generally renal clearance is affected by glomerular filtration rate (GFR), active tubular secretion, and tubular reabsorption. GFR normalized to body surface area is lower in neonates compared with children and adults, with lowest values seen in premature neonates.[179] The kidneys of the newborn receive only 15-20% of the

cardiac output, in contrast to the 25% observed in the adult.[193] This hypoperfusion in combination with a severely limited filtration surface are the basic reasons of the very low GFR of the neonate. Thus, in a healthy term neonate the GFR at birth is just 20 mL/min/1.73m^2 and about 10-15 mL/min/1.73m^2 in a premature one.[194] This low GFR limits all renal functions, especially with regard to water and electrolyte homeostasis and drug excretion. During the first month of life, GFR increases rapidly due to a rise of systemic BP and a concomitant fall in renal vascular resistance, but it hardly exceeds 40 mL/min/1.73m^2.[193]

Term neonates experience a rapid increase in GFR during the first 2 weeks of life, followed by a steady rise to adult values by 6–12 months of age.[195] Premature infants demonstrate similar trends, with an initial rise in GFR that is less steep due to nephrogenesis not being complete until 34 weeks gestation.[174,179] Active tubular secretion and tubular reabsorption are also immature at birth and are about 20–30% of adult values.[179] Maturation occurs gradually, reaching adult values by 7–12 months of life.[195] Maturation of tubular reabsorption continues slowly into adolescence, with the steepest rise occurring between 1 and 3 years of age.[196]

2.4 PHARMACOKINETICS

Pharmacokinetics is concerned with the time-course of drug movement through the body. This involves the absorption, distribution, metabolism and excretion of drugs and their metabolites. The pharmacokinetic of a drug can now be studied in patient populations (population pharmacokinetics).

2.4.1 TRADITIONAL PHARMACOKINETIC STUDIES

In traditional pharmacokinetics studies, few subjects are intensively sampled over a given post-dose period using a fixed design. Pharmacokinetic parameter estimates can be obtained with a "two-stage approach".[197] In the first stage, values of pharmacokinetic parameters (clearance and volume of distribution) in each individual are calculated. The second stage involves estimation of descriptive statistics, usually the mean or geometric mean and standard deviation for each parameter. Deficiencies with traditional studies includes the inability to handle sparse data and ability to identify covariates, such as age and weight as sources of pharmacokinetic variability.[197] The imprecision in estimating the parameter values is also unidentified when fitting the model to the data. This uncertainty leads to inter-individual variability being overestimated.

Another method of estimating pharmacokinetic parameters is the "naive pooled data". In this approach data from all participants are pooled as though they had been collected from one 'super-subject'. However, this approach ignores the sources of variability within and between individuals. It is not recommended even if there are numerous participants and the inter-individual pharmacokinetic variability is relatively small.

2.4.2 POPULATION PHARMACOKINETIC STUDIES

Population pharmacokinetics (Pop PK) can be defined as the study of variability in plasma drug concentrations in a population. Pop PK tries to identify and measure sources of variability responsible for differences in drug concentration among patients. This variability can be sub-divided into inter-individual and residual variability. The inter-

individual variability stems from the simple fact that every person is biologically different from all others. Residual variability is a combination of sources of variation, such as intra-individual differences, inter-occasional differences, and errors made in measurement, dosing and modeling.

One of the major areas of the application of Pop PK is the analysis of drug concentration measurements in pediatric populations. The first Pop PK analyses in pediatric patients were performed after the introduction of the non-linear mixed effects modeling methodology to clinical pharmacology.[198] The subsequent widespread application of this modeling technique in pediatric studies can particularly be ascribed to its ability to analyze sparse and unbalanced PK data. These are frequent features in pediatrics because of ethical, as well as logistic constraints inherent in many aspects of studies in such populations, such as limitations of multiple blood sampling.

2.4.2.1 Population pharmacokinetic modeling

Mathematical models can now be used to describe the PK profile of a drug or experimentally collected data. Both the model structure and the model parameters are derived from the experimental data.[199,200] In PK modeling, the body is typically divided into one, two, or three compartments, that represent distributional spaces with identical kinetics. The most frequently used models are depicted in Fig. 2.2. The simplest model is the one-compartment model which depicts the body as one large container where drug distribution between blood and tissues occurs instantaneously. Drug is introduced into the compartment by infusion (ko), absorption (ka), or IV bolus; distributes immediately into

a volume of distribution (V); and is eliminated from the body via an elimination rate constant (ke). The simplest multi-compartment model is a two-compartment model which represents the body as a central compartment into which drug is administered and a peripheral compartment into which drug distributes. The central compartment (1) is composed of blood and tissues which equilibrate rapidly with blood. The peripheral compartment (2) represents tissues that equilibrate slowly with blood. Rate constants (k_{12}, k_{21}) represent the transfer between compartments and elimination from the body (k_{10}).

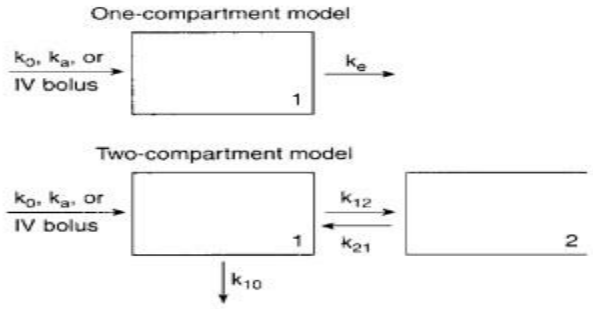


Fig. 2.2: Pharmacokinetic compartment models (Adopted from Bauer[210])

The compartments are interconnected by first-order rate constants, while input rate constants may follow zero-order kinetics. Elimination is typically assumed to occur from the central compartment based on either a linear or a saturable Michaelis-Menten process.[200] The rate of change of drug concentration after an IV bolus dose is described by the following equation:

$$V \frac{d}{dt} C_1 = - CL \times C_1 \quad \text{..... } \textit{Eqn 2.1}$$

With initial condition

$$C_1 (t = 0) = \frac{dose}{V} \quad \text{..... } \textit{Eqn 2.2}$$

From these equations (Eqns. 2.1 and 2.2), V is defined as an apparent volume into which the total amount of drug would have to distribute in order to produce the observed plasma concentration. CL denotes clearance, and is defined as the volume of body fluid that is cleared from the drug per unit time.[200] C_1 is the serum concentration of a drug at any given time, and at time (t) zero, C_1 can easily be calculated with a known V of the drug.

To account for an IV infusion or extravascular drug administration, Eqn. 2.2 is amended to;

$$V \frac{d C_1}{dt} = - CL \times C_1 + r_{admin} \quad \text{..... } \textit{Eqn 2.3}$$

with $C_1 (t = 0) = 0$

where r_{admin} describes the mass inflow per unit time

Two- or three-compartment models are used to describe the PK of a drug if drug disposition is rate-limited so that the whole body cannot be considered as a single, homogenous distributional space. For each compartment, the rate of change of drug concentration is described by an equation. For example, a two-compartment model accounting for drug concentration in the central, C_1, and peripheral compartment, C_2, is given by:

$$V_1 \frac{d C_1}{dt} = q \times C_2 - q \times C_1 - CL \times C_1 + r_{admin} \quad \text{..... } \textit{Eqn 2.4}$$

$$V_2 \frac{d C_2}{dt} = q \times C_1 + q \times C_2 \quad \text{..... } \textit{Eqn 2.5}$$

Where $r_{admin} = r_{IV}$ or r_{abs}, rate of IV infusion and rate of absorption respectively, depending on the route of administration. Drug elimination is assumed to occur from the

central compartment with clearance, CL, and q denoting the inter-compartmental clearance.

2.4.2.2 Analyzing population pharmacokinetic models

One of the most widely used methods in analyzing population pharmacokinetic models is the non-linear mixed effects approach. Non-linear because pharmacokinetic parameters - rate constants, clearance rates, etc, occur non-linearly in a model function. The term "mixed-effect" refers to a model that contains a fixed structure and a randomness block.[201] The fixed structure, quantifies mean pharmacokinetic parameters (structural model) and associated effects of covariates (covariate model) on parameters, whiles the randomness block quantifies the magnitude of inter-individual and intra-individual variability (statistical model). Mixed-effects models can describe experimental observations involving multiple subjects, yielding correct estimates of the standard errors and estimating no more parameters than necessary. Using a Bayesian approach, mixed-effects models can be "individualized" as observations that capture unique characteristics of the individual.

Parameters of mixed-effects models can be estimated by application of the maximum likelihood principle. According to this principle, the best estimates for intra- and inter-individual variability terms ($\sigma^2$and ω^2) are those that would yield the greatest likelihood of observed values. This requires knowledge of the type of probability distribution functions for the inter-individual variability of the parameters, which need to be postulated.[202]

For a basic structural model, individual pharmacokinetic parameters (CL_i and V_i) are related by:

$CL_i = CL_p \times EXP(\eta_{CLi})$ ..... *Eqn 1*

$V_i = V_p \times EXP(\eta_{Vi})$ ..... *Eqn 2*

Population mean parameters: CL_p and V_p; Inter-individual variability with CL and V: $\eta_{CLi\ and}\ \eta_{Vi}$

Software products that implement non-linear mixed-effects modeling include WinNonmix, NONMEM, and Monolix. The development of a model with NONMEM takes into account both fixed and random effects. Model built of a fixed structure has two stages: development of a base model, and the introduction of covariates into the base model to obtain the final model. The randomness block of the model will quantify the magnitude of all kinds of variability.

2.4.2.3 Assessing model performance

A principle of pharmacokinetic and pharmacodynamics modeling is that a model can never be proven, only disproved. The more tests a model passes, the greater the credibility of the model. It is now recognized that there is not a single statistic or graphic method for assessing model performance. Hence, several diagnostic processes should be used together to estimate model performance. Commonly used tools include numerical and graphical based diagnostics.

Numerical diagnostics

A common numerical diagnostic measure used in model performance is the objective function value (OFV). OFV measures the difference between observed and predicted

values for a group of patients, describing how good a model is at fitting the observed data. It does this by assuming that the model is correct, and questions the probability of getting observed data if the model is true. It employs the -2log likelihood (-2LL) value. By minimizing this value, one increases the likelihood of the model being a good fit for the data. NONMEM looks for parameter estimates that will give the smallest possible -2LL.

The difference between -2LL values follows a chi squared distribution, with the degrees of freedom being the difference in the number of parameters. For example, with a probability of 0.05, and degree of freedom of 1, the value of the chi distribution is 3.84. Thus, if the difference in -2LL (ΔOFV) values for two models differ by only 1 parameter and less than -3.84, then the parameter is significant at $p < 0.05$ level.[204] Another numerical diagnostic, standard error, can be used in evaluating model performance. It has been reported that standard error for structural model parameters and random effects parameters should not exceed 25% and 50%, respectively.[205]

Graphical diagnostics

Goodness-of-fit (GOF) plots are graphical diagnostics used to show different aspects of population data that are described by a selected model. GOF plots are created to detect potential bias or problems in the structural model and/or the random effects models, and are generated based on: observations (DV), population predictions (PRED), individual model predictions (IPRED), residuals (RES), weighted residuals (WRES), conditional weighted residuals (CWRES) and time (TIME).[206] Plots of these give a general

impression of the performance of a model. The most common of these diagnostic plots is a plot of DV versus PRED. A line of identity, and sometimes a regression line, is included to illustrate how well the observations and predictions agree. This diagnostic may give a useful impression of the extent of variability in the data that is explained by the structural and covariate components of the model.

2.4.2.4 Model evaluation

Most of the evaluation methods used in Pop PK modeling are based on simulation diagnostics. It includes bootstrapping and predictive checks. Bootstraping, a resampling method,[207] involves repeatedly generating pseudo-samples distributed according to the same distribution as the original sample. Predictive check is the name given to the multiple simulations that are made from the model and reference distributions created for features of the observed data. Within this diagnostic procedure is the visual predictive check (VPC). VPC refers to a plot of time course of the observations together with the time course of prediction intervals for simulated values. This approach is used for both fixed and random effects.[208] Additionally, prospective validation (confirm model for the same drug but other patients) and cross validation (confirm model for other drugs and other patients) can be done.[209]

2.5 POPULATION PHARMACOKINETICS OF AMINOGLYCOSIDES IN NEONATES

The PK of AMGs in neonates is marked by large inter-individual variability necessitating an individualized therapeutic approach. A number of studies have described the Pop PK of AMGs in neonates, but few with amikacin. The Pop PK of tobramycin was evaluated

within the first two weeks of life of 140 neonates with gestational age between 30 to 42 weeks and currrent body weight between 0.8 to 4.25 kg.[210] A one-compartment model with zero-order absorption and first-order elimination was found to best describe the population. The effect of covariates: gender, height, birth weight, current weight, gestational age, postnatal age, postconceptional age, and serum creatinine concentration on CL and V was investigated. Current weight was found as an important determinant in tobramycin CL and V. Interindividual variability for CL and V was 25.8% and 21.9%, respectively, and residual variability was 19.2%.[210]

In a study that had 200 premature neonates, the Pop PK of gentamicin was evaluated. A two-compartment open model was found to best describe the PK of gentamicin among the neonates. Covariates that influenced gentamicin disposition in this population were birth weight, postnatal age and creatinine clearance.[211] The Pop PK of netilmicin was studied in 74 neonates of gestational ages ranging between 27 to 42 weeks and weight between 0.8 to 5.0 kg. A two-compartment model best described the PK of this population. CL was found to depend on body weight, gestational age, and postnatal age. The V of the central and peripheral compartments was also related to body weight.[55] Others have also reported the Pop PK of the following AMG in neonates; gentamicin,[212-218] tobramycin,[219] nitilmicin,[220] and arbekacin.[221]

A number of Pop PK studies of amikacin in neonates have been described.[59,60,61,222] Major differences among these studies include varying birth weight, postnatal age, gestational age, and size of the population being studied. Botha *et al*, used data from 53

neonates of African origin, with mean (SD) birth weight of 2.1 ± 0.8 kg, mean gestational age of 35.1 ± 3.6 weeks, and mean postnatal age of 3.1 ± 3.1 days and 6.3 ± 3.3 days at the start of treatment and at the time of sampling respectively.[60] Sherwin *et al* on the other hand used data from 80 neonates with median (range) birth weight of 0.97 (0.44 – 4.4) kg, median gestational age of 28 (24 – 41) weeks, and median postnatal age of 9 (3 – 64) days.[61] Sherwin *et al* assessed a number of covariates in their model building process, and these included; gestational age, postnatal age, current weight, sepsis, serum creatinine, sex, Apgar scores and post-menstrual age.[61] The current study also assessed similar covariates in addition to the influence of co-administration of aminophylline and acute phase reactants (PCT and CRP) on the disposition of amikacin.

Most of these Pop PK studies of amikacin in neonates have found body weight to be a an important determinant of amikacin clearance.[59,60,61] Reports suggest that birth weight and postnatal age are two main determinants of amikacin clearance, representing prenatal and postnatal kidney maturation.[223-225] Additionally, it has been reported that the use of non-steroidal anti-inflammatory drugs (NSAIDs), either aspirin or ibuprofen, and perinatal asphyxia can reduce amikacin CL in the neonate.[226] Overall, the most important determinant of amikacin distribution volume is body weight.[60,61]

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a longitudinal study done to evaluate the effect of treatment with amikacin on outcome and selected pharmacokinetic and pharmacodynamic characteristics of neonates with suspected sepsis at a teaching hospital in Accra, Ghana.

3.2 STUDY SITE

The study was conducted at the Neonatal Intensive Care Unit (NICU) of Department of Child Health, Korle-Bu Teaching Hospital (KBTH), from November 2013 to June 2014. The NICU is a 60-bed unit that admits pre-term and sick newborns. It is located in close proximity to the obstetrics and labor wards of KBTH. The unit admits an average of 2000 neonates annually of which about 60% are preterm births. There is also a Kangaroo Mother Care Unit for stable low birth weight neonates. Admissions at the unit usually peak during the months of May to July. Full clinical staff strength of the unit comprises 9 medical doctors and 30 nurses. There is a 24 hour staff cover each day of the week.

3.3 STUDY POPULATION

The NICU at KBTH admits neonates referred from health facilities in the southern regions of Ghana. Eighty percent of the neonates are in-born at KBTH, while the others are out-born referrals. The gestational age of patients range from 24 weeks to 43 weeks. Admission is restricted to neonates aged less than 48 hours unless in exceptional

circumstances. Older newborns or those who have received poliomyelitis vaccination, usually given at birth in Ghana, are managed in another unit of the Department of Child Health, KBTH. The major causes of admission at the NICU include; prematurity, low birth weight, birth asphyxia, neonatal jaundice, and neonatal infections.

3.4 ADMISSION AND RECRUITMENT PROCEDURE

In brief, neonates suspected of having sepsis and in whom a decision to treat with amikacin ± other therapy were recruited after initial examination and assessment by a NICU pediatrician. A suspected case of neonatal sepsis included a combination of maternal and/or perinatal risk factors and clinical signs, examples of which include: prolonged premature rapture of membranes (> 18 hours), chorioamnionitis, offensive liquor, prematurity (GA < 37 weeks), low birth weight (< 2.5 kg), pus from umbilical cord, recurrent vomiting, tachycardia (heart rate > 160 bpm) or bradycardia (heart rate < 100). Neonates who were known to have received any aminoglycoside prior to admission, those with major congenital anomaly or whose parents refused informed consent were excluded.

3.5 DATA COLLECTION

Standard case record forms were used to collect information from each recruited neonate. Data collected included: demographic information, perinatal data, reason for referral to NICU, record of clinical assessment and management at point of admission including exact drug dose administered, date and time of drug administration, blood sampling dates and times.

3.6 BLOOD SAMPLING

3.6.1 FBC, Clinical chemistry, CRP and PCT

Blood was taken from each recruited neonate by study pediatrician (Day 0), before first drug dose, for blood culture, full blood count (FBC), clinical chemistry (serum creatinine, total bilirubin and urea), CRP and PCT. For culture, blood sample (1–2 mL) from each neonate was collected into pediatric culture vial (BACTEC Peds plus/F) using aseptic procedures including cleaning venous site with iodine and 70% alcohol. For FBC analysis, 500 μL of blood was aseptically drawn from each neonate into ethylenediaminetetraacetic acid (EDTA) microtainer tubes. Additionally, 500 μL of venous blood was drawn into gel microtainer tubes and immediately centrifuged to obtain serum. Forty microliter aliquots of serum was used for the determination of total bilirubin, urea, creatinine, CRP and PCT.

3.6.2 Amikacin levels

Two blood samples, 500 μL each, were drawn from each neonate at various times after steady state was attained following amikacin administration. Samples were taken at 1, 2, 4, 6, 8 and 11.5 hours after the 3rd and 7th amikacin doses. This schedule was selected in order to obtain drug levels at different time points to describe the entire PK profile of amikacin. Blood samples were centrifuged within 10 minutes after collection, and serum kept frozen at -80ºC until time for analysis. Summary of daily schedule at NICU and drug sampling design are shown in Tables 3.1 and 3.2 respectively.

TABLE 3.1 DAILY SCHEDULE AT NICU, KBTH

DAY OF ADMISSION	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	TILL CLINICALLY INDICATED
1. Neonate recruited if inclusion criteria is met 2. Case record filled 3. Initial blood sample for Culture, Clinical Chemistry, FBC, CRP & PCT 4. Drug therapy (Amikacin + other supportive therapy)	1. Drugs administered as per schedule 2. Blood sample for drug assay (*According to schedule)* 3. Follow-up clinical assessment every 12 hours	1. Drugs administered as per schedule 2. Follow-up clinical assessment every 12 hours	1. Drugs administered as per schedule 2. Blood sample for drug assay (*According to schedule)* 3. Follow-up clinical assessment every 12 hours	1. Drugs administered as per schedule 2. Blood sample for FBC and clinical chemistry (follow-up) 3. Follow-up clinical assessment every 12 hours	1.Drugs administered as per schedule 2. Follow-up clinical assessment every 12 hours	1.Drugs administered as per schedule 2. Follow-up clinical assessment every 12 hours

TABLE 3.2 DRUG SAMPLING SCHEDULE FOR RECRUITED NEONATES

45 Neonates	45 Neonates	45 Neonates	45 Neonates	45 Neonates	45 Neonates
• 1 hour after 3rd dose • 1 hour after 7th dose	• 2 hours after 3rd dose • 2 hours after 7th dose	• 4 hours after 3rd dose • 4 hours after 7th dose	• 6 hours after 3rd dose • 6 hours after 7th dose	• 8 hours after 3rd dose • 8 hours after 7th dose	• 30 mins before 4th dose • 30 mins before 8th dose

3.7 DRUG ADMINISTRATION

Amikacin (Bristol-Myers Squibb, USA), 15 mg/kg body weight loading dose, followed by a maintenance dose of 7.5 mg/kg body weight was administered 12 hourly in combination with cloxacillin, 50 mg/kg body weight 12 hourly. Aminophylline, 8 mg/kg body weight loading dose followed by a maintenance dose of 3 mg/kg body weight, was administered 8 to 12 hourly to neonates who required it.[227] These dosing schedules were standard guidelines at NICU, KBTH at time of study. Amikacin, cloxacillin and/or aminophylline vials containing drug solutions were diluted with saline to respective volumes corresponding to weight/kilogram dose required for each neonate. Each drug was administered as a slow bolus and a single push through an intravenous (IV) line over 2 minutes. This was then flushed with 2 mL of normal saline immediately after drug administration.

3.8 LABORATORY ANALYSIS

3.8.1 Full blood count

Sysmex KXT 21N, an automated hematology analyzer (Sysmex Corporation Kobe, Japan), was used for full blood count analysis. Briefly, analysis of white blood cell count (WBC) was based on a semi-conductor laser that analyses physiological and chemical characteristics of cells. Red blood cell (RBC) and platelet (PLT) counts were analyzed by RBC and PLT detectors using hydro-dynamic focusing. Hemoglobin concentration (HB) was analyzed by the HB detector.

3.8.2 Clinical chemistry, CRP and PCT

3.8.2.1 Clinical chemistry

Total bilirubin was assayed by a modification of Ehrlich's method.[228] This is based on reaction between a stabilized diazonium salt, 3,5-dichlorophenyldiazonium tetrafluoroborate (DPD), and bilirubin to form azobilirubin. Serum urea was determined by the coupled-enzyme reaction.[229] The enzyme urease hydrolyses urea in the sample and the ammonium ion produced is quantified. Serum creatinine level determination was by Jaffe method. [230] Creatinine in an alkaline solution reacts with picric acid to form a colored complex. The rate of color development is directly proportional to the concentration of creatinine in the sample. All these assays were run using an automated clinical chemistry analyzer (Randox Laboratories Ltd, USA). Creatinine clearance (CRCL) for all recruited neonates was calculated using the Schwartz equation.[231]

The equation is as follows:

$$CRCL\ (mL/min\ per\ 1.73\ m^2) = \frac{K \times L\ (cm)}{SCR\ (mg/dL)} \qquad \ldots\ldots\ldots\ Eqn\ 3.1$$

where K is 0.33 for pre-term neonates, and 0.45 for term neonates
L is length of neonate
SCR is serum creatinine

3.8.2.2 C-reactive protein level determination

CRP assay was performed with a BNII automated system (Dade-Behring Inc., Newark, Del), according to manufacturer's instructions. The assay uses particle-enhanced immunonephelometry to quantify CRP in serum samples.[232] Briefly, polystyrene particles

coated with monoclonal antibodies against CRP become agglutinated when mixed with samples containing CRP. The intensity of light scattering due to the agglutination reaction is measured by the nephelometer and is directly related to the CRP concentration. Samples were automatically diluted 20-fold by the instrument prior to analysis. The assay was standardized against the reference preparation, CRM 470. Limit of detection of assay was 0.17 mg/L.

3.8.2.3 Procalcitonin level determination

Electrochemiluminescence immunoassay, which is based on a sandwich principle,[233] was used to analyze PCT in serum of neonates. An automated Elecsys (Roche Diagnostics, Rotkreuz, Switzerland) was used for this purpose according to manufacturer's instructions. Briefly, antigen in the sample, a biotinylated monoclonal PCT-specific antibody, and a monoclonal PCT-specific antibody labeled with a ruthenium complex react to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. Reaction mixture is aspirated into a measuring cell where the microparticles are magnetically captured onto the surface of an electrode. Unbound substances are then removed with ProCell. Application of voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Limit of detection of assay was 0.1 ng/mL.

3.8.3 Bacteria identification and antibiogram

Blood culture was done using a fully automated BACTEC 9240 blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md).[234] Each culture vial had a sensor which responds to the concentration of CO_2 produced by the metabolism of microorganism or the consumption of oxygen needed for the growth of microorganism. The sensor is monitored for an increase in its fluorescence, which is proportional to increasing amount of CO_2 or the decreasing amount of O_2 present in the vial. A positive reading indicates the presumptive presence of viable microorganism. Positive cultures were flagged by an indicator light on the front of the BACTEC instrument and also displayed on an LCD screen. Vials stayed in the BACTEC for at least 5 days before declaring a negative result.

Sub-cultures were made for positive cultures on sheep blood agar and MacConkey agar and incubated at 35°C. Gram-positive bacteria isolates were biochemically identified by catalase, slide and tube coagulase test, and Gram-negative by cytochrome oxidase and confirmed by API 20E and 20NE (BioMerieux France).

Antibiotic susceptibility tests were performed according to the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (Oxoid UK), in accordance with Clinical Laboratory Standards Institute (CLSI) criteria.[235] Classes of antibiotics used included cephalosporins, penicillins, aminoglycosides, beta-lactamase inhibitors, carbapenems, fluoroquinolones, glycopeptides, macrolides, lincosamides, and others (co-trimoxazole and rifampicin).

3.8.4 Serum amikacin measurement

Serum amikacin concentration was measured by particle-enhanced turbidimetric immunoassay (Indiko, Thermo Fisher Scientific Inc., Finland) according to manufacturer's instructions. Briefly, this assay is based on competition between drug in the sample and drug coated onto microparticles for antibody binding sites of the amikacin antibody reagent. The lower limit of quantification of the assay was 0.8 µg/mL, and coefficient of variation was less than 6% over the entire calibration range, 1.5 to 50 µg/mL. Concentrations below the lower limit were not included in the analysis because numbers were few (n = 8 concentration time points).[236]

3.9 POPULATION PHARMACOKINETICS OF AMIKACIN

3.9.1 Data exploration

For the Pop PK aspect of this study, sampling procedure used was an experimental Pop PK design or full pharmacokinetic screen.[237] Data from 247 eligible neonates was available for this aspect of study. Prior to PK modeling, exploratory graphs and tables were generated to gain an understanding of the data to be modeled. Scatterplots were used for all continuous covariates in order to investigate potential correlations existing among them and plots of amikacin concentration versus time in order to investigate the general trend.

3.9.2 Model building

3.9.2.1 Software used

PK parameters for the study population were estimated by a nonlinear mixed-effects modeling approach, using NONMEM Version 7.3 (ICON Development Solutions, Ireland) together with Pirana Version 2.8.2 (Pirana Software and Consulting BV). R Software Version 3.0.2 and R Package Xpose (Uppsala University, Sweden) were used for post-processing of data, graphical analysis of model outputs, and exploratory covariate analysis.

3.9.2.2 Structural model

One- and two compartment models with linear elimination and zero order input were tested using specific subroutines from NONMEM. One-compartment model subroutine used was; ADVAN1 TRANS2, parameterized as total drug clearance (CL) and volume of distribution (V). Two-compartment model subroutine used was; ADVAN3 TRANS4, parameterized as total drug clearance (CL), central compartment volume of distribution (V_1), peripheral compartment volume of distribution (V_2) and inter-compartmental clearance (Q).

3.9.2.3 Statistical model

A statistical model was developed by assessing between subject variability (BSV) within the population. The effect of administration of amikacin only and amikacin with aminophylline were tested as covariates in the model building process. Additionally the influence of other covariates, birth weight, gestational age, gender, positive blood

cultures with pathogenic bacteria, acute phase proteins (PCT and CRP), Apgar score at 5 minutes and creatinine clearance, on individual PK parameters was assessed by examining parameter-versus-covariate plots. All potential covariates were systematically incorporated into the structural model and retained if they improved fit of the model. Both proportional and combined (proportional plus additive) error models were tested during the process of model building. First-order conditional estimation with interaction (FOCEI) was used as the approximation method.

3.9.2.4 Initial estimates of parameters

Initial estimates for the structural model were mean PK values obtained from a similar study conducted in neonates of African origin.[58] Estimates for variance (ω^2) on BSV (η), and variance (σ^2) on residual errors (ε) were also selected considering an associated error of 50%. Since the coefficient of variation is the square root of the variance parameter, the initial estimates of ω^2 or σ^2 (when modeled proportionally) were set at 0.25.

$$\omega^2 = (0.5)^2 = 0.25 \qquad \ldots\ldots\ldots\ldots \text{Eqn. 3.2}$$

3.9.2.5 Model comparison

The main criteria for model comparison were: i) the likelihood ratio test, which is represented in NONMEM as a difference in objective function value (ΔOFV) of -3.84, corresponding to a significance level of < 0.05 [237] ii) the values of the variance of random effects and iii) basic goodness of fit plots; plots of observed (DV) versus population predicted and individual predicted (PRED and IPRED) concentrations, and plots of conditional weighted residuals (CWRES) versus PRED concentrations.

3.9.3 Model evaluation

The final model was evaluated using: i) bootstrap; one thousand data set of the same size as the original was reconstructed by re-sampling from the original data and final model fitted to each replicate data set allowing parameter estimates to be obtained for each one.[237,238]

3.10 BRAINSTEM AUDITORY EVOKED POTENTIAL (BAEP)

A group of neonates: i) with suspected sepsis and treated with amikacin in combination with cloxacillin (n = 20) and ii) who did not require amikacin treatment (n = 5) were evaluated with BAEP. The group who did not require amikacin included three neonates who were referred to the NICU on account of respiratory distress but did not require drug treatment after initial evaluation, one neonate (34 weeks gestation) with respiratory distress who required only aminophylline and one neonate with congenital pneumonia who was treated with cefotaxime in combination cloxacillin. Neonates wit birth weight <1500g, birth asphyxia and hyperbilirubinemia were excluded.

Baseline brainstem auditory evoked potential (BAEP) was done by a certified audiologist before first drug dose (Day 0), and a follow-up done after an average of 7 days post-first-dose for both test and comparison subjects under similar conditions. Method of BAEP assessment was similar to one reported by Langhendries *et al*,[239] as described below. Tests were recorded with a portable BAEP unit (Smart USB Lite; Intelligent Hearing System, Miami, Florida, USA) connected to a laptop computer by a USB port. All

neonates were tested while asleep, without sedation, and in supine position. Signal was obtained with 100 µsec alternating polarity broadband click stimuli calibrated to normal hearing level (dBnHL).

Briefly, stimulus was delivered via insert earphone (10 Ohms) with foam tips and signal recorded through a single channel obtained from three electrode arrays (vertex to earlobe with contralateral earlobe serving as ground). Responses were averaged for 2048 sweeps with a 24 ms time base at a rate of 21.7 Hz and latencies of peak I, III, and V measured. The inter-peak latencies between waves were also measured. Response to each intensity was replicated, and latency of peak V measured till the threshold (lowest level at which wave V occurred) was obtained. Tests were run with an initial intensity of 50 dBnHL, and when a clear response was identified, intensity was reduced until the lowest level with no response. However, if at 50 dBnHL the waveform was not clear, the intensity was increased till a clear waveform was obtained. The frequency range of recordings was 2 – 4 kHz.

3.11 PCT VERSUS CRP AS DIAGNOSTIC TOOLS FOR NEONATAL SEPSIS

Neonates admitted with suspected sepsis and deemed eligible for this sub-component of the study, diagnostic utility of PCT versus CRP, were categorized into 3 groups; highly probable, probable and less probable, based on a septic score similar to one reported by Gitto *et al*,[119] and Ali *et al*,[120] and shown in Table 3.3. Those presenting with birth asphyxia, meconium aspiration, congenital anomalies, or after resuscitation, were

excluded from this sub-component of the study.[27,48] In all, data from 62 neonates was available for this aspect of study.

Table 3.3: Septic score criteria for groupings

I	Positive blood culture (pathogenic microorganism)
II	Neonatal history: Prematurity (< 37 weeks gestation) or Low birth weight (< 2.5 kg)
III	Maternal risk (prolonged premature rapture of membranes > 18 hours)
IV	Tachycardia (Heart rate > 160 bpm) or Bradycardia (Heart rate < 100 bpm)
V	Respiratory distress (Rate > 60 cpm)
VI	CRP > 5 mg/L
VII	Leukopenia (WBC < 5 x 10^9/L) or Leukocytosis (WBC > 25 x 10^9/L)
VIII	Thrombocytopenia (platelet < 150 x 10^9/L)

Highly probable (Group 1): At least 2 of the first 3 criteria (I, II & III), plus > 2 of remaining criteria; **Probable (Group 2)**: Either II or III (excluding I), plus $\geq$ 2 of remaining criteria;
Less probable (Group 3): Either II or III, with none of the remaining criteria

3.12 Statistical analysis

A minimum sample size of 15 was required to detect a 2-fold difference in the mean PCT and CRP levels among neonates. This minimum sample size (n = 15) would also be sufficient to detect a difference in the proportion of neonates with elevated PCT and CRP levels. A minimum sample size of 270 was required in order to obtain at least 50 neonates with positive blood culture. This 18% (50/270) is not an assumed prevalence, but an estimate to get enough positive blood cultures to be used as a covariate during population pharmacokinetic modeling. Continuous variables were summarized as means with accompanying standard deviations, and compared with either student's *t*-test or one-way analysis of variance (ANOVA). Proportions were compared with an independent *t*-

test for proportions. Chi square tests were performed to identify association between categorical variables. Baseline and follow-up BAEP results (threshold of hearing) of test and comparison group were compared using Wilcoxon signed rank test. A p-value < 0.05 was considered statistically significant.

3.13 ETHICAL ISSUES

The Ethical and Protocol Review Committee of the University of Ghana School of Medicine and Dentistry approved the study (Protocol ID: MS-Et/M.8-P.5.3/2011-2012). Data was handled anonymously, and all forms of identifiers removed from all reports. Written informed consent was administered, explained and signed by all parents/guardians of recruited neonates. Information about study was explained in Ghanaian dialects that parents/guardians understood, when required. It was made clear that there was no immediate direct benefit of the study to the child. The potential benefit as explained, however, was that data from study would contribute to scientific knowledge about aminoglycoside use in neonates and possibly improve treatment for future patients.

The consent form was either signed or thumb-printed in the presence of a witness. All procedures were done by clinically qualified and experienced staff. No therapeutic or life-saving procedure was delayed for the purpose of completing case record form or blood sampling. Only minimal blood volumes were collected, and sample volume was tailored to clinical condition and size of neonate. Only authorized personnel had access to data sheets.

CHAPTER FOUR

4.0 RESULTS

4.1 CHARACTERISTICS OF RECRUITED NEONATES

4.1.1 Baseline admission characteristics and laboratory parameters

Three hundred and forty-one neonates (341) were recruited over the study period. Complete demographic and perinatal data was available for 322 neonates. One hundred and sixty-three (50.6%) of the recruited were preterm (GA < 37 weeks). Of the 163 neonates that were preterm, 14 (4.3%) were extreme preterm (GA < 28 weeks) and these extreme preterms had a median weight of 1.05 (0.5 – 1.8) kg. At the point of admission, 126 (39%) of the recruited neonates had axillary body temperature less than 36.5°C. Seventy-six, 23.5%, had mild hypothermia (36 – 36.4°C), while 50 (15.5%) showed moderate hypothermia (32 – 35.9°C). Out of the 65 neonates that had heart rates greater than 160 bpm, 42 (64.6%) were preterm. Those with heart rate less than 100 bpm had a median Apgar score at 5 minutes of 5 (4 – 7). Eighty-eight neonates (27.3%) had respiratory rate greater than 80 cpm, out of which 69 (78.4%) were preterm. The baseline admission characteristics of recruited neonates (n = 322) are shown in Table 4.1. Provisional diagnoses for the neonates in whom sepsis was suspected (n = 322), showed that a majority, 79 (24.5%) had birth asphyxia, and 83 out of the 163 preterm neonates (50.9%) had respiratory distress syndrome. Table 4.2 summarizes the provisional diagnoses of recruited neonates.

Complete full blood count (FBC), urea, total bilirubin, creatinine, CRP and PCT data was available for 273 neonates. Neonates with hemoglobin level less than 10 g/L were 13

(4.8%). A WBC less than 5 x 10^3/μL was recorded in 24 neonates (8.8%). Neonates whose platelet count was less than 150 x 10^3/μL were 97 (35.5%). Neonates that had CRP > 5 mg/L and PCT > 2 ng/L were 82 (30%) and 93 (34%) respectively. Gestational age-related differences were observed in the median (range) levels of PCT and CRP in this cohort of neonates. No specific trend was observed in the admission laboratory investigations based on gestational age, however, neutrophil number and CRP level were found to increase with increasing gestational age. Results of relevant admission laboratory investigations are summarized (according to gestational age) in Table 4.3.

4.1.2 Selected outcome measures

In all, 282 (87.5%) of the recruited neonates were discharged and 39 (12%) died. The median (range) of admission duration for those discharged was 9.5 (4 – 44) days. One baby was transferred to the pediatric surgical ward on account of paraphimosis. Mortality was highest among extreme preterm neonates (n = 10, 71.4%), and those with birth weight less than 1 kg (n = 8, 88.9%). The proportion of neonates with CRP greater than 5 mg/L that died was 8/39 (20.5%). Neonates who died having PCT greater than 2 ng/mL were 18 out of 39 (46.2%). Independent *t*-test of proportions showed that there was no statistical difference ($p > 0.05$) between the overall mortality (12%) and mortality associated with CRP > 5 mg/L (20.5%). However, there was a statistical difference ($p < 0.001$) when the proportion of neonates with PCT > 2 ng/mL was compared with overall mortality (12%). Characteristics of neonates that died are summarized in Table 4.4.

Table 4.1: Admission demographic and clinical data of recruited neonates (n = 322)

PARAMETER	**NUMBER: PERCENT**				
Sex	Male = 160 (49.7%)				
Gestational Age (weeks)	**> 42**	**37 – 42**	**32 – 36**	**28 – 31**	**< 28**
	6 (1.9%)	153 (47.5%)	90 (28%)	59 (18.3%)	14 (4.3%)
Birth weight (kg)	**> 4**	**2.5 – 4**	**1.5 – 2.4**	**1 – 1.4**	**< 1**
	14 (4.3%)	148 (46%)	105 (32.6%)	46 (14.3%)	9 (2.8%)
Apgar Score at 5 mins	**8 – 10**	**4 – 7**	**< 4**		
	127 (39.4%)	191 (59.4%)	4 (1.2%)		
Fasting blood glucose (mmol/L)	**> 5.6**	**2.2 – 5.6**	**< 2.2**		
	39 (12.1%)	264 (82%)	19 (5.9%)		
Temperature (°C)	**> 37.4**	**36.5 – 37.4**	**< 36.5**		
	4 (1.2%)	192 (59.8%)	126 (39%)		
Heart rate (bpm)	**> 160**	**100 – 160**	**< 100**		
	65 (20.2%)	252 (78.3%)	5 (1.5%)		
Respiratory rate (cpm)	**> 80**	**60 – 80**	**< 60**		
	88 (27.3%)	128 (39.8%)	106 2.9%)		

Table 4.2: Provisional diagnosis of recruited neonates (n = 322)

Provisional diagnosis	Number (percent)
Prematurity + SNS	54 (16.8%)
Prematurity + BA + SNS	18 (5.6%)
Prematurity + IUGR +SNS	3 (0.9%)
Prematurity + BA + IUGR + SNS	1 (0.3%)
Prematurity + BA + jaundice + SNS	3 (0.9%)
Prematurity + RDS + SNS	74 (23%)
Prematurity + RDS + BA + SNS	4 (1.2%)
Prematurity + RDS + HIV exposed + SNS	1 (0.3%)
Prematurity + RDS + IUGR + SNS	1 (0.3%)
Prematurity + RDS + jaundice + SNS	3 (0.9%)
Prematurity + severe anemia + SNS	1 (0.3%)
SNS	101 (31.4%)
BA + SNS	52 (16.2%)
BA + severe anemia + SNS	1 (0.3%)
Respiratory distress (uncertain) + SNS	2 (0.6%)
Subgaleal bleed + SNS	1 (0.3%)
Congenital pedal edema + SNS	1 (0.3%)
Rhesus incompatibility + SNS	1 (0.3%)

BA = Birth asphyxia, IUGR = Intrauterine growth retardation, RDS = Respiratory distress syndrome, SNS = Suspected neonatal sepsis

Table 4.3: FBC, clinical chemistry, CRP and PCT of recruited neonates based on gestational age

PARAMETER	**> 37 weeks (n = 140)**	**32 – 36 weeks (n = 77)**	**28 – 31 weeks (n = 48)**	**< 28 weeks (n = 8)**
Hemoglobin (g/dL)	17 (6.6 – 23.7)	16.8 (8.2 – 24.9)	17 (8.1 – 23.9)	15.1 (11.4 - 20)
WBC ($x10^3/\mu L$)	13.75 (3.4 – 40)	11.5 (2.6 – 36.4)	11.65 (2.9 – 49.8)	14.5 (7.8 – 31)
Neutrophil number	8.9 (2.3 – 27)	5.2 (2 – 21.7)	4.8 (1.3 – 25.9)	4.65 (2.6 – 13.6)
Lymphocyte number	4.8 (1.7 – 29.9)	4.4 (1.2 – 25.9)	6.3 (1.8 – 36.2)	7.7 (2.5 – 18.4)
Platelets ($x10^3/\mu L$)	191 (20.5 – 774)	217 (37 – 548)	190 (42 – 439)	182 (87 – 292)
Urea (mmol/L)	5.42 (1.76 – 36.9)	6.87 (1.9 – 26.1)	6.11 (1.3 – 64.72)	7.14 (3.32 – 20.7)
Creatinine (μmol/L)	85.1 (36 – 233.9)	89 (36.8 – 232.7)	78 (22.1 – 213.7)	116 (69.4 – 249.2)
Total bilirubin (μmol/L)	42 (14.9 – 233.9)	52.5 (22 – 285.2)	51 (13.29 – 308)	71 (26.6 – 126.6)
CRP (mg/L)	4.3 (0.27 – 45.6)	2.1 (0.29 – 56.6)	1.41 (0.18 – 24.9)	1.25 (0.13 – 15)
PCT (ng/mL)	1.26 (0.12 – 26.6)	9.98 (0.19 – 47.8)	1.34 (0.2 – 13.2)	5.3 (2.27 – 8.22)

CRP = C-reactive protein

PCT = Procalcitonin

Table 4.4: Characteristics of recruited neonates that died

	NUMBER: PERCENT				
Number	39/322 (12%)				
Sex	Male = 17/39 (43.6%)				
Culture positivity	8/39 (20.5%)				
Gestational age-related case fatality	**> 42 wks**	**37 – 42 wks**	**32 – 36 wks**	**28 – 31 wks**	**< 28 wks**
	0	4/153 (2.6%)	7/90 (7.8%)	18/59 (30.5%)	10/14 (71.4%)
Birth weight-related case fatality	**> 4 kg**	**2.5 – 4 kg**	**1.5 – 2.4 kg**	**1 – 1.4 kg**	**< 1 kg**
	0	7/148 (4.7%)	5/105 (4.7%)	19/46 (41.3%)	8/9 (88.9%)

4.2 POPULATION PHARMACOKINETICS OF AMIKACIN

4.2.1 Characteristics of patients

There were 247 neonates with complete data for Pop PK analysis. The mean GA and BW of neonates (± SD) were 35.3 ± 4.62 weeks and 2.5 ± 0.98 kg respectively. Male neonates constituted 131 (53%), while female neonates were 116 (47%). Thirteen out of the 247 (5.26%) had positive blood culture results. In all, 93 neonates (38%) received amikacin with aminophylline and 154 (62%) received amikacin only. The mean creatinine clearance calculated from serum creatinine for the population was 20.7 ± 10 mL/min. Median (range) postnatal age of neonates was 1.23 (0.16 – 21.75)

4.2.2 Serum amikacin concentration

The mean amikacin serum concentration (± SD), one hour after the 3rd and 7th amikacin doses (Cpeak) was 20.56 ± 8.7 µg/mL. The proportion of neonates with Cpeak > 35 µg/mL was 9/88 (10.2%). Neonates with Cpeak < 20 µg/mL were 55 out of 88 (62.5%) measurements. The mean amikacin serum concentration, 30 minutes before the 4th and 8th amikacin doses (Ctrough) was 6.68 ± 3.86 µg/mL. The proportion of neonates with Ctrough < 5 µg/mL was 42/111 (37.8%). Neonates with Ctrough > 10 µg/mL were 18 out of 111 (16.2%) measurements. Serum amikacin concentration of neonates at various sample times after the 3rd and 7th amikacin doses is shown in Fig. 4.1. A plot of trough amikacin concentration against serum creatinine levels in neonates (Fig. 4.2) showed minimal correlation ($R^2 = 0.0231$, $p = 0.158$).

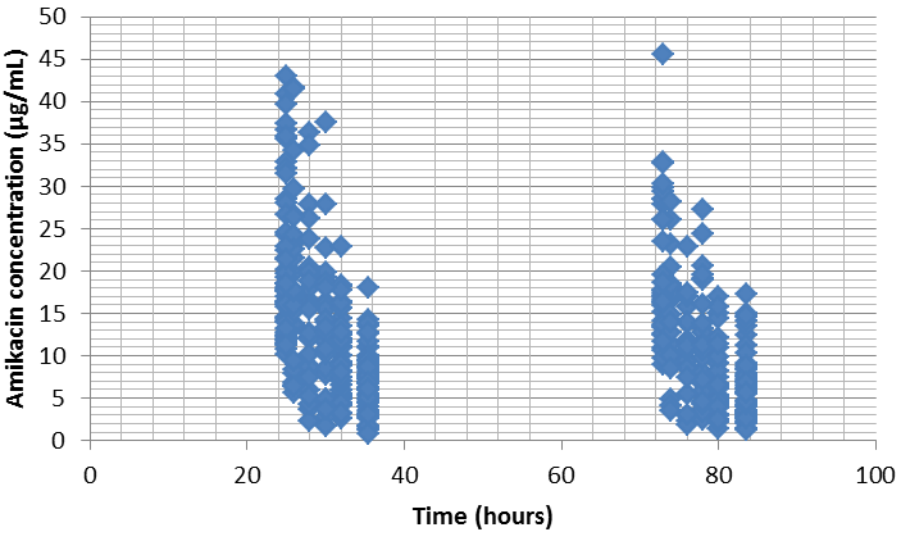


Fig. 4.1: Serum amikacin concentration of neonates sampled according to full pharmacokinetic screen after 3rd and 7th amikacin doses

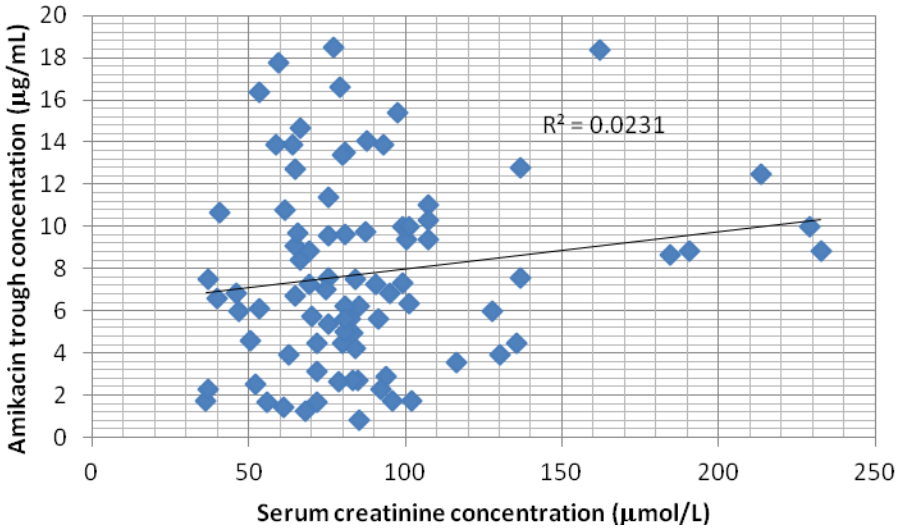


Fig. 4.2: A plot of serum amikacin trough concentration against serum creatinine levels of recruited neonates

4.2.3 Model building process

A comparison of structural models, one- and two-compartment showed that a one-compartment best described data. A change in OFV of +17.916 was obtained when the two-compartment model was compared with one-compartment. Population values of CL and V (CL_{TV} and V_{TV}) in the base model were modeled as though they were the same for all individuals ($CL_{TV}= \theta_1$, $V_{TV}= \theta_2$). After this, incorporation of covariates into the base model was done. Neonates were categorized into those who received amikacin only (Group 0), and those who received amikacin with aminophylline (Group 1). Incorporation of this covariate (administration of amikacin with or without aminophylline) showed varying clearance (CL) and distribution volume (V) for both groups (Group 0 and Group 1). Population CL (CL_{TV}) was 0.187 L/h and 0.07 L/h for amikacin only (Group 0) and amikacin with aminophylline (Group 1) respectively. Population V (V_{TV}) was also 4.11 L and 1.38 L for Group 0 and Group 1 respectively. Pharmacokinetic parameter covariate plots for those who received amikacin $\pm$ aminophylline are shown in Fig 4.3: A and B. Although ΔOFV was significant, incorporation of amikacin $\pm$ aminophylline into the model did not improve ω^2 on BSV when compared to the base model.

Initial analysis of PK parameter versus covariate plot showed that birth weight (BW) would explain some of the variability in CL and V (Fig 4.4: A and B). It was found that BW as a power function would correlate better with CL and V, than linear function. Incorporation of BW in the model was found to significantly improve its fit (ΔOFV < -3.84). BW also showed a low variance (ω^2) on BSV when its effect on CL and V was

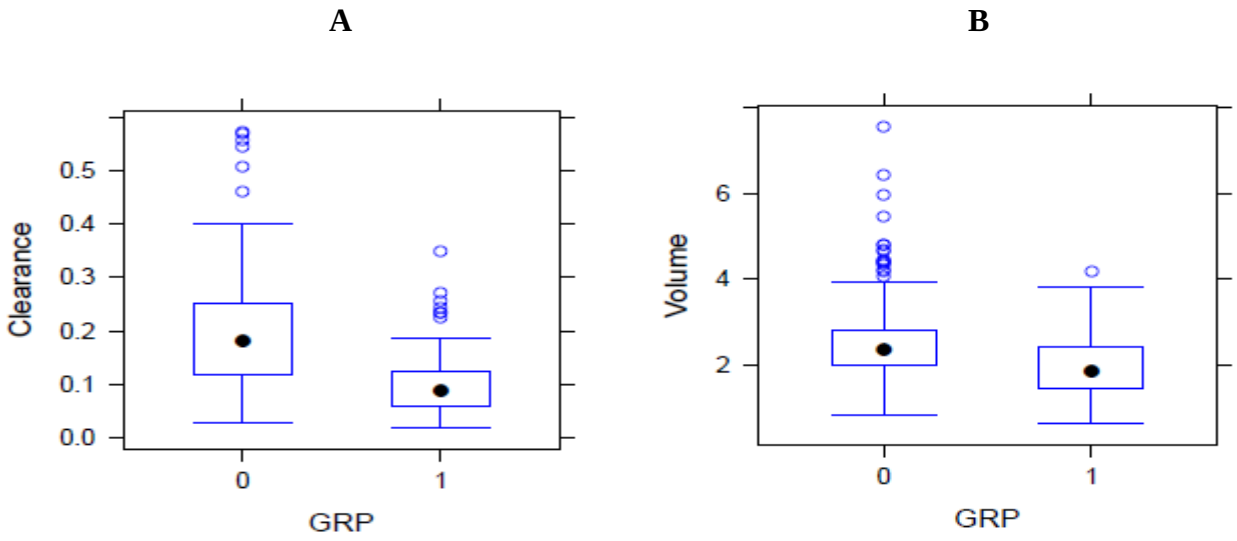
assessed. Although some correlation was identified in the parameter covariate plots (Fig 4.5: A and B), incorporation of gestational age did not significantly improve model (based on ω^2 on BSV).

Addition of the covariate, culture proven pathogenic bacteria, did not improve the model (ΔOFVs -1.255 and -0.357 on CL and V respectively). Similarly, the addition of gender and the acute phase proteins, CRP and PCT as covariates, did not have any significant influence on the model, as evident in initial parameter-covariate plots [Fig. 4.6 (A and B), 4.7 (A and B), and Fig 4.8 (A and B) respectively]. There was no significant influence of postnatal age when it was incorporated as a covariate in the model, parameter-covariate plots shown in Fig 4.9 A and B. Incorporation of Apgar score at 5 minutes and creatinine clearance did not improve ω^2 on BSV during model building process.

The influence of birth weight combined with creatinine clearance as covariates on CL did not improve the model (ΔOFV = -0.244). The influence of birth weight combined with Apgar score at 5 minutes as covariates on CL appeared to improve the model (ΔOFV < -3.84). However, this additional parameter did not improve ω^2 on BSV. A similar trend, little improvement in ω^2 on BSV, was observed when these same combined covariates (birth weight and Apgar score at 5 minutes) were assessed for their influence on V.

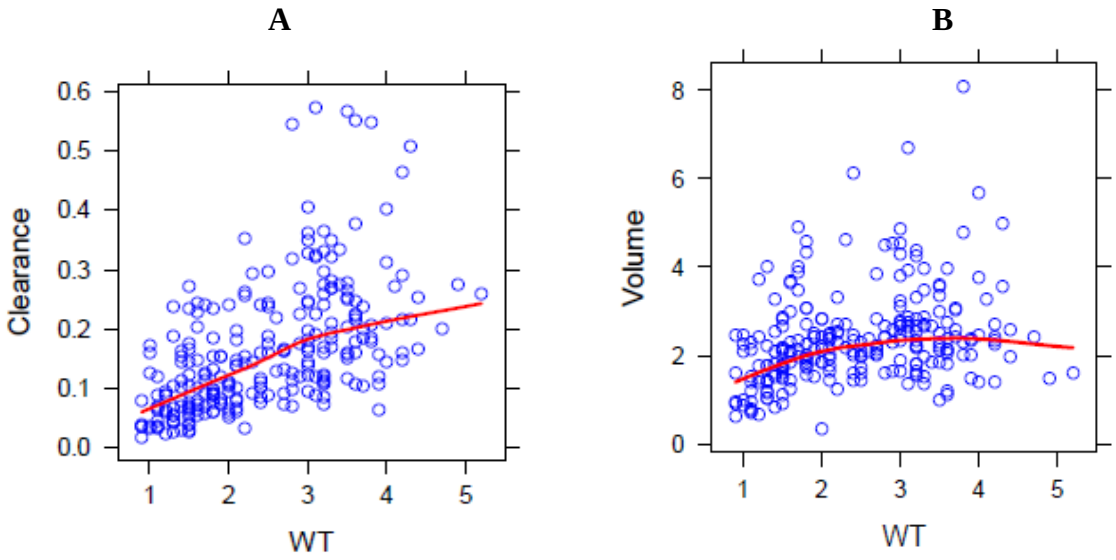
Models that had birth weight showing most influence on CL and V and explaining part of the BSV were subsequently merged. A block structure was added to separate the influence of ω^2 on BSV on CL and V. This showed a significantly better fit of the model.

The use of first order conditional estimation with interaction (FOCEI) resulted in better precision in the PK parameters estimated. Proportional error resulted in a better fit for the final model compared with combined error model (additive and proportional). A summary of the process of model building from the base model (Model 1) to the final model (Model 18) is shown in Table 4.5.



GRP 0 = Amikacin alone group GRP 1 = Amikacin + aminophylline group

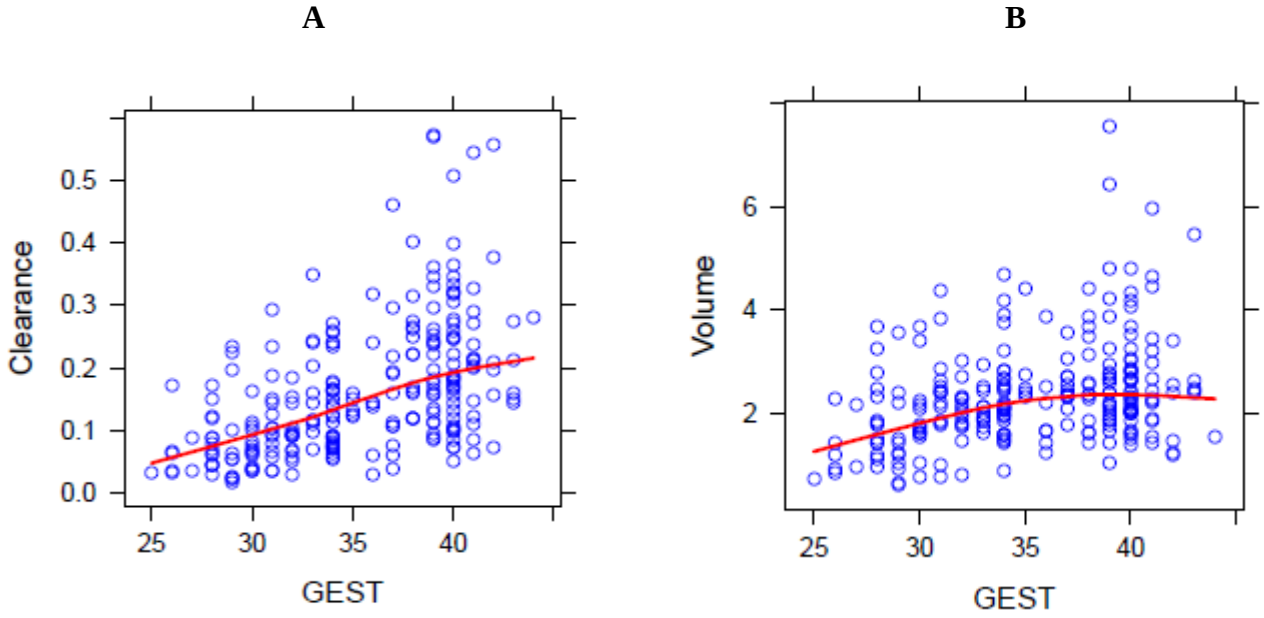
Fig. 4.3 A: Clearance (L/h) derived from the base model (Model 1) versus GRP (0:1)
Fig. 4.3 B: Volume (L) derived from the base model (Model 1) versus GRP (0:1)



WT = Birth weight

Fig. 4.4 A: Clearance (L/h) derived from the base model (Model 1) versus birth weight (kg)

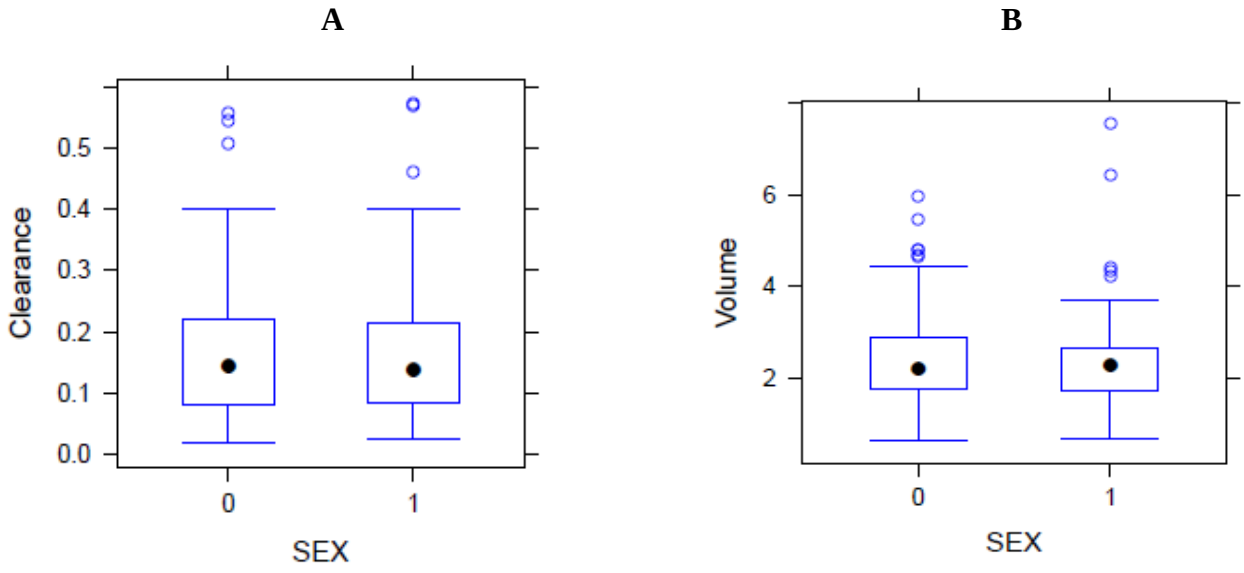
Fig. 4.4 B: Volume (L) derived from the base model (Model 1) versus birth weight (kg)



GEST = Gestational age

Fig. 4.5 A: Clearance (L/h) versus gestational age (weeks)

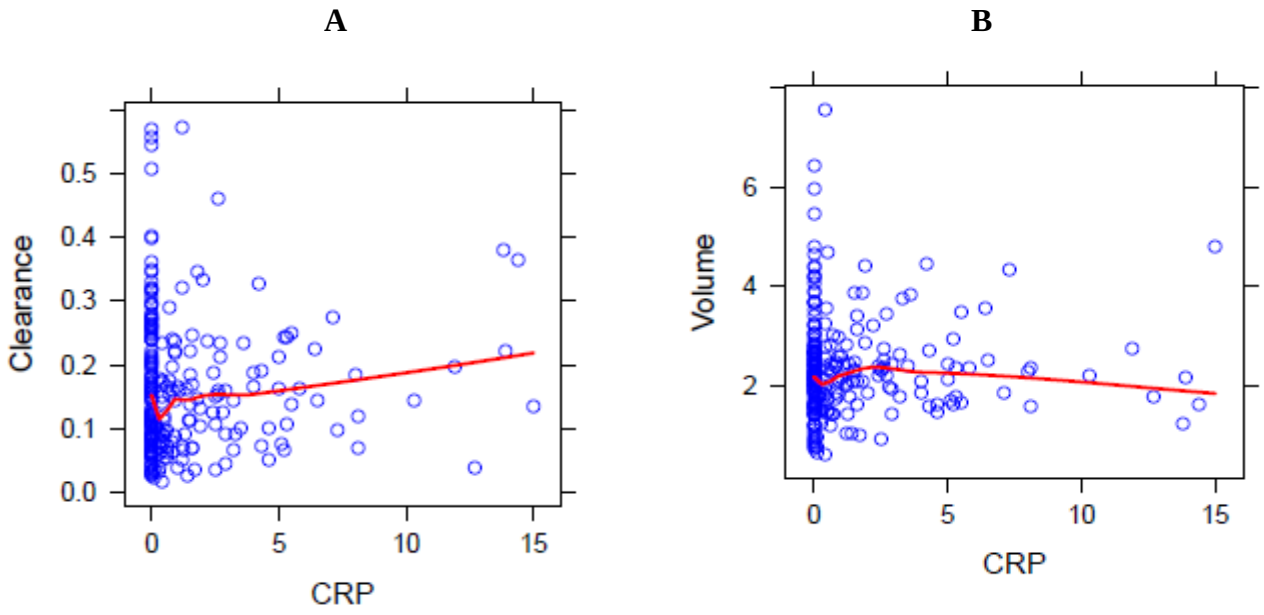
Fig. 4.5 B: Volume (L) versus gestational age (weeks)



0 = Male, 1 = Female

Fig. 4.6 A: Clearance (L/h) derived from the base model (Model 1) versus sex

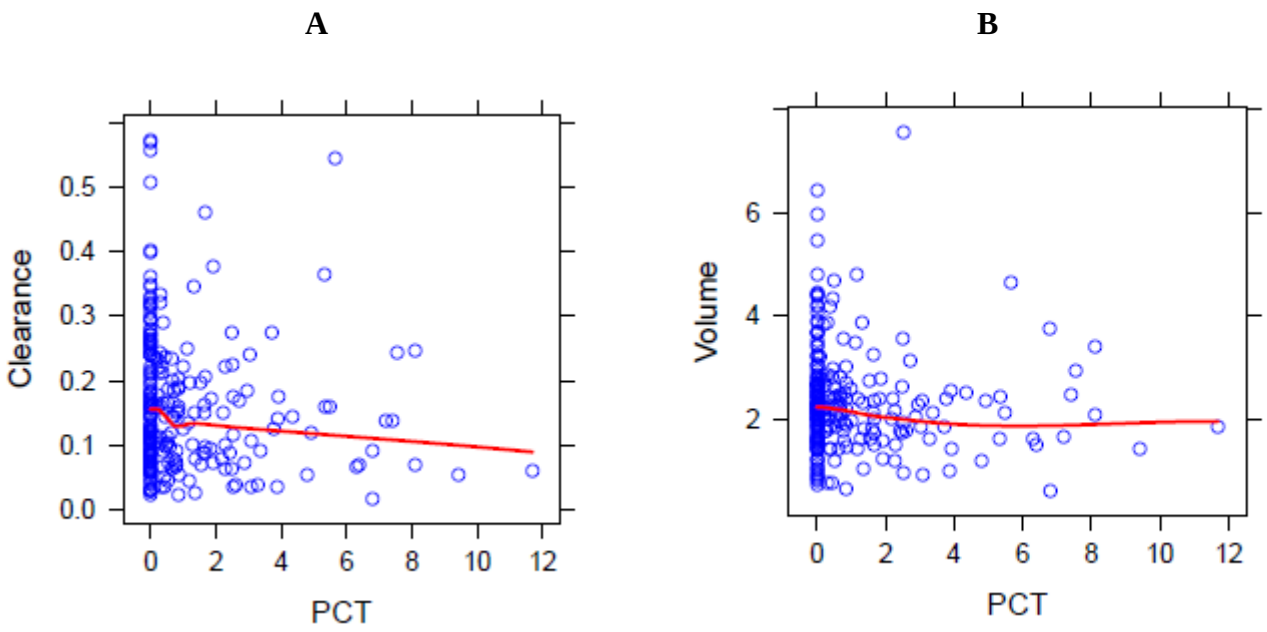
Fig. 4.6 B: Volume (L) derived from the base model (Model 1) versus sex



CRP = C-reactive protein

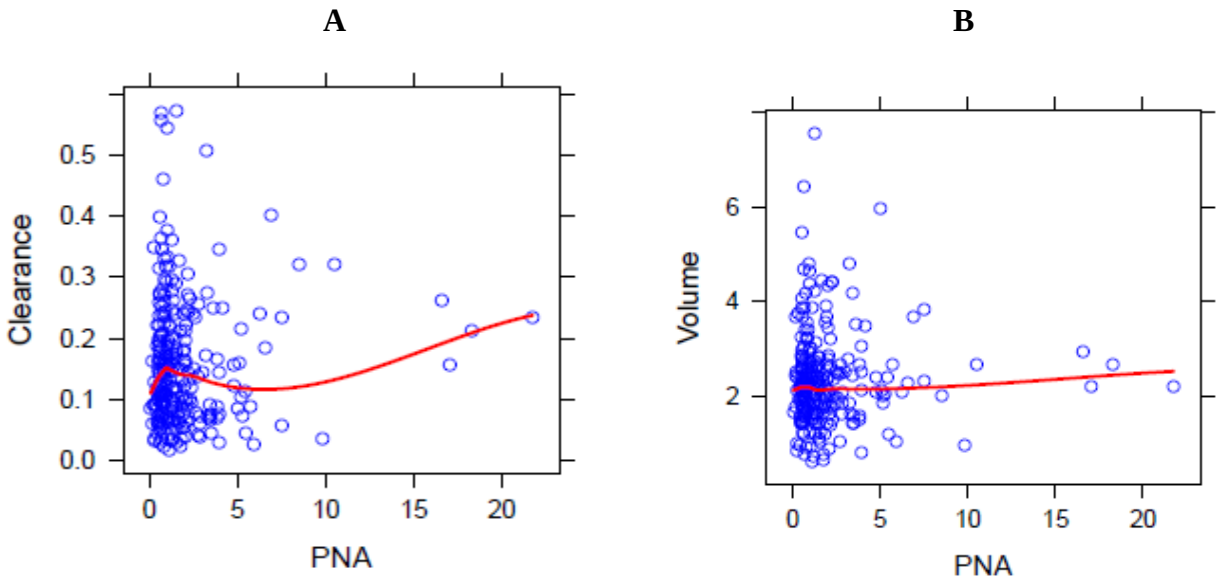
Fig. 4.7 A: Clearance (L/h) derived from the base model (Model 1) versus CRP (mg/L)

Fig. 4.7 B: Volume (L) derived from the base model (Model 1) versus CRP (mg/L)



PCT = Procalcitonin

Fig. 4.8 A: Clearance (L/h) derived from the base model (Model 1) versus PCT (ng/mL)
Fig. 4.8 B: Volume (L) derived from the base model (Model 1) versus PCT (ng/mL)



PNA = Postnatal age

Fig. 4.9 A: Clearance (L/h) derived from the base model (Model 1) versus PNA (hrs)
Fig. 4.9 B: Volume (L) derived from the base model (Model 1) versus PNA (hrs)

Table 4.5: Model development process

Model No.	Model description	OFV	Ref. Model	ΔOFV	ω^2 on BSV (%) CL, V
1	One-compartment (ETA on CL, V)	2232.1	-	-	96.5, 99.7
2.	Two-compartment (ETA on CL, V_1)	2250.0	1	+17.916	105.3, 163.4
3.	One-compartment, $CL_{TV} = \theta_1$: GRP= 0 $CL_{TV} = \theta_2$: GRP=1	2161.3	1	-70.749	74.6, 103.5
4.	One-compartment, $V_{TV} = \theta_1$: GRP=0 $V_{TV} = \theta_2$: GRP=1	2196.9	1	-35.147	99.1, 81.8
5.	One-compartment, $CL_{TV} = \theta_1 (BW/2.5)^{\theta 2}$	2094.4	1	-137.693	54.6, 92.9
6.	One-compartment, $CL_{TV} = \theta_1 (GEST/35.3)^{\theta 2}$	2128.6	1	-103.481	64.7, 97
7.	One-compartment, $CL_{TV} = \theta_1 (CRCL/20.7)^{\theta 2}$	2157.9	1	-74.132	72.5, 102.9
8.	One-compartment, $CL_{TV} = \theta_1 (APG5/7.2)^{\theta 2}$	2204.1	1	-27.943	88.6, 90
9.	One-compartment, $CL_{TV} = \theta_1$: SEP=0 $CL_{TV} = \theta_2$: SEP=1	2230.8	1	-1.255	96.2, 99.2
10.	One-compartment, $V_{TV} = \theta_1 (BW/2.5)^{\theta 2}$	2148.8	1	-83.265	71.8, 91.9
11.	One-compartment, $V_{TV} = \theta_1 (GEST/35.3)^{\theta 2}$	2179.0	1	-53.117	101,59.6
12.	One-compartment, $V_{TV} = \theta_1 (APG5/7.2)^{\theta 2}$	2217.5	1	-14.6	98.4, 84.1
13.	One-compartment, $V_{TV} = \theta_1$: SEP=0 $V_{TV} = \theta_2$: SEP=1	2231.7	1	-0.357	96.5, 99.7
14.	One-compartment, $CL_{TV} = \theta_1 (BW/2.5)^{\theta 2} \times \theta_3 (CRCL/20.7)^{\theta 4}$	2094.1	5	-0.244	54.6, 93.4
15.	One-compartment, $CL_{TV} = \theta_1 (BW/2.5)^{\theta 2} \times \theta_3 (APG5/7.2)^{\theta 4}$	2089.8	5	-4.63	55.1, 87.4
16.	One-compartment, $V_{TV} = \theta_1 (BW/2.5)^{\theta 2} \times \theta_3 (APG5/7.2)^{\theta 4}$	2145.2	10	-3.615	72.2, 85.8
17.	One-compartment, $CL_{TV} = \theta_1 (BW/2.5)^{\theta 3}$, $V_{TV} = \theta_2 (BW/2.5)^{\theta 4}$	2058.1	5	-36.264	59.5, 54.6
18.	One-compartment, $CL_{TV} = \theta_1 (BW/2.5)^{\theta 3}$, $V_{TV} = \theta_2 (BW/2.5)^{\theta 4}$, + Block ($\eta_{CL}$, η_v)	2044.4	17	-13.708	58.9, 50.7

GRP=0: Amikacin only, GRP=1: Amikacin + aminophylline, BW: birth weight, GEST: gestational age, CRCL: creatinine clearance, APG5: Apgar score at 5 minutes, SEP: culture proven pathogenic bacteria

4.2.4 Model comparison and evaluation

Goodness of fit plots, population and individual predictions versus observations, and conditional weighted residuals versus population predictions, of the final model are depicted in Fig. 4.10 A and B, respectively. Parameter estimates for the final model obtained after bootstrapping are shown in Table 4.6. The table also shows the means and 95% confidence intervals (CIs) of PK parameter estimates from a 1000 bootstrap sampling. Mean values from bootstrapping were close to the population mean for all the parameters. Individual observations (DV) and individual predictions (IPRED) versus time as well as individual observations (DV) and population predictions (PRED) versus time after final model are shown at Appendix 1.

From the final model, the individual CL and V are related by the equations:

$CL_i = [\theta_1 (BW/2.5)^{\theta 3}] \times EXP (\eta_{CLi})$ …….. *Eqn 4.1*

$V_i = [\theta_2 (BW/2.5)^{\theta 4}] \times EXP (\eta_{Vi})$ ..... *Eqn 4.2*

A **B**

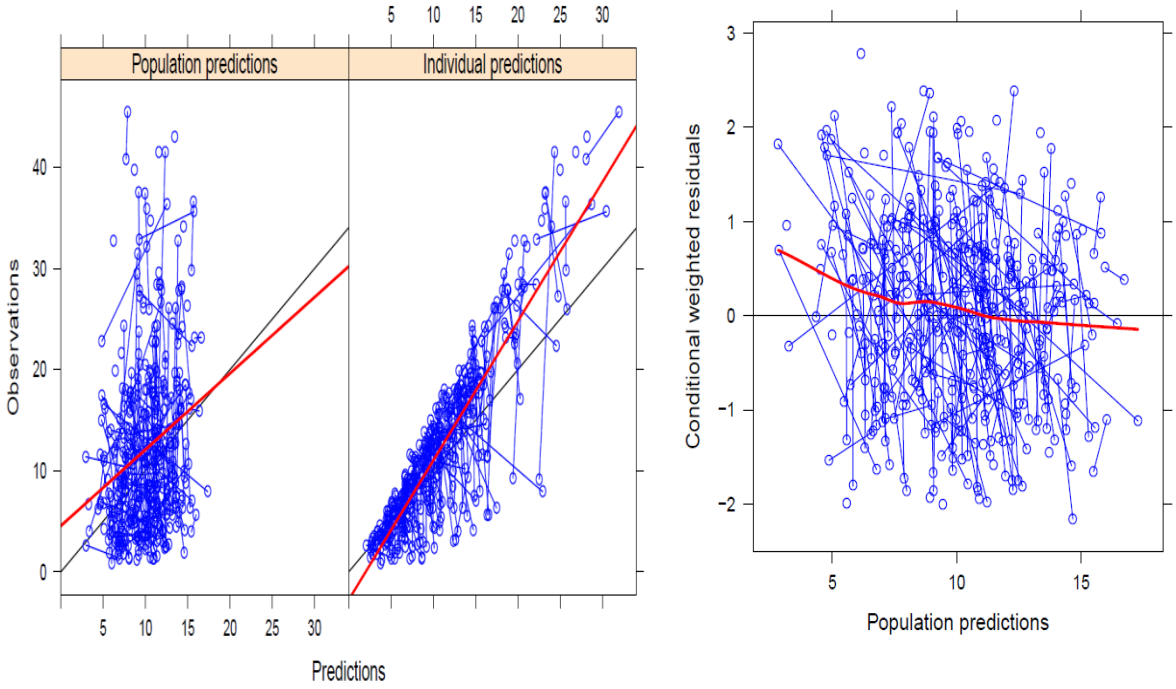


Fig. 4.10 A: Observed data (mg/L) versus population and individual predictions (mg/L) from the final model. Black line is line of identity and the red line is data smooth

Fig. 4.10 B: Conditional weighted residuals (SD) versus population predictions (mg/L) plot. Black line is a zero line and red line is a data smooth

Table 4.6: Estimates of final Pop PK model

Parameter	**Unit**	**Estimate**	**RSE (%)**	**After Bootstrap: Mean (95% CI)**
θ_1	L/h	0.153	4	0.153 (0.139 – 0.167)
θ_2	L	2.94	9	2.97 (2.5 – 3.47)
θ_3		1.31	8	1.32 (1.11 – 1.55)
θ_4		1.18	16	1.18 (0.737 – 1.53)
BSV				
CL	%	58.9	7	58.0 (47.8 – 67.9)
V	%	50.7	28	50.8 (31.0 – 84.2)
Residual variability				
Proportional	%	46.1	4.5	46.1 (41.4 – 51.0)

θ_1 = Population clearance, θ_2 = Population volume θ_3 = Power function on weight with clearance, θ_4 = Power function on weight with volume, BSV = Between subject variability RSE = Relative standard error, CL = Clearance, V = Volume of distribution

The mean half-life (95% CI) of amikacin calculated from the final model was 13.6 (13.3 – 13.8) hours.

4.3 BRAINSTEM AUDITORY EVOKED POTENTIAL

4.3.1 Characteristics of patients

The BAEP was done in 20 neonates administered amikacin (test subjects), and 5 neonates who did not receive amikacin (comparison group). The mean gestational age, postnatal age and birth weight of test subjects (n = 20) were 36.0 $\pm$ 3.52 weeks, 6.76 $\pm$ 2.15 hours and 2.7 $\pm$ 0.93 kg respectively. The comparison group (n = 5) had mean gestational age, postnatal age and weight of 37.0 $\pm$ 3.54 weeks, 4.28 $\pm$ 2.56 hours and 2.5 $\pm$ 0.83 kg respectively. The mean gestational age, postnatal age and birth weight between the groups were comparable.

4.3.2 BAEP threshold

At a frequency range of 2 – 4 kHz, the BAEP threshold at baseline and follow-up of test subjects and comparison groups are shown in Fig 4.11 and 4.12 respectively. Analysis with Wilcoxon signed rank test showed no significant difference between the baseline and follow-up BAEP thresholds ($p = 0.209$) of the left ear of test subjects. No significant difference was found in the baseline and follow-up BAEP thresholds ($p = 0.225$) of the right ear of test subjects. No statistical difference also existed in the baseline and follow-up BAEP thresholds of the left ears ($p = 0.70$) of comparison group. The difference in baseline and follow-up BAEP threshold of the right ear of comparison group was not statistically significant ($p = 0.11$).

Five of the test subjects, numbers 2, 7, 11, 18 and 20, showed BAEP thresholds > 30 dBnHL at baseline. A BAEP threshold > 30 dBnHL was also observed for the

comparison subject number 2 (Fig. 4.12). However, follow-up audiograms showed lower BAEP thresholds for these subjects. At a frequency range of 2 – 4 kHz, test subject number 17 showed a baseline BAEP threshold of 30 dBnHL and follow-up 40 dBnHL. Overall, five out of 20 neonates (25%) who received amikacin treatment including test subject number 17 had mean (after 3rd and 7th doses) serum amikacin trough concentrations > 10 μg/mL. The clinical characteristics of these five neonates are shown in Table 4.7. The BAEP reading of test subject number 17 is shown at Appendix 2.

Table 4.7: Clinical characteristics of neonates with high (> 10 μg/mL) amikacin trough concentrations

Test subject	Trough amikacin level (μg/mL)	Gestational age (weeks)	Birth weight (kg)	Clinical outcome (length of stay)
7	11.97	38	3	Discharged (6 days)
8	12.69	34	1.6	Discharged (5 days)
12	14.65	42	3.7	Discharged (5 days)
17	12.22	40	3.2	Discharged (6 days)
19	10.56	34	1.9	Discharged (5 days)

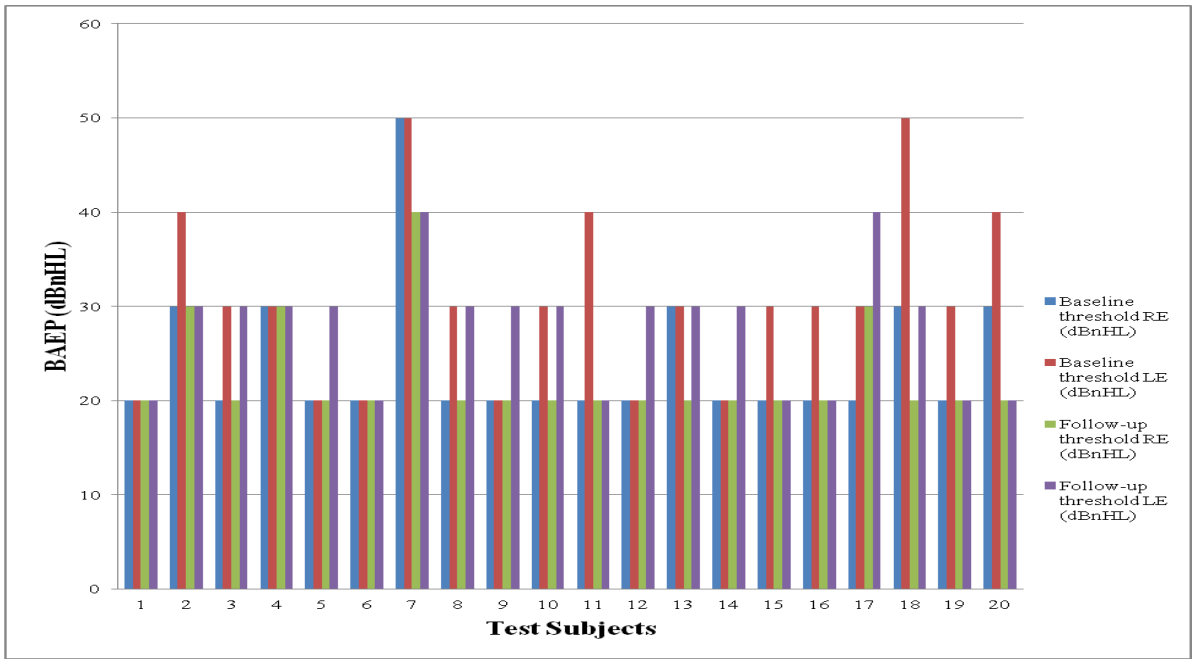


Fig. 4.11: BAEP threshold of test subjects at baseline and follow-up in both ears

**RE = right ear, LE = left ear*

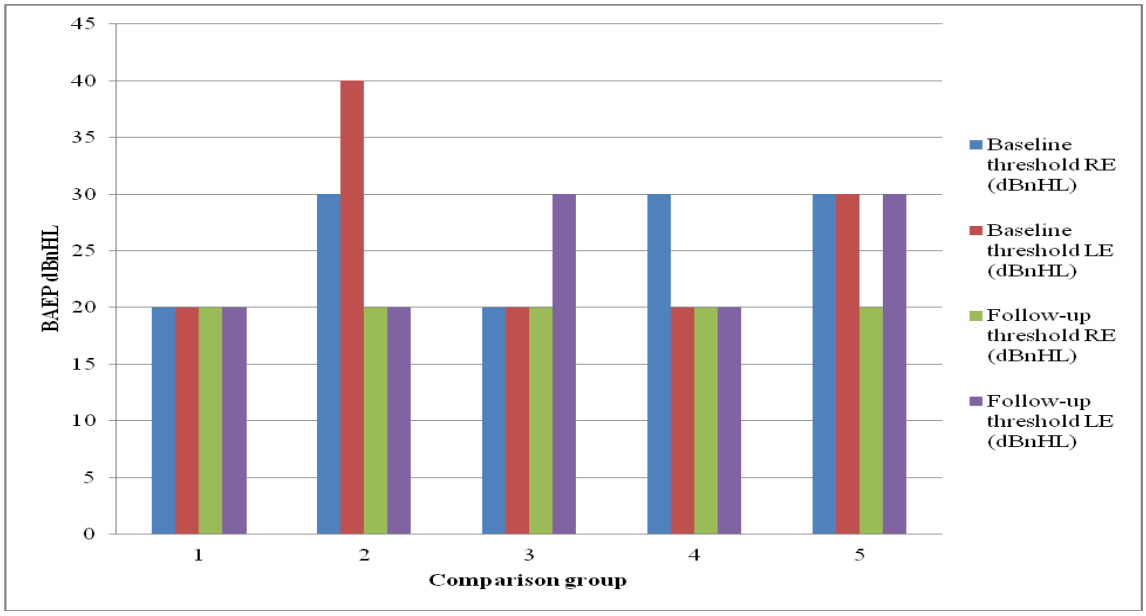


Fig. 4.12: BAEP threshold of comparison group at baseline and follow-up in both ears

** RE = right ear, LE = left ear*

4.4 PCT VERSUS CRP AS DIAGNOSTIC MARKERS OF NEONATAL SEPSIS

4.4.1 Patient characteristics

The 62 neonates whose data was available for this component of the study were categorized into three groups based on a modified septic score described by Gitto *et al*,[119] and Ali *et al*,[120] into: highly probable (22 neonates), probable (19 neonates) and a less probable group (21 neonates). There was no statistical difference ($p > 0.05$) in gestational age, birth weight and sampling time (postnatal hours) between the three groups. A significant difference ($p < 0.001$) was found in mean PCT levels between the three groups. These baseline characteristics of neonates and mean CRP and PCT values (pre-antibiotic treatment observations) in each category are shown in Table 4.8.

The mean CRP of the highly probable group was more than 2-fold that of the probable or less probable groups. However, the random variability around the mean was high in the highly probable group, probably explaining the between-group difference which tends towards, but did not attain statistical significance.

There were 91% (20/22) and 31.6% (6/19) neonates with elevated PCT levels (> 2 ng/mL) in the highly probable and probable groups. An independent *t*-test for proportions showed the difference between 91% and 31.6% was statistically significant ($p < 0.001$). There were 54.5% (12/22) and 26.3% (5/19) of neonates with elevated CRP levels (> 5 mg/L) in the highly probable and probable groups, a test for proportions showed difference was not statistically significant ($p = 0.067$).

Table 4.8: Baseline characteristics of study patients with mean PCT and CRP (n = 62)

Characteristic	Highly probable (n = 22)	Probable (n = 19)	Less probable (n = 21)	p-value
Gestational age (wks)	33.18±4.27	32.53±4.18	34.76±4.27	0.234
Birth weight (g)	2.09±0.90	1.69±0.67	2.35±1.11	0.080
Sex (Female)	15(68.2%)	9(47.4%)	10(47.6%)	0.294
Sampling time (Postnatal hrs)	5.45±3.28	4.13±2.79	6.03±2.23	0.102
PCT (ng/mL)	4.15±2.16	1.52±1.18	0.75±0.55	<0.001
	95% CI (-0.08, 8.38)	95% CI (-0.79, 3.83)	95% CI (-0.33, 1.83)	
CRP (mg/L)	7.43±13.35	2.91±3.95	1.96±1.81	0.079
	95% CI (-18.74, 33.6)	95% CI (-4.83, 10.65)	95% CI (-1.59, 5.5)	

4.4.2 Comparison of PCT and CRP of neonates in Group 1 (highly probable) with positive and negative blood culture results

Eight neonates from the highly probable group (Group 1) had positive blood cultures. Pathogenic bacteria isolated included *Streptococcus agalactiae* (n = 2), *Klebsiella pneumonia* (n = 2), *Acinetobacter baumannii* (n = 1), *Streptococcus viridians* (n = 1), *E. coli* (n = 1), and *Burkholderia cepacia* (n = 1). A comparison of mean PCT and CRP levels in those with either positive or negative blood culture in the highly probable group is shown in Table 4.9. There was no statistically significant difference in the mean PCT ($p > 0.05$) between those with positive and negative blood culture. The mean CRP of those with positive cultures was 2-fold higher than that of the negative blood culture

group. However, the difference was not statistically significant, probably on account of limited number of sample.

4.4.3 Comparison of outcome measures between groups

The number of deaths in each group was small and would not allow for meaningful statistical comparison, as shown in Table 4.10.

Table 4.9: Comparison of CRP and PCT levels between subjects in the highly probable group according to blood culture results

Comparison	Positive Blood Culture (n = 8)	Negative Blood Culture (n = 14)	p-value
CRP (mg/L)	11.25±7.68	5.24±1.19	0.322
PCT (ng/mL)	4.25±0.85	4.10±0.56	0.880

Table 4.10 Comparison of outcome measures between the 3 groups

Outcome	Highly probable (n = 22)	Probable (n = 19)	Less probable (n = 21)
Died	3(13.6%)	2(10.5%)	1(4.8%)
Discharged	19(86.4%)	17(89.5%)	20(95.2%)

4.4.4 Receiver operator characteristics (ROC) of PCT and CRP in neonates

A comparison of the area under curve (AUC) of the receiver operator characteristics (ROC) of PCT and CRP is shown in Fig. 4.13. Although the AUC for PCT (0.646) was larger than that of CRP (0.569), the difference did not attain statistical significance. A chi-squared test yielded a *p* value of 0.088 [$\chi^2(1) = 2.91$]. A summary of ROC analysis:

sensitivity, specificity, PPV, NPV and AUC, at cut-off points of 2 ng/mL and 5 mg/L for PCT and CRP respectively is shown in Table 4.11. PCT showed higher sensitivity, PPV and NPV, but lower specificity compared to CRP.

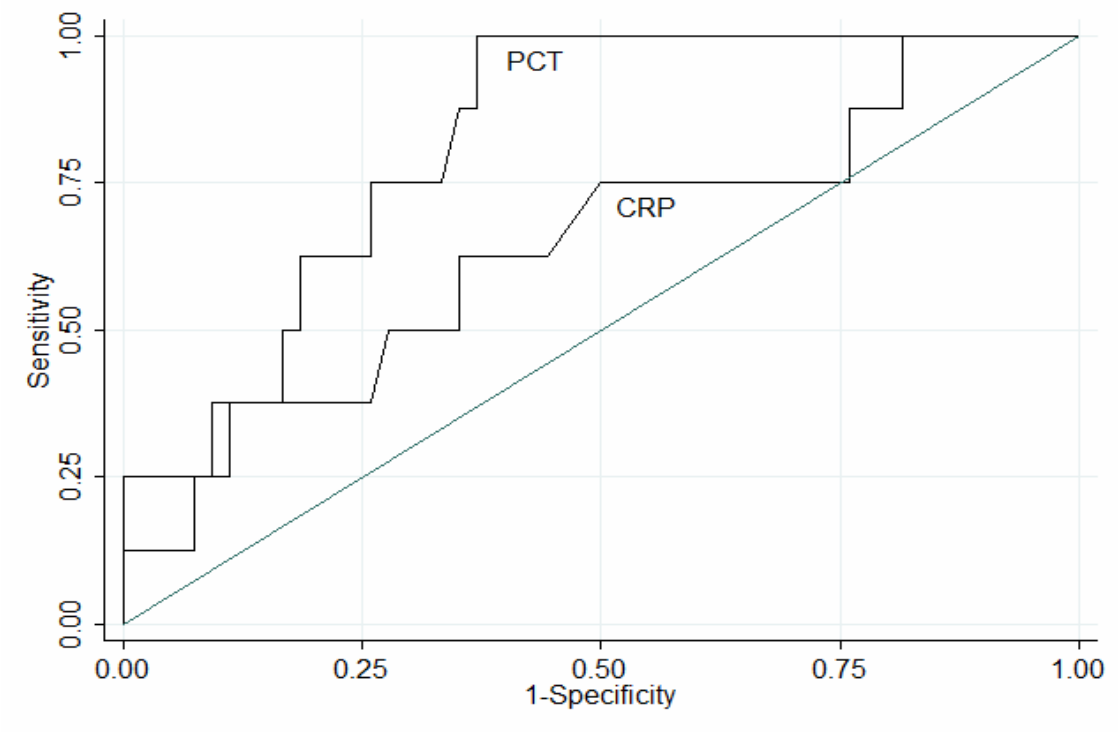


Fig. 4.13: Comparison of ROC curves of PCT and CRP

Table 4.11: ROC at specific cut-off points for PCT and CRP

	Cut-off value	Sensitivity	Specificity	PPV	NPV	AUC
CRP	5mg/L	50.0%	72.2%	37.5%	81.3%	0.569
PCT	2ng/mL	87.5%	63.0%	44.1%	93.8%	0.646

4.5 BACTERIA IDENTIFICATION AND ANTIBIOGRAM

4.5.1 Blood Culture and antimicrobial susceptibility

There was 13.6% (41/302) culture positivity (Table 4.12). The predominant isolate was CoNS (22/41). All Gram-negative bacteria showed sensitivity to the carbapenems (imipenem and meropenem). With the exception of Coliform bacillus and *Enterobacter aerogenes*, all other Gram-negative bacteria were sensitive to amikacin. *Burkholderia cepacia* and *Pseudomonas stutzeri* showed sensitivity to cephalosporins, but the other Gram-negative bacteria were resistant. The isolated CoNS showed 100% susceptibility to clindamycin and vancomycin, but not the other antimicrobials. All Gram-positive bacteria showed 100% susceptibility to penicillin, with the exception of CoNS and *Staphylococcus aureus*. Vancomycin resistance was not observed among any of the Gram-positive bacteria. The susceptibility pattern of isolated bacteria to tested antimicrobials is shown in Table 4.13.

4.5.3 Mortality and associated blood culture results

There was a 12.9% (39/302) mortality rate among recruited neonates who had blood culture data. Mortality among neonates with positive blood culture was 19.5% (8/41). A comparison of the proportion of mortality associated with blood culture positivity (8/39) and the overall mortality (39/322) showed no statistical difference ($p > 0.05$). Bacteria isolated from the 8 neonates who died were CoNS (n=6), Coliform bacillus (n=1) and *E. coli* (n=1).

Table 4.12: Distribution of positive blood culture isolates (n = 41) based on pathogenicity

Isolate	**Number (percent) of total**
Known pathogen (n = 16)	
Escherichia coli	1 (2.4%)
Klebsiella pneumoniae	2 (4.8%)
Serratia odorifera	1 (2.4%)
Burkholderia cepacia	1 (2.4%)
Coliform bacillus	1 (2.4%)
Pantoea sp.	1 (2.4%)
Enterobacter aerogenes	1 (2.4%)
Pseudomonas stutzeri	1 (2.4%)
Staphylococcus aureus	2 (4.8%)
Streptococcus agalactiae	4 (9.8%)
Streptococcus viridans	1 (2.4%)
Potential pathogen (n = 25)	
Bacillus sp.	2 (4.8%)
Corynebacterium sp.	1 (2.4%)
Coagulase negative *Staphylococcus* (CoNS)	22 (54%)
Total	41 (100%)

Table 4.13: Susceptibility (%) of isolated bacteria (n = 41) to tested antimicrobials

Antimicrobial	Gram-positive species (n=32)						Gram-negative species (n=9)							
	CoNS (n=22)	Ba s (n=2)	Cor s (n=1)	Sta A (n=2)	Stre V (n=1)	GBS (n=4)	Pseu S (n=1)	Kleb P (n=2)	Coliform (n=1)	Ente A (n=1)	Sera O (n=1)	Pant S (n=1)	*E. coli* (n=1)	Burk C (n=1)
Penicillin	36	100	100	50	100	100								
Amoxi-Clav	55			50					0	0	0	0	0	
Clindamycin	100			100	100	75								
Vancomycin	100	100	100	100	100	100								
S + T	14	50	100	50			100			0		0		
Rifampicin	68	50	100	100	100									
Erythromycin	73			100		75								
1st Gen Ceph				50				0	0		0	0	0	
Cefuroxime	55							0	0	0	0	0	0	
Ceftriaxone		100	100				100	0	0		0		0	100
Cefepime							100	0	0	0	0	0	0	100
Meropenem		100	100				100	100	100	100	100	100	100	100
Imipenem							100	100	100	100	100	100	100	100
Amikacin							100	100	0	0	100	100	100	100
Ciprofloxacin							100		0	0	100	100	100	100

0 = Resistant , 100 = Sensitive

S + T: Sulphadoxine and Trimethoprim, Amoxi-Clav: Amoxicillin and Clavulanic acid 1st Gen Ceph: First generation cephalosporin

Ba s: *Bacillus sp*., Cor s: *Corynebacterium sp*., Sta A: *Staphylococcus aureus*, Stre V: *Streptococcus viridans*, GBS: *Streptococcus agalactiae*, Pseu S: *Pseudomonas stutzeri*, Kleb P: *Klebsiella pneumoniae*, Coliform: Coliform bacillus, Ente A: *Enterobacter aerogenes*, Sera O: *Serratia odorifera*, Pant S: *Pantoea sp.*, Burk C: *Burkholderia cepacia*

CHAPTER FIVE

5.0 DISCUSSION

5.1 PATIENT CHARACTERISITICS

Amongst neonates with suspected sepsis, 163 (50.6%) were preterm. Preterm neonates that presented with respiratory distress constituted 50.9%. Provisional diagnoses also showed that 24.5% of neonates had birth asphyxia. The overall mortality in the current study was 12%. Mortality was highest among extreme preterm neonates (71.4%) as well as neonates with elevated PCT (46.2%). Neonates with positive blood cultures that died were 19.5%. Neonates with CRP greater than 5 mg/L that died were 20.5%, and those with PCT greater than 2 ng/mL that died were 46.2%. In a study that described demographics and clinical characteristics of neonates in a NICU in Uganda, the most common diagnoses were neonatal infections, prematurity, respiratory distress and asphyxia.[240] The mortality rate in this study was relatively lower than reported in a NICU in Tanzania, where mortality rate for neonatal sepsis was 19%.[241] Gebrihewot *et al* reported that, death among neonates with suspected sepsis was significantly associated with positive blood culture and multidrug resistant Gram-negative bacteria.[242]

5.2 SERUM AMIKACIN CONCENTRATION

In this current study, the mean peak and trough serum concentration of amikacin was 20.56 $\pm$ 8.7 µg/mL and 6.68 $\pm$ 3.86 µg/mL respectively. Relatively higher mean trough concentrations were reported in term (9.97 $\pm$ 5.67 µg/mL) and preterm neonates (14.4 $\pm$ 9.28 µg/mL) in a drug monitoring study conducted in Pakistan.[243] It has been reported elsewhere that the normal range for multiple therapeutic regimens of amikacin should be

a trough concentration of less than 10 µg/mL, and a peak of 20 – 30 µg/mL.[244] Some neonates in this current study, 18 out of 111 (16.2%), had serum trough amikacin concentration > 10 µg/mL, which has been reported to be potential toxic concentrations.[244]

5.3 POPULATION PHARMACOKINETICS OF AMIKACIN

5.3.1 Compartmentalization

This study showed that concentration-time data of amikacin in neonates best fitted a one-compartment model. This is consistent with other studies that have also reported a one-compartment model for amikacin disposition in neonates.[60,61,226,245] On the contrary, others have reported two-compartment models for amikacin disposition, but these studies were conducted in adult patients.[246,247] After parenteral administration of aminoglycosides, they are primarily distributed within the extracellular fluid and eliminated almost completely unchanged in the urine primarily by glomerular filtration. Aminoglycosides ability to penetrate biologic membranes is poor because of the drug's polar nature, and intracellular concentrations are usually low. For these reasons, the drug is likely to achieve near-homogenous distribution, typical of a drug that shows a one-compartment drug disposition.

5.3.2 Co-administration of amikacin with or without aminophylline

In the current study, there was a difference in the clearance of neonates who received amikacin only and those who received amikacin with aminophylline. The mean clearance among those who received amikacin only (CL_{TV} = 0.187 L/h) was higher compared with

those who received amikacin with aminophylline (CL_{TV} = 0.07 L/h). Neonates who received aminophylline were of gestational ages ≤ 34 (range: 25 – 34), whiles those who did not receive aminophylline were of GA > 34 weeks. Generally renal clearance of drugs increases with increasing gestational age, postnatal age, and body weight.[177] Hence, the difference observed in clearance could be due to the greater weight of the amikacin only group, even though gestational age and postnatal age did not appear to have an effect on the current model. There is limited information of the interaction between amikacin and aminophylline, however *in vitro* studies suggest methylxanthines can potentiate or inhibit certain antimicrobial agents.[48,49] Also, a study that also looked at the effect of concomitant administration of NSAIDs with amikacin reported that NSAIDs reduced amikacin clearance on the first day of life.[226]

In contrast, the mean distribution volume of the amikacin only group (V_{TV} = 4.11 L) was unexpectedly higher than those who received amikacin with aminophylline (V_{TV} = 1.38 L). Preterm neonates have a higher total body water content than term neonates,[177] hence it may be expected that for a polar drug like amikacin, distribution volume could be higher in preterm neonates compared with term neonates. Even though it is possible to speculate that this unexpected result could be due to aminophylline co-administration, a greater variation was observed in the box plot (Fig. 4.3 B) for the amikacin only group, and this could have accounted for the higher population mean. The data showed no overall significant effect of co-administration of aminophylline with amikacin, as evident in no improvement in the variance of between subject variability. These reasons could

explain the overall little improvement in the variance of the between subject variability in model building process.

5.3.3 Effect of other covariates on amikacin clearance

From the final model (Eqn. 4.1) obtained in this study; i) a population with mean birth weight of 2.1 kg will have a clearance of 0.058 L/h/kg and this is comparable with clearance for a population with same weight reported elsewhere (0.048 L/h/kg).[60] ii) a population with mean birth weight of 1.38 kg will have a clearance of 0.85 mL/kg/min, and this is comparable to that reported by Kenyon *et al*, 0.84 mL/kg/min, for a population with a mean body weight of 1.38 kg.[248] Data from this study showed that birth weight was the most important determinant of amikacin clearance. Aminoglycoside clearance has been found to correlate well with glomerular filtration rate (GFR).[249] Furthermore, body size (related to weight) and renal maturation (related to gestational age) have been found to correlate with GFR.[250] Studies have shown a direct relation between birth weight and the number of nephrons, with 250,000 more glomeruli per kidney per kilogram increase in birth weight.[251,252] Additionally, the between subject variability (coefficient of variation = 58.9%) on clearance was relatively high, indicating that there is approximately 59% of variability not explained with clearance. Gender, postnatal age, postconception age, and postmenstrual age have also been found to be important determinants of amikacin clearance in other studies.[59,60,61] There is a likelihood that these other covariates could have accounted for the high variability observed, although the model did not show this. However, our selection of patients in the first 24 hours of life precludes conclusions to be drawn about the effect of postnatal age.

Estimated creatinine clearance was not found to be an important determinant in amikacin clearance. Additionally, amikacin trough concentration was not found to correlate with serum creatinine level (R^2 = 0.0231, p = 0.158) in this current study. In neonates (first few days postnatal), the estimated creatinine clearance often reflects maternal renal function.[253] Therefore, the estimated creatinine clearance does not actually represent the actual rate at which the neonate is clearing the drug, making it likely that it is maternal rather than the neonate's clearance. However in adults, creatinine clearance has been found to be an important determinant of amikacin clearance.[254]

5.3.4 Effect of other covariates on amikacin volume of distribution

Birth weight was found to be the only determinant of amikacin distribution volume in this study. In general, the body composition of a neonate has a higher percentage of total body water than adults. As such, it is expected that aminoglycosides, which are polar in nature, will have a relatively larger distribution volume in neonates than in adults. It has been reported that total body water of a neonate contributes to about 75% - 80% of the total body weight.[255] Aminoglycosides are distributed in total body water, and total body water correlates positively with body weight, thus the determining effect of birth weight on amikacin distribution is consistent with these considerations. Other studies have also found body weight to influence amikacin distribution in neonates. [60,61,236] A population with an average birth weight of 2.1 kg will have a distribution volume of 1.15 L/kg based on the final model of this study (Eqn. 4.2). This is relatively high compared with 0.434 L/kg reported by Botha *et al.*[60] Also with the final model obtained from this study, a population with an average birth weight of 2.0 kg will have a distribution volume of 1.14

L/kg. When compared with a related study where infants had a similar average weight but with postnatal age between 1 to 28 days (V = 0.65 L/kg),[256] this study's volume of distribution appeared large.

Data from neonates with culture positive isolates (13 out of 247), was incorporated as a covariate during the process of model building, but this did not have an influence on the distribution volume of amikacin. It is, however, known that sepsis, particularly septic shock, is characterized by vasodilatation and increased vascular permeability leading to capillary leak syndrome.[257] This capillary leak is responsible for a fluid shift from the intravascular compartment to the interstitial space, resulting in edema formation.[258] Edema formation can contribute to an increase in the volume of distribution of hydrophilic antimicrobials like aminoglycoside.

The half-life obtained in this study for a 2.1 kg population, 13.62 hours, was longer than the duration (6.4 hours) reported by Botha *et al*.[60] The half-life of amikacin in low-birth neonates is however estimated to range between 7 – 9 hours. In term neonates, however, the half-life is estimated to be between 4 – 5 hours.[157] Generally, studies have reported large volumes of distribution and long elimination half-lives of aminoglycosides in lower gestational aged neonates. The higher proportion of preterm neonates (50.6%) could have accounted for the overall long half-life. Additionally, based on first-order kinetics, the distribution volume of a drug is directly related to its elimination half-life, hence a large volume of distribution would imply a relatively long half-life. The implication of the large volume of distribution of amikacin observed in this population is that, drug dose

may have to be increased to achieve the desired target concentration. Furthermore, for a drug showing a long half-life in a population, an extended dosing interval may be recommended to avoid adverse drug reactions.

Furthermore, the lack of pediatric clinical trial data directly results in a variation and uncertainty of drug therapy in pediatric clinical practice. Findings from this study and others conducted in the neonatal period show continuous developmental changes in this age category. Thus, neonatal specific pharmacokinetic studies in this population is required.

5.4 BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEP)

This study evaluated BAEP before treatment, and at specified times post-treatment in a randomly selected subset of recruited neonates. The BAEP readings before and after treatment in both the amikacin and non-amikacin treated neonates at baseline and at 7 days post-treatment showed no significant difference. An exception being one subject out of the amikacin exposed neonates whose baseline BAEP was 30 dBnHL and follow-up was 40 dBnHL, who also had a trough amikacin concentration > 10 μg/mL. This follow-up was higher than the range 20 – 30 dBnHL reported as normal elsewhere.[259,260] In-utero infections, craniofacial anomalies, birth weight <1500g, hyperbilirubinemia, and postnatal asphyxia are known risk factors for hearing loss in infants.[261,262] This current study excluded neonates in whom some of these risk factors were present.

Furthermore, mutations in the human 12S rRNA gene, specifically mutations at the 961, 1494, and 1555 loci have also been implicated in aminoglycoside-induced

ototoxicity.[263,264] The current study did not conduct genetic studies to determine the genotypic profile(s) of recruited neonates.

The neonate with elevated follow-up BAEP had a trough amikacin concentration of 12.22 µg/mL. The remaining (n = 4), with amikacin trough concentration > 10 µg/mL did not show an elevated follow-up BAEP, hence, the current study cannot relate serum amikacin trough concentration with the elevated follow-up BAEP observed in one subject.

There are reports where delayed onset of hearing loss in infants is described,[265,266] with recommendation of follow-up after 6 months of age of infants. It is possible that hearing screening performed in infants before discharge might be too early to detect aminoglycoside-induced hearing loss. Also, the current study tested subjects at a frequency range of 2 to 4 kHz. Aminoglycoside-induced hearing loss usually starts at higher frequencies (> 8 kHz), which might not be detected by routine hearing screening methods.[267] These factors imply that aminoglycoside related hearing loss could be underreported, and that other methods targeting the known frequency for aminoglycoside ototoxicity might be indicated.

Most studies in infants and children show that hearing loss is a rare complication of aminoglycoside therapy.[268-270] A pilot study done by Langhendries and colleagues to compare once and twice a day amikacin dosing in neonates showed that at day 9 after commencement of therapy there was no case of auditory toxicity in both groups.[239] In another study to evaluate the relationship of serum amikacin levels with hearing in very

low birth weight neonates, it was concluded that in the absence of any other risk factors for hearing impairment the use of amikacin in therapeutic doses for 14 days is safe.[271]

In contrast, a study that consisted of 79 neonates who received gentamicin reported that 1.3% of the cohort had evidence of hearing impairment.[272] Another prospective study evaluated 820 patients who had received aminoglycosides during neonatal period.[273] Hearing was evaluated by either the transient evoked otoacoustic emission or the brainstem evoked response audiometry, and it was reported that 1.6% of patients had sensorineural hearing loss.

5.5 DIAGNOSTIC UTILITY OF PCT AND CRP IN NEONATAL SEPSIS DIAGNOSIS

A comparison of mean PCT between the high probability of sepsis, probability of sepsis and less probability of sepsis groups showed that the difference in PCT levels between the groups was statistically significant. The difference in CRP levels between groups was not statistically significant, possibly due to the large random variability around the mean. Additionally, a test of proportions for neonates with elevated PCT in the highly probable and probable groups showed statistical significance difference, but this was not found with CRP. It is noteworthy that all neonates recruited in this current study were suspected of having early onset neonatal sepsis, since they were less than 24 hours postnatal age. Generally, serum PCT levels rise 4 hours after exposure to bacterial endotoxin, and remain raised for at least 24 hours.[110] On the other hand, CRP starts to increase 4–6 hours later than PCT and reaches its peak 36 hours later.[274] CRP is not the earliest marker to

rise in the event of an infection, hence, not a good screening test for sepsis at an early stage.[105] Monneret *et al*,[28] reported that the levels of both PCT and CRP increased in early onset neonatal sepsis, but PCT levels rose earlier. Gendrel *et al* also concluded that CRP is released much later in early onset neonatal sepsis.[275] Thus, the finding of higher PCT in the highly probable group is consistent with others. In contrast, others have reported that PCT was not a better marker than CRP because PCT was affected by perinatal factors within the first 48 hours of life.[29]

In this study neonates who died having PCT greater than 2 ng/mL were 18 out of 39 (46.2%). Ali *et al* demonstrated that mortality was significantly increased in the group that had the highest mean PCT, especially neonates with PCT greater than 10 ng/mL.[120]

A comparison of mean PCT levels between subjects of the high probability of sepsis group based on blood culture results showed no significant difference between those that had positive and negative blood culture. PCT levels above 2 ng/mL are highly suggestive of an infectious process with systemic consequences. Concentrations above 10 ng/mL are almost exclusively found in patients with severe sepsis or septic shock.[276] Generally, blood culture has a low sensitivity in neonates, and a number of studies that have had neonates with clinical signs of sepsis but negative culture have termed this clinical or probable sepsis,[119] so did this study. This goes to emphasize the fact that a negative blood culture alone does not warrant a withdrawal of antibiotics, especially if the patient's clinical condition indicates ongoing sepsis.[92]

The AUCs obtained after ROC curve analysis in this study were 0.646 and 0.569 for PCT and CRP respectively. There was no statistically significant difference between the two AUCs, although that of PCT appeared higher. A number of studies have reported ROC analysis showing a greater AUC for PCT.[277,278] In a study to estimate the usefulness of PCT in diagnosing early onset neonatal sepsis, the AUC of PCT and CRP were 0.616 and 0.602 respectively.[277] This difference was also not statistically significant, despite the fact that the study reported PCT to be a better diagnostic tool than CRP. In another study where 67 neonates were randomly recruited and divided into groups; highly probable sepsis, probable sepsis, possible sepsis and no sepsis, the AUC for PCT and CRP using mean PCT and CRP levels were 0.77 and 0.64 respectivly.[278] They concluded that serum PCT seemed to be superior to serum CRP levels in early diagnosis of neonatal sepsis. Park *et al* retrospectively reviewed medical data of 269 neonates with suspected infection and evaluated serum PCT as a diagnostic marker of neonatal sepsis.[279] Their ROC analysis showed an AUC of 0.803 for PCT and 0.951 for CRP, and concluded that both had a great diagnostic value, but CRP showed more usefulness. The study by Park *et al* [279] used data from neonates with postnatal ages greater than 4 and less than 30 days old, whereas this study recruited neonates who were less than 24 hours postnatal age. Hence, sepsis in that study cannot be said to be early onset, but rather late onset.

At a cut-off point of 2 ng/mL, the sensitivity, specificity, PPV and NPV for PCT in this study were 87.5%, 63%, 44.1% and 93.8% respectively. Additionally, this study, using a cut-off point of 5 mg/L found the sensitivity, specificity, PPV and NPV for CRP to be 50%, 72.2%, 37.5% and 81.3% respectively. These values, higher sensitivity, PPV and

NPV, show PCT to be a better diagnostic tool for early neonatal sepsis than CRP, although the specificity for PCT was lower than CRP. Also with a cut-off value of 2 ng/mL, Boo *et al* showed that the sensitivity, specificity, PPV and NPV of PCT were 88.9%, 65.2%, 40% and 95.7% respectively.[280] In a systematic review and meta-analysis by Vouloumanou *et al* that included 16 studies with 1,959 neonates, they found PCT sensitivity and specificity to range between 74–87% and 69–87%, respectively.[27] Altunhan *et al*, found that the sensitivity, specificity, PPV, and NPV of PCT at 24 hours of life were 88.3%, 88.6%, 83.33%, and 88.57%, respectively, using a cut-off value of 5.38 ng/mL.[281] They also found that the sensitivity, specificity, PPV, and NPV of CRP at 24 hours of life were 76.4%, 78.9%, 79.75%, and 81.62%, respectively, using a cutoff value of 12 mg/L, and concluded that PCT might be a more sensitive diagnostic parameter within the first 24 hours of life than CRP.

In spite of these affirmations of PCT as a sensitive diagnostic tool in neonatal sepsis diagnosis, Ballot *et al* suggests that PCT alone is not sufficient to confirm neonatal sepsis.[282] Another study also affirms based on ROC analysis that PCT is a useful indicator in the diagnosis of early onset neonatal sepsis than CRP, but at an expensive cost.[275]

Although this current study has shown PCT to be a more reliable marker for early diagnosis of neonatal sepsis than CRP, it did not have an influence on amikacin clearance or distribution. For a neonate with suspected infection, it is expected that PCT will decrease 24–48 hours later after antibiotics are initiated and its level return to normal 5 days after.[283]

5.6 MICROBIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA ISOLATES

Findings from this study showed that CoNS was the most common bacteria isolate (54%). A previous study from the same NICU (KBTH) done a decade earlier also reported CoNS as the most common isolate (31.9%).[8] Some studies have also reported a high percentage of CoNS out of the total number of bacteria isolated from neonates with infection.[284-286] Others in developed countries have reported CoNS as the dominant LOS pathogen.[83,84]

A total of 4 (9.8%) GBS were isolated throughout the duration of this study. The study done by Enweronu-Laryea and Newman [8] at same NICU (KBTH) a decade ago did not report any GBS. However, Anyebuno and Newman [287] reported that GBS constituted 1.8% of the total isolates in a similar study done at NICU, KBTH in 1991/1992. Indeed, microorganisms that cause neonatal infection may differ significantly from time to time, hence the need for continuous monitoring. Although the epidemiology of GBS in the developed world is well documented, its contribution to the burden of neonatal infection in the developing world is less clear.[288] With the exception of *Burkholderia cepacia* and *Serratia odorifera*, all other Gram-negative bacteria isolated have been reported in previous studies done at the NICU.[8,287]

At the NICU, KBTH, empirical treatment of suspected sepsis involves a combination of amikacin with cloxacillin. Antimicrobial susceptibility tests indicated that all Gram-negative bacteria were sensitivity to amikacin except Coliform bacillus and *Enterobacter*

aerogenes. However, the previous studies at the NICU, KBTH, reported bacteria susceptibility to aminoglycosides and cephalosporins but not penicillin(s).[8,287] Among the Gram-positive isolates, vancomycin susceptibility was preserved, and among the Gram-negatives, carbapenems were preserved. Other similar studies have reported little or no resistance of bacteria isolates to vancomycin and carbapenems.[288,289]

5.7 CONCLUSION

In this study, 322 neonates were admitted with sepsis. One hundred and sixty-three (50.6%) out of the 322 of the recruited neonates were preterm. Provisional diagnoses showed that 24.5% of neonates with suspected sepsis had birth asphyxia. The proportion of preterms that presented with respiratory distress syndrome was 50.9%. Overall mortality was 12%. Mortality was highest among extreme preterms and those with birth weight less than 1 kg. The proportion of neonates who died with positive blood culture, CRP and PCT were 20.5%, 20.5% and 46.2% respectively. At the NICU, KBTH, amikacin together with cloxacillin are used as first-line for suspected neonatal sepsis. The population studied showed that a one-compartment model best described amikacin disposition. Although population values showed variation in clearance and distribution volume among neonates administered amikacin $\pm$ aminophylline, it does not appear addition of aminophylline improved fit of model. Birth weight was found as important predictor of amikacin clearance and volume of distribution. The half-life and distribution volume of neonates in this study were relatively longer and larger compared with other studies. Based on these, it appears that a higher dose and extended dosing interval may be an optimal schedule for amikacin administration in this population. PCT may be a better

diagnostic tool for early onset neonatal sepsis, however, its low specificity relative to CRP means neonates without infection might be incorrectly identified as having an infection.

5.8 LIMITATIONS OF STUDY

Although two blood-samples per neonate was projected for population pharmacokinetic studies, on certain occasions samples could not be taken due to difficulty in locating peripheral vein of neonate or sample volume was too low. Blood sampling for drug assay was affected in some neonates due to extravasation at venous sites. Neonates administered amikacin with or without aminophylline varied in gestational age, < 35 weeks and ≥ 35 weeks respectively. BAEP done for neonates at early postnatal ages could have been hampered by middle ear fluid retention. Additionally, the number of positive blood culture isolates was low for any influence on the pharmacokinetic model.

5.9 RECOMMENDATIONS

Based on large volume of distribution and prolonged half-life estimates from this study, a higher dose and an extended interval of amikacin is recommended in this population. A future study that assesses the pharmacokinetics of amikacin with or without aminophylline in neonates with relatively similar gestational age characteristics (< 37 weeks) is recommended. Further studies that involve a reliable PD measure for PK-PD modeling of amikacin is recommended. Studies to evaluate the effect of amikacin treatment on cochlear function in neonates using higher BAEP frequencies and behavioral observation audiometry (BOA) is recommended. Additionally, a 6-month

follow-up BAEP and BOA after NICU discharge is recommended. Due to its high sensitivity, PCT is recommended as a diagnostic measure in early onset neonatal sepsis, although it has a low ability to correctly identify patients without an infection.

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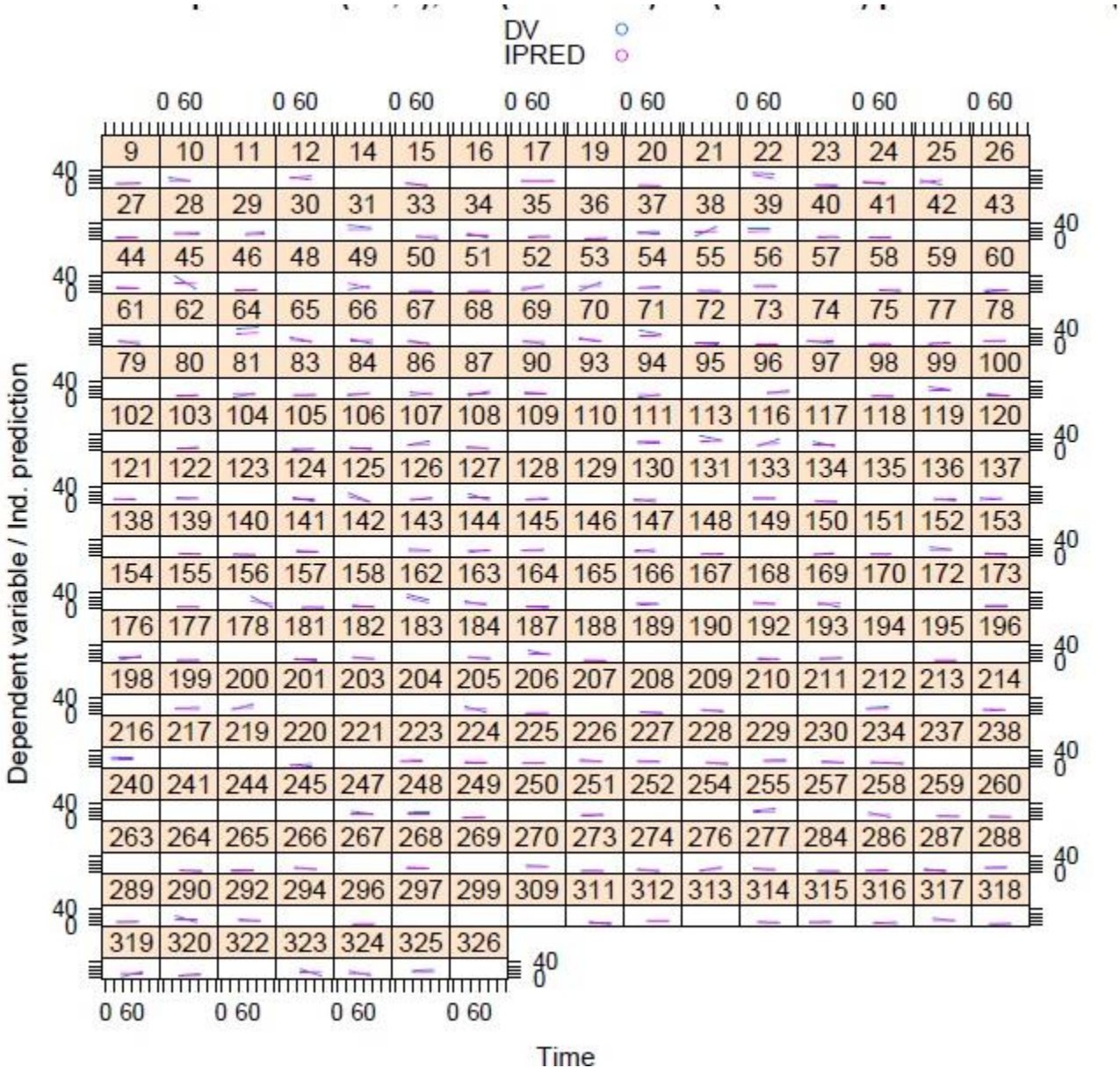
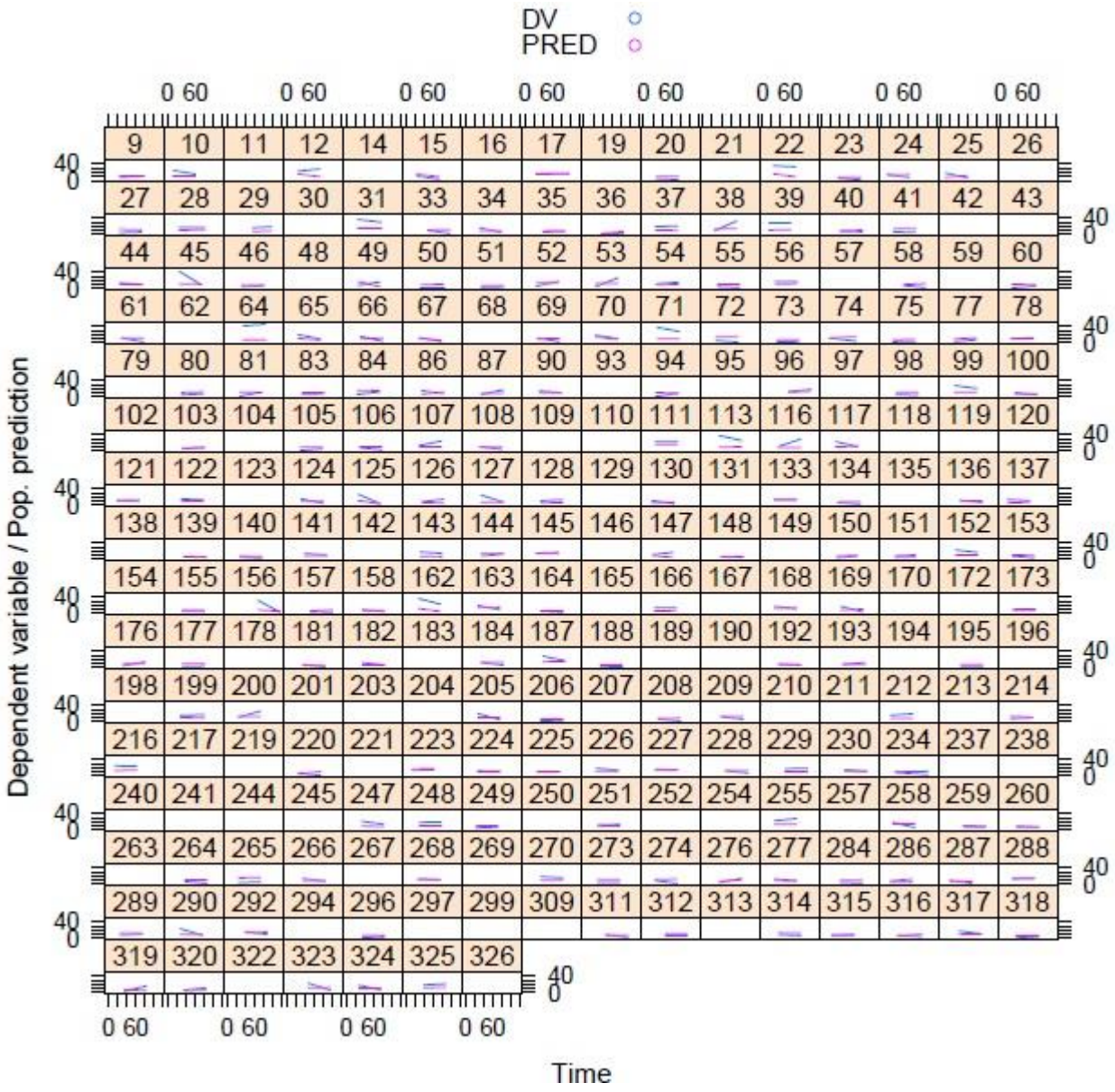
APPENDIX 1

DV AND IPRED VERSUS TIME OF RECRUITED NEONATES

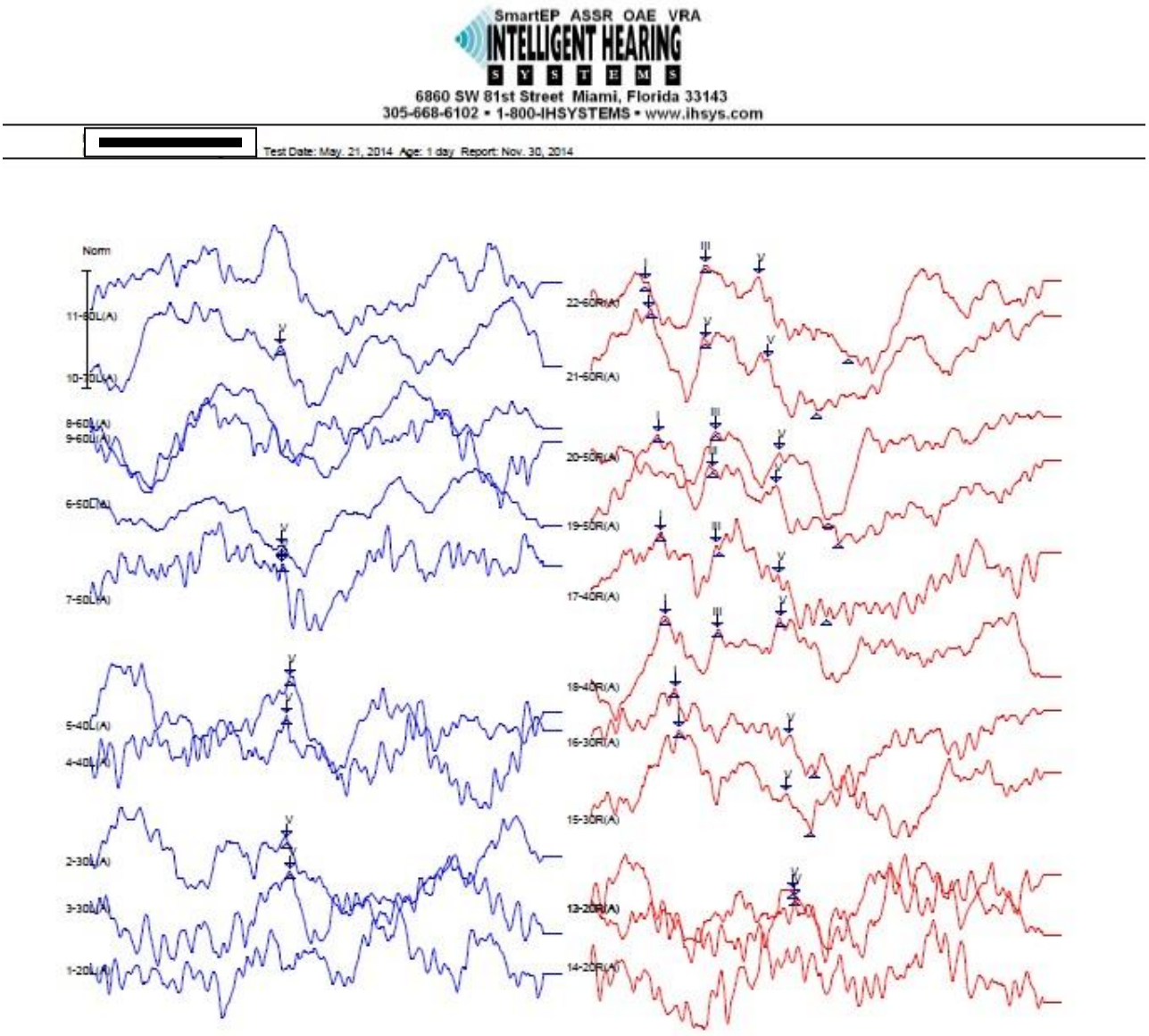


DV AND PRED VERSUS TIME OF RECRUITED NEONATES



APPENDIX 2

BAEP READING OF TEST SUBJECT 17 (BASELINE)



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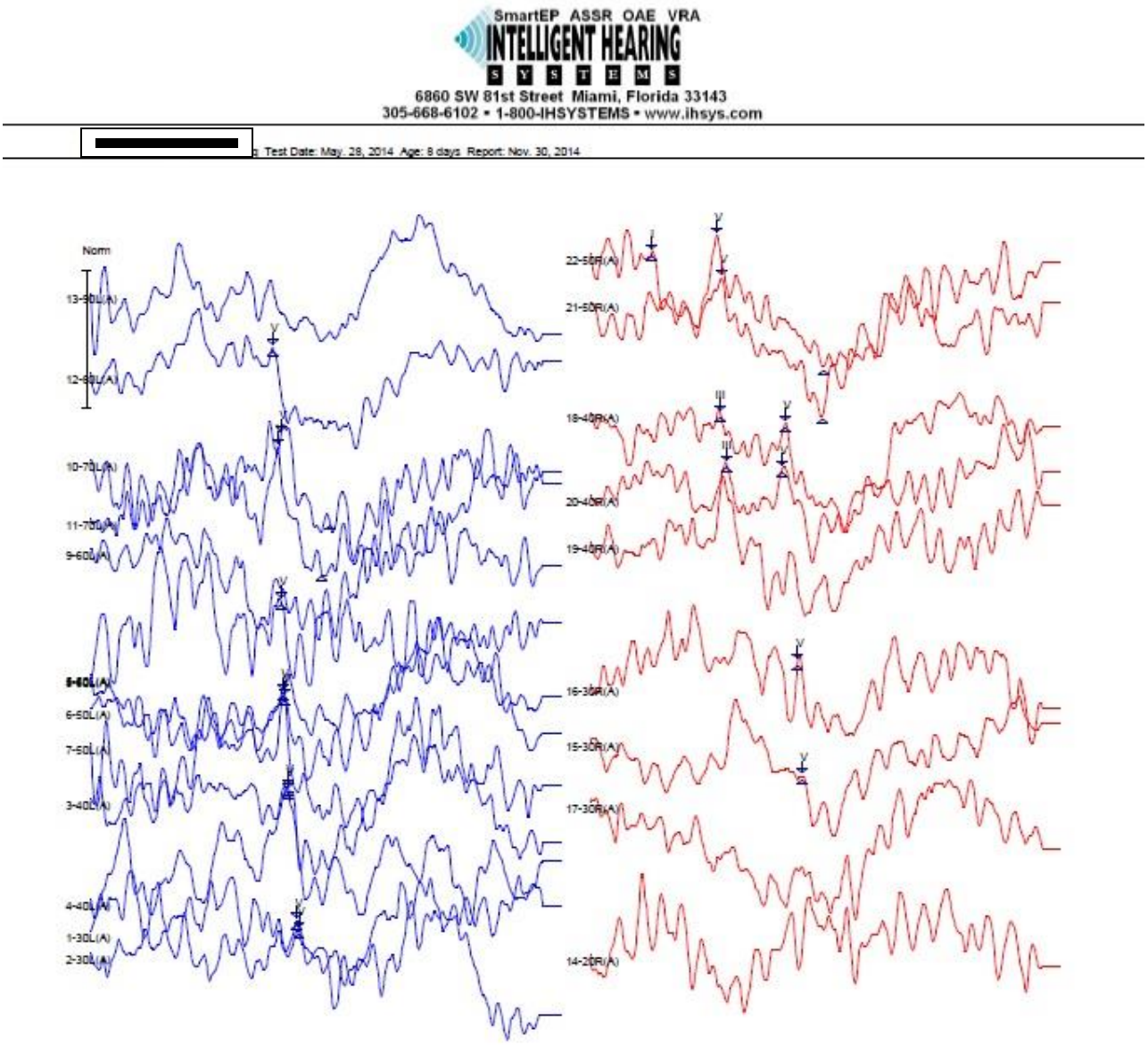
Test Date: May. 21, 2014 Age: 1 day Report: Nov. 30, 2014

Num	Filename	Int	Ear	Stim.	Type	Swps/Art	Rate	Mode	PP Amp	SNR	RN	Gain	Filters
1	BNLA20A.1	20nHL	L	Inst	Click	2048/92	21.7	Altr	0.13	0.28	0.12	100	30-1500Hz
2	BNLA30A.1	30nHL	L	Inst	Click	2048/112	21.7	Altr	0.20	0.27	0.23	100	30-1500Hz
3	BNLA30A.4	30nHL	L	Inst	Click	2048/20	21.7	Altr	0.13	0.30	0.10	100	30-1500Hz
4	BNLA40A.3	40nHL	L	Inst	Click	2048/14	21.7	Altr	0.12	0.50	0.05	100	30-1500Hz
5	BNLA40A.4	40nHL	L	Inst	Click	2048/162	21.7	Altr	0.21	0.11	0.25	100	30-1500Hz
6	BNLA50A.1	50nHL	L	Inst	Click	2048/72	21.7	Altr	0.51	0.31	0.28	100	30-1500Hz
7	BNLA50A.2	50nHL	L	Inst	Click	2048/20	21.7	Altr	0.14	0.38	0.10	100	30-1500Hz
8	BNLA60A.1	60nHL	L	Inst	Click	2048/53	21.7	Altr	0.40	1.72	0.12	100	30-1500Hz
9	BNLA60A.2	60nHL	L	Inst	Click	2048/38	21.7	Altr	0.17	0.35	0.15	100	30-1500Hz
10	BNLA70A.1	70nHL	L	Inst	Click	2048/47	21.7	Altr	0.29	0.30	0.21	100	30-1500Hz
11	BNLA80A.1	80nHL	L	Inst	Click	2048/9	21.7	Altr	0.20	0.50	0.09	100	30-1500Hz
12	BNRA20A.1	20nHL	R	Inst	Click	2048/71	21.7	Altr	0.14	0.48	0.07	100	30-1500Hz
13	BNRA20A.2	20nHL	R	Inst	Click	2048/36	21.7	Altr	0.15	0.60	0.06	100	30-1500Hz
14	BNRA20A.3	20nHL	R	Inst	Click	2048/2	21.7	Altr	0.13	0.50	0.09	100	30-1500Hz
15	BNRA30A.1	30nHL	R	Inst	Click	2048/10	21.7	Altr	0.20	0.70	0.10	100	30-1500Hz
16	BNRA30A.2	30nHL	R	Inst	Click	2048/12	21.7	Altr	0.21	1.12	0.05	100	30-1500Hz
17	BNRA40A.1	40nHL	R	Inst	Click	2048/47	21.7	Altr	0.18	0.45	0.13	100	30-1500Hz
18	BNRA40A.2	40nHL	R	Inst	Click	2048/20	21.7	Altr	0.26	0.49	0.13	100	30-1500Hz
19	BNRA50A.2	50nHL	R	Inst	Click	2048/0	21.7	Altr	0.26	1.18	0.07	100	30-1500Hz
20	BNRA50A.3	50nHL	R	Inst	Click	2048/22	21.7	Altr	0.36	1.09	0.12	100	30-1500Hz
21	BNRA60A.1	60nHL	R	Inst	Click	2048/44	21.7	Altr	0.27	0.99	0.10	100	30-1500Hz
22	BNRA60A.2	60nHL	R	Inst	Click	2048/245	21.7	Altr	0.30	0.64	0.18	100	30-1500Hz

System SNR & RN Region: 4.00 - 9.00 ms (*)-Indicates different region used

Num	Int	Ear	Peaks: Latency(ms) | Amp(uV) (AR=Amp Ratio)					
1	20	L						
2	30	L		V: 10.70 | 0.00				
3	30	L		V: 10.90 | 0.00				
4	40	L		V: 10.65 | 0.00				
5	40	L		V: 10.85 | 0.00				
6	50	L		V: 10.45 | 0.00				
7	50	L		V: 10.45 | 0.00				
8	60	L						
9	60	L						
10	70	L		V: 10.35 | 0.00				
11	80	L						
12	20	R		V: 11.15 | 0.00				
13	20	R		V: 11.00 | 0.00				
14	20	R						
15	30	R	I: 4.85 | 0.00	V: 10.65 | 0.07				V-I: 5.80 |
16	30	R	I: 4.65 | 0.00	V: 10.80 | 0.07				V-I: 6.15 | 465.92AR
17	40	R	I: 3.85 | 0.00	III: 6.85 | 0.01	V: 10.25 | 0.07	III-I: 3.00 |	V-III: 3.40 | 11.20AR	V-I: 6.40 |
18	40	R	I: 4.05 | 0.00	III: 6.90 | 0.00	V: 10.35 | 0.00	III-I: 2.85 |	V-III: 3.45 |	V-I: 6.30 |
19	50	R	I: 3.70 | 0.00	III: 6.70 | 0.00	V: 10.10 | 0.13	III-I: 3.00 |	V-III: 3.40 |	V-I: 6.40 |
20	50	R		III: 6.80 | 0.00	V: 10.30 | 0.22		V-III: 3.50 |	
21	60	R	I: 3.20 | 0.00		V: 9.70 | 0.13			V-I: 6.50 | 48.41AR
22	60	R	I: 3.05 | 0.00	III: 6.30 | 0.00	V: 9.15 | 0.22	III-I: 3.25 |	V-III: 2.85 |	V-I: 6.10 | 103.91AR

BAEP READING OF TEST SUBJECT 17 (FOLLOW-UP)



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ID [redacted] g Test Date: May. 26, 2014 Age: 8 days Report: Nov. 30, 2014

Num	Filename	Int	Ear	Stim.	Type	Swps/Art	Rate	Mode	PP Amp	SNR	RN	Gain	Filters
1	BNLA30A.1	30nHL	L	Inst	Click	2048/54	21.7	Altr	0.18	0.46	0.10	100	30-1500Hz
2	BNLA30A.3	30nHL	L	Inst	Click	2048/86	21.7	Altr	0.19	0.45	0.10	100	30-1500Hz
3	BNLA40A.1	40nHL	L	Inst	Click	2048/43	21.7	Altr	0.16	0.26	0.07	100	30-1500Hz
4	BNLA40A.2	40nHL	L	Inst	Click	2048/46	21.7	Altr	0.25	1.13	0.07	100	30-1500Hz
5	BNLA50A.1	50nHL	L	Inst	Click	2048/140	21.7	Altr	0.24	0.59	0.08	100	30-1500Hz
6	BNLA50A.3	50nHL	L	Inst	Click	2048/52	21.7	Altr	0.19	0.46	0.08	100	30-1500Hz
7	BNLA50A.4	50nHL	L	Inst	Click	2048/32	21.7	Altr	0.13	0.71	0.07	100	30-1500Hz
8	BNLA60A.1	60nHL	L	Inst	Click	2048/50	21.7	Altr	0.11	0.63	0.09	100	30-1500Hz
9	BNLA60A.2	60nHL	L	Inst	Click	2048/34	21.7	Altr	0.17	1.14	0.06	100	30-1500Hz
10	BNLA70A.1	70nHL	L	Inst	Click	2048/39	21.7	Altr	0.15	0.34	0.12	100	30-1500Hz
11	BNLA70A.2	70nHL	L	Inst	Click	2048/56	21.7	Altr	0.14	0.16	0.25	100	30-1500Hz
12	BNLA80A.1	80nHL	L	Inst	Click	2048/41	21.7	Altr	0.26	0.47	0.12	100	30-1500Hz
13	BNLA90A.1	90nHL	L	Inst	Click	2048/33	21.7	Altr	0.31	0.25	0.33	100	30-1500Hz
14	BNRA20A.1	20nHL	R	Inst	Click	2048/46	21.7	Altr	0.16	0.79	0.09	100	30-1500Hz
15	BNRA30A.1	30nHL	R	Inst	Click	2048/62	21.7	Altr	0.29	1.24	0.09	100	30-1500Hz
16	BNRA30A.2	30nHL	R	Inst	Click	2048/93	21.7	Altr	0.17	0.68	0.06	100	30-1500Hz
17	BNRA30A.3	30nHL	R	Inst	Click	2048/180	21.7	Altr	0.19	0.56	0.08	100	30-1500Hz
18	BNRA40A.1	40nHL	R	Inst	Click	2048/225	21.7	Altr	0.27	0.63	0.09	100	30-1500Hz
19	BNRA40A.2	40nHL	R	Inst	Click	2048/39	21.7	Altr	0.19	0.94	0.06	100	30-1500Hz
20	BNRA40A.3	40nHL	R	Inst	Click	2048/47	21.7	Altr	0.24	0.96	0.06	100	30-1500Hz
21	BNRA50A.1	50nHL	R	Inst	Click	2048/59	21.7	Altr	0.23	0.47	0.11	100	30-1500Hz
22	BNRA50A.2	50nHL	R	Inst	Click	2048/34	21.7	Altr	0.20	0.20	0.33	100	30-1500Hz

System SNR & RN Region: 4.00 - 9.00 ms (*)-Indicates different region used

Num	Int	Ear	Peaks: Latency(ms) | Amp(uV) (AR=Amp Ratio)				
1	30	L			V: 11.40 | 0.00		
2	30	L			V: 11.25 | 0.00		
3	40	L			V: 10.75 | 0.00		
4	40	L			V: 10.75 | 0.00		
5	50	L					
6	50	L			V: 10.55 | 0.00		
7	50	L			V: 10.50 | 0.00		
8	60	L			V: 10.40 | 0.00		
9	60	L					
10	70	L			V: 10.40 | 0.09		
11	70	L			V: 10.20 | 0.13		
12	80	L			V: 9.95 | 0.00		
13	90	L					
14	20	R					
15	30	R			V: 11.55 | 0.00		
16	30	R			V: 11.35 | 0.00		
17	30	R					
18	40	R		III: 7.15 | 0.00	V: 10.65 | 0.00		V-III: 3.50 |
19	40	R					
20	40	R		III: 7.45 | 0.00	V: 10.50 | 0.00		V-III: 3.05 |
21	50	R			V: 7.20 | 0.23		
22	50	R	I: 3.40 | 0.00		V: 6.90 | 0.19		V-I: 3.50 |