

Comparison of the trough levels of two vancomycin formulations in a
selected preterm infant population

H.A. Griesel

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Magister Pharmaceuticae School of Pharmacy, University of the Western Cape,
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SUPERVISOR: Prof. P. Mugabo

CO-SUPERVISOR: Dr. R. Dippenaar



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Heletje Aletta Griesel



Keywords:

Trough levels

Vancomycin

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Neonatal sepsis

Volume of distribution

Renal clearance

Therapeutic drug monitoring (TDM)

Generic formulations

Abstract

Comparison of trough levels of two vancomycin formulations in a selected preterm infant population

Heletje Aletta Griesel

M.Pharm thesis, School of Pharmacy, University of Western Cape.



Infections contribute 26% of the 4 million neonatal deaths occurring annually in developing countries.

Late onset nosocomial sepsis is often caused by gram positive bacteria including *Staphylococcus aureas*, *Staphylococcus epidermidis*; coagulase-negative staphylococci (CONS), *Enterococcus* species and methicillin resistant *Staphylococcus aureas* (MRSA).

The antibiotic of first choice for MRSA is vancomycin, a glycopeptide with bactericidal activity against gram-positive bacteria.

There are limited studies in premature infants on the equivalence of generic formulations of vancomycin.

The aim of this study was to compare the trough plasma levels of Aspen-Vancomycin® (AV); and Sandoz-Vancocin CP® (SV) in premature infants with suspected Methicillin Resistant *Staphylococcus aureus* (MRSA) infection.

The study was designed as a prospective, double blind, randomised trial involving male and female premature infants admitted in the Neonatal Intensive care Unit (NICU) at Netcare Blaauwberg and N1-city Hospitals for treatment of suspected MRSA-infection between April 2012 and June 2013.

The inclusion criteria were: 29-35 weeks postmenstrual age (PMA), informed and written consent from parents of each premature infant enrolled in the study.

Blood samples (0.3-0.4ml) were collected for renal function test and vancomycin trough levels determination.

Blood samples for vancomycin trough level assay were collected thirty minutes prior to the administration of the third dose of vancomycin.

Statistical analysis was performed and estimation was made giving an indication of how many infants will be needed to make the study statistically significant.

Wilcoxon Two-Sample test was performed to determine the p-values and Spearman correlation coefficients were used to determine the correlation between trough levels and variables. P-values < 0.05 were considered significant.

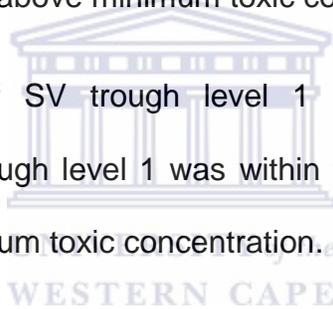
A total of 19 premature infants met with study criteria, 10 (5 females and 5 males) received AV and 9 (6 females and 3 males) received SV.

There was no statistical significant difference between the demographic (GA, BW, PMA, PNA, weight at trial entry, height at trial entry) and biological (albumin, serum creatinine concentration and glomerular filtration rate) parameters of the premature infants in the AV and SV group.

There were no statistical significant difference between trough level 1 of AV and SV, although trough level 1 had a lower trend in the SV group ($p=0.118$).

No AV trough level 1 was below the minimum effective concentration ($<5\mu\text{g/ml}$). It was found that 30% of AV trough level 1 was within the therapeutic range ($5\text{-}10\mu\text{g/ml}$) and 70% of AV trough level 1, were above minimum toxic concentration ($>10\text{mg/l}$).

It was found that 22.2% of SV trough level 1 was below minimum effective concentration, 44.4% of SV trough level 1 was within therapeutic range and 33.3% of trough level 1 was above minimum toxic concentration.



No correlation was found between trough level 1 and the demographic and biological parameters of the premature infants in the AV group. SV had a positive correlation with GA, BBW, PMA and a negative correlation with PNA.

Vancomycin serum trough concentrations are dependent on demographic, biological and pharmacokinetic parameters. Since the first two parameters was of no definitive value one needs to investigate the effect of pharmacokinetic parameters on serum vancomycin trough levels.

It was found that a premature infant's renal function drives vancomycin trough level concentrations and that age or weight are the most relevant covariates of vancomycin

clearance and should be considered in individual vancomycin dosing schedule in premature infants by means of therapeutic drug monitoring.

This study suggests that Sandoz Vancocin CP® should be used when treating premature infants for a suspected MRSA infection. Sandoz Vancocin CP® offered lower vancomycin serum trough levels which ensure a lower risk of toxicity.



Dedications

“Everything that happens in this world happens at the time God chooses.

He sets the time for birth and the time for death,

The time for planting and the time for pulling up, the time for killing and the time for healing,

The time for tearing down and the time for building,

He sets the time for sorrow and the time for joy,

The time for mourning and the time for dancing,

The time for making love and the time for not making love,

The time for kissing and the time for not kissing.



He sets the time for finding and the time for losing,

The time for saving and the time for throwing away,

The time for tearing and the time for mending,

The time for silence and the time for talk.

He sets the time for love and the time for hate,

The time for war and the time for peace.

What do we gain from all our work?

I know the heavy burdens that God has laid on us. He has set the right time for everything. He has given us a desire to know the future, but never gives us the satisfaction of fully understands what He does. So I realized that all we can do is to be happy and do the best we can while we are still alive.”

Ecclesiastes 3: 1-12



Declaration

I declare that the dissertation titled “*Comparison of the trough levels of two vancomycin formulations in a selected preterm infant population*” is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Heletje Aletta Griesel

May 2014

University of Western Cape

Bellville

South Africa



Signed: _____

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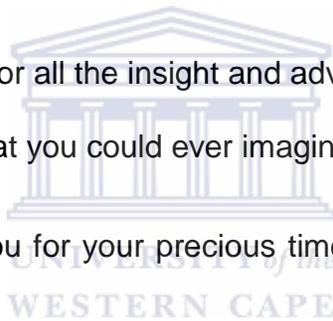
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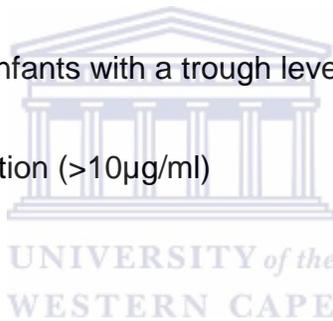
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Neonatal age terminology

The following terminologies will be used to describe the age of the premature infant:

Gestational age:

The gestational age (GA) is the age from the first day of the last menstrual period until the day of delivery, measured in weeks (Engle, 2004). The gestational age can be calculated by using an ultrasound, measuring the first day of the last menstrual period (LMP), and physical examination of the infant according to the new Ballard score (Fenton & Kim, 2013).



Post natal age (PNA):

It is the time from birth measured in days, weeks, months and years (Engle, 2004).

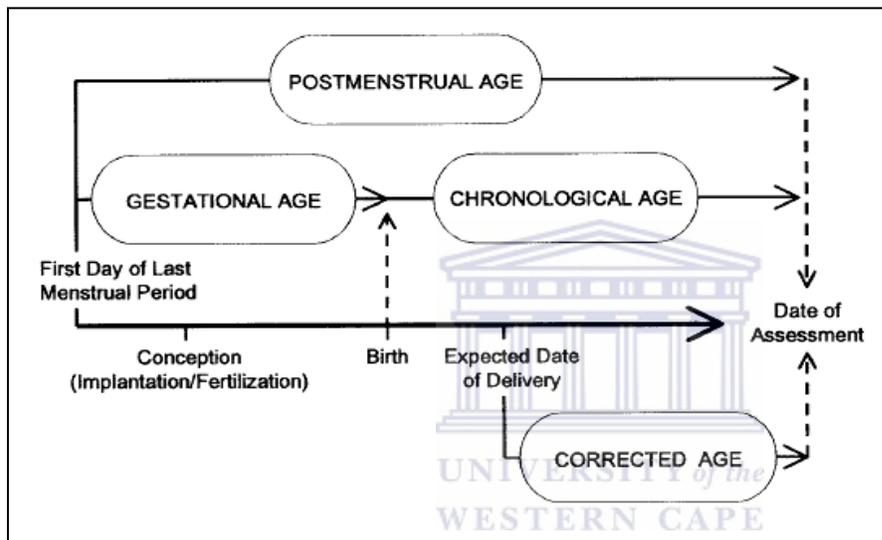
Post menstrual age (PMA):

The age of the infant from the first day of the last menstrual cycle plus the time elapsed from birth (post natal age), measured in weeks (Engle, 2004).

Corrected age:

The corrected age is the post natal age minus the amount of weeks before 40 weeks gestation, measured in weeks and months (Engle, 2004).

Figure 2.1: Age terminology during the perinatal period (Engle (2004))



Low birth weight premature infants (LBW):

A premature infant is defined as LBW if her/his birth weight is < than 2500g at birth (Beers et al, 2006).

Very low birth premature infant (VLBW):

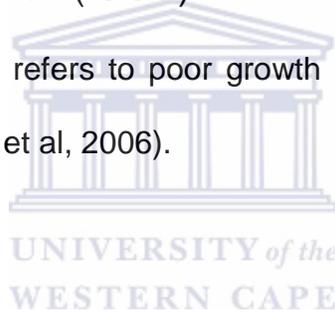
A premature infant is defined as LBW if her/his birth weight is < than 1500g at birth (Beers et al, 2006).

Small for gestational age (SGA):

Premature infants whose weight is less than the 10th percentile for gestational age (Beers et al, 2006).

Intra-uterine growth retardation (IUGR):

Intra-uterine growth retardation refers to poor growth of a baby while in the mother's womb during pregnancy (Beers et al, 2006).



Chapter 1 Introduction

1.1 Background information

Increased mortality and morbidity in neonatal intensive care units (NICUs) is a concern, especially in the developing countries in the world according to Satar & Ozlu (2012). Lawn et al (2005) note that 95% of neonatal deaths arise from low- and middle-income countries, such as south-central Asia and sub-Saharan Africa.

According to Qazi & Stoll (2009) and Lawn et al (2005), there are three major causes which contribute to neonatal deaths: infection (36%), consequences of premature birth/low birth weight (28%) and birth asphyxia (23%).

Satar & Ozlu (2012) found that the occurrence of neonatal sepsis in developed countries varies from one to five cases per 1000 live births, while in developing countries some reports state clinical sepsis rates to range from 49 to 170 cases per 1000 live births.

It is a challenge to identify and treat of neonatal sepsis, particularly in developing countries. If the correct antibiotic is not prescribed within an hour of suspected sepsis it

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might lead to a prolonged neonatal inflammatory response and undesired neurodevelopment (Qazi & Stoll, 2009, Sherman, 2010).

Late onset nosocomial sepsis is often caused by gram positive bacteria and the *Staphylococcus* species account for 30 to 50% of all infections (Satar & Ozlu, 2012).

Rana et al (2012) found that during the period that they conducted their study the incidence of Methicillin resistant *Staphylococcus aureus* (MRSA) cultures increased from 13.7- 24.77 per 1000 admissions ($p=0.01$). Kayange et al (2010) found in their study in Sub-Saharan Africa that 28% of *Staphylococcus aureus* positive cultures were MRSA.

MRSA infections can be treated with linezolid, teicoplanin and vancomycin. That is the opinion of Beers (2006). The first choice antibiotic, however, is vancomycin.

Tattevin et al (2013) mentioned that Elli Lilly, the originator of vancomycin, discontinued the manufacturing of vancomycin towards the end of 2004. Nambiar et al (2012) note that since then generic vancomycin products have emerged in the market and are being used all over the world. With the introduction of these generic formulations into the market, multiple cases of impurities and sub-optimal antimicrobial activity have been reported such as β -lactam agents, antifungal agents and glycopeptides e.g. vancomycin. Snyman et al (2009) refer in their study that there is criticism around the world regarding the bioequivalence of generic products.

Chapter 1: Introduction

1.2 Motivation for this study

In South Africa sepsis and pneumonia account for 36% of neonatal deaths, according to Jeena et al (2008). This makes it critical that the antibiotic therapy of choice is effective against the bacterial pathogens and that toxicity is avoided at all costs. This opinion is shared by Pritchard et al (2010) and Kayange et al (2010).

The efficacy of vancomycin has been questioned since the emergence of vancomycin resistance in isolates of *Staphylococcus aureus* (Howden, 2010).

Various studies have been performed in adults in order to determine the efficacy of generic formulations of vancomycin, but no study has been done in the premature infant population. This is found by Vesga et al (2010). On a broader level it becomes necessary to test the assumption that generic formulations are not only pharmaceutically equivalent, but also therapeutic equivalent to the innovator (Tattevin et al, 2013).

It is against this background that this study seeks to investigate whether there is a difference between two generic formulations of vancomycin which are available in South Africa, with regards to trough levels measured in a selected premature infant population.

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Aim of this study

The aim of the study is to compare vancomycin serum trough levels (C_{\min}) of the two vancomycin preparations available in South Africa: (a) Aspen-Vancomycin®; vancomycin 500mg vial (Aspen Pharmaceuticals) and (b) Sandoz-Vancocin CP; vancomycin 500mg vials (Sandoz Pharmaceuticals) in a selected premature infant population presenting with suspected MRSA infection.



Chapter 2 Literature review

2. Neonatal sepsis

2.1 A global perspective

The increase of neonatal morbidity and mortality, especially in developing countries are of great concern. Almost 99% of the four million neonatal deaths annually occur in developing countries (Thaver& Zaidi , 2009). The highest number of neonatal deaths is in low-and middle-income countries such as south-central Asia and sub-Saharan Africa (Lawn et al, 2005).

There are three major causes that contribute to neonatal deaths: infection (36%), birth asphyxia (23%) and consequences of premature birth/low birth weight (28%) (Qazi & Stoll, 2009) (Lawn et al, 2005).

Neonatal infections are contributing to the global burden of child mortality. The mortality rate of neonatal infections is as high as 40-50 per 1000 live births in the poorest parts of the world. The main reason for such high neonatal infection rates can be attributed to

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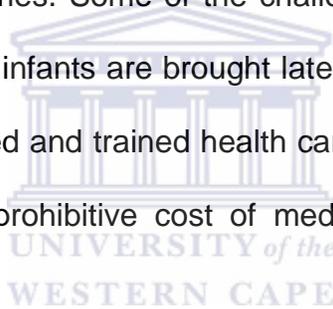
the poor hygiene during labour and delivery and lack of resources for good postnatal care (Zaidi et al, 2005).



2.2 The incidence of neonatal sepsis in developing countries

Sepsis is defined as a syndrome with systemic signs of infection accompanied by bacteraemia (Satar & Ozlu, 2012). The incidence of sepsis in developing countries varies from 49-170 per 1000 live births, whereas in developed countries 1-5 cases are reported per 1000 live births (Satar & Ozlu, 2012). In South Africa sepsis and pneumonia account for 36% of neonatal deaths (Jeena et al, 2008).

The identification of neonatal sepsis and the treatment thereof is challenging, particularly in developing countries. Some of the challenges are: lack of recognition of the subtle symptoms of sepsis; infants are brought late to the health care facility by the family; lack of access to qualified and trained health care workers, lack of quality health care facilities as well as the prohibitive cost of medical treatment in resource-poor countries (WHO, 2011).



2.3 Consequences of neonatal sepsis

Neonatal sepsis results in significant morbidity and mortality in premature infants in neonatal intensive care units (NICUs) (Cooke et al, 1997). The mortality rate of neonatal sepsis where premature infants have not been treated adequately is as high as 50%. Neonatal sepsis is a major cause of neonatal deaths in the first month of life, attributing 13-15% (Kermorvant-Duchemin et al, 2008).

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In a large cohort study, Stoll et al (2004) note that premature infants that had survived neonatal sepsis in comparison to those who had not been infected, were more likely to develop cerebral palsy ($p \leq 0.01$), had lower mental developmental index (MDI) scores ($p \leq 0.01$), lower psychomotor development index (PDI) scores ($p \leq 0.001$) and suffered from visual ($p \leq 0.001$) and growth impairment ($p \leq 0.001$).

If the correct antibiotic is not prescribed within an hour of suspected sepsis it may lead to a prolonged neonatal inflammatory response and undesired neurodevelopment as described by (Qazi & Stoll, 2009) (Sherman, 2010).

2.4 Neonatal sepsis: Early-onset sepsis and late-onset sepsis



Neonatal sepsis can be divided into two groups according to the postnatal age of the infant: Early-onset sepsis (EOS) that occurs within 48 hours to 6 days of birth; and late-onset sepsis (LOS) occurring 6 days after birth (Satar & Ozlu, 2012).

Klebsiella spp. accounted for 28.2% and *Staphylococcus aureus* 14.3% of all isolated obtained from blood cultures in Africa. Methicillin Resistant *Staphylococcus aureus* (MRSA) contributes to 25% of LOS in Africa and 38% in all developing countries (Zaidi et al, 2009).

2.5 Risk factors for neonatal sepsis

2.5.1 The premature infant's immune system

Neonatal immune systems are immature and more susceptible to infection (gram positive- and negative bacteria and fungi) (Soltau & Schelonka, 2008).

Premature infants have a poorly developed skin barrier and decreased development of pro-inflammatory cytokines e.g. interleukin (IL)-1 β , tumour necrosis factor (TNF)- α , and interferon- γ and therefore their immune system is compromised (Satar & Ozlu, 2012). Further risk factors can be divided into intrinsic, extrinsic, and risk factors related to institutional infrastructure.

2.5.2 Intrinsic risk factors

The intrinsic risk factors can be described as factors that cannot be influenced and it relates to the premature infant's biological status. The factors include birth weight, gestational age and conditions at birth (Downey et al, 2010).



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2.5.3 Extrinsic risk factors

Extrinsic factors relates to the treatment the premature infant receives including mechanical ventilation, central venous devices and parenteral nutrition (TPN) (Downey et al, 2010).

2.5.4 Infrastructure related risk factors

Lastly, infrastructure-related risk factors include environmental cleanliness, hand washing, isolation facilities and nursing staff (Downey et al, 2010).

2.6 Clinical signs and symptoms of neonatal sepsis

Symptoms of neonatal sepsis are often minor and include lethargy, apnoea, hypotonia and feeding intolerance (Cooke et al, 1997). Clinical signs include hypotension, glucose dysregulation, an elevated white cell count (WCC), thrombocytopenia and an increase in infection markers e.g. C-reactive protein (CRP) and procalcitonin (PCT) (Ghazal et al, 2013). Because the symptoms could easily go unrecognised, it is crucial that neonatal sepsis is diagnosed as early as possible.

2.7 Diagnosis of neonatal sepsis

Untreated sepsis can account for more than 50% mortality rate. Physicians find it too great a risk to wait for positive cultures to confirm sepsis, therefore they will initiate therapy while waiting for culture results (Kermorvant-Duchemin et al, 2008). Late-onset neonatal sepsis is best diagnosed with a positive blood culture. Blood, urine and cerebrospinal fluid (CSF) should be collected if sepsis is suspected, before commencing

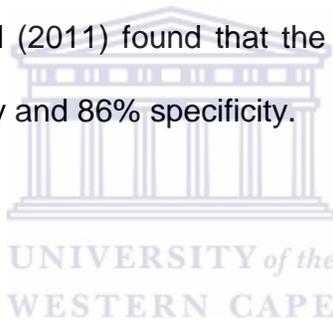
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antimicrobial treatment (Satar & Ozlu, 2012). Various biomarkers are available to aid in the diagnosis of sepsis (Chiesa et al, 2011) as discussed below.

2.7.1 Clinical biomarkers

2.7.1.1 C-reactive protein (CRP)

CRP, the most widely used diagnostic infection biomarker, is synthesised by the liver after six hours of onset of inflammation and tissue necrosis and peaks at 36-50 hours. It is also used in neonates to diagnose bacterial sepsis (Nabulsi et al, 2012 and Chiesa et al 2011). Mahbuba Meem et al (2011) found that the cut-off point for a positive CRP was 17mg/L with 66% sensitivity and 86% specificity.



2.7.1.2 Procalcitonin (PCT)

PCT is a precursor peptide from the hormone calcitonin (Satar & Ozlu, 2012 and Vazzalwar et al, 2005). Serum PCT concentrations increase in systemic inflammation particularly when the inflammation is caused by bacterial infection (Vazzalwar et al, 2005). When compared with CRP, PCT has a higher sensitivity in identifying late-onset sepsis in VLBW premature infants (Vazzalwar, 2005). According to Auriti et al (2012) a serum PCT value of $> 2.4\text{ng/ml}$ in VLBW premature infants increase the accuracy for neonatal sepsis before a blood culture confirms neonatal sepsis.

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2.7.1.3 Complete blood count (CBC)

A complete blood count (CBC) is a rapid, inexpensive and readily available diagnostic test to determine late onset neonatal sepsis. CBC results include a white blood cell (WBC) count, absolute neutrophil count (ANC), immature-to-total neutrophil count (I/T ratio), and platelet counts. Hornik et al (2012) found that a WBC count $< 1000\text{cells/mm}^3$ or $> 50\,000\text{cells/mm}^3$ and a platelet count of $< 50\,000\text{cells/mm}^3$ were associated with late-onset sepsis. (Hornik et al, 2012).



2.8 Gram positive neonatal sepsis

Late onset nosocomial sepsis is often caused by gram positive bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*; coagulase-negative staphylococci (CONS); *Enterococcus* species and methicillin resistant *Staphylococcus aureus* (MRSA) (Ghazal, 2013).

Staphylococci infection account for 30-50% of late-onset infections and can be attributed to intravascular devices e.g. umbilical artery, intravascular catheters (Satar & Ozlu, 2012) as well as endo-tracheal intubation and other invasive lines (Anderson, 2006) (Behrman, 2000).

2.9 Methicillin-resistant *Staphylococcus aureus* (MRSA)

MRSA is a well known nosocomial pathogen that is responsible for serious bacterial infections in premature infants and outbreaks in NICUs (Maraqa, 2011). MRSA developed resistance to beta-lactam antibiotics such as cephalosporins and penicillins (oxacillin, nafcillin and cloxacillin) (Haddadin, 2002).

The major route of transmission is from one premature infant to another by means of contact via healthcare workers (Maraqa, 2011). Active surveillance by means of routine MRSA screening, isolation of premature infants and rigorous attention to environmental decontamination and hand wash has proven to reduce the incidence of MRSA infections in NICUs (Maraqa, 2011).

Rana et al (2012) found that during their study period the incidence of MRSA cultures increased from 13.7- 24.77 per 1000 admissions ($p=0.01$). A sub-Saharan Africa study

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found that 28% of their *Staphylococcus aureus* positive cultures were MRSA (Kayange et al, 2010).

Maraqqa et al (2011) stated that risk factors contributing to MRSA infection include delivery by Cesarean section, low birth weight, multiple gestation and longer length-of-stay in the NICU. The factors that predicts a positive blood culture are cyanosis, lethargy, premature rupture of membrane (PROM), meconium stained liquor and convulsions (Kayange, 2010).

Treatment of suspected MRSA

MRSA infections can be treated with gram positive antibiotics such as linezolid, teicoplanin and vancomycin (Beers, 2006). The first choice antibiotic is vancomycin (Lutsar & Metsvaht, 2010).



2.10 Vancomycin

Vancomycin is a glycopeptide antibiotic with bactericidal activity against gram-positive bacteria (Hardman et al, 2001). It was originally produced by an Actinomycete isolated from soil samples obtained in Indonesia and India, called *Streptococcus orientalis* (Hardman et al, 2001). Vancomycin is a fermentation product and to produce the active pharmaceutical ingredient (API) at industrial level requires complex processes for biosynthesis and purification (Tattevin et al, 2013). Since Eli Lilly has stopped the production of the originator, Vancocin CP, the market has seen an increased in generic vancomycin formulations, all with debatable efficacy. (Vesga et al, 2010).

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2.10.1 Mechanism of action of vancomycin

The bactericidal activity of vancomycin is based on the inhibition of bacterial cell wall synthesis through hydrogen bonding to the C-terminal D-Ala-D-Ala residue portion of peptidoglycan precursor. It forms a non-covalent complex, which inhibits the precursor for cell wall synthesis (Howden, 2010). Any process that interferes with vancomycin binding to the D-Ala-D-Ala residue decreases the binding potency of the drug (Howden, 2010).

2.10.2 Side effects of vancomycin

According to Hardman (2001), one of the most common side effects of vancomycin administration is the so-called “Redman syndrome”. It is associated with erythematous or urticarial reactions, flushing, tachycardia and hypotension. This phenomenon is caused by the rapid infusion of vancomycin. Therefore vancomycin should be infused over a period of at least 60 minutes to avoid the symptoms associated with “Redman syndrome”. Hypersensitivity reactions, including skin rashes may occur in 5% of patients. Reversible neutopenia and eosinophilia have been reported (Gibbon, 2005).

2.10.3 Toxicity

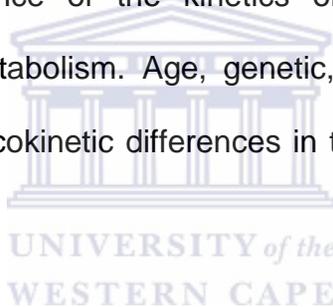
Auditory impairment is associated with high concentrations of vancomycin in plasma. It is seldom permanent and is worsened when co-administered with other ototoxic drugs e.g. aminoglycosides (Hardman, 2001).

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Nephrotoxicity is defined as an increase of $\geq 50\%$ in serum creatinine levels from baseline during vancomycin therapy (Pritchard et al, 2010). Nephrotoxicity has become an unusual side effect in premature infants when administered at appropriate doses. Caution must be exercised when administered with other nephrotoxicity drugs e.g. amikacin and gentamycin (Hardman, 2001).

2.11 Pharmacokinetic parameters of vancomycin

Pharmacokinetics is the science of the kinetics of drug absorption, distribution, elimination (excretion) and metabolism. Age, genetic, gender and ethnic differences may also contribute to pharmacokinetic differences in the general population (Shargel, 2005).



The following pharmacokinetic parameters are of value: peak plasma concentrations (C_{max}), trough concentrations (C_{min}), the apparent volume of distribution (V_d), renal clearance (Cl), elimination half life ($t_{1/2}$), metabolism, protein binding and the area under the curve (AUC) (Shargel et al, 2005).

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2.11.1 Peak plasma concentration (C_{\max})

The peak plasma concentration can be defined as the concentration achieved after a single dose. The measured plasma peak concentration should be between 30-40mg/L (Horn, 2007, De Hoog et al, 2000 and Rodvold et al, 1995).

2.11.2 Trough plasma concentration (C_{\min})

The trough level presents the minimum amount of the drug present in the blood. (Ulldemolins et al, 2011). The desired trough concentration for vancomycin is 5-10mg/L (McDougal et al, 1995, Rodvold et al, 1995 and Marsot et al, 2012). In meningitis the trough level should reach 10-15mg/L (Horn et al, 2007). There is conflicting evidence in the literature regarding the timing of trough levels. Some studies suggest (1) half an hour before the third dose (De Hoog et al, 2000), (2) half an hour before the fourth dose (Rybak et al, 2009).

There seem to be various recommendations in literature with regards to the dosing of vancomycin in premature infants. In a study done by Koren & James (1987), it was found that if the dosing of vancomycin is based on the PMA, 75.5% of the trough concentrations taken were within therapeutic range of < 10mg/l.

McDougal et al (1995) did a study where it was found that for premature infants with a PMA of 31-36 weeks, a vancomycin dosing schedule of 18mg/kg eighteen hourly and trough levels collected 30min before the third dose the mean trough levels were $3.4 \pm$

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0.6mg/l. No bacteriologic treatment failures were reported during this study despite the low though levels obtained.

In another study done by Machado et al (2007) the premature infants were divided into two groups with a statistical difference in PMA and PNA. All infants were dosed according to standard guidelines. It was found that group 1 with a statistically lower PNA ($p=0.02$) and PMA ($p<0.001$) had lower trough levels than group 2. It can be attributed to the increased extracellular fluid in group 1. It was also found that the Vd differed significantly between the two study groups ($p=0.01$) and that the volume of distribution can be inversely correlated to the PNA and PMA of premature infants (Machado et al, 2007).



2.11.3 Volume of distribution (Vd) UNIVERSITY of the WESTERN CAPE

The volume of distribution can be defined as the theoretical volume of fluid into which the total drug that was administered would be diluted, to produce a drug concentration in the plasma (Beers et al, 2006). Hydrophilic drugs, e.g. vancomycin, predominantly distribute into intravascular and interstitial fluid. These drugs do not penetrate the intracellular space in meaningful concentrations, due to the fact that it is unable to passively cross the lipid cellular membrane (Ulldemolins et al, 2011).

In premature infants the volume of distribution of hydrophilic drugs is affected by the total body water and the extracellular fluid volume (Ulldemolins et al, 2011 and Marsot et al, 2012). According to one study the volume of distribution for a neonatal study

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population (PMA = 34.6weeks; weight=1700g) was 0.572L/kg^{-1} (Marqués-Minana et al, 2010).

Vd in premature infants differ from that of adults and young children (Marqués-Minana et al, 2010) (Noya, 1998). The Vd in premature infants is larger than in adults (Marqués-Minana, 2010 and De Hoog et al, 2004). In premature infants (<2kg) approximately 80% of their body composition is extracellular fluids and water (Noya et al, 1998). Premature infants' extracellular body fluid decreases postnatally with a resultant in a decrease in vancomycin's Vd, resulting in higher trough levels than anticipated (Marqués-Minana et al, 2010). It was also found that a premature infant's current body weight has a strong positive correlation to the volume of distribution (Rocha et al, 2006). This finding were also supported by McDougal et al (1995) where it was found that the absolute Vd correlates with PMA ($p<0.0001$), but when compared with normalised Vd there was no correlation with PMA ($p=0.59$). This suggests that with an increased PMA, the absolute Vd decreases and that it is a function of body weight and not maturation of other physiological systems (McDougal et al, 1995).

2.11.4 Metabolism

Vancomycin is degraded into CDP-1 (a biological inactive degradation product (Vollmerhaus et al, 2003) when exposed to heat over time. Theoretically the accumulation of CDP-1 could be responsible for therapeutic failure (due to under dosing of vancomycin) and drug induced toxicity (Somerville et al, 1999).

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2.11.5 Elimination

Vancomycin is eliminated 80-90% unchanged by the kidneys (Gibbon et al, 2005). A small amount of vancomycin is eliminated via non-renal mechanisms of unknown origin (Marsot et al, 2012).

2.11.6 Vancomycin clearance (CL)

Vancomycin clearance (CL) is defined as the quantification of irreversible loss of drug form from the body's metabolism and excretion mechanisms (Uildemolins et al, 2011).

Given vancomycin's route of elimination via the kidneys, it is logical to assume that there is an association between the glomerular filtration rate (GFR) and the drug's clearance. The correlation between serum creatinine and the GFR with vancomycin clearance has been discussed in various studies according to the review article done by De Hoog et al (2004). Furthermore James et al (1987) found a positive linear correlation between the PMA of the premature infant and vancomycin clearance. This finding was supported by a study done by Reed et al (1987). It was also found in the same study done by Reed et al that the premature infant's body weight had a strong positive correlation with vancomycin clearance. This finding was also supported by Rocha et al (2006) where it was found that the premature infant's body weight at trial entry had a strong positive correlation with vancomycin clearance.

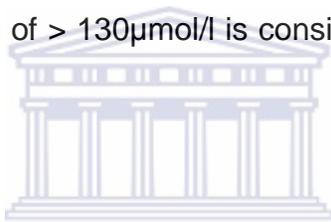
According to McDougal et al. (1995) the mean absolute clearance (L/h) of vancomycin increases with an increase in PMA ($p < 0.0001$). When the PMA is compared with normalised clearance (L/kg/h) there still exists a correlation between PMA and

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normalised clearance ($p < 0.005$) (McDougal et al, 1995). This suggested that the increase of absolute clearance with an increased PMA was not due to body weight alone, but also due to maturation of the renal function (McDougal et al, 1995). Thus the variables that have a strong influence on vancomycin clearance are the premature infant's body weight at trial entry, the PMA and the serum creatinine concentration.

2.11.6.1 Serum creatinine concentration a marker of renal function

Serum creatinine concentrations are widely used to describe the renal function of premature infants. Serum levels of $> 130 \mu\text{mol/l}$ is considered to be an indicator of renal impairment.



James et al (1987) did a study where it was found that serum creatinine concentration had a positive correlation with the half life of vancomycin and that the serum creatinine concentration had an irreversible correlation with vancomycin clearance. This finding was supported by Grimsley & Thomson (1999). They also found that if vancomycin were dosed according to serum creatinine concentrations, 75% of the trough levels were within the desired range of $5-12 \mu\text{g/ml}$. Thayyil et al (2008) reported that serum creatinine levels are influenced by gestational age (GA) and the post natal age.

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2.11.6.2 Glomerular filtration rate (GFR) of premature infants

Vancomycin is excreted primarily through the kidneys by means of glomerular filtration (Marqués-Minana et al, 2010).

Renal mechanisms are physiologically immature in newborn infants and the glomerular filtration rate (GFR) is 25% of that of adults and even lower in very premature infants (Noya, 1998)

Nephrogenesis starts at 5-6 weeks of gestation and is only fully completed at 36 weeks of age (De Cock et al, 2012 and Schreuder et al, 2009). Infants that were born prematurely (before the completion of nephrogenesis) have been shown to have less nephrons (Schreuder et al, 2009).

It was found that the measurement of GFR is unreliable in premature infants, because it reflects that of maternal serum creatinine concentrations. Grimsley & Thompson (1999) reported that the pharmacokinetics of vancomycin, including clearance, depended on both the current weight of the infant, as well as the serum creatinine levels.

The GFR in premature infants can be calculating using the following equation:

$GFR = 0.33 \times \text{height (cm)} / \text{Serum creatinine concentration } (\mu\text{mol/l})$. The GFR is expressed as ml/min/1.73m². Normal values for premature infants 30-32 weeks is 0.3-1.3 ml/min/1.73m² and for premature infants 33-35 weeks is 0.5-1.5 ml/min/1.73m² (Brion et al, 1986).

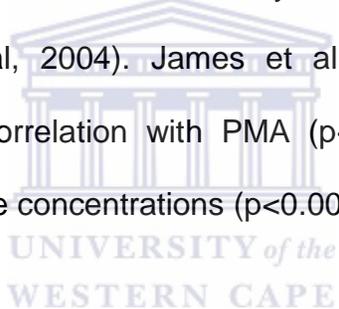
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2.11.7 Vancomycin half life ($t_{1/2}$)

The half life ($t_{1/2}$) can be defined as the time required for the plasma concentration of a drug to be eliminated by 50% (Ulldemolins et al, 2011). The $t_{1/2}$ of vancomycin is approximately 4-8 hours in adults with normal renal function (Hardman, 2001 and De Hoog et al, 2004). In a study done by McDougal et al it was reported that the $t_{1/2}$ in premature infants was 5.59 ± 0.36 hours for premature infants with a PMA of 31-36 weeks. The $t_{1/2}$ of most drugs, especially hydrophilic drugs, is longer in premature infants, due to their increased percentage of body water (De Hoog et al, 2000). In various studies it was found that the $t_{1/2}$ of vancomycin in premature infants is between 3.5-10 hours. (De Hoog et al, 2004). James et al (1987) found that the $t_{1/2}$ of vancomycin has a negative correlation with PMA ($p < 0.0001$) and a positive linear correlation with serum creatinine concentrations ($p < 0.0001$).

2.11.8 Steady state

Steady state is observed when the elimination rate of a drug equals the administration rate. Steady state is approximately five times the drug's $t_{1/2}$ (Shargel et al, 2005).

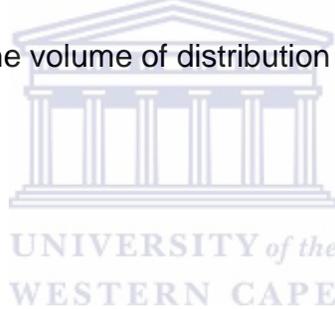


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2.11.9 Protein binding

Protein binding refers to the proportion of the drug that binds to plasma proteins (Uildemolins et al, 2011).

Vancomycin is 50-55% plasma protein bound, mainly to albumin and Immunoglobulin A (IgA) (Rybak et al 2009 and Sun et al 1993) and not to α -1 acid glycoprotein. While albumin is synthesised in the liver, its concentration is often low in premature infants in the first few weeks of life (Sun et al, 1993). Reduced plasma protein binding e.g. albumin bounding is associated with differences in body compartments (extracellular fluid) and frequently influence the volume of distribution (Jackson et al, 1999).



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Table 2.1: Results of pharmacokinetic studies in premature infants (Pacifci & Allegaert, 2012)

Reference	Reed et al (1987)	McDougal et al (1995) (Group 2)	De Hoog et al (2000) Retrospective study	Asbury et al (1993)
Sample size (n)	15	15	108	19
Gestational age (weeks)	28.4 ± 2.6	29.4 ±	28.9 ^a	29.3 ± 4.2
Post natal age (days)	20.5 ± 10.4	23 ± 14	14 ^b	33.9 ± 19.9
Postmenstrual age (weeks)	31.4	32.9	N/A ^c	34.2
Weight (g)	1069 ± 435	1194 ± 412	1045	1780 ± 1080
Dose (mg/kg)	12.6 Q24h	18 Q18h	15 Q12h	note A
Clearance (CL) (L/h)	1.22 ± 0.7	1.19 ± 0.08	3.4 ± 0.11	1.22 ± 0.54
Volume of distribution (Vd) L/kg	0.53 ± 0.13	0.56 ± 0.02	0.43 ± 0.013	0.52 ± 0.08
Half life (t1/2)	6 ± 2.0	5.6 ± 0.36	6 ± 0.27	5.6 ± 1.2
Peak levels (µg/ml)	31.2± 12	27.9 ± 1.2	34.3 ± 7.7	27.8 ± 0.7
Trough level (µg/ml)	9.5 ± 3.5	3.9 ± 0.6	8.2 ± 2.2	7.5 ± 1.2

^amean; ^bmedian; ^c NA: Not available

2.12 Pharmacodynamics of vancomycin

Pharmacodynamics (Pd) refers to the study of the relationship between the drug concentration in the human body and the effect it exhibits. The pharmacokinetic/pharmacodynamic characteristics of a particular drug seek to establish a relationship between the dosage of a drug and its pharmacological effect in the human body (Ulldemolins et al, 2011).

2.12.1 Area under the curve (AUC)

The activity of vancomycin has a time-dependant killing effect on gram positive bacteria. It has, however, been shown that under the same experimental conditions the higher the vancomycin concentration, the longer the post antibiotic effect (Vandecasteele et al, 2013). Therefore its efficacy is best predicted by the 24 hour area under the time curve (AUC_{0-24}) divided by the minimum inhibitory concentration (MIC) (Pacifici & Allegaert, 2012). The MIC is defined as the lowest concentration of an antimicrobial agent that is required to inhibit the visible growth of a microorganism *in vitro*, after overnight incubation (Gould et al, 2008).

Lutsar et al (2010) state that the target AUC_{0-24}/MIC ratio for vancomycin is 400 and can only be achieved with a dose of 60mg/kg/day if the MIC for *S.aureus* is ≤ 2 mg/l (Lutsar & Metsvaht, 2010). If the MIC of *S.aureus* is found to be >2 mg/ml alternative glycopeptide therapy should be considered to avoid possible resistance (Lutsar & Metsvaht, 2010).

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The susceptibility breakpoint for MRSA lays within a minimum inhibitory concentration (MIC) of 2µg/ml, therefore the risk for resistance increases as the MIC of vancomycin falls below the 2µg/ml breakpoint (Gould et al, 2008).



2.13 Therapeutic drug monitoring of vancomycin

The above mentioned pharmacokinetic and pharmacodynamic parameters play an important role in the therapeutic drug monitoring (TDM) of vancomycin.

Pharmacokinetic studies demonstrate a variability of TDM that is partially explained by weight, age and creatinine levels (Pacifci & Allegaert, 2012). This variability explains the reasoning behind the implementation of TDM of vancomycin trough levels - ensure the effectiveness of therapy as well as the minimisations of toxicity (Pacifci & Allegaert, 2012).

Therapeutic drug monitoring of vancomycin is crucial due to its nephrotoxic potential when the trough concentration is above 10µg/l (Marqués-Minana et al, 2010). Trough values below 5µg/l have the potential to cause micro-organism resistance (Marqués-Minana et al, 2010). Nephrotoxicity is associated trough levels above 10µg/ml, concurrent therapy with aminoglycosides and prolonged therapy exceeding 21 days. Concurrent treatment with amphotericin B and furosemide, existing renal failure and a high total dose of vancomycin also plays a role in nephrotoxicity (Pacifci & Allegaert, 2012). These two studies performed in premature infants and children showed that with the correct vancomycin TDM the glomerular and tubular nephrotoxicity could be reduced (Marqués-Minana et al, 2010 and Pacifci & Allegaert, 2012)

2.14 Vancomycin impurities

Before the 1970s, vancomycin was referred to as “Mississippi mud”, due to its impurities. These impurities (crystalline degradation product) were responsible for the ototoxicity and nephrotoxicity associated with vancomycin (Somerville et al, 1999). Once these were removed the side effects associated with vancomycin were eliminated (Somerville et al, 1999).

When exposed to heat over time, vancomycin breaks down into crystalline degradation product (CDP-1). Two isomers of CDP-1 exist, CDP-1m (minor) and CDP-1M (major). Both of which have no antimicrobial activity.

In vitro at 20-25°C, 50% of the total vancomycin starting weight will be broken down to CDP-1 in 16 hours, and 90% is converted to CDP-1 in 40 hours. To date no pharmacokinetic study was performed to determine the half-lives and route of elimination of CDP-1 (Somerville et al, 1999).

The USP (United States Pharmacopoeia) state that vancomycin should not contain less than 88% factor B (active ingredient) and not more than 4% impurities of which CDP-1 < 2% (Somerville et al, 1999).

2.15 Controversy around generic products

According to the World Health Organisation (WHO) two products are therapeutically equivalent “if they are pharmaceutically equivalent and after administration in the same molar dose, their effects with respect to both efficacy and safety are the same, as determined from appropriate bioequivalence, pharmacodynamic, clinical and *in vitro* studies.” (Vesga et al, 2010).

With the introduction of generic formulations into the market, multiple reports of impurities and sub-optimal antimicrobial activity have been reported for example, β -lactam agents, antifungal agents and glycopeptides e.g. vancomycin. In addition there is criticism around the world regarding the bioequivalence of generic products (Snyman et al, 2009).

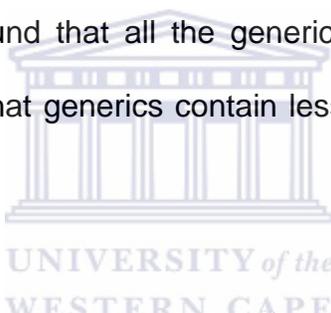
In a South African study done by Lowman et al (2011) they found that when comparing the generic meropenem with the originator, the generic was equivalent *in vitro* in terms of microbiological activity using the minimum inhibitory concentration (MIC).

Elli Lilly, the originator of vancomycin, discontinued the manufacturing of vancomycin end of 2004 (Tattevin et al, 2013). Generic vancomycin products have since emerged in the market and are being used all over the world (Nambiar et al, 2012).

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The efficacy of vancomycin has been questioned since the emergence of vancomycin resistance in isolates of *Staphylococcus aureus*; the increase in vancomycin MICs and failure for treating MRSA where the vancomycin MIC > 1.5µg/ml (Tattevin et al, 2013).

In a study done by Vesga et al (2010) it was stated that pharmaceutical equivalence does not imply therapeutic equivalence. The study compared three generic formulations of vancomycin with the innovator. The neutropenic mouse thigh infection model was used to compare the pharmacodynamic properties of the generic formulations of vancomycin and the innovator *in vivo*. *In vitro* testing was done by both microdilution and time-kill curves. It was found that all the generics failed *in vivo* to kill *S.aureus* ($p < 0.0001$). It was postulated that generics contain less factor B and three times more CDP-1 (Vesga et al, 2010).



This study caused intense debate amongst scientific communities, the public and drug regulatory authorities.

In the light of the concerns raised with regards to the quality of parenteral vancomycin products the US Food and Drug Administration (FDA) investigated the quality of vancomycin and its impurities. The investigation focused on high pressure liquid chromatography (HPLC) method as stated by the British (BP) and United States Pharmacopeia (USP) monographs. The FDA investigation found that factor B was between 90-95% and that the impurities were 5-10% and no single impurity was more than 2%. The conclusion from this investigation was that all generics used in this study

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surpassed USP standard for factor B and impurity levels of vancomycin. It was reported that the CDP-1 amount present in the generic formulations is of such small amount that it will be unlikely to affect the activity of vancomycin, given the antagonistic properties of competing for the binding site to D-Ala-D-Ala on the bacterial cell wall (Nambiar et al, 2012).

A newly published study done by Tattevin et al (2013) compared six generic vancomycin formulations on a rabbit model of aortic valve endocarditis induced with a MRSA strain. *In vitro* no significant difference was observed in time-killing curve studies with the six generic vancomycin formulations.

It was found that the mean peak concentration was above 35µg/ml and the mean trough concentration was below 10µg/ml. Treatment guidelines stipulate that for adults the serum trough levels must range between 15-20µg/ml and for premature infants between 5-10µg/ml. The AUC/MIC₍₀₋₂₄₎ was 369 with a dosage of 60mg/kg 12 hourly. This is below the recommended AUC/MIC₍₀₋₂₄₎ ≥400 for clinical efficacy (Tattevin et al, 2013).

It is necessary to test the assumption that generic formulations are not only pharmaceutically equivalent, but also therapeutic equivalent to the innovator. Pharmacokinetic studies in premature infants have not been performed to determine the therapeutic equivalence of generic formulations.

2.16 Experimental hypothesis

There is a difference between the trough levels of two commonly used vancomycin formulations with regards to:

- a) the correlation with clinical parameters,
- b) the therapeutic range of trough plasma concentrations of vancomycin, and
- c) and clinical treatment outcomes.

2.17 The Null hypothesis

There is no difference between the trough levels of two commonly used vancomycin formulations with regards to:

- a) the correlation with clinical parameters,
- b) the therapeutic range of trough plasma concentrations of vancomycin, and
- c) and clinical treatment outcomes.

2.18 Research questions

2.18.1 Is there a correlation between trough level 1 and clinical parameters observed, in the two vancomycin formulations?

2.18.2 What is the difference between the therapeutic ranges of the trough levels of the two vancomycin formulations?

2.18.3 What is the difference in clinical treatment outcomes between the two vancomycin formulations?

2.19 Objectives of the study

The objectives of this study are:

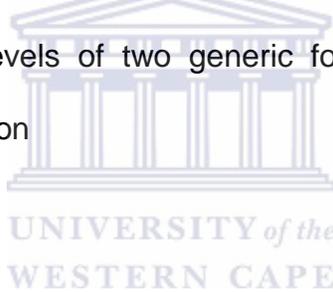
1. To compare vancomycin serum trough levels between the two generic formulations of vancomycin in a selected premature infant population with regards to:
 - d) the correlation with clinical parameters,
 - e) the therapeutic range of vancomycin serum trough levels of vancomycin, and
 - f) the clinical treatment outcomes.



Chapter 3 Research design and methodology

3.1 Study design

The study was designed as a prospective, comparative, double blinded randomised study comparing the trough levels of two generic formulations of vancomycin in a selected preterm infant population



3.2 Study site

The study was conducted at Netcare Blaauwberg and N1City hospitals. Netcare is the largest provider of health care in both South Africa and the United Kingdom and was founded in 1996.

3.3 Inclusion criteria

Infants were included in the study if they complied with the following criteria:

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- a. All Infants with a corrected post menstrual age between 29-35 weeks admitted in the Neonatal Intensive care unit (NICU) with suspected nosocomial infection warranting vancomycin as per the discretion of the attending physician (28).

Early antenatal ultrasound was used to determine gestational age, and when not available, the new Ballard score was used.

- b. Informed and written parental consent for each infant enrolled in the study

3.4 Exclusion criteria

An infant was excluded from the study when he/she had the following:

Renal dysfunction

Chromosomal abnormalities or in extremis

Concomitant treatment with a drug known to interact with vancomycin

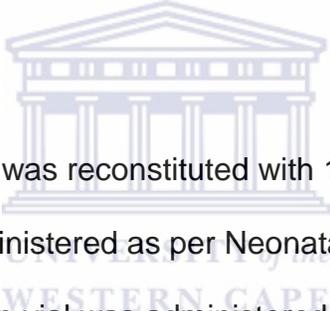
Refusal of consent by parents (Die numering het hier vreemde goed gedoen...)

3.5 Randomisation

The two study groups were divided into vancomycin A and vancomycin B. Each vial's original label was obscured and a new label was created to state the expiring date, the batch number and the strength of the vancomycin vial. As per computer generated (<http://www.random.org/sequences/>), numbers were randomly allocated to each of the two study groups by an external person. The envelopes contained 1x500mg vial vancomycin "A" or "B", three sticky labels indicated that the patient was on the

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vancomycin trial to be stuck onto the patient's prescription chart, and one non-sticky label indicating that the patient was on the vancomycin trial and to be placed into patient's medication file. The envelopes were sealed and two independent signatures signed the envelopes, therefore preventing from being tampered. Each envelope was given a number that corresponded with the computer generated number. The envelopes were randomised and sealed, thereby allocating the infant to either one of the two formulations of vancomycin. The attending physician drew an envelope as an infant met with the inclusion criteria. The physician was blinded to the vancomycin formulation. Each participant was allocated a study number (envelope number) to ensure patient confidentiality.



Each vial of 500mg vancomycin was reconstituted with 10ml water for injection to make a solution of 50mg/1ml and administered as per Neonatal Intensive Care Unit (NICU) protocol. The 500mg vancomycin vial was administered at 15mg/kg twelve hourly as per formulary via peripheral intravenous line over 60 minutes by a registered nurse at the NICUs.

3.6 Anthropometrics

The collected demographics of the study population included the post menstrual age (PMA), chronological age, also referred as postnatal age (PNA), gestational age (GA), gender, birth weight, weight at trial entry, height at trial entry, serum creatinine levels, GFR and albumin levels.

3.7 Biological parameters to be assessed

All blood sampling took place under standard clinical guidelines and no additional blood was taken for study purposes.



When a MRSA infection was suspected, C-reactive protein (CRP) levels were collected. Procalcitonin (PCT) levels were taken upon discretion for the physician.

Albumin and serum creatinine levels were collected as per NICU protocol.

3.8 Blood sampling procedures

Blood sample of 0.3-0.4ml was collected in a BD Mirotrainer SST™ tube (Becton, Dickinson & Company, Franklin Lakes, NJ, 07417, USA), allowed to clot and then centrifuged to separate the clot from the serum.

Sampling for vancomycin trough levels was done thirty minutes before the administration of the third dose of vancomycin by a registered nurse from both the NICUs at Netcare Blaauwberg Hospital and N1City Hospital. The samples were transported to the Ampath-Davies laboratory at N1City, Cape Town for analysis.

3.9 Plasma trough concentrations determination

The Architect™ c1600 System from Abbott Laboratories was used to do the particle-enhanced turbidimetric inhibition immunoassay (PETINIA). The lower quantification limit is 1.1µg/l. The serum trough levels were interpreted by attending microbiologist at Ampath-Davies, N1 City, Cape Town. The results were sent to the attending physician for continued clinical management and for data collation by the study group.

Data were collected in the NICUs at Netcare Blaauwberg and N1 City. Data were obtained from the each infant's patient file, nursing and physician notes.

3.10 Treatment outcomes

When a premature infant showed signs of sepsis the physician prescribed meropenem and vancomycin concurrently. Whereas vancomycin was to treat suspected MRSA and meropenem was to treat suspected gram negative bacteria.

Vancomycin treatment was discontinued after two negative CRP values and a negative blood culture. Premature infants were discharged from the NICUs on the physicians' discretion and clinical improvements.

3.11 Statistical analysis

3.11.1 Sample size

Using the statistical analysis computer program SAS® 9.3, 2011, USA, estimation was made giving an indication of how many infants will be needed to make the study statistically significant.

Assuming a difference of three units is clinically important. , that a difference will be detectable with better than 80% power based on a two-sample t-test with a two-sided alternative hypothesis and a significance level of 0.05 when the actual standard deviation is 1.5 units. Therefore the number of patients needed is nine in each study group.

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3.11.2 Data analysis

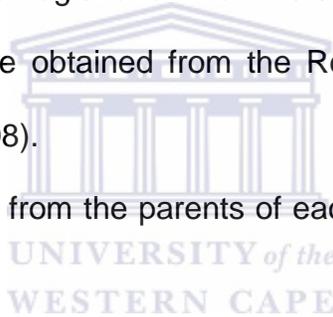
Data analysis was performed by the SAS® 9.3 program and Pearson correlation coefficients were used to compare the trough levels with variables and pooled t-test were performed to determine the p-values. Given $p=0.05$ being scientifically significant.

3.12 Ethical consideration

Ethical approval was obtained from the Ethics Committee of the University of Western Cape for this study (Certificate registration no. 12/2/21). Permission to conduct the study in Netcare hospitals were obtained from the Research Department of Netcare (Reference no. (UNIV-2012-0008).

Informed consent was obtained from the parents of each premature infant enrolled into the study.

The study was done in accordance with Helsinki Declaration and confidentiality was observed.



3.13 Dissemination of research results

The study results would be disseminated through the following:

1. Presentation of results at the School of Pharmacy, University of Western Cape,
2. Poster presentation at the Three's Company Conference 2013.
3. Publication in a relevant scientific journal
4. Thesis for Master's degree at the University of the Western Cape.



Chapter 4 Results

4.1 Study population

4.1.1 Patient demographics

The study was conducted from April 2012 to July 2013. It involved 19 premature infants who met study inclusion criteria. The patient demographics, dose/body weight, serum albumin levels, serum creatinine levels and the glomerular filtration rate (GFR) are summarised in Table 4.1 (n=10) for the Aspen Vancomycin CP® group (AV) and in table 4.2 (n=9) for the Sandoz Vancocin CP® group (SV).

Ten premature infants (50% male and 50% female) were randomly included in AV group and 9 premature infants (33% male and 66% female) SV group.

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Table 4.1: Summary of Aspen Vancomycin® patient demographics, dose/body weight, serum albumin levels, serum creatinine levels and glomerular filtration rate.

Study number	Gender	Gestational age (Weeks)	Birth body weight (g)	Post natal age (days)	Post menstrual age (weeks)	Body weight at trial entry (g)	Height at trial entry (cm)	Dose/Body weight (15mg/kg)	Serum albumin levels (g/l)	Serum creatinine (mmol/l)	Glomerular filtration rate (GFR) (ml/min)
1	M	32	2130	7	33	2080	45	31.95	24	0.39	38
2	F	32	2690	18	34.57	2615	45	35	32		
3	M	31	1710	9	32.29	1660	44	25.6	27	0.22	65
6	M	30	1640	17	32.43	1850	36	27.8	24		
7	M	28	940	18	30.57	1080	38	16.2	19	0.26	48
11	F	32	1390	7	33	1490	45	22.4	34	0.28	53
12	F	32	1230	7	33	1250	41	18.8	34	0.38	35
15	F	33	1170	3	33.42	1170	46	26.5	28	0.21	72
9	M	28	1270	14	30	1300	28	19.5	24	0.12	74
17	F	32	1585	7	33	1585	48	23.8	32	0.33	47
Median		32	1487.5	8	33	1537.5	44.5	24.7	27.5	50.5	0.27
Range		28-33	940-2690	3-18	30-34.6	1080-2615	28-48	18.8-31.95	19-34	35-74	0.12-0.39

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Table 4.2: Summary of Sandoz Vancocin CP® patient demographics, dose/body weight, serum albumin levels, serum creatinine levels and glomerular filtration rate.

Study number	Gender	Gestational age (Weeks)	Birth body weight (g)	Post natal age (days)	Post menstrual age (weeks)	Body weight at trial entry (g)	Height at trial entry (cm)	Dose/body weight (15mg/kg)	Serum albumin levels (g/l)	Serum creatinine (mmol/l)	Glomerular filtration rate (GFR) (ml/min)
4	F	34	2015	5	34.71	1855	45	30		50	0.297
5	F	29	925	36	34.14	1115	39	16.7	25	60	0.214
8	F	28	775	30	32.28	925	32	13.8	34	50	0.211
10	M	26	680	43	32.14	980	36	14.7			
13	F	26	910	35	31	1165	36	17.5	37	39	0.304
14	M	33	1160	3	33.42	1150	40	17.4	23	52	0.254
16	M	30	1450	11	31.57	1480	42	22.2	22	53	0.262
18	F	23	630	58	31.28	1830	40	27.5	27	26	0.507
19	F	31	1590	18	33.57	1640	43.5	24.5	23	61	0.235
Median		29	925	30	32.28	1165	40	17.5	25	51	0.258
Range		26-34	630-2015	3-58	31-34.7	925-1855	32-45	13.8-27.5	22-37	26-61	0.211-0.507

The results are presented as the median and range.

In the AV group the median (range) gestational age (GA), post natal age (PNA) and postmenstrual age (PMA) were 32 (28-33) weeks, 8 (3-18) days and 33 (30-34.6) weeks respectively. The median (range) birth body weight, body weight at trial entry and height at trial entry were 1487.5 (940-2690) g, 1537.5 (1080-2615) g and 44.5 (28-48) cm respectively.

In the SV group it was found that the median (range) GA, PNA and PMA were 29 (26-34) weeks, 30 (3-58) days and 32.28 (31-34.7) respectively. The median (range) birth body weight, weight at trial

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entry and height at trial entry were 925 (630-2015) g, 1165 (925-1855) g and 40 (32-45) cm respectively.

For the AV group the median (range) albumin, serum creatinine and GFR are 27.5 (19-34) g/l, 50.5 (35-74) mmol/l and 0.27 (0.12-0.39) ml/min/1.73m² respectively.

The median (range) albumin, serum creatinine and GFR are 25 (22-37), 51 (26-61) and 0.26 (0.21-0.51) respectively for the SV group



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Table 4.3: Comparison of patient demographics as per study group.

Demographics	n	Aspen-Vancomycin (AV) (Median)	Range	N	Sandoz-Vancocyn (SV) (Median)	Range	p-value
GA (weeks)	10	32	28-33	9	29	23-34	0.202
BBW (g)	10	1488	940-2690	9	925	630-2015	0.061
PNA (days)	10	8	3-18	9	30	3-58	0.102
PMA (weeks)	10	33	30-34.6	9	32.28	31-34.7	0.936
Weight at trial entry (g)	10	1538	1080-2615	9	1165	925-1855	0.195
Height at trial entry (cm)	10	44.5	28-48	9	40	32-45	0.179

Key: GA=gestational age; BBW=birth body weight; PNA=postnatal age; PMA=postmenstrual age

Table 4.3 compares the patient demographics as per study group. Non-parametric tests are used when the sample sizes are small, since these techniques are distribution-free techniques and therefore do not have any assumptions of underlying distributions of the data. Wilcoxon Two-Sample Test (Two-sided) was used to determine the p-values. The p-value is indicated for each parameter compared. Since all p-values are higher than 0.05 (5% significant level), there is no significant difference between the two study groups regarding GA, BBW, PNA, PMA, weight at trial entry and height at trial entry.

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4.1.2 Biological parameters

Table 4.4: Comparison of biological parameters as per study group.

Biological parameters	Aspen-Vancomycin CP® (AV)			Sandoz – Vancocin® (SV)			p-value
	n	(Median)	Range	n	(Median)	Range	
Albumin (g/l)	10	27.5	19-34	7	25	22-37	0.696
Serum creatinine concentration (mmol/l)	8	50.5	35-74	8	51	26-61	0.757
Glomerular filtration rate (GFR) (ml/min/1.73m ²)	8	0.27	0.12-0.39	8	0.26	0.21-0.51	1.00

Normal values: Albumin – PMA: 29-33 weeks = 22-35g/l; Serum creatinine - >130µmol/l indicative of renal impairment;

GFR: PMA 30-32 weeks = 0.3-1.3 and PMA 33-35 = 0.5-1.5

Table 4.4 compares the biological parameters *viz.* the albumin levels, serum creatinine concentration and GFR, of each of the study groups. The results are presented as the median (range).

Since p-values are higher than 0.05 (5% significant level), there is no significant difference between the two groups regarding the albumin levels, serum creatinine concentration and GFR.

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Figure 4.1: Comparing albumin levels with trough level 1 in AV and SV

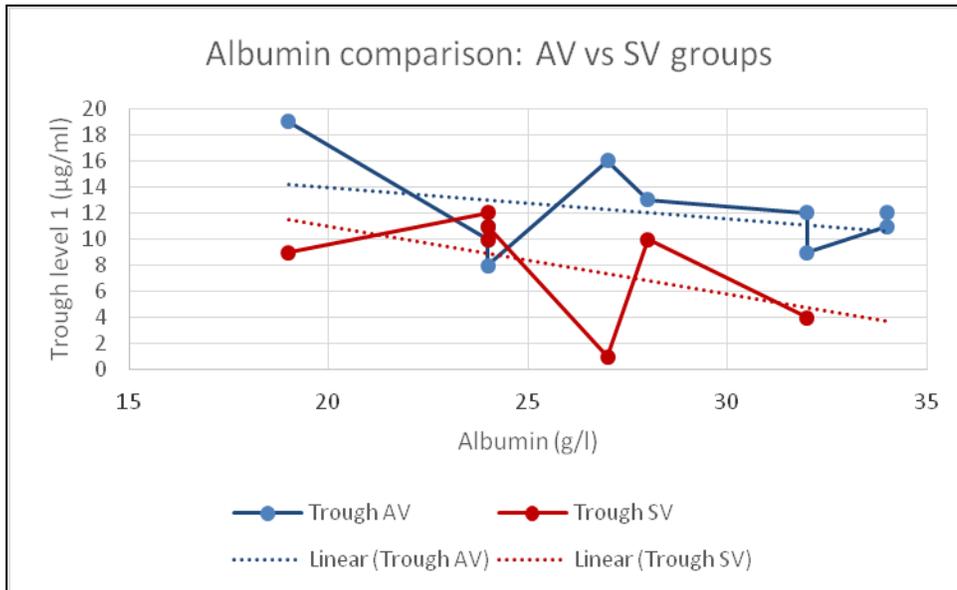


Figure 4.1 compares the relationship between trough level 1 and the albumin levels taken per premature infants in both study groups. The premature infants in the AV group is represented by the blue line, and those in the SV group with the red line. Eighty four percent of premature infants had an albumin level taken. It can be observed that there is a lower trend towards albumin levels observed in the SV group.

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Comparison of trough levels assessed

Table 4.5: Comparison of trough level 1 as per study group

Trough levels	Aspen – Vancomycin (AV)			Sandoz-Vancomycin (SV)			p-value
	n	(median)	Range	N	(median)	Range	
Trough 1 (µg/ml)	10	11.5	8-19	9	10	1-14	0.118

In table 4.5 trough levels 1 of both study groups were compared. Trough levels were collected for each premature infant depending on the severity of the illness and the duration of the vancomycin therapy, as per discretion of the attending physician. The results are presented as median (range). Wilcoxon Two-Sample Test (Two-Sided) was used to determine the p-value. Since the p-value is larger than 0.05 (5% significant level), there is no significant difference between trough level 1 of the two study groups.

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Figure 4.2: Comparing trough level 1 in AV and SV

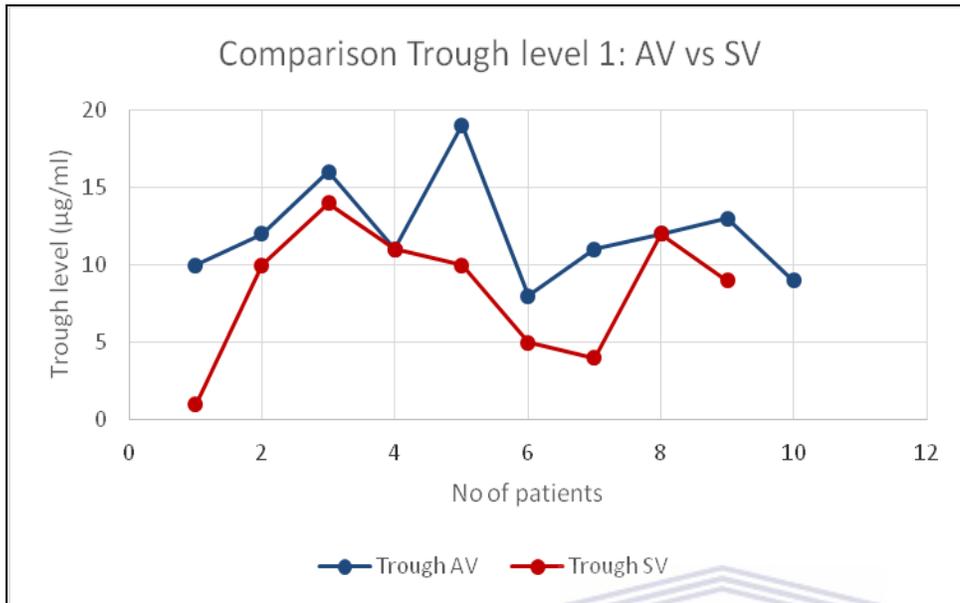


Figure 4.2 compares trough level 1 observed in premature infants in AV (blue line) and SV (red line). There is a trend towards a lower trough level 1 in the SV group when compared to with those in the AV group.

Trough level 2 and 3 were measured at the physician's discretion and not 60 minutes before the sixth and ninth dose respectively, as per study protocol. Therefore it could not be included in this study.

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4.3 Correlations between parameters assessed and trough levels 1

Table 4.6: Correlations between clinical parameters and trough level 1 in each study group.

Clinical parameters	Aspen Vancomycin (n=10)		Sandoz Vancomycin (n=9)	
	r-value	p-value	r-value	p-value
GA (weeks)	0.01	0.971	0.89	0.0013
BBW (g)	-0.28	0.432	0.71	0.031
PNA (days)	0.19	0.594	-0.73	0.026
PMA (weeks)	0.06	0.877	0.903	0.0008
Body weight at trial entry (g)	-0.34	0.344	0.017	0.966
Height at trial entry (cm)	-0.02	0.960	0.39	0.291
Albumin (g/l)	-0.07	0.845	-0.37	0.410
Serum Creatinine (mmol/l)	-0.02	0.955	0.43	0.291
GFR (ml/min/1.73m ²)	-0.12	0.779	-0.46	0.257

Key: GA=gestational age; BBW=birth body weight; PNA=postnatal age; PMA=postmenstrual age; r=correlation coefficient

The actual trough levels 1, not the average, were correlated by Spearman's rank order correlation for GA, BBW, PNA, PMA, bodyweight at trial entry, height at trial entry, serum albumin levels, serum creatinine levels and GFR. Table 4.6 describes the correlations found.

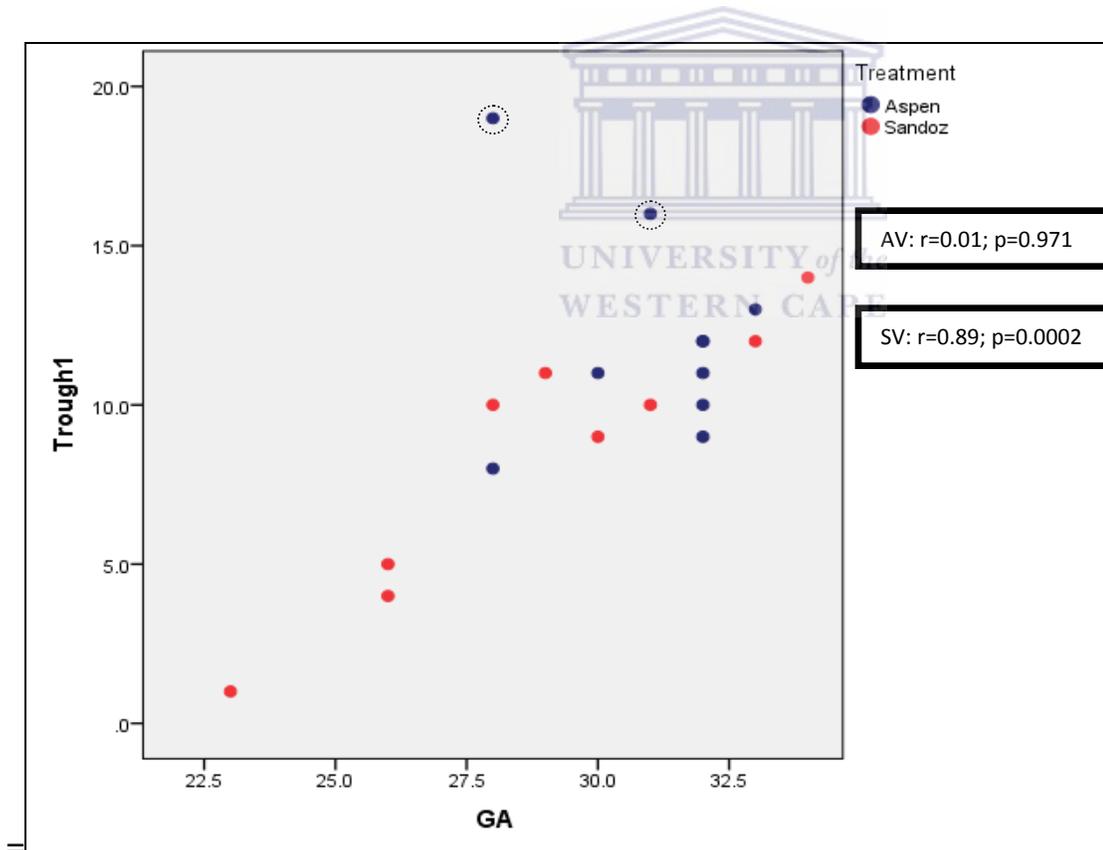
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There was no correlation between any of the parameters assessed and AV trough level 1.

Though it was found that there is a strong positive correlation between SV trough level 1 and GA ($r=0.89$; $p=0.0013$), BBW ($r=0.71$; $p=0.031$), and PMA ($r=0.903$; $p=0.0008$), a negative correlation was found between SV trough level 1 and PNA ($r=-0.73$; $p=0.021$). No correlation was found between the body weight and height at trial entry ($r=0.017$; $p=0.961$ and $r=0.39$; $p=0.291$) respectively.

No correlation was found between SV trough level 1 and the following biological parameters albumin ($r=-0.37$; $p=0.41$) serum creatinine ($r=0.43$; $p=0.291$) and GFR ($r=-0.63$; $p=0.257$).

Figure 4.3: Scatter plots comparing trough level 1 and gestational age (GA) of both study groups.



When SV is considered (the red dots), it is clear that there is a good positive trend. For this reason, a significant correlation ($r=0.89$; $p=0.0002$) is found between GA and trough level 1 for the SV group. In

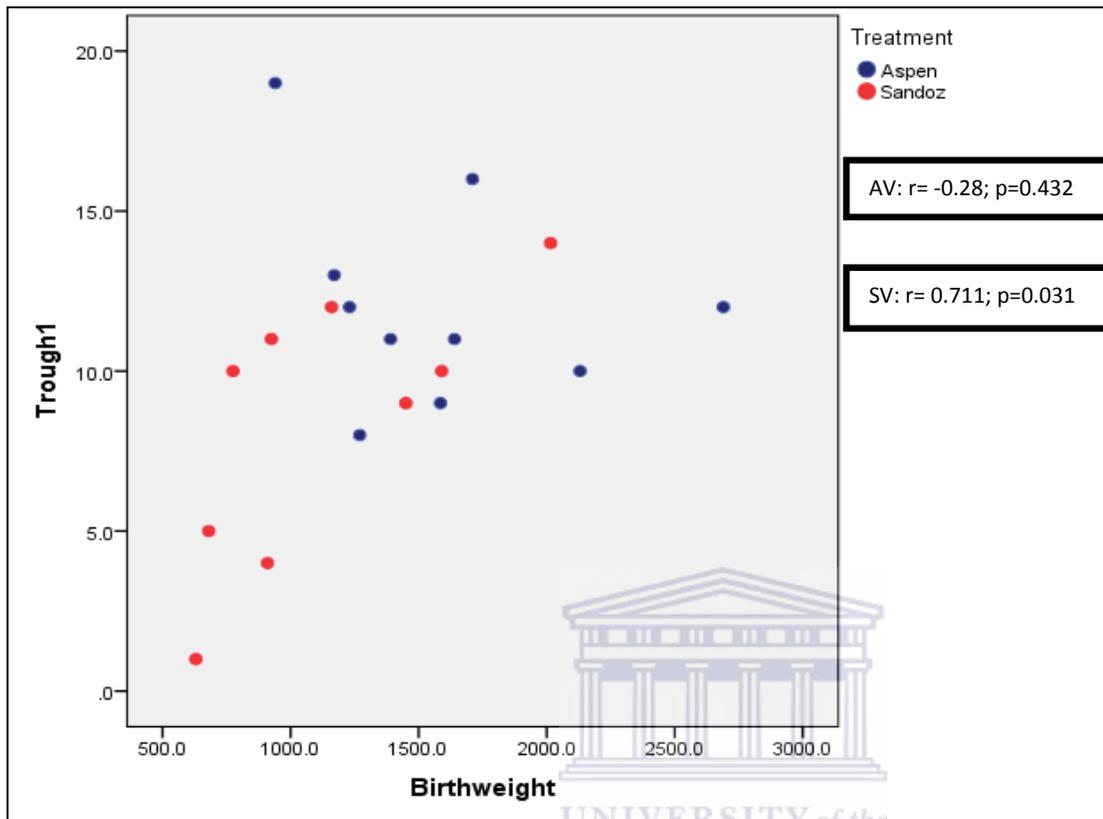
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the AV group, two observations have much higher values for trough level 1 than the rest (see encircled blue dots). These two values ensure that the correlation between these two variables are not significant ($r=0.01$; $p=0.971$) for the AV group.



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Figure 4.4: Scatter plots comparing trough level 1 and birth weight (BW) of both study groups.

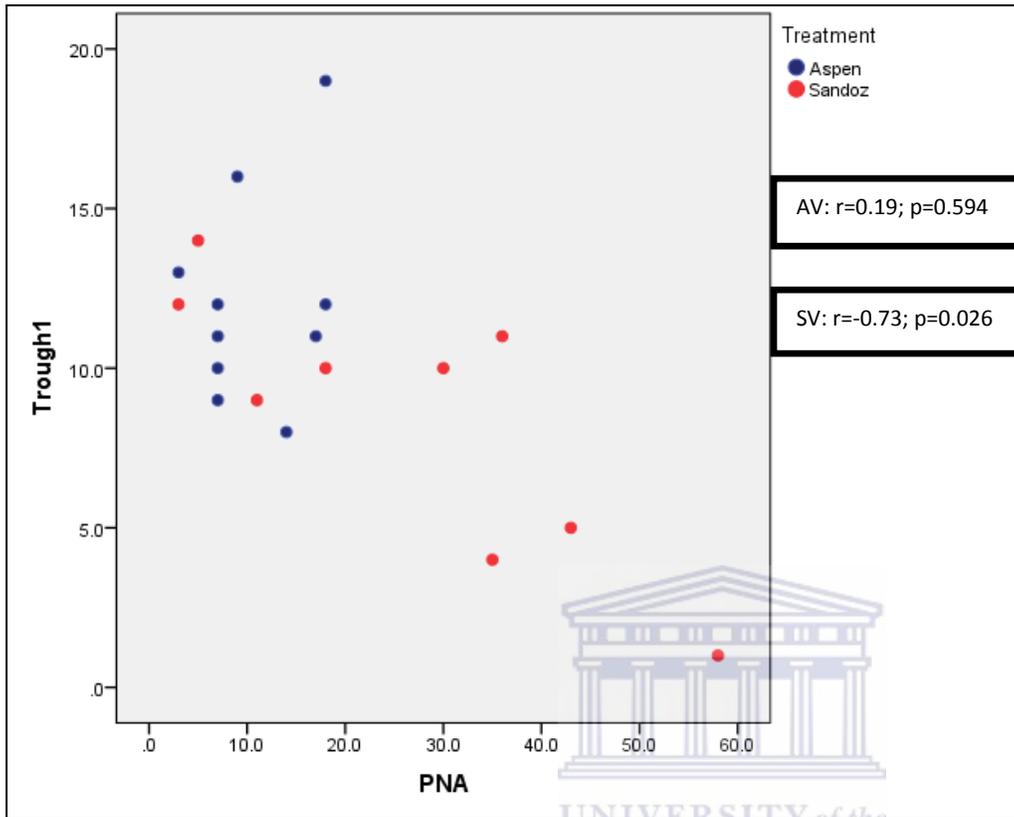


The values of AV are scattered, thus no good correlation ($r = -0.28$; $p = 0.432$) is found for this group between the birth weight and trough level 1.

There is a positive correlation ($r = 0.711$; $p = 0.031$) between birth weight and trough level 1 in the SV group.

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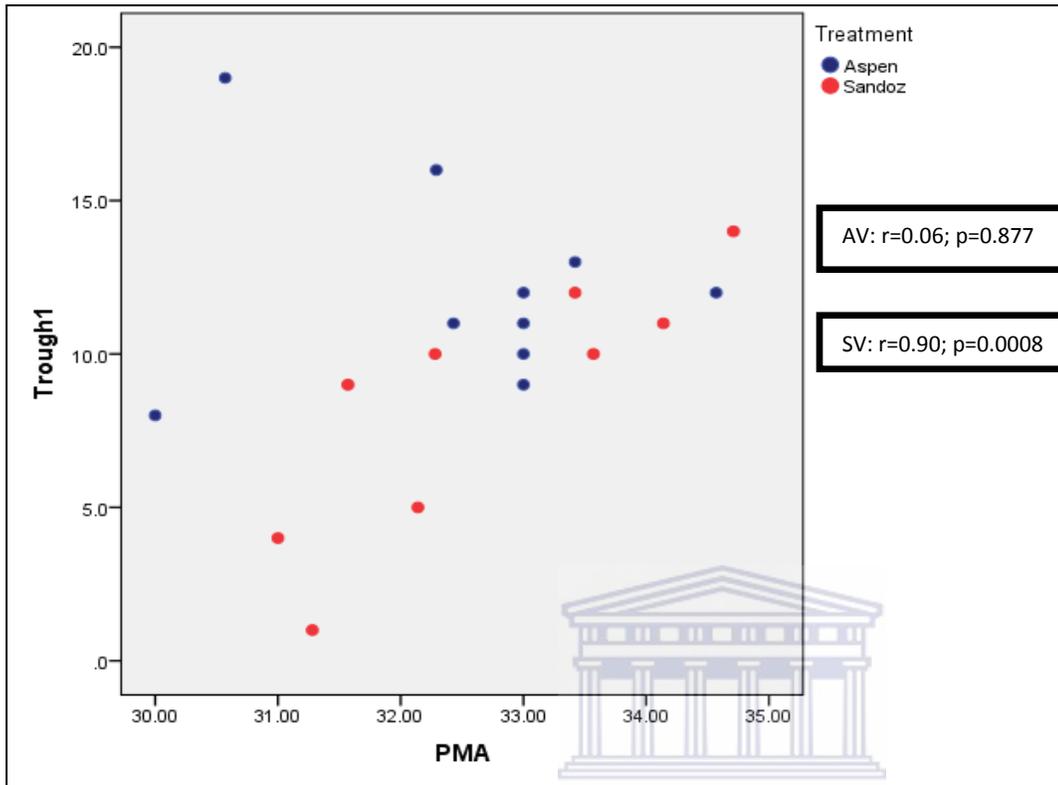
Figure 4.5: Scatter plots comparing trough level 1 and PNA of both study groups



The values of AV are scattered, no clear trend is visible, thus no significant correlation ($r=0.19$; $p=0.594$) is found for this group between PNA and trough level 1. It is clear that there is a negative correlation ($r=-0.73$; $p=0.026$) between these two variables for SV.

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Figure 4.6: Scatter plots comparing trough level 1 and PMA of both study groups.



The presence of the two outliers in the AV group is the reason that there was no correlation found between the PMA and trough level 1.

A strong positive correlation ($r=0.90$; $p=0.0008$) was found between the PMA and trough level 1 in the SV group.

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4.4 Therapeutic ranges of trough level 1 in both study groups

Table 4.7: Distribution of premature infants with regards to trough level 1 being below minimum therapeutic serum concentration (<5 µg/ml I), within serum therapeutic range (5-10 µg/ml) and above minimum toxic serum concentrations (>10mg/l) in both study groups

	<5 µg/ml	5-10 µg/ml	>10 µg/ml	Total
Aspen Vancomycin® (AV)	0 0%	3 30%	7 70%	10
Sandoz Vancomycin CP® (SV)	2 22.22%	4 44.44%	3 33.33%	9
Total	2	7	10	

Table 4.7 above describes the number (top) and percentage (bottom) of premature infants whose trough level 1 were below minimum effective concentration (<5 µg/ml), within therapeutic range (5-10 µg/ml) and above minimum toxic serum concentration (>10 µg/ml) in both study groups.

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Figure 4.7: Distribution of premature infants that had a trough level 1 below the minimum effective concentration (<5 µg/ml) for each study group

Distribution of premature infants that had a trough level 1 below the minimum therapeutic concentration (<5 µg/ml) for each study group (n=2).

(Solid blue line with circles=AV and dotted green line with squares = SV)

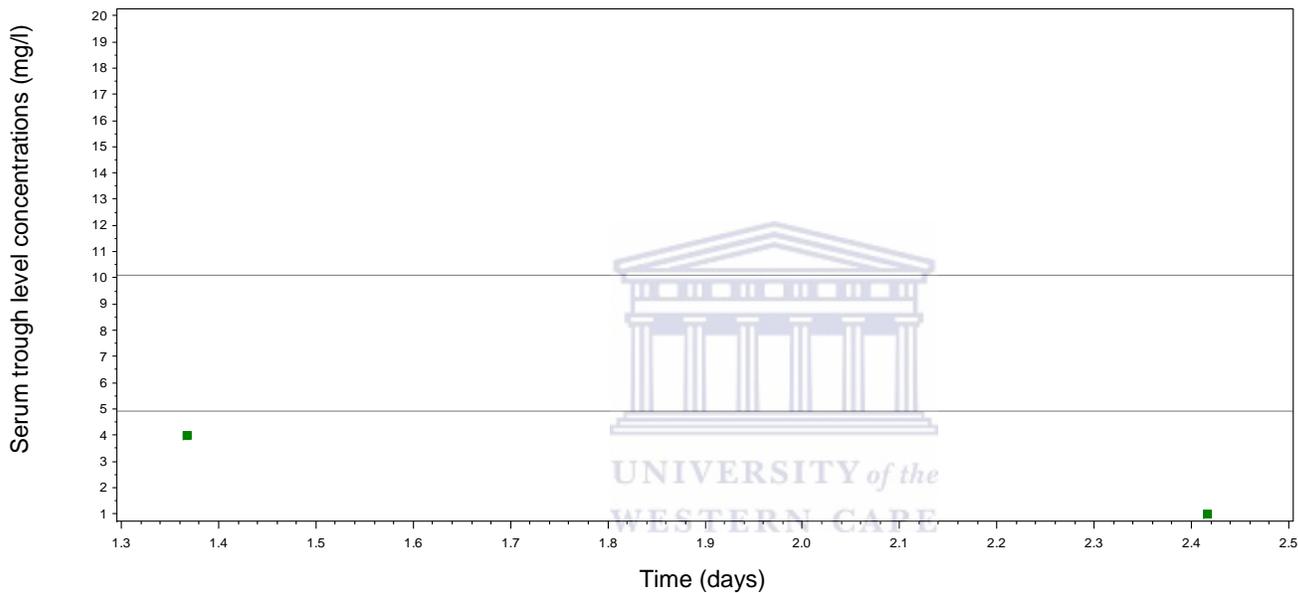


Figure 4.7 describes those premature infants with trough level 1s that were below the minimum therapeutic serum concentration. The two squares indicate that both the premature infants received SV. No additional trough levels were taken for these two premature infants as per the physician's discretion. Therapy was discontinued after trough level 1 in both premature infants.

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Figure 4.8: Distribution of premature infants that had a trough level 1 within therapeutic range (5-10 $\mu\text{g/ml}$) for each study group

Distribution of premature infants that had a trough level 1 within the therapeutic range (5-10 $\mu\text{g/ml}$) for each study group (n=7).

(Solid blue line with circles=AV and dotted green line with squares = SV)

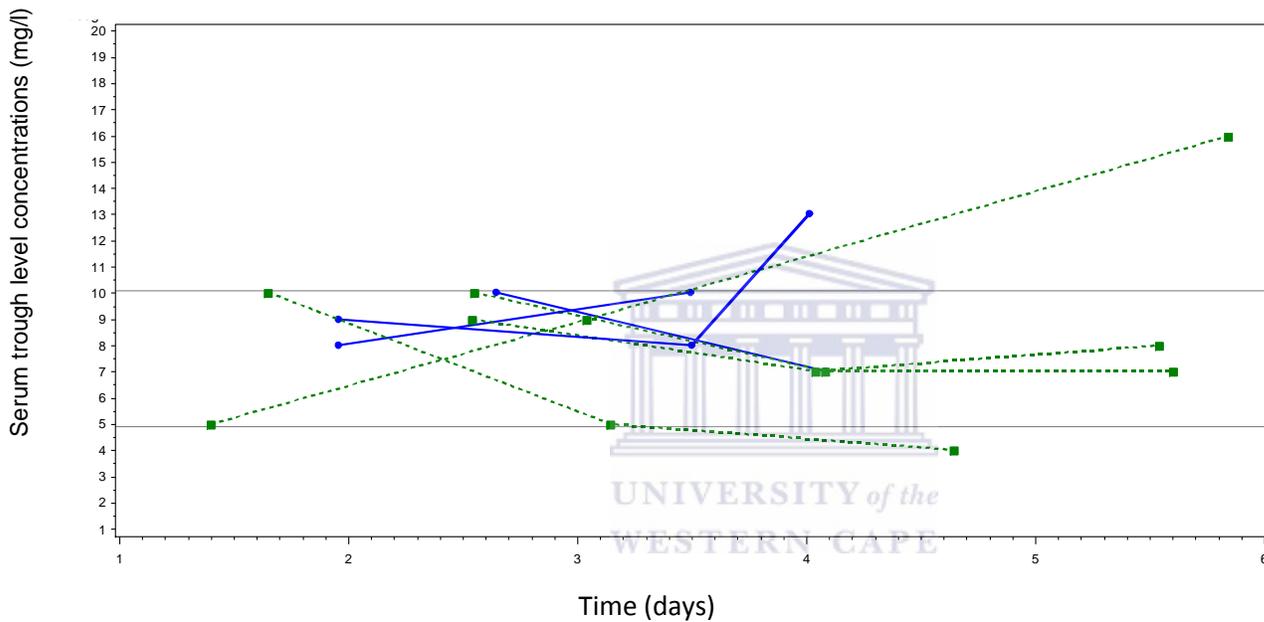


Figure 4.8 describe those premature infants that had a trough level 1 within therapeutic range and the placement of trough level 2 and 3 respectively.

The dotted green line and squares indicate those premature infants that received SV and the solid blue line with the circles indicates those infants that received AV.

For the study population it was found that all seven premature infants (4 received SV and 3 received AV) had though level 1 within therapeutic range also had the second trough level within therapeutic range. Four out of the seven (57%) premature infants (3 SV and 1 AV) had the third trough level within therapeutic range. Two of the seven (29%) premature infants (1 SV and 1 AV) had the third

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trough level above the minimum toxic concentration and one premature infant (SV) (14%) had the third trough level below the minimum effective concentration.



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Figure 4.9: Distribution of premature infants that had a trough level 1 above the minimum toxic serum concentration ($>10\mu\text{g/ml}$) $n=10$.

Distribution of premature infants that had a trough level 1 above minimum toxic serum concentration ($>10\mu\text{g/ml}$) for each study group ($n=10$).

(Solid blue line with circles=AV; Dotted green line with squares = SV)

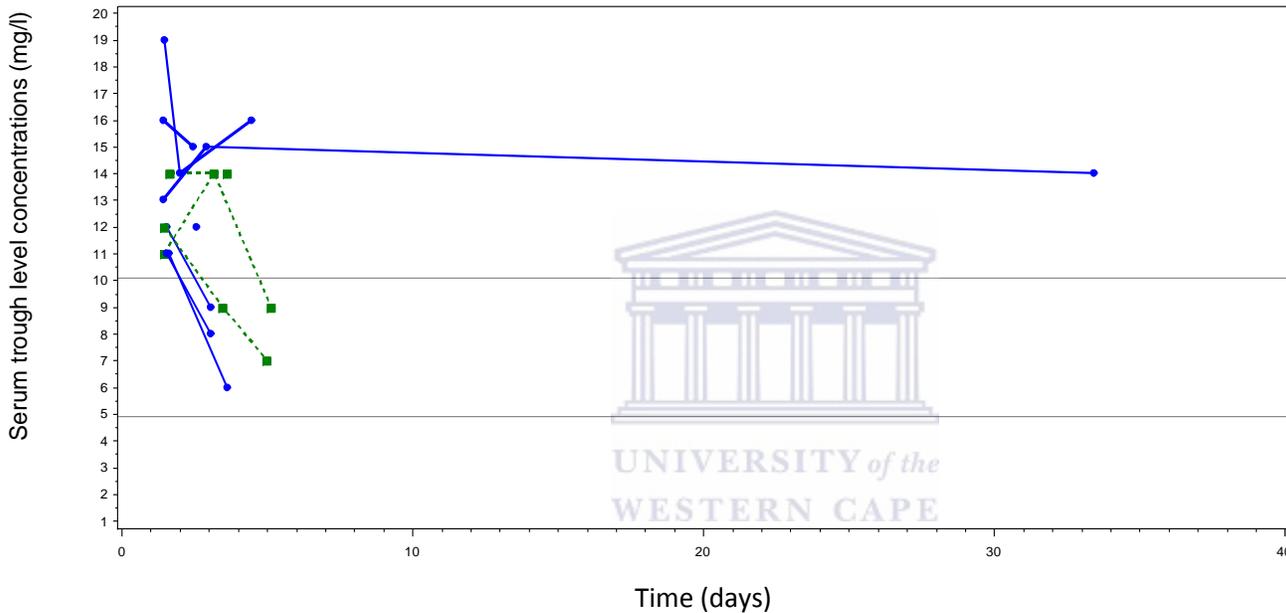


Figure 4.9 describes premature infants that had a trough level 1 above the minimum toxic serum concentration ($>10\mu\text{g/ml}$) and the results of trough level 2 and 3 respectively.

It was found that ten out of 19 (52%) premature infants had trough level 1 above the minimum toxic serum concentration. Seven (36%) premature infants received AV and three (15%) received SV.

Out of the seven premature infants that received AV, five had a second trough level taken. Therapy was discontinued as per the physician's discretion in two of those seven premature infants before a second trough level could have been taken. Out of the five premature infants that had a second

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trough level taken, two had a second trough level within therapeutic range and three had the second trough level above the minimum toxic serum concentration. Out of the seven premature infants that received AV, only two had a third trough level taken and it was also above the minimum toxic concentration. Both those premature infants had all three trough levels above the minimum toxic serum concentration.

The three premature infants that received SV had a second trough level taken. Two premature infants' second trough level was above the minimum toxic concentration and one was within therapeutic range. Both those premature infants had a third trough level within therapeutic range. Therapy was discontinued as per the physician's discretion in one premature infant after the second trough level was taken.



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4.5 Treatment outcomes of the both study groups

Table 4.8: Treatment outcomes of Aspen-Vancomycin® premature infants

Premature infant study no.	LOS (days)	Corrected GA at discharge (weeks)	Body weight at discharge (g)	Blood culture	Reason for vancomycin treatment	Treatment duration (doses)	CRP (NV:<5)				PCT (NV:0-0.05ng/ml)		
							Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3
1	26	36	2390	Negative	Presumed line related sepsis	6	3	<1	<1		0.2	0.35	
2	27	36	3100	Negative	Presumed line related sepsis	3	3		<1		0.17		
3	31	35	2110	Negative	Sepsis, ileus	6				<2	0.45	0.24	
6	167	54	4040	Negative	NEC	9	35		31	29			
7	61	37	1900	Negative	Abdominal mischief	7	2	2			0.3	0.43	
11	42	39	2055	Negative	Abdominal mischief	7	<2	<2					
12	42	39	1835	Negative	Abdominal mischief	7	<2				0.16		
15	24	36	2030	Negative	Presumed line related sepsis	7	4	3					
9	55	36	2145	Negative	Presumed line related sepsis	6			7		0.33		0.29
17	37	37	2155	Negative	Abdominal mischief	6	8	<5	3				

Median	39.5	36.5	2127.5
Range	(26-167)	(36-54)	(1835-4040)

Key: LOS=Length of stay; GA= Gestational age; NEC= necrotising enterocolitis CRP=C-reactive protein; PCT=Procalcitonin; NV=normal value

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Table 4.8 describes the LOS, corrected GA at discharge and the premature infant's weight at discharge for study group AV. It also describes blood culture, the reason for treatment, duration of treatment, the CRP (Day1-4) and PCT values (Day1-3). It was found that four (40%) infants were treated for presumed line related sepsis, four (40%) were treated for abdominal mischief, one (10%) was treated for NEC and one (10%) was treated for sepsis associated with ileus.

The median (range) length of stay (LOS) was 39.5 (26-167) days, corrected gestational age and the body weight at discharge for AV was 36.5 (36-54) weeks and 2127.5 (1835-4040) g respectively.



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Table 4.9 Treatment outcomes of Sandoz-Vancocin CP® premature infants

Premature infant study no.	LOS (days)	Corrected GA at discharge (weeks)	Body weight at discharge (g)	Blood culture	Reason for vancomycin treatment	Treatment duration (doses)	CRP (NV:<5)				PCT (NV:0-0.05ng/ml)		
							Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3
4	13	36	1930	Negative	Presumed IV line sepsis	8	14				0.41		
5	77	40	2000	Negative	Sepsis and abdominal distention	9	107	82	30				
8	75	38	1825	Negative	Abdominal mischief	10	<1	<1			0.14	0.16	
10	82	37	1822	Negative	Abdominal mischief	9	7	2			0.16		
13	70	36	1925	Negative	Abdominal mischief	5	<2				0.17		
14	37	38	1820	Negative	Presumed IV line sepsis	9	44	10	8		0.53	0.26	0.16
16	39	35	2000	Negative	Abdominal mischief	7	4	9	6		0.34	0.15	
18	122	41	2495	Negative	Pneumonia	5	<2				0.1		
19	35	36	2120	Negative	Abdominal mischief	10	23	37	15		2.8		

Median	70	37	1930
Range	13-122	35-41	1820-2495

Key: LOS=Length of stay; GA= Gestational age; CRP=C-reactive protein; PCT=Procalcitonin; NV=normal value

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Table 4.9 describes the LOS, corrected GA at discharge and the premature infant's weight at discharge for study group SV. It also describes blood culture, the reason for treatment, duration of treatment, the CRP (Day1-4) and PCT values (Day1-3).

It was found that two (22%) infants were treated for presumed line related sepsis, five (55%) were treated for abdominal mischief, one (11%) was treated for pneumonia and one (11%) was treated for sepsis and abdominal distension.

The median (range) LOS is 70 (13-122) days; corrected gestational age and body weight at discharge for SV were 37 (35-41) weeks and 1930 (1820-2495) g respectively.



Chapter 5 Discussion

The aim of this prospective, double blind and randomised study is to compare the trough plasma levels (C_{\min}) of two vancomycin preparations available in South Africa (Aspen-Vancomycin and Sandoz-Vancocin) in a selected premature infant population presenting with suspected MRSA infection. Similarities between patients' demographic and pathophysiological profiles have been confirmed.

5.1 Patient demographics

The sample size ($n=19$) and demographic parameters of premature infants involved in this study are similar to that of premature infants included in previous studies. The studies conducted by Reed et al, McDougal et al, De Hoog et al and Asbury et al involved 15, 15, 22 and 19 premature infants respectively.

In this study the median (range) GA was 32 (28-33) weeks in the AV group and 29 (23-34) weeks in the SV group. This is similar to the GA of premature infants in previous studies, (Reed et al 28.4 ± 2.6 weeks, McDougal et al 29.4 ± 2 and De Hoog et al 29 (25-42) and Ashbury et al 29.3 ± 4.2 weeks).

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The median (range) PNA of the premature infants in the AV group was 8 (3-18) days and in the SV group was 30 (3-58) days. This is similar that was found in previous studies where the PNA was 20.5 ± 10.4 in Reed et al , 23 ± 14 in McDougal et al, 11 (7-21) in De Hoog et al, and 33.9 ± 19.9 days in Asbury et al.

The median (range) PMA in this study was 33 (30-34.6) and 32 (31-34.7) weeks for AV and SV respectively. This is similar to premature infants involved in previous studies (Asbury et al, 1993; McDougal et al, 1995).

The weight at trial entry for the premature infants in the AV and SV group was 1537 (1080-2615) g and 1165 (925-1855) g respectively and is similar to that of premature infants included in previous studies done by Reed et al. (1069 ± 435 g), McDougal et al (1194 ± 412 g), De Hoog et al (1160 (730-3420g) and Ashbury et al (1780 ± 1080 g).

The recommended dose for premature infants with a PMA of 29-34 weeks is 15mg/kg twelve hourly (Horn et al, 2007; Gibbon et al 2005). Dosing schedules in the literature do however differ - De Hoog et al (10mg/kg Q8h), Reed et al (12.6mg twenty four hourly) and McDougal et al (18mg/kg eighteen hourly).

As indicated in table 4.3 there was no significant statistical difference between GA, BW, PMA, PNA and weight at trial entry in the premature infants enrolled in the AV group when compared with those of the SV group.

5.2 Patient biological parameters

As indicated in table 4.4 there was no significant statistical difference between serum creatinine concentration, GFR and albumin, of the premature infants enrolled in AV group and those of the SV group.

The median (range) serum creatinine for the premature infants in the AV group was 50.5 (35-74)mmol/l and 51 (26-61)mmol/l for those in the SV group. This serum creatinine concentration is similar to that found in premature infants by Grimsley & Thomson (1999) where the median serum creatinine concentration was 49 (18-172) mmol/l.

Albumin levels were measured for eighty four percent of all study premature infants. Although no statistically significant difference ($p=0.696$) could be found between the AV ($n=10$) and SV ($n=7$) group, there was a trend towards a lower albumin level in the SV group (see figure 4.1). Vancomycin is 50-55% albumin bound and it was found that with an increase in serum albumin levels, the lower the vancomycin serum trough levels, (Sun et al,1993, Jackson et al, 1999)

5.3. Comparison of trough levels assessed as per study group

There was no significant statistical difference ($p=0.118$) between trough level 1 in the AV and SV groups.

Trough levels 2 and 3 were collected, but were not statistically assessed due to failure to study protocol. The levels were not collected strictly sixty min before the sixth and ninth dose but at the physician's discretion. Vancomycin dosing schedules were amended by the attending physician due to the previous trough level being considered inappropriate. The following dose was omitted therefore the next trough level being not in line with study protocol.

The median vancomycin serum trough level 1 was 11.5 (8-19) $\mu\text{g/ml}$ and 10 (1-14) $\mu\text{g/ml}$ for AV and SV respectively. This correlates with what was found by De Hoog et al ($8.2 \pm 2.2\mu\text{g/ml}$ with a dose of 15mg/kg twelve hourly). McDougal et al found serum trough levels of $3.9 \pm 0.6\mu\text{g/ml}$ with a dose of 18mg/kg eighteen hourly.

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5.4 Correlations between clinical parameters and trough level 1 in each study groups

No correlation between any clinical parameters, (demographic or biological) was found in the AV group. A strong positive correlation was observed between trough level 1 and the GA ($r=0.89$; $p=0.0013$), BBW ($r=0.71$; $p=0.031$), PMA ($r=0.903$; $p=0.0008$) and a strong negative correlation was observed between trough level 1 and PNA ($r=-0.73$; $p=0.026$) in the SV group.

A possible reason for this non-correlation between trough level 1 and biological and demographic parameters in the AV group may be the two outlying trough levels when compared to these parameters in two study premature infants, as indicated in figures 4.3-4.6.

Vancomycin trough levels are dependent on demographic factors (GA, BBW, PMA, PNA), biological parameters (S-Cr, Alb, GFR) as well as pharmacokinetic parameters (protein binding, Vd, clearance) (De Hoog et al, 2004). This study has shown that the first two sets of parameters were not of definitive value in determining trough levels. Therefore the possible effect of pharmacokinetic parameters on the vancomycin serum trough levels was assessed.

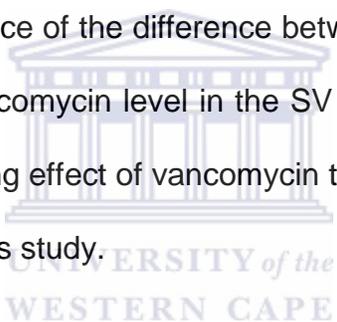
In literature the protein binding, vancomycin clearance and volume of distribution have an effect on vancomycin serum trough concentration (De Hoog et al, 2004; Bauer et al, 2008; Pacifici & Allegaert, 2012)

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5.4.1 Protein binding

Vancomycin is bound to albumin 50-55% (Sun et al, 1993; Rybak et al, 2009) and to serum immunoglobulin A (s-IgA). The total fraction (bound and unbound) of vancomycin is measured in the serum, although only the free fraction is dependent on drug clearance (Sun et al, 1993).

Albumin levels were assessed in this study, but no significant statistical difference was observed between the AV and SV group ($p=0.686$). However, as shown in figure 4.1 there is as expected, a decreasing vancomycin trough level with an increasing albumin level. Despite the non-significance of the difference between SV and AV there seems to be a trend towards a lower vancomycin level in the SV group as compared to AV for a similar albumin level. The binding effect of vancomycin to other plasma proteins such as s-IgA was not investigated in this study.



5.4.2 Vancomycin clearance

Vancomycin is 80-90% eliminated by the kidneys via glomerular filtration (Rocha et al, 2006). Renal function and glomerular filtration increases linearly with PMA, due to maturation of kidney functions (Rocha et al, 2006; McDougal et al, 1995) and this result in an increase in vancomycin clearance (Grimsley & Thompson, 1999) with lower vancomycin serum trough levels as a result. Vancomycin clearance increased with PMA and was associated with a greater elimination rate constant and a shorter half life (McDougal et al, 1995).

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Grimsley & Thompson (1999) also found that vancomycin clearance has a positive linear correlation with weight and PMA and an inverse relation to the serum creatinine.

This study found a positive linear correlation ($r=0.89$; $p=0.0013$) between trough level 1 and GA in the SV group. This would clinically imply an increase in renal function maturation and therefore a lower trough level 1.

A negative linear correlation was found ($r=-0.73$; $p=0.026$) between trough level 1 and PNA in the SV group. Thus with an increase in PNA, trough level 1 decreases. This finding can be due to the increase in renal function maturation and therefore an increase in vancomycin clearance as PNA increases, therefore a lower trough level 1.

This finding correlates with what was found in literature (Pacifci & Allegaert, 2012).

No correlation was found between trough level 1 and either serum creatinine concentration or GFR in the current study. Literature has shown that vancomycin clearance is inversely related to serum creatinine level by Grimsley & Thompson (1999)

5.4.3 Volume of distribution

Due to vancomycin's hydrophilic properties it distributes into total body water and extracellular fluid (Uildemolins et al, 2011). Premature infants have large extracellular fluid volume (80%) thus a large volume of distribution (Marques-Minana et al, 2010; De Hoog et al, 2004) resulting in lower than expected serum trough levels. McDougal et al found a positive linear correlation between the absolute Vd and the PCA ($r=0.79$; $p<0.001$), but no difference when Vd was standardized for weight. No correlation with

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weight at trial entry was found ($r=0.09$; $p=0.59$). This finding was supported by Grimsley & Thompson (1999) who also found that PMA and weight influenced the Vd with the weight having a more significant effect on the Vd than PMA.



5.5 Therapeutic ranges of trough level 1

In AV group (n=10) no trough level 1 was below the minimum therapeutic serum concentration, three (30%) were within therapeutic range and seven (70%) were above the minimum toxic serum concentration.

In SV group (n=9), two (22.2%) premature infants had trough level 1 below the minimum therapeutic serum concentration, four (44.4%) were within therapeutic range and three (33.3%) were above the minimum toxic serum concentration.

5.5.1 Distribution of premature infants with a trough level 1 below minimum therapeutic serum concentration (<5µg/ml)

Premature infants that had trough level 1 below the minimum therapeutic serum concentration were born at 26 and 23 weeks GA respectively. Their weight at trial entry was 1165g and 1830g respectively. The PMA at trial entry was 31 and 31.5 weeks respectively. The PNA was 35 and 58 days respectively. Both premature infants received SV. No additional trough levels were taken for these premature infants and therapy was stopped as per physician's discretion and per clinical signs. A possible explanation for this is the improved renal function due to the advanced PNA that may have required an adjustment in the dosing schedule. In a retrospective study done by De Hoog et al (2004), 17.6% of infants were found to have vancomycin serum trough levels 1 below therapeutic levels, irrespective of dosing schedule used. The study

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offered no explanation for this finding. It can however only be assumed to be dosing schedule related.



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5.5.2 Distribution of premature infants with a trough level 1 within therapeutic range (5-10 μ g/ml)

The study will discuss these premature infants by means of two case studies.

5.5.2.1. Case study 1

Premature infant number 1 had a trough level 1 (5 μ g/ml) within therapeutic range. Trough level 2 (9 μ g/ml) stayed within therapeutic range, but trough level 3 (16 μ g/ml) was measured above maximum therapeutic serum concentration (>10 μ g/ml). This male 43 days PNA premature infant was admitted into the study at PMA of 32.1 weeks and a weight at trial entry of 980g. The infant was intra-uterine growth retarded (IUGR) at birth and remained small for gestational age (SGA) at time of study entry. The infant received SV.

A possible explanation for this phenomenon is the IUGR/ SGA (Fenton et al, 2003) status of the infant. Renal function in SGA infants has been shown to be immature (Aly et al, 2013) thereby increasing the vancomycin trough levels.

5.5.2.2. Case study 2

Premature infant number 2 had a trough level 1 (10 μ g/ml) that was on the border of the therapeutic range and decreased to border-line sub-therapeutic at trough level 2 (5 μ g/ml) before becoming sub-therapeutic at trough level 3 (4 μ g/ml). The female premature infant was admitted to the trial at a PNA of 18 days and a PMA of 33.5 weeks and a trial entry weight of 1640g. The infant received SV.

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A possible explanation for this finding is the relative maturity of this premature infant. She was on full feeds receiving additional protein and calorie supplementation which may have altered the serum protein levels (Van den Akker et al, 2007), thereby increasing the bound vancomycin level and decreasing the serum trough levels in time. The infant was also approaching the 35 week of vancomycin dosing schedule change (8 hourly instead of 12 hourly) (Horn et al, 2007). Due to renal maturity, an increase in renal clearance and therefore a decreased half-life may also have contributed to the decrease of vancomycin trough levels over time (McDougal et al, 1995).



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5.5.3 Distribution of premature infants with a trough level 1 above the minimum toxic serum concentration ($>10\mu\text{g/ml}$)

The study will discuss these premature infants by means of two case studies

5.5.3.1 Case study 3

This male premature infant received a vancomycin dose of 17mg twelve hourly. Trough level 1 ($12\mu\text{g/ml}$) was above the minimum serum toxic concentration, trough level 2 ($9\mu\text{g/ml}$) and 3 ($7\mu\text{g/ml}$) was within therapeutic range. This premature infant was admitted to the study with a PNA of 3 days, GA of 33 weeks and his birth weight and weight at trial entry was 1160g and 1150g respectively. This premature infant received SV.

Furthermore the premature infant's albumin level was 23 g/l at trial entry which can also contribute to the high trough level 1 observed, because of more unbound vancomycin in the serum (Sun et al, 1993, Jackson et al, 1999)

Due to the correlation found between trough level 1 and PNA in the SV group, one can say that with a decrease in PNA there will be an increase in trough level 1. This finding is attributed to the premature infant's immature renal function associated with a small PNA (De Hoog et al, 2004).

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5.5.3.2 Case Study 4

This premature infant received 16.2mg vancomycin twelve hourly and had a trough level 1 (19 µg/ml), trough level 2 (14 µg/ml) and 3 (16 µg/ml) above minimum toxic serum concentration.

This male premature infant was admitted to the study with a PNA of 18 days, GA of 28 weeks, and PMA of 30.5 weeks. His body birth weight and weight at trial entry was 940g and 1080g respectively. This premature infant received AV. The dose after trough level 1 was omitted by the attending physician in order to avoid serious toxicity.

Possible reasons for this phenomenon are that he had a serum albumin level of 19 g/l on trial entry. This may attribute to the high serum trough level concentrations found due to more unbound vancomycin (Sun et al, 1993, Jackson et al 1999).

This premature infant just made the twelve hourly, instead of twenty four hourly dosing schedules (Horn et al, 2007) due to PMA of 30.5 weeks. This can also contribute to the high trough level 1 concentrations in this premature infant.

Factors other than demographic (age, weight) and biological (s-Alb, S-creatinine concentration, and renal sufficiency) parameters that may influence vancomycin serum trough levels (discussed below) could not be excluded.

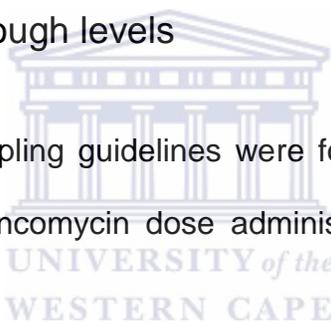
5.5.4 Factors that can influence vancomycin serum trough levels

Factors that may contribute to vancomycin serum trough levels not being within the therapeutic range are:

- 5.5.4.1 The timing of trough levels
- 5.5.4.2 The presence of impurities e.g. vancomycin crystalline degradation product (CDP-1)
- 5.5.4.3 The dosing schedule of vancomycin in premature infants
- 5.5.4.4 Mode of vancomycin administration

5.5.4.1 The timing of trough levels

In this study, international sampling guidelines were followed by collecting the trough level thirty minutes prior to vancomycin dose administration (de Hoog et al, (2000); McDougal et al, 1995).



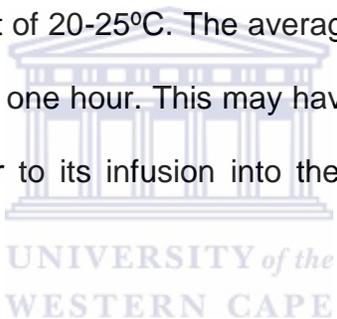
Conflicting evidence exists in literature regarding the sampling time of vancomycin serum trough levels. De Hoog et al, (2004) and McDougal et al, (1995) suggests thirty minutes prior to the third dose (23h30min), while Rybak et al, (2009) suggests thirty minutes prior the fourth dose (35h30min). Time to reach steady state plasma concentrations equals five half lives. Vancomycin's half life in premature infants is approximately six hours, and therefore will reach steady state within approximately thirty hours (Shargel et al, 2005). In order to prevent the ototoxicity and nephrotoxicity associated with vancomycin, it is preferable to measure vancomycin trough levels

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before steady state is reached. This allows for dose adjustments to be made and prevent and limit adverse effects.

5.5.4.2 The presence of impurities in vancomycin formulations

Vancomycin breaks down to crystalline degradation product (CDP-1) and factor B (active ingredient) when exposed to heat over time (Somerville et al, 1999). The United States Pharmacopoeia (USP) states that any generic formulation of vancomycin may not have less than 88% factor B and may not have more than 4% of CDP-1 (Somerville et al, 1999). The *in vivo* and *in vitro* studies showed that CDP-1 breakdown was 50% at sixteen hours in an environment of 20-25°C. The average NICU temperature is 24-26°C and vancomycin is infused over one hour. This may have an implication on the external breakdown of vancomycin prior to its infusion into the neonate. This implication also needs further research



It can be speculated that Sandoz-Vancocin CP® may break down quicker than AV and therefore have more than the required 4% CDP-1 present. This finding may lead to lower vancomycin serum trough levels than anticipated.

5.5.4.3 The dosing schedule of vancomycin in premature infants

In this study a dose of 15mg/kg 12hourly was used for premature infants with a PMA of 29-35 weeks (Horn et al, 2007) regardless of GA and PNA.

Many studies have been performed using different dosing strategies of vancomycin in premature infants. Koren & James, (1986) did a study where they divided the premature infants into two groups. Group 1 received vancomycin at a dose of 30mg/kg

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twelve hourly (PNA=0-7days) and eight hourly (PNA>7days). Group 2 received vancomycin at 18mg/kg twelve hourly for premature infants with a PMA of 31-36 weeks and a body weight of 1200-2000g. They found that for Group 1, 39.6% of trough levels were within therapeutic range (60.4% were > 10µg/ml) compared with 75.5% (p=0.002) of those in group 2 (22% were > 10µg/ml). This finding by Koren & James, 1987 supports the findings in this current study where 36.8% of trough levels 1 were within therapeutic range and 57% were > 10µg/ml. Koren & James, 1987 attributed this finding to age-dependant maturation of renal function and proposed the dosing schedule on both body weight and PMA.

To further illustrate the difficulties of optimum dosing of vancomycin in premature infants, it was also reported by Pacifici & Allegaert (2012) that when vancomycin is dosed at 15mg/kg twelve hourly that only 51% of premature infants attained trough levels within therapeutic range and that 33% of trough levels were below <5µg/ml.

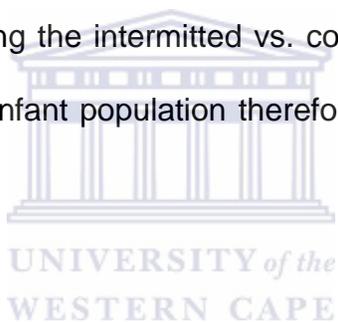
Grimsley & Thompson (2012), found that when vancomycin was dosed according to the premature infant's serum creatinine concentration, 72% of first trough levels taken were within therapeutic range (5-12µg/ml)

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5.5.4.4 Intermittent vs. continuous administration of vancomycin

Vancomycin shows time-dependent killing properties and therefore to maintain the vancomycin serum trough concentrations above the given MIC of $2\mu\text{g/ml}$, it has been suggested that vancomycin can be administered as a continuous infusion (Pacifci et al, 2012; Rybak et al, 2009). In a study conducted by Pawlotsky et al (1998) they administered vancomycin to premature infants with a PMA of 28-51.5 weeks, a loading dose of 7mg/kg and followed by a continuous infusion of $10\text{-}40\text{mg/kg/24hours}$ according PMA. The mean serum trough levels at steady state were $15.4\mu\text{g/ml}$.

Limited literature exist comparing the intermitted vs. continuous infusion administration of vancomycin in a premature infant population therefore this method of administration was not used in this study.



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5.6 Treatment outcomes

It was found that all premature infants that were admitted to this study and that received AV was cured and discharged from the NICU's as per clinical outcomes and physician's discretion. Despite that vancomycin treatment was initiated for suspected MRSA infection no premature infant presented with a positive blood culture. Thus treatment was initiated as per clinical findings and not microbiological confirmation.

All premature infants that were admitted to the SV group was cured and discharged from the NICU's as per clinical outcomes and the physician's discretion. Despite that vancomycin treatment was initiated for suspected MRSA infection, no positive blood culture was collected.

The median (range) LOS for the premature infants in AV was 39.5 (26-167) days. The LOS of the premature infants that received SV was 70 (13-122) days. The increased LOS for the SV group cannot only be attributed to the choice of vancomycin formulation. Other factors such as severity of prematurity, respiratory distress syndrome (RDS), surgical complications and the presence of a patent ductus arteriosus (PDA) can also contribute to the LOS of the premature infants.

Biological infection markers were used as guide to initiate and to stop vancomycin therapy. C-reactive protein (CRP) and procalcitonin (PCT) were both used to evaluate the severity of the presumed infection, though literature suggests that a PCT value is more accurate for LOS in the premature infant (Chiesa et al, (2011); Nabulsi et al, (2012); Vazzalwar et al, (2005).

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Therapy was discontinued after a negative blood culture and two negative CRP-values (Pacifici & Allegaert, (2012).



5.7 Were the study objectives achieved?

Yes the study objectives were achieved. Firstly it was found that there was no statistical significant difference between the trough levels 1 of each premature infant in the Aspen-Vancomycin CP® and those in the Sandoz-Vancocin® group. Despite this finding the Sandoz-Vancocin® group had a lower trough level 1 when compared to the Aspen-Vancomycin CP® group. Thirty six percent of the premature infants in the Aspen-Vancomycin CP® group had trough level 1 above the minimum toxic concentration ($>10\mu\text{g/ml}$) when compared to the fifteen percent of premature infants of Sandoz-Vancocin®.

Secondly it was found that there exists no correlation between the demographic and biological parameters and trough level 1 in the AV group. A correlations was found in the SV group between trough level 1 and BBW, GA, PMA and PNA.

Thirdly, it was found that both vancomycin formulations had the same treatment outcomes – no therapeutic failure was observed in any premature infant.

5.8 Study limitations

This study was conducted as a randomised prospective study where data collection was done according to study protocol and dose scheduling adjustments were done by the attending physician, according to the first vancomycin serum trough level. Therefore trough level 2 and 3 were not strictly collected thirty minutes before the sixth and ninth dose.

A major limitation was that the sample size was too small, despite that the statistical analysis determined nine premature infants per study arm.

Vancomycin peak serum concentrations and steady state serum trough concentration could not be collected due to NICU protocol (limited blood collection protocol) thus this study had not enough data points to determine the pharmacokinetic parameters of vancomycin in the premature infant population.

The concurrent treatment with meropenem made it difficult to determine the independent efficacy of vancomycin on treatment outcomes.

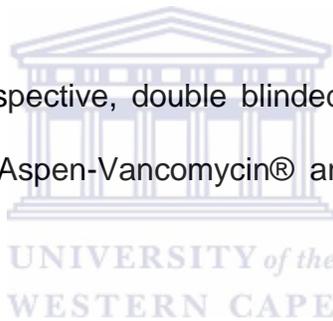
High-performance liquid chromatography (HPLC) was not performed to determine the composition of vancomycin crystalline degradation products in the AV and SV group, therefore its effect on vancomycin serum trough levels could not be confirmed.

Vancomycin was not administered as a continuous infusion in this study due to limited literature available that tested this method in premature infants.

Chapter 6 Conclusion and Recommendations

6.1 Conclusion

The main objective of this prospective, double blinded and randomised study was to compare the trough levels of Aspen-Vancomycin® and Sandoz Vancocin CP® in a premature infant population



Demographic and biological parameters were assessed and no statistical significant difference was found between the two study populations.

No statistical significant difference were found between trough level 1 in both study groups, but the Sandoz Vancocin CP® group had a trend towards lower vancomycin serum trough level 1 concentrations.

When trough level 1 was compared with demographic and biological parameters it was found that the Aspen-Vancomycin® group showed no correlation with any of the parameters, this may have been due to outlying trough level 1 values. In the Sandoz

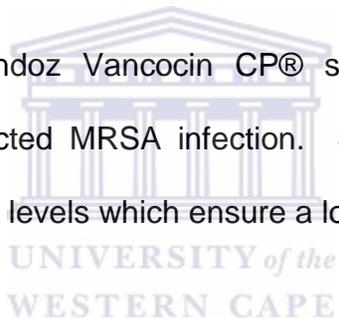
Chapter 6: Conclusion and Recommendations

Vancocin CP® group correlations were found between trough level 1 and GA, PMA, PNA.

It was found that a premature infant's renal function drives vancomycin trough level concentrations and that age or weight are the most relevant determinants of vancomycin clearance.

This study suggests that vancomycin trough levels should be collected 30min prior to the third dose to limit toxic serum trough levels and to ensure optimum efficacy.

This study suggests that Sandoz Vancocin CP® should be used when treating premature infants for a suspected MRSA infection. Sandoz Vancocin CP® offered lower vancomycin serum trough levels which ensure a lower risk of toxicity.



Chapter 6: Conclusion and Recommendations

6.2 Recommendations

Vancomycin serum peak and steady state trough levels need to be collected in order to determine and compare the pharmacokinetic parameters in each study group in a premature infant population.

Therapeutic drug monitoring needs to be applied per individual premature infant in order to optimise vancomycin dose adjustments and limit toxicity.

Studies need to be conducted where a bigger sample size is collected.

Further studies need to be performed to determine the concentration of CDP-1 by means of HPLC method in both Aspen Vancomycin® and Sandoz-Vancocin CP® and its effect on a premature infant population.

More randomised studies should be performed to determine the efficacy and toxicity of continuous and intermitted vancomycin administration in a premature infant population.

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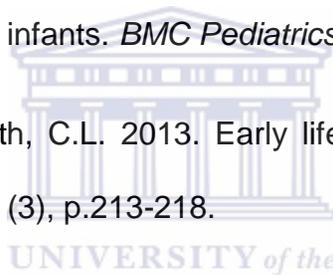
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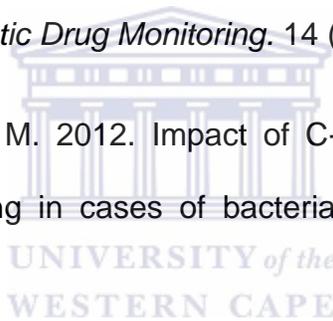
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