

# Descriptive and retrospective investigation into clinical health outcomes of HIV patients on AZT-based regimens

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***Opgedra aan:***

∞W.J. van Graan∞

**(22 June 1922 ~ 25 June 2010)**

*Oupa het my passie vir medisyne gedeel en my aangemoedig om nooit op te hou leer  
nie, maar in my soeke na kennis, die Here in alles te sien en Hom altyd te dien.*

∞ Joshua 1:9 ∞

**“Be strong. Be Brave. Be fearless. You are never alone.”**

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# ABSTRACT

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The number of people living with human immunodeficiency virus / acquired immune deficiency syndrome (HIV/AIDS) in South Africa keeps growing, those succumbing to the virus adding up to almost a third of all deaths in South Africa. This dissertation aimed to improve the clinical knowledge of zidovudine-based antiretroviral therapy (ART) and to contribute supportive evidence of the implementation of ART by different types of health care providers in different facilities in the City of Matlosana as part of Dr Kenneth Kaunda district in the North West province.

Antiretroviral therapy for the treatment of HIV is therefore a major priority for all health care systems worldwide. Initially, ART programmes in South Africa followed a physician-initiated and managed model. However, the limited number of physicians in the public sector forced a task-shifting approach from physicians to nurses to respond to the challenge to deliver ART programmes to a greater number of people. This led to the implementation of revised treatment guidelines, enabling nurse-initiated management of antiretroviral therapy (NIMART) in primary health care settings. Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, is widely used as part of second line regimens to treat HIV. Haematological disorders such as anaemia can occur within 4-12 weeks after AZT initiation and it mainly presents as macrocytosis but can also present as leukopenia or neutropenia. The monitoring of haematologic parameters is crucial for AZT-based regimens and full blood counts are currently advised at baseline, month 3, 6 and then 12 monthly thereafter.

HIV disease progression markers (CD4 cell counts & viral load (VL)), haematological markers (haemoglobin (Hb) & mean corpuscular volume (MCV)) and possible associated risk factors of disease progression (body mass index (BMI), hospital admissions & frequency of opportunistic infections(OIs)) were retrospectively extracted over a 12-month period from health records of adult patients on AZT-based treatment in a hospital clinic (n = 100) and NIMART cohort (n = 100) in the sub-district of City of Matlosana, North West province, in order to compare these two approaches. Other primary health outcomes variables such as time to undetectable VL suppression, time from enrolment to death, time since HIV diagnosis, total time spent on ART and AZT were also included. One hospital clinic, two primary health care clinics and one community health centre clinic were included. Ethics approval was obtained from the Human Research Ethics Committee (HREC), North-West University (NW-00362-15-A1), Wits HREC medical (M160267), PPRM&E directorate (North West Department of Health), the CEO of Tshepong-Klerksdorp hospital complex and the acting PHC manager. Descriptive statistics were used to present the

demographic results of the two cohorts. Linear mixed models which adjusted for age, total duration on ART and duration since HIV diagnosis were incorporated to analyse disease progression marker (CD4 cell count), haematological parameters (Hb & MCV) and BMI as an associated risk factor for disease progression. Kaplan-Meier survival analysis was used to determine the effect of time on undetectable VL suppression in both cohorts over time.

The mean (SD) age for hospital, clinic and NIMART patients was 42.4 ( $\pm$  8.92) and 50.30 ( $\pm$  1.82) years respectively, with females comprising the biggest proportion of patients in both cohorts (59% and 69% respectively). Both OIs and hospital admissions occurred more frequently for the hospital clinic cohort.

After **adjusting for the three confounding covariates**, CD4 cell count increased significantly overall in both cohorts, but indicated no significant differences ( $p > 0.05$ ) over time ( $p = 0.562$ ,  $F = 0.582$ ), between the cohorts ( $p = 0.091$ ,  $F = 2.899$ ) or for the time\*cohort interaction ( $p=0.927$ ,  $F = 0.076$ ). Haemoglobin for both groups also reacted similarly and relatively unchanged over time with no interaction (time\*cohort;  $p = 0.835$ ,  $F = 0.180$ ) to report, thus not achieving anaemic status ( $< 8$  g/dL) after 12 months on AZT-based regimen. The MCV, however, increased significantly over time ( $p = 0.009$ ,  $F = 5.255$ ), more specifically between baseline and month 12 ( $p = 0.008$ ), but showed no statistical differences between the cohorts ( $p = 0.227$ ,  $F = 1.476$ ) or indicated an interaction between time\*cohort ( $p = 0.128$ ,  $F = 2.152$ ). Macrocytosis was already evident in the NIMART group at baseline and continued up to 12 months compared to being present only at 6 and 12 months in the hospital clinic cohort. Macrocytic anaemia (macrocytosis and Hb  $< 8$  g/dL) was only observed in 1% (one in each cohort) of the study population. Body mass index values were almost unchanged over the first year on AZT-based therapy for both cohorts and did not differ significantly between the two cohorts.

In total, 58% of all patients (both cohorts) achieved successful viral suppression, although NIMART patients (80%) were considerably more successful in achieving VL suppression compared to the hospital clinic patients (36%) possibly due to the median baseline VL already  $< 20$  copies/ml in 44% of the NIMART cohort. It is important to emphasise that although the two survival curves were not equivalent they followed the same trend for both cohorts at 6 and 12 months of analysis. The estimate mean time for NIMART subjects to have reached VL suppression ( $5.7 \pm 5.4$  months) were almost twice as fast compared to hospital clinic subjects ( $10.4 \pm 3.6$  months).

Both cohorts showed improved clinical treatment outcomes with significantly elevated CD4 cell counts and viral suppression 12 months after AZT-based initiation. The parameters (CD4, Hb, MCV, BMI) measured were similar between the two cohorts over the 12-month period however

the NIMART cohort reached VL suppression quicker compared to the hospital clinic cohort possibly due to better VL suppression at baseline. Based on these results we conclude that NIMART was non-inferior to physician-managed ART in this study setting. There is a need for more comparative research studies where these approaches can be measured and investigated. Considering the most recent change in HIV treatment guidelines, enabling the initiation of all HIV positive patients on ART irrespective of their CD4 cell count and considering the immense burden this places on the public health care systems and human resources, this study contributed to the existing knowledge of implementing NIMART especially where second line regimens are concerned.

**Key words:** NIMART, disease progression markers, AZT, macrocytosis, ARVs

# OPSOMMING

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Die aantal mense in Suid-Afrika met die menslike immuniteitsgebrekvirus / verworwe immuniteitsgebreksindroom (MIV/VIGS) neem steeds toe. Diegene wat beswyk as gevolg van die virus maak byna 'n derde van alle sterftes in Suid-Afrika uit. Hierdie verhandeling het ten doel om die kliniese kennis van zidovudien-gebaseerde behandeling uittebrei asook om ondersteunende bewyse te lewer met betrekking tot die implementering van antiretrovirale behandeling (ARB) deur verskillende tipe gesondheidsorg verskaffers en fasiliteite in die stad van Matlosana wat deel vorm van die Dr Kenneth Kaunda distrik in die Noordwes provinsie.

Antiretrovirale behandeling vir die behandeling van MIV is 'n prioriteit vir alle gesondheidsorgstelsels wêreldwyd. Aanvanklik het die ARB-programme in Suid-Afrika 'n geneesheer-geïnisieerde en -bestuurde model gevolg. Die beperkte aantal geneesheer in die openbare sektor het egter 'n taakverskuiwingsbenadering meegebring waar die fokus van geneesheer na verpleegkundiges verskuif het in reaksie op die uitdaging om ARB-programme aan groter hoeveelhede mense beskikbaar te stel. Dit het gelei tot die implementering van hersiene behandelingsriglyne wat verpleegkundige-geïnisieerde bestuur van antiretrovirale behandeling (*nurse-initiated management of antiretroviral therapy* - NIMART) in primêre gesondheidsorg (PGS) konteks moontlik te maak. Zidovudien (AZT), 'n nukleosied trutranskriptase-inhibeerder word algemeen gebruik as deel van die tweede behandelingslinie teen MIV. Hematologiese afwykings soos anemie kan dikwels binne 4-12 weke na AZT-inisiasie presenteer as makrositose maar ook as leukopenie of neutropenie. Die monitering van hematologiese parameters is belangrik met AZT-gebaseerde behandeling en volbloedtellings word huidiglik aanbeveel op basislyn, 3, 6 en 12 maande na aanvang van die terapie.

MIV-siekteprogressiemerkers (CD4-seltellings, virale lading (VL)), hematologiese merkers (hemoglobien (Hb) en gemiddelde korpuskulêre volume (GKV)) en moontlik geassosieerde risiko faktore van die siekte se progressie (liggaamsmassa-indeks (LMI), hospitaalopnames, en frekwensie van opportunistiese infeksies (OIs)) is retrospektief verkry vanuit volwasse pasiënt rekords oor 'n 12-maande periode. Die data is vergelyk in volwasse pasiënte wat op AZT-gebaseerde behandeling in 'n hospitaalkliniek (n = 100) en 'n verpleegkundige-geïnisieerde (NIMART, n = 100) kohort in die Matlosana subdistrik van die Noord-Wes provinsie was. Ander primêre gesondheidsuitkomstes soos tydperk tot virale deteksielimit, tydperk vanaf aanvang van AZT-gebaseerde behandeling tot dood, tydperk sedert MIV diagnose, totale tydperk op ARB en AZT was ook ingesluit. Een hospitaalkliniek, twee primêre gesondheidsorgklinieke (PGK) en een

gemeenskapsgesondheidsentrumkliniek was ingesluit. Etiekklaring is verkry van die Human Research Ethics Committee (HREC), Noordwes-Universiteit (NW-00362-15-A1), Wits (HREC) (medies) (M160267), die PPRM&E direktoraat (Noordwes Departement van Gesondheid), die Tshepong-Klerksdorp hospitaalkompleks en die waarnemende primêre gesondheidsorgbestuurder.

Beskrywende statistiek was gebruik om die demografiese resultate van die twee kohorte voortestel. Liniêre gemengde modelle wat aanpassings gemaak het vir ouderdom, tydsverloop sedert aanvang op ARB en tydsverloop sedert MIV-diagnose is geïnkorporeer in die analyses van die siekteprogressiemerker (CD4-seltelling), hematologiese parameters (Hb & GKV) en LMI as 'n geassosieerde risiko faktor van siekte progressie. 'n Kaplan-Meier oorlewingsanalise is gebruik om die invloed van tyd op verborge deteksielimiet van virale lading te vergelyk in die twee kohorte.

Die gemiddelde (SD) ouderdom vir hospitaalkliniek- en NIMART-pasiënte was 42.4 ( $\pm$  8.92) en 50.30 ( $\pm$  1.82) jaar onderskeidelik, met vroulike pasiënte as die grootste gedeelte van beide kohorte (59% en 69% respektiewelik). Beide Ols en hospitaalopnames het meer dikwels onder die hospitaalkliniekkohort plaasgevind.

Nadat aanpassings gemaak is vir die drie strengelingsveranderlikes, het die CD4-seltellings beduidend oorhoofs toegeneem, maar het oor tyd ( $p = 0.562$ ,  $F = 0.582$ ) geen beduidende verskille ( $p > 0.05$ ) getoon tussen die twee kohorte ( $p = 0.091$ ,  $F = 2.899$ ) of vir die tyd\*kohort-interaksie ( $p = 0.927$ ,  $F = 0.076$ ) nie. Hemoglobien het ook in beide groepe eenders gereageer en was relatief onveranderd oor tyd, met geen interaksie (tyd\*kohort;  $p = 0.835$ ,  $F = 0.180$ ) om te rapporteer nie, dus is anemiese status ( $< 8$  g/dL) nie binne 12 maande op AZT-gebaseerde terapie bereik nie. Die GKV het egter oor tyd beduidend toegeneem ( $p = 0.009$ ,  $F = 5.255$ ), meer spesifiek tussen basislyn en maand 12 ( $p = 0.008$ ), maar daar was geen statisties beduidende verskil tussen die twee kohorte nie ( $p = 0.2227$ ,  $F = 1.476$ ), of enige interaksie tussen tyd\*kohort nie ( $p = 0.128$ ,  $F = 2.152$ ). Makrositose was reeds duidelik in die NIMART groep op basislyn en deurgaans oor die 12 maande tydperk teenwoordig teenoor die hospitaalkliniek groep waar dit eers op 6 en 12 maande teenwoordig was. Makrositêre anemie (makrositose en Hb  $< 8$  g/dL) was slegs in 1% (een in elke kohort) van die studie populasie waargeneem. Liggaamsmassa-indeks het bykans onveranderd gebly gedurende die eerste jaar op AZT-terapie en het ook nie beduidend verskil tussen die twee kohorte nie.

Oorhoofs het 58% van alle pasiënte (beide kohorte) suksesvol virale onderdrukking bereik, alhoewel NIMART-pasiënte (80%) beduidend meer suksesvol virale onderdrukking bereik het vergeleke met die hospitaalkliniekpasiënte (36%), dit kan moontlik toegeskryf word aan die NIMART-kohort wat reeds op basislyn mediaan (20 kopieë/ml) virale onderdrukking getoon het

by 44% van die pasiënte. Die geskatte gemiddelde tyd vir NIMART-pasiënte om virale onderdrukking te bereik ( $5.7 \pm 5.4$  maande) was byna twee maal vinniger vergeleke met die hospitaalkliniekpasiënte ( $10.4 \pm 3.6$  maande).

Beide kohorte het verbeterde kliniese behandelingsuitkomst getoon met beduidende verhoogde CD4-tellings en virale onderdrukking 12 maande na AZT-gebaseerde terapie aanvang, ongeag die behandelingskohort. Die meetbare parameters (CD4, Hb, GKV, LMI) was goed vergelykbaar tussen die twee kohorte oor die 12 maande tydperk, die NIMART kohort het vinniger virale lading onderdrukking getoon in vergeleke met die hospitaalkliniek kohort, moontlik weens die groter virale onderdrukking wat reeds met basislyn teenwoordig was. Die gevolgtrekking van die studie is dat die NIMART-kohort nie ondergeskik was aan geneesheer-geïnisieerde en -bestuurde ARB nie. Daar is steeds 'n behoefte vir hierdie tipe vergelykende navorsing waartydens die verskillende benaderings gemeet en ondersoek moet word. Inaggenome die mees onlangse MIV-behandelingsriglyne wat die aanvang van alle MIV-positiewe pasiënte op ARB aanbeveel ongeag hulle CD4-seltelling en die gevolglike enorme las op die openbare gesondheidsorgstelsel en menslike hulpbronne het die studie bygedra tot reeds bestaande kennis van die implementering van NIMART maar meer spesifiek waar tweede-linie behandeling van toepassing is.

**Sleuteltermes:** NIMART, siekteprogressiemerkers, AZT, makrositose, antiretrovirale behandeling

# CONFERENCE PROCEEDINGS

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*Results obtained from the main research study and the additional quality improvement study were presented as follows:*

- a) VAN GRAAN, R., VILJOEN, M., RHEEDERS, M., MOTARA, F. **A retrospective analysis of adverse drug reactions (ADRs) of antiretrovirals (ARV) in the Tlokwe District (Jan 2010 – Dec 2014).**
  - Presented as a podium presentation at the Dr Kenneth Kaunda District research day, Department of Health, North West, held in Orkney, South Africa on 24 August 2016.
  
- b) VAN GRAAN, R., VILJOEN, M., RHEEDERS, M., MARTINSON, N., VARIAVA, E. **Descriptive and retrospective investigation into the clinical health outcomes of HIV patients on AZT-based regimens.**
  - Presented as a podium presentation at the All Africa Congress on Basic and Clinical Pharmacology and Pharmacy, held at the Misty Hills Hotel and Conference Centre, Muldersdrift, Gauteng, South Africa from 5-8 October 2016 (3<sup>rd</sup> place in SASBCP Young Pharmacologists category).
  
- c) VAN GRAAN, R., VILJOEN, M., RHEEDERS, M., MARTINSON, N., VARIAVA, E. **Comparing clinical health outcomes of adult HIV patients in a hospital clinic vs NIMART cohort.**
  - Presented as a podium presentation at the Soweto Matlosana Collaborating Centre for HIV/AIDS and TB (SoMCHAT) conference, held at the Chris Hani Baragwanath Hospital Learning Centre, Gauteng, South Africa on 18 November 2016.

# ABBREVIATIONS

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## A

ABC	Abacavir
ADR	Adverse drug reaction
AE	Adverse event
AFD	Abnormal fat distribution
AIC	Akaike's Information Criterion
AIDS	Acquired immune deficiency syndrome
ANCOVA	Analysis of covariance
APV	Amprenavir
AR-1	Autoregressive order 1
ART	Antiretroviral therapy
ARV	Antiretroviral
ARV-DSP	Antiretroviral distal-sensory polyneuropathy
ATV	Atazanavir
AZT	Zidovudine

## B

BMI	Body mass index
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## C

cART	Combination antiretroviral therapy
CDC	Centers for Disease Control and Prevention
CD4	CD4 T lymphocyte cell count
CFU-E	Colony forming unit - Erythrocyte
CFU-GM	Colony forming unit - Granulocyte-macrophage
CG	Cockcroft-Gault
CHC	Community health center
CI	Confidence intervals

CIPRA	Nurse versus doctor management of HIV-infected patients receiving ART
CMI	Cell-mediated immune
CNS	Central nervous system
CrCl	Creatinine clearance
CRF	Case report form

**D**

DDC	Zalcitabine
ddl	Didanosine
DKKD	Dr Kenneth Kaunda district
DLV	Delavirdine
DNA	Deoxyribonucleic acid
DSP	Distal-sensory polyneuropathy
d4t	Stavudine
DOB	Date of birth
DoH	Department of Health
DRV	Darunavir

**E**

EFV	Efavirenz
eGFR	Estimate glomerular filtration rate
ETV	Etravirine

**F**

FBC	Full blood count
FDA	Food and Drug Administration
FDC	Fixed dose combination
Fe <sup>2+</sup>	Iron
FTC	Emtricitabine
FPV	Fosamprenavir
FRS	Fat redistribution syndrome

## H

HAART	Highly Active Antiretroviral Therapy
Hb	Haemoglobin
HBV	Hepatitis B
HIV	Human immunodeficiency virus
HIV-SN	Human immunodeficiency virus associated sensory neuropathies
HL	Hyperlactatemia
HOSP	Hospital based out-patients initiated on ARV therapy by doctors
HREC	Human Research Ethics Committee

## I

IDV	Indinavir
i.e.	id est - namely
IF	Impact factor
INSTI	Integrase strand transfer inhibitor
IQR	Inter quartile range

## K

KZN	Kwazulu-Natal
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## L

LPV/r	Lopinavir/ ritonavir combination
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## M

MCC	Medicines Control Council
MCV	Mean corpuscular volume
MDR-TB	Multi-drug resistant tuberculosis
MPV	Mean platelet volume
MSH	Management Sciences for Health

**N**

NADEMC	National Adverse Drug Event Monitoring Centre
NDoH	National Department of Health
NFV	Nelfinavir
NHLS	National Health Laboratory Service
NIMART	Nurse-Initiated Management of Antiretroviral Therapy
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NPC	National Pharmacovigilance Centre
NRTI	Nucleoside reverse transcriptase inhibitor
NS	Not specified
n.s.	Not significant
NVP	Nevirapine
NW	North West Province

**O**

OI	Opportunistic infection
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**P**

pCr	Plasma creatinine
PDW	Platelet distribution width
PHC	Primary healthcare
PHCN	Primary healthcare nurse
PHRU	Perinatal HIV Research Unit
PI	Protease inhibitors
PN	Peripheral neuropathy
PPRM&E	Policy, Planning, Research, Monitoring and Evaluation
PV	Pharmacovigilance

**Q**

QIP            Quality improvement project

**R**

RBC            Red blood cell(s)  
RNA            Ribonucleic acid  
RPN            Registered professional nurse  
RTV            Ritonavir  
RVD            Retroviral disease

**S**

SA            South Africa  
SCr            Serum creatinine  
SD            Standard deviation  
SQR            Saquinavir  
SSA            Sub-Saharan Africa  
STRETCH      Streaming Tasks and Roles to Expand Treatment and Care for HIV  
SoMCHAT      Soweto Matlosana Collaborating Centre for HIV/AIDS and TB

**T**

TB            Tuberculosis  
TDF            Tenofovir  
3TC            Lamivudine  
TPV            Tipranavir

**U**

UNAIDS        Joint United Nations Programme on HIV/AIDS

**V**

VF            Virological failure

VL            Viral load

**W**

WBC            White blood cell

WHO            World Health Organization

# SYMBOLS AND UNITS

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## C

cells/ul      Cells per microliter

copies/ml      Copies per millilitre

## F

fL      Femtolitres

## G

g/dL      Gram per decilitre

## K

kg      Kilogram

kg/m<sup>2</sup>      Kilogram per square metre

## M

mg/day      Milligrams per day

ml/min      Millilitre per minute

μMol/ml      Micromole per millilitre

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# CHAPTER 1: INTRODUCTION

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## 1.1 Overview

For the purpose of this dissertation, the following study was conducted:

- Descriptive and retrospective investigation into the clinical health outcomes of HIV patients on AZT-based regimens in a NIMART and hospital clinic cohort respectively.

This was a comparative study where the clinical health outcomes of a group of patients in a nurse-initiated management of antiretroviral therapy (NIMART) and hospital clinic cohort was investigated.

## 1.2 Problem Statement

There are contradictory reports in the literature about the difference in efficiency between the antiretroviral therapy (ART) programmes initiated in a hospital clinic setting by a physician and those initiated in a primary healthcare (PHC) clinic by a NIMART (Fatti *et al.*, 2010). The two approaches have been compared in some ways in South Africa (Fairall *et al.*, 2012, Nyasulu *et al.*, 2012 & Sanne *et al.*, 2010), but not specifically for zidovudine-based (AZT) regimens. In order to compare the two approaches (hospital clinic cohort versus NIMART cohort), it is important to compare diagnostic parameters, disease progression markers and clinical treatment outcomes.

The use of ART is associated with an increase in haemoglobin (Hb) concentrations and a decrease in the prevalence of human immunodeficiency syndrome / acquired immune deficiency syndrome-induced (HIV/AIDS) anaemia (Sharma, 2010). According to a recent study performed by Assefa and colleagues (2015), although the prevalence of anaemia decreased considerably after ART was initiated, a substantial number of patients still remained anaemic after 12 months of ART. Zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI) and one of the first drugs to be approved by the Food and Drug Administration (FDA) for the treatment of HIV/AIDS (Sharma, 2010), is possibly the most common cause of anaemia in patients infected with the HIV virus (Doweiko & Groopman, 1998; Wandeler *et al.*, 2013). It is known to cause severe anaemia that resolves promptly when the drug is stopped (Rajesh *et al.*, 2011). Zidovudine currently forms part of second line ART regimens in South Africa although commonly used as part of first line therapy in case of contraindications to other ARVs (NDoH, 2014) (see ADDENDUM C1).

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A key impediment to ART expansion in South Africa has been the limited human resource capacity of specialised HIV physicians in the public sector in a country with the largest ART programme worldwide (Sanne *et al.* 2010). From the 6.19 million people living with HIV in South Africa, 3.4 million people were receiving ART in 2015 (UNAIDS, 2016; Kanabus, 2016). However, the time saving benefits of NIMART and the increasing burden of HIV patients on the public healthcare systems, especially hospitals, support the view that successful ART can be realised by transferring the task to nurses in PHC settings. However, there is limited research on the exploration of care provided by NIMART graduates within clinical management of ART (Green *et al.*, 2014). Despite being an important public health problem, data collected on HIV induced anaemia in sub-Saharan Africa are limited and few studies have documented - Hb levels among patients on ART in resource-restricted settings (Takuva *et al.*, 2013), and even more so in the Dr Kenneth Kaunda district (DKKD) in the North West. Although the haematological effects of AZT on HIV patients are well known, the ultimate goal of the study was to investigate the effect of AZT-based ART on HIV patients over time between a hospital clinic and NIMART cohort to assess treatment outcomes in different study settings. The use of AZT-based regimens is less prescribed as it forms more part of second line regimens and AZT-based regimens have not specifically been investigated when comparing physician-managed and NIMART approaches.

### 1.3 Study objectives

The general objective of this study was to investigate retrospectively if there was a difference in the clinical outcomes of patients on AZT-based regimens, initiated as either first or second line therapy, in a hospital clinic setting (under physician care) compared to NIMART twelve months post-initiation.

*This hospital clinic setting did not include patients that were hospitalised. It refers only to outpatient-based visits to the hospital clinic either for their monthly follow-up visits or prescription repeats.*

#### 1.3.1 Primary objectives

The primary objectives of this study were:

- to compare the disease progression markers (CD<sub>4</sub> and VL) that can predict treatment outcomes of AZT-based regimen between the two groups of patients (hospital clinic and NIMART cohorts) and
- to compare haematological related factors for AZT-based regimen, specifically Hb and MCV, between the two groups (hospital clinic and NIMART cohorts).

## CHAPTER 1: INTRODUCTION

### 1.3.2 Secondary objectives

The secondary objectives were:

to investigate the possible associated risk factors for disease progression such as body mass index (BMI), hospital admissions, the frequency of opportunistic infections especially TB, creatinine clearance and WHO staging in both these groups.

### 1.3.3 Primary outcomes

The primary outcomes for this study were to specifically investigate the following:

- 1) Time to undetectable viral load (VL) suppression on AZT-based therapy for up to 12 months in both study groups by means of a Kaplan-Meier survival analysis.
- 2) The number of hospital admissions for each patient on AZT-based therapy in both the hospital clinic and NIMART setting within the first 12 months post-AZT initiation.
- 3) Frequency of OIs for each patient on AZT-based therapy in both study groups within the first 12 months post-AZT initiation
- 4) Time from enrolment to death (if applicable) within the first 12 months for both the hospital clinic and NIMART groups post AZT-based initiation.

## 1.4 Hypothesis

It was anticipated that the clinical health outcomes of HIV patients initiated on AZT-based regimens would be similar between the hospital clinic (outpatient-based) and NIMART initiated cohorts, specifically with regard to major markers for HIV disease progression and possible associated risk factors for disease progression.

## 1.5 Structure of the dissertation

This dissertation is presented in chapter format. Each chapter includes its own reference list. Chapters are arranged as follows:

- Chapter 1: Introduction
- Chapter 2: Literature review
- Chapter 3: Research methodology

## CHAPTER 1: INTRODUCTION

Chapter 4: Results

Chapter 5: Discussion of results

Chapter 6: Conclusions, study limitations and recommendations

ADDENDA: A to F - Various ethics approval and certifications, conference proceedings, case report forms, additional results and a manuscript which formed part of an additional study and a quality improvement project.

### 1.6 Contributions of authors to the study and manuscript presented in the dissertation

The table below lists the roles and responsibilities of the various role players and collaborators who were involved in the studies and manuscripts presented in this dissertation.

Author	Affiliation	Role
Miss R van Graan	Pharmacien, Division of Pharmacology, North-West University, Potchefstroom Campus, South Africa	<ul style="list-style-type: none"><li>• First author: Manuscript A (ADDENDUM F1)</li><li>• Writing of study protocols and ethics applications</li><li>• Clinical data collection</li><li>• Statistical analysis of both studies</li><li>• Writing of dissertation, feedback report and manuscript</li><li>• Oral feedback to Potchefstroom Hospital on additional research project as part of a QIP – 18 August 2016</li><li>• Oral presentation of at AAC - 6 October 2016</li><li>• Oral presentation of at SoMCHAT conference – 18 November 2016, Johannesburg.</li></ul>
Dr M Viljoen	Pharmacien, Division of Pharmacology, North-	<ul style="list-style-type: none"><li>• Supervisor for R van Graan</li><li>• Conceptualisation of study designs</li></ul>

## CHAPTER 1: INTRODUCTION

	West University, Potchefstroom Campus, South Africa	<ul style="list-style-type: none"> <li>• Guidance with the writing of protocols, ethics applications, abstracts, manuscript and dissertation.</li> <li>• Collaboration meetings and setting up networks for these studies</li> <li>• Oral feedback presentation at DKK Research Day – 24 August 2016</li> <li>• Funding of projects</li> <li>• Final feedback reports to Hospital managers and ethics monitoring reports to NWU-HREC and NW DoH.</li> </ul>
Dr M Rheeders	Pharmacien, Division of Pharmacology, North-West University, Potchefstroom Campus, South Africa	<ul style="list-style-type: none"> <li>• Co-supervisor for R van Graan</li> <li>• Guidance with the writing of protocols, conference preparation, manuscript and dissertation</li> </ul>
Me F Motara	Potchefstroom Hospital Pharmacy, Department of Health, North West, South Africa	Collaborator and co-author on additional research project intended as a quality improvement project (QIP).
Dr N Martinson	Perinatal HIV Research Unit, MRC Soweto Matlosana Collaborating Centre for HIV/AIDS and TB, University of the Witwatersrand, South Africa	Collaborator and co-author (All Africa Congress abstract)
Prof E Variava	Department of Internal Medicine, Klerksdorp Tshepong Hospital Complex, Department of Health, North-West & Perinatal HIV Research Unit, MRC Soweto Matlosana Collaborating Centre for HIV/AIDS and	Part of concept development for NIMART

## CHAPTER 1: INTRODUCTION

	TB, University of the Witwatersrand, South Africa	
Me M Cockeran	Medicine usage in South Africa (MUSA), North-West University Potchefstroom Campus, South Africa	General statistical consultation and assistance with analysis and modeling of data

### 1.7 Additional research project as part of a quality improvement project

An additional study titled:

Retrospective clinical analysis of pharmacovigilance reports in the Tlokwe subdistrict (Jan 2010 - Dec 2014).

This study was performed as part of a quality improvement project (QIP) and is presented in ADDENDUM F1 in the form of a full-length manuscript (A). This manuscript only focuses on adverse drug reactions (ADRs) pertaining to ARVs as a final report was submitted to the Potchefstroom Hospital manager during October 2016 which consisted of all the reported ADRs during the reported period.

## CHAPTER 1: INTRODUCTION

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NDoH **see** South Africa. National Department of Health

## CHAPTER 1: INTRODUCTION

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## CHAPTER 2: LITERATURE REVIEW

---

### 2.1 Introduction

This literature review provides a general overview of HIV (type 1 and 2) and related clinical pharmacology (mechanism of action, side effects and toxicity) of ARVs in general. The role of NIMART in South Africa is also addressed. More specifically, the role of anaemia is investigated with a focus on AZT-induced macrocytic anaemia. The literature review also appraises the two major markers for HIV disease progression and possible associated risk factors such as BMI, WHO-staging and OIs.

### 2.2 General overview

The estimated number of people living with HIV/AIDS in South Africa reached 6.19 million in 2015. Those succumbing to the virus made up to 30.5% of all deaths in SA (Kanabus, 2016). HIV/AIDS refers to the most progressive stages of HIV infection and is the largest known pandemic, with an estimated 36.7 million people infected and 15 million receiving ART worldwide (WHO, 2015). Sub-Saharan Africa (SSA) is the worst affected, accounting for almost 70% of HIV-infected people globally (Hegdahl *et al.*, 2016). Since the introduction of the first national antiretroviral treatment guidelines in South Africa in 2004, the first and second line regimens and guidelines have been revised and adapted in 2004, 2010, 2013, and 2014.

The efficacy and success of highly active antiretroviral treatment (HAART) has been proven and it has improved the life expectancy of HIV-infected patients since its first introduction in 1996 (CDC, 2013). By the middle of 2014, 2.6 million people had been initiated on ART in South Africa (NDoH, 2014). It is important that any national health department keeps up to date and changes its treatment guidelines (see ADDENDUM C1) according to the most recently updated relevant scientific knowledge, the feasibility to improve access and treatment to the majority of the communities they serve and the available human resource capabilities of healthcare workers.

### 2.3 Nurse-initiated management of antiretroviral treatment

Initially, ART programmes in South Africa followed a physician-initiated and managed ART model. However, the limited number of physicians in the public sector forced task shifting from physicians to nurses to respond to the challenge to deliver ART programmes to a greater number of people and to provide better access in rural areas (Georgeu *et al.*, 2012). The South African government implemented revised treatment guidelines in October of 2010, enabling primary healthcare nurses (PHCNs) to initiate ART for both treatment and prevention (Long *et al.*, 2011).

Cameron *et al.* (2012) state that South African professional nurses with the necessary training to initiate and change ART were authorised to do so from the first of April, 2010. The Streaming Tasks and Roles to Expand Treatment and Care for HIV (STRETCH) study contributed greatly to supportive evidence in task shifting from doctors to nurses and contributed to the policy change in 2010 (although the implementation of NIMART in South Africa only commenced beginning 2011) to promote NIMART (Fairall *et al.*, 2012). NIMART in essence refers to the initiation of HIV-positive patients on ART in a public healthcare setting (clinic) by nurses, with these nurses monitoring and re-prescribing ART for stable patients and appropriately referring complicated patients' cases to physicians (Georgeu *et al.*, 2012).

In a study performed by Green *et al.* (2014), the focus was placed on the hypothesis that nurse-initiated ART and monitoring in South Africa is not subordinate to physician-initiated therapy. Green and colleagues went on to conclude that NIMART was successful in the majority of patients, enabling physicians to handle complex patient cases and ultimately lightening the burden of HIV on public health services and provided fundamental proof for task-shifting efficiency. A similar study conducted by Long *et al.* (2011) confirmed that physician-initiated patients down-referred to primary healthcare clinics managed by primary healthcare nurses, showed outcomes similar or even better compared to hospital-initiated care. Nurses in all primary healthcare clinics in the Tlokwe and City of Matlosana sub-districts have been initiating ART and monitoring therapy outcomes since 2011.

This includes managing virological failure, deciding when to switch to second line regimens, and using nurse-based care as described in the South African National Guidelines in 2010 and initiated in selected clinics in 2011 (Zuber *et al.*, 2014). At the end of 2011, 10 541 nurses had already attended NIMART training courses (Swart *et al.*, 2013). The South African Department of Health has since set the goal of having nurses initiate 85% of patients eligible for ART by 2016 (Green *et al.*, 2014). In October 2016, there was a major boost for specialised healthcare with the roll-out of more than a thousand nurses in KZN, most of whom will be enabled by NIMART to help ease the massive HIV-burden placed on the region and its physicians. Ultimately, NIMART will

## CHAPTER 2: LITERATURE REVIEW

contribute a great deal to successfully implement the new policy change of initiating all newly diagnosed HIV patients on ART, irrespective of their CD4 cell counts. Colvin *et al.* (2010) stated that the medical and moral imperative to provide ART to all HIV-infected people does not have to be justified. According to Child (2016), a significantly increased number of patients will benefit from this collaborative approach, allowing earlier access to ART with more effective outcomes.

The Joint United Nations Programme on HIV/AIDS (UNAIDS) launched a global fast-track strategy known as the 90-90-90 targets to be achieved by 2030 (AVERT, 2016):

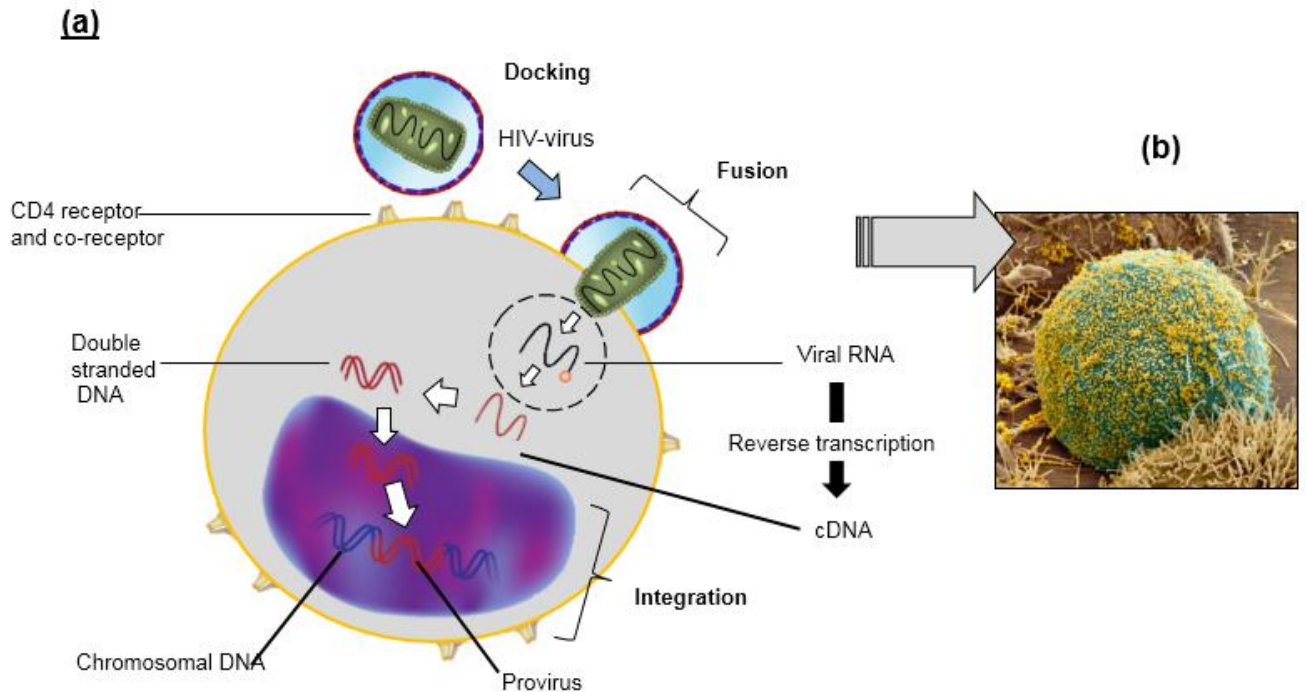
- a) 90% of all people living with HIV will know their status;
- b) 90% of all people living with HIV will receive ART; and
- c) 90% of all patients receiving ART, will have reached viral suppression.

This strategy is especially important for low and middle-income countries with focus on South Africa with the greatest HIV burden and ART programme worldwide (AVERT, 2016). Increasing deployment of nurses (NIMART) in combination with physician-managed ART (Sanne *et al.*, 2010) is essential to reach the 90-90-90 targets, improve health outcomes and deliver quality care (Evans, 2013).

### 2.4 Pathology of HIV/ AIDS

Both HIV-1 and HIV-2 are related to RNA human lentiviruses (Popper *et al.*, 1999), and they infect their host by binding or docking to CD4 receptors on the surface of suitable helper T lymphocyte cells, macrophage or dendritic cells (Spencer, 2005). They then merge with the cell membrane in a process known as fusion (Cox & Siliciano, 2014) (Figure 2-1). Reverse transcription of viral RNA to DNA and integration of viral DNA into the host cell genome follows, leading to the destruction of cell-mediated immune (CMI) system (Cox & Siliciano, 2014).

The HIV-2 variant is a close transmutation of the HIV-1 virus, being biologically similar (Marlink *et al.*, 1994), but less pathogenic and endemic to West Africa (Popper *et al.*, 1999). Independent of ART, HIV-1 infection demonstrates lifelong persistence of the virus (Hall *et al.*, 2011), being characterised by a high rate of viral replication whereas HIV-2, with a lower death rate, takes longer to reach symptomatic HIV/AIDS (Popper *et al.*, 1999).



**Figure 1-1:** (a) Productive infection of a CD4 T-cell with the HIV virus, reverse transcription of viral RNA to DNA and integration into the host cell genome (adapted by Cox and Siliciano (Nature, 2014); (b): Electron microscope image of a host CD4 T-cell infected by the HIV virus (Hayden, 2014).

## 2.5 Antiretroviral therapy and pharmacology

The use of HAART remains the only effective treatment to decrease viral replication in patients with HIV/AIDS (Sharma, 2010). A brief overview is presented here on the first and second line regimens that have been used in South Africa since 2010 and their respective mechanisms of actions.

### 2.5.1 First and second line regimens

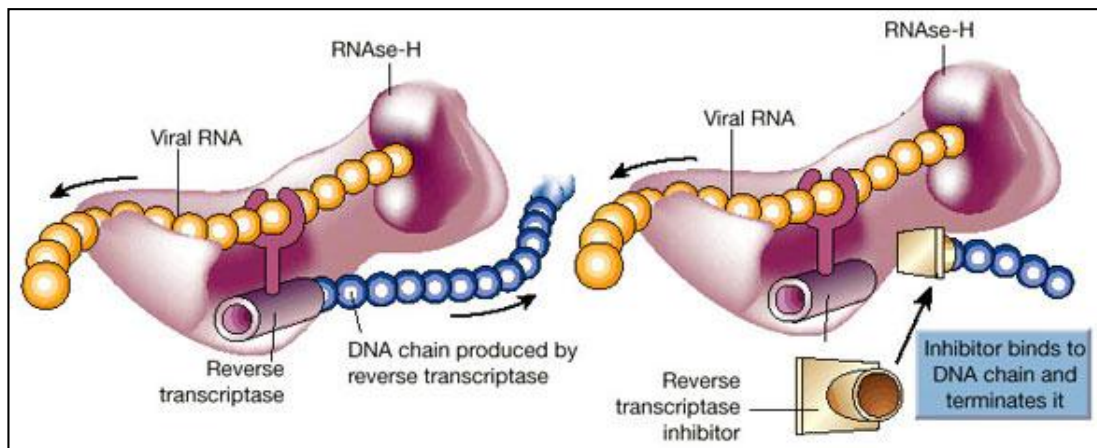
Several changes have occurred for first and second line regimens from 2010-2015. In 2010, stavudine (d4t) was still considered first line therapy. From 2013, fixed dose combination (FDC) (tenofovir (TDF) + emtricitabine (FTC)/ lamivudine (3TC) + efavirenz (EFV)) was preferred, with zidovudine (AZT), 3TC and lopinavir/ritonavir combination (LPV/r) acting as second line therapy, AZT primarily replacing TDF in cases of contraindications or failure on TDF-based regimens. By 2014, new guidelines forced the phasing-out of d4t. According to the WHO (2011) and Kerkhoff

*et al.* (2014), AZT forms part of first line regimens where TDF/ 3TC/ EFV is either contraindicated or not available. Zidovudine is primarily indicated as a second line drug in South Africa (NDoH, 2014). Abacavir based regimens were preferred for patients presenting with anaemia and renal failure (see *ADDENDUM C1 for a full summary on the changes in the NDoH consolidated guidelines for ART from 2004-2015*).

## 2.5.2 Mechanism of action of nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors

### 2.5.2.1 Nucleoside reverse transcriptase inhibitors

Nucleoside reverse transcriptase inhibitors (NRTI) work *in vitro* by inhibiting the replication of the HIV-1 virus. These analogues must undergo intracellular phosphorylation to create synthetic substrates for the enzyme (Acosta and Flexner, 2011). Phosphorylated analogues inhibit HIV replication and infection of new cells (Richman, 2001) by preventing incorporation of native nucleotides, terminating elongation of proviral DNA (Dudley, 1995). Ultimately, DNA from the viral genome is thus rendered incomplete and replication is inhibited. Zidovudine was the first approved antiretroviral agent and NRTI for HIV-1 treatment in 1987 (Arts & Hazuda, 2012) (Figure 2-2). However, the drug has no effect on already-infected cells (Acosta & Flexner, 2011; Bhushanam, 2014).



**Figure 1-2:** The mechanism of action of AZT *in vitro*.

Zidovudine incorporates itself into the DNA of the HIV-1 virus (refer to the encircled viral DNA in Figure 2-1) that is competing with natural nucleotides, thereby stopping the building transcription process from RNA to DNA (Taken from Richman, 2001).

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Intracellular AZT is phosphorylated by thymidine kinase to AZT 5' – monophosphate and by thymidylate kinase to AZT 5' – diphosphate. Finally, through nucleoside diphosphate kinase, AZT 5' – diphosphate is phosphorylated to AZT 5' – triphosphate responsible for the termination of proviral DNA elongation because it is incorporated by reverse transcriptase into nascent DNA (Acosta & Flexner, 2011). Although no longer part of first line adult regimens, AZT is still used as second line in adults in South Africa (NDoH, 2014). Initiating AZT-based regimens in patients with progressive HIV infections decreases the severity, prevalence and frequency of OIs, increases CD4 cells (Brooker, 2010) and ultimately prolongs survival (Fischl *et al.* 1990). Other NRTIs available in South Africa include abacavir (ABC), didanosine (ddl), emtricitabine (FTC), lamivudine (3TC), stavudine (d4t) and tenofovir (TDF) (SAMF, 2016).

### 2.5.2.2 Non-nucleoside reverse transcriptase inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are chemical substrates that bind to a hydrophobic pocket in the p66 subunit of the HIV-1 reverse transcriptase enzyme. They induce conformational changes in the three-dimensional enzyme structure that decreases its activity and act as non-competitive inhibitors. Unlike NRTIs, intracellular phosphorylation is not required to attain activity (Harris & Montaner, 2000; Acosta & Flexner, 2011). The binding site for these NNRTIs are virus specific resulting in activity against HIV-1 but not HIV-2 infection (Harris and Montaner, 2000). The most common NNRTIs available in South Africa include efavirenz (EFV), etravirine (ETV) nevirapine (NVP) and rilpivirine (SAMF, 2016). Delavirdine (DLV) is however not yet registered in South Africa (Safrin, 2012).

### 2.5.2.3 Protease inhibitors

Peptide-like protease inhibitors (PIs) competitively inhibit the viral action of the aspartyl protease enzyme. Human aspartyl proteases contain only one polypeptide chain and are not significantly inhibited by the viral PIs (Flexner, 1998). Protease inhibitors include saquinavir (SQV), indinavir (IDV), ritonavir (RTV), nelfinavir (NFV), amprenavir (APV), LPV/r, atazanavir (ATV), fosamprenavir (FPV), tipranavir (TPV) and darunavir (DRV) (Acosta & Flexner, 2011).

## 2.5.3 Fixed dose combination as part of ART

South Africa commenced with FDC therapy in adults in April 2013. The earlier available two-combinations were 3TC/ABC (300/600 mg) and AZT/3TC (300/150 mg). The current first line ART regime for adults consists of a fixed combination tablet containing three standard ARV drugs: TDF + FTC or 3TC + EFV (NDoH, 2014). Second line regimes (not given as FDCs) consist of AZT +

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3TC + LPV/r (when failing on a TDF-based first line regimen) or TDF + 3TC/FTC + LPV/r (when failing on a stavudine (d4T) or AZT-based first line regimen) (NDoH, 2014).

### 2.6 Major markers used in clinical monitoring of HIV/AIDS disease progression

The main focus in the evaluation of clinical treatment outcomes of HIV patients on ART is the measurement of two key markers; CD4 cell counts and VL monitoring. The prognostic value of immunological markers (CD4 cell counts and CD4%) and virological marker (VL suppression as function of immune improvement) are crucial to evaluate and monitor treatment response or progression of HIV in clinical practice (Govender *et al.*, 2014; Kim *et al.*, 2013) as a function of immune system augmentation. In infected patients, HIV RNA plasma VL in concurrence with CD4 cell counts are used as routine laboratory markers to guide initiation of HAART and monitor the efficacy and feasibility of HIV/AIDS disease progression (Hall *et al.*, 2011).

#### 2.6.1 CD4 (lymphocyte) cell count

CD4 cells, also referred to as helper T cells (lymphocytes), are the main target of HIV. These cells harmonise and regulate the body's immune response, assisting B cells in antibody production and increasing the immune response to antigens on a cellular level (WHO, 2007). Diminishing CD4 cell counts have been essential in the development of HIV/AIDS, but also central to judgements and conclusions regarding clinical management (Ford *et al.*, 2015). Human immunodeficiency virus is known for the gradual and persistent direct and indirect destruction of CD4 cells, ultimately inhibiting a specific immune response to the virus. CD4 glycoproteins are the receptor for the HIV-1 virus and while normally long-lived, once infected the half-life changes to 1.6 days with a loss of up to 5% CD4 cells due to destruction (Beck, 2008). Loss of T-cell function *in vitro* predicts progression to AIDS (Rosenberg *et al.*, 1997). In South Africa, ART initiation was based on CD4 cell counts used as clinical and immunological evaluation (Govender *et al.*, 2014; WHO, 2007). According to 2014/2015 guidelines, ART is recommended for HIV-positive patients with a CD4 count  $\leq 500$  cells/ $\mu$ l (NDoH, 2014).

However, after the recommendation from the WHO to initiate all patients that test positive for HIV on ART, a policy change was announced in May 2016, enabling South African guidelines to provide ARVs to all South Africans, irrespective of their CD4 cell count (Child, 2016). All patients currently in HIV clinical stage III or IV (as defined by the WHO – see *ADDENDUM C2*), should start ART irrespective of their CD4 cell count (NDoH, 2014). The reduction of CD4 cells reflects the inhibition of immune system function and capability ultimately resulting in a reduced immune response to any pathogens. Initial HIV infection causes a 20-40% reduction in CD4 cell counts,

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but in response to successful and good adherence to ART, CD4 cell counts augmented by > 50 cells/ $\mu$ l within weeks after viral suppression was achieved. CD4 cell counts increased annually by an estimated 50-100 cells/ $\mu$ l and stabilised after a threshold was reached (WHO, 2007).

### 2.6.2 Viral load

According to guidelines released by the WHO (2007), CD4 cell counts remained the most important indicator for treatment initiation and monitoring. However, since better access to improved equipment has increased, the use of VL monitoring has become more frequent but not a requirement at baseline. This resulted in the release of newer guidelines by the WHO, recommending VL as the preferred marker for disease progression and ART monitoring (Ford *et al.*, 2015). Viral load monitoring in South Africa is done 12-monthly for patients with a VL of < 400 copies/ml and 6-monthly for VLs between 400-1000 copies/ml. For patients with a VL of > 1000 copies/ml, another VL is repeated after two months after adherence has been addressed. If the second VL is still > 1000 copies/ml, patients should be switched to second line therapy (NDoH, 2014). Continuous detectable VL is a risk factor for resistance development, morbidity and mortality. A sustained VL of < 50 copies/ml is the most robust value for ART success within the first 6 months of ART initiation with the focus on sustaining this suppression. VL blips or viral rebound refer to low-level increases in HIV VLs followed by return to suppression without a change in therapy (Meintjes *et al.*, 2015) and are not an indicator for an augmented risk for virological failure.

#### 2.6.2.1 Virological failure

Virological failure (VF), a type of HIV treatment failure, occurs when ART is not able to suppress and sustain a patient's VL < 1000 copies/ml (NDoH, 2014; WHO, 2015). Failure can be attributed to poor patient adherence, drug toxicity and resistance. The definition of virological failure changed during the period of 2004 to 2014 and has recently been redefined by the NDoH (2014) as two VLs > 1000 copies/ml taken at least 3 months apart after adherence has been addressed. If patients fail on first line regimens, second line therapy is introduced (which should contain a PI – mostly LPV/r). Virological failure on second line therapy is defined as a VL > 1000 copies/ml for at least one year where after patients qualify for specialist referral and genotype testing (NDoH, 2014). According to Hassan *et al.* (2014), a systematic review done on virological efficacy and drug resistance outcomes on patients receiving ART in SSA, revealed only 76% virological suppression after 12 months and 67% after 24 months. The main aim at the start of ART is to achieve viral suppression so that the VL is below the level of detection (LDL, < 20 copies/ml of blood) (Zhou *et al.*, 2010).

### 2.6.3 Association between CD4 cell counts, viral loads and disease progression (survival)

HIV-infection is marked by a progressive decline in the number of circulating CD4 cells leading to immune failure over a period of years and ultimately resulting in advanced lymphodegeneration and impaired CD4 cell replenishment (Arnaout *et al.*, 1999). As HIV multiplies, CD4 cells ultimately become depleted (Beck, 2008) resulting in no defence against viral HIV copies and an increase in VLs. Outcomes of ART are thus associated with an increase in CD4 cell counts and a decrease in VLs. A study done by Zhou *et al.* (2010) found that after combined ART (cART) initiation, CD4 continued to increase even after the concomitant HIV VL was detectable. However, Arnaout *et al.* (1999) determined that CD4 cell counts are inaccurate as an indicator for VLs as higher CD4 cell counts did not extend predicted survival times while higher VLs were associated with faster decline and shorter predicted survival times.

### 2.7 Anaemia and HIV/AIDS

Anaemia is characterised by either a decrease in Hb or red blood cells (erythrocytes) which then result in diminished oxygen carrying capacity of the blood. Anaemia can be further classified on the basis of the morphology, aetiology or pathophysiology of the red blood cells (RBCs) (Brooker, 2010 & Dipiro *et al.*, 2013). Major causes of anaemia include a decrease in the number of RBC, a diminished concentration of Hb per RBC or a combination of both. Other causes may include blood loss, chronic infections, malnutrition, certain drugs or hereditary factors (Widmaier *et al.*, 2006 & Dipiro *et al.*, 2013).

Anaemia and impaired haematopoiesis, neutropenia, lymphopenia and thrombocytopenia (Moyle, 2001) are well-known haematological abnormalities of HIV/AIDS infection, affecting 60-80% of patients in the latter stages of the disease (Meidani *et al.*, 2012). These abnormalities are related to diminished quality of life, most commonly due to the association with fatigue, accelerated disease progression and decreased survival rates (Ferri *et al.*, 2001). Full blood count (FBC) tests almost always show alterations and almost any irregularity can be found (Beck, 2008). Anaemia and malnourishment both prominently occur in SSA and this region has the greatest population of HIV/AIDS-infected people in the world (Takuva *et al.*, 2013).

Few studies, however, have studied anaemia in developing countries (Meidani *et al.*, 2012). Anaemia is common in HIV-infected patients and is associated with a greater risk of death and this risk increases as the severity of the anaemia increases (Moore *et al.*, 1998). Early in the HIV/AIDS pandemic, anaemia was acknowledged as a prognostic marker of future disease development or death, independent of CD4 counts and VL, a statement that is still true in the

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HAART era (Moyle, 2001). HIV itself can also be a leading cause of anaemia (Sullivan *et al.*, 1998). Risk factors associated with anaemia in HIV-infected persons include low CD4 count, increased VL, female gender, African American ethnicity, black race, AZT use, increased age, low BMI, history of bacterial pneumonia, WHO stage III or IV, oral candidiasis and a history of fever (Volberding *et al.*, 2004; Takuva *et al.*, 2013).

According to Volberding *et al.* (2004), the pathophysiology of HIV/AIDS-induced anaemia involves three basic mechanisms: decreased red blood cell (RBC) production, increased RBC destruction and ineffective RBC production. Underlying these three mechanisms is reduced hematopoietic growth factor production, malabsorption, impaired iron recycling and decreased erythropoiesis due to inflammatory cytokine release (Takuva *et al.*, 2013). Masaisa and co-workers (2011) also state that infections caused by OIs, especially tuberculosis (TB), caused by *Mycobacterium tuberculosis*, contribute to the development of anaemia.

Volberding *et al.* (2004) stated that some HIV/AIDS-infected individuals are more likely to develop anaemia, especially women (with a 71% greater prevalence among women than among men) and African American patients. Meidani *et al.* (2012) report that anaemia in HIV-infected individuals may also result from nutritional deficiencies. In some patients, anaemia may present in the early stages of HIV/AIDS infection as a simple laboratory irregularity where in others, typical symptoms (e.g. decreased functionality, dyspnoea and fatigue) directly related to a reduction in Hb levels, may be visible (Meidani *et al.*, 2012). ART has reduced the prevalence of severe anaemia since its introduction, although mild to moderate anaemia continues to be common (Volberding *et al.*, 2004).

Kiragga and colleagues (2010) reported that antiretroviral therapy resulted in substantial increases in mean Hb levels. A South African longitudinal study of more than five years (conducted from April 2004 to August 2009) in 10 259 HIV-infected patients showed the prevalence of anaemia (< 10 g/dL) at 25.8% when ART was initiated (Takuva *et al.*, 2013). Other studies in SSA indicated baseline anaemia (< 9.5 g/dL) ranging from 12-18.2% and 21% (< 10 g/dL) (Zhou *et al.*, 2012). According to Ferri *et al.* (2002), one of the most important treatment goals in managing anaemia in HIV-infected persons is to improve normal Hb levels. Takuva and colleagues (2013) suggest that monitoring Hb levels and prompt diagnosis of anaemia can result in improved morbidity and mortality of patients initiated on ART.

The current South African guidelines suggest FBCs at baseline, month 3, 6 and then 12-monthly when on AZT-based regimen (NDoH, 2014). Point of care devices such as HemoCue® is available at some PHC facilities but it is unclear how frequently the sifting finger prick tests to determine

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Hb are performed at PHC facilities. Patients presenting with baseline anaemia (Hb  $\leq$  9.5 g/dL) and baseline severe anaemia (Hb  $\leq$  8 g/dL) after ART initiation, should be switched to an ABC-containing second-line regimen. AZT is known to cause anaemia especially in the early stages of treatment. However, the results of two South African studies by Takuva *et al.* (2013) (n = 322 on AZT-based) and Hoffmann *et al.* (2008) (n = 853 on AZT-based), showed improvement in the mean Hb levels over time, despite concerns for potentially worsening anaemia as a result of ART toxicity.

### 2.7.1 Definition of anaemia

In most populations, normal Hb levels fluctuate between 12 g/dL (120 g/L) and 16-18 g/dL (160-180 g/L) (Spencer, 2005). The NDoH (2014) defined anaemia as Hb levels  $<$  8 g/dL (80 g/L) (men and women) and  $<$  7 g/dL (70 g/L) in pregnant woman, and is said to be present when the Hb value is below the reference interim for the sex and age (Table 2-1) of the patient (Hughes-Jones *et al.*, 2008). The NDoH (2014) and the AIDS Clinical Trials Group (ACTG) definition of a severity grading of anaemia was adopted in this investigation as shown in Table 2-2.

Anaemia in women infected with HIV/AIDS has been associated with a low BMI, increasing age, a history of pneumonia or fevers, the presence of oral thrush (candidiasis), the use of AZT-based ART and a CD4 count  $<$  200 cells/ $\mu$ l (Spencer, 2005).

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**Table 2-1:** Reference ranges for Hb levels to diagnose anaemia (WHO, 2011) in adults.

<b>Reference ranges for non-anaemic and anaemic Hb values: WHO</b>				
	<b>Hb concentration (g/dL)</b>			
		<b>Anaemia</b>		
<b>Individual</b>	<b>Non-anaemic</b>	<b>Mild</b>	<b>Moderate</b>	<b>Severe</b>
<b>Adult females (non-pregnant ≥ 15 years)</b>	≥ 12 g/dL	11-11.9 g/dL	8-10.9 g/dL	< 8 g/dL
<b>Adult females (pregnant ≥ 15 years)</b>	≥ 10 g/dL	10-10.9 g/dL	7-9.9 g/dL	< 7 g/dL
<b>Adult males (≥ 15 years)</b>	≥ 13 g/dL	11-12.9 g/dL	8-10.9 g/dL	< 8 g/dL

**Table 2-2:** Grading of the severity of anaemia in adults based on Hb values (NDoH, 2014).

<b>Laboratory grading of anaemia according to the NDoH</b>			
<b>Grade I</b>	<b>Grade II</b>	<b>Grade III</b>	<b>Grade IV</b>
8-9.4 g/dL	7-7.9 g/dL	6.5-6.9 g/dL	< 6.5 g/dL

### 2.7.2 Indicators for the diagnosis and monitoring of anaemia

#### 2.7.1.1 Haemoglobin

According to Brooker (2010), Hb is defined as the “red, respiratory pigment in red blood cells. It combines with oxygen and releases it to the tissues.” Hb tests are therefore used to monitor the

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status of Hb in patient's RBCs. Haemoglobin is a complex metalloprotein that is synthesised in the developing red blood cells in bone marrow (Blakemore & Jennett, 2001). It consists of four subunits bound together; each consisting of a protein (or polypeptide chain) that envelops a heme group. The four polypeptides of a Hb molecule are cooperatively called globin. Each of the four heme groups contains one atom of iron ( $\text{Fe}^{2+}$ ) (responsible for the red colour of blood), which binds a molecule of oxygen, although the absorption of iron into the blood is estimated to be only 10% of that which is ingested (Widmaier *et al.*, 2006). The oxygen available to body tissue is directly related to the number of oxygen molecules in the RBCs and local perfusion of the tissues (DeMoranville & Best, 2003).

*Refer to table 2-1 for the reference ranges of Hb according to WHO and to table 2-2 for the grading of anaemia according to the NDoH in South Africa.*

### 2.7.1.2 Mean corpuscular volume

Mean corpuscular volume (MCV) is commonly used as a marker to differentiate between several types of anaemia i.e. microcytic and macrocytic anaemia (Moyle, 2001) and is a indicator of RBC size (Abshire, 2001; Lesperance *et al.*, 2002). Microcytosis, indicated by smaller than normal cell size and associated with defective Hb production and iron deficiency. Whereas macrocytosis presents with RBCs larger than normal and associated with vitamin B12 or folic acid deficiency (Cohen, 1996; Neufeld, 2002). Anaemia expressed as elevated MCV with a decreased reticulocyte count suggests that the overall count of RBCs generated, is low (Abshire, 2001; Hermiston & Mentzer, 2002; Lesperance *et al.*, 2001). According to Kim *et al.* (2013), MCV is the standard diagnostic test to assess and monitor HIV patients and indicate the average volume of RBCs present. MCV is measured in femtolitres (fL) with normal values ranging from 80-100 fL and values from 100-150 fL being interpreted as high to extremely high (Aslinia *et al.*, 2006).

### 2.7.3 Zidovudine induced anaemia

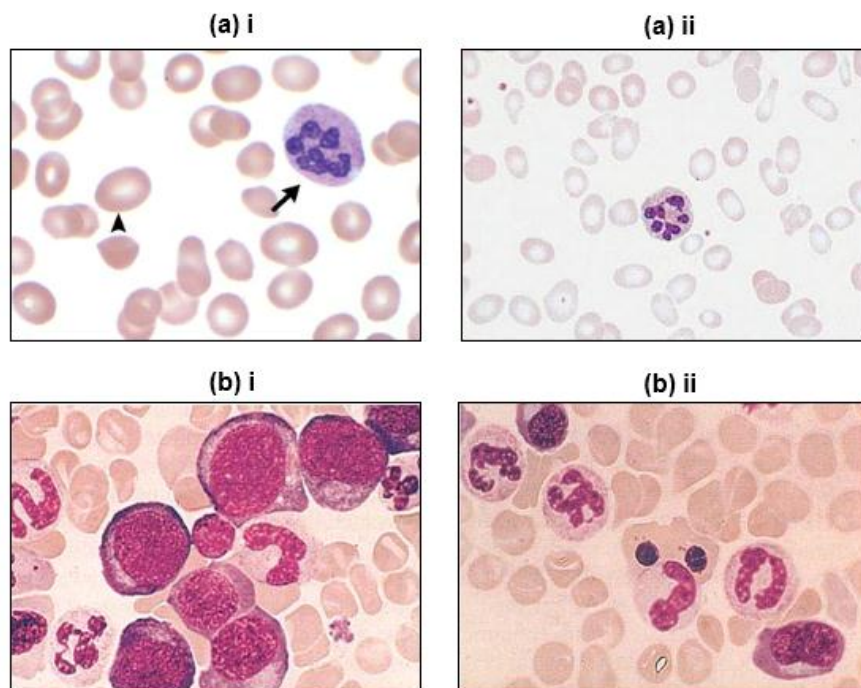
It is known that although AZT is highly effective, it has been associated with dangerous adverse drug reactions (ADRs), including myelotoxicity (bone marrow suppression) manifesting clinically as anaemia (Agarwal *et al.*, 2010). Zidovudine-induced haematological disorders often occur within four to twelve weeks of drug initiation (Meidani *et al.*, 2012). The mechanism of AZT-induced anaemia is attributed to 50-70% inhibition and proliferation of blood cell progenitor cells, including CFU-E (colony forming unit – erythrocytes) and CFU-GM (colony forming unit - granulocyte-macrophage) (Calabresi *et al.*, 1990). Inhibition and proliferation occurs in a time-dose-dependent fashion, showing cytotoxicity to myeloid and erythroid precursor colonies (Beck,

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2008). The main target area includes normal human bone marrow as at drug concentrations close to those associated with optimal antiviral effect *in vitro* (Sharma, 2010).

A recent study in several Southern African countries with combined cohorts indicated that AZT is associated with inferior immunological recovery compared to the other backbone regimens (Wandeler *et al.*, 2013). This can be possibly attributed to the hyper-segmentation effect of AZT on neutrophils by inhibiting GFU-GM. This is also possibly the main reason why its use in adults has decreased. Severe anaemia manifested in 25% of HIV/AIDS patients on a dose of 1500 mg/day (Beck, 2008).

Abnormally large RBCs in peripheral blood flow is referred to as macrocytosis (Aslinia *et al.*, 2006). Macrocytic anaemia can be divided into megaloblastic and non-megaloblastic anaemia based on the appearance of developing erythroblasts in the bone marrow (Hoffbrand *et al.*, 2006). Zidovudine is responsible for the development of macrocytic anaemia, more specifically megaloblastic anaemia, or rather a lesser degree of macrocytosis (Tefferi, 2003) (Figure 2-4).



**Figure 2-3:** Photomicrographs representative of a peripheral blood smear. Severe macrocytic megaloblastic anaemia (Figure **a-i** and **a-ii**) and bone marrow smears indicating severe drug-induced macrocytic megaloblastic anaemia (Figure **b-i**, **b-ii**) (Luzzatto & Notaro, 2002).

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Megaloblastic anaemia induced by AZT therapy can be attributed to cytotoxicity to myeloid and erythroid precursors in the bone marrow (Sharma, 2010), resulting in impaired red cell maturation (Beck, 2008). Manifesting macrocytosis caused by the AZT therapy is not treatable with vitamin B<sub>12</sub> or the administration of folate, as is the case with megaloblastic anaemia caused by non-drug related factors (Beck, 2008).

*Macrocytosis without the occurrence of anaemia may be a normal variant as large circulating erythrocytes are not always associated with a pathologic condition (Aslinia et al., 2006).*

### 2.8 Possible risk factors associated with disease progression

#### 2.8.1 Body mass index

The use of HAART lessens the wasting effect of HIV/AIDS on body weight (Wanke *et al.*, 2000). Van der Sande *et al.* (2004) found BMI to be a strong predictor of survival in HIV/AIDS patients and mentioned more than ten per cent (>10%) loss in body mass as a frequent symptom of this infection. Since the introduction of more effective combinations of PIs and other ARVs, it was anticipated that a rapid reduction in HIV-associated weight loss and wasting would occur (Wanke *et al.*, 2000). Body mass index is derived from a patient's weight and height is an indirect measure of body fat.

Body mass index is defined as the body mass (weight in kilograms) divided by the square of the body height (in meter) and is expressed in kg/m<sup>2</sup> (Rothman, 2008). Phe *et al.* (2013) confirms a direct correlation between increased AZT-induced haematological toxicity and low baseline weight among HIV-positive patients and state that low BMI is a risk factor associated with AZT induced anaemia. Takuva *et al.* (2013) supported this by stating that incident anaemia (anaemia due to HIV or other HIV-related illnesses) is associated with lower BMI in individuals affected by HIV/AIDS, females, zidovudine and patients with lower CD4 cell counts and is most likely to imitate malnourishment as iron deficiency and other nutritional deficits contribute to anaemia. This can also relate to the fact that women are more prone to anaemia than men due to their lower BMI.

#### 2.8.2 World Health Organisation HIV clinical stage

All HIV-positive patients suffering from severe HIV disease classified by the WHO (2013) as clinical stage II or IV, irrespective of the CD4 cell count, are eligible to start ART according to South African guidelines (Meintjes *et al.*, 2015). The WHO clinical stage given at the time of HIV diagnosis is retained for life throughout the patients' therapy. No up or down staging occurs

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(Variava, 2016). Phe *et al.* (2013) found that an advanced stage of HIV disease is a risk factor associated with AZT-induced anaemia. A study by Takuva *et al.* (2013) indicated, as was the case with lower BMI and AZT-induced anaemia that advanced WHO stage (III or IV) was also linked to incidence anaemia (due to severe OIs). Progressive immunosuppression in WHO stage III and IV with an increased risk of anaemia in HIV patients was also reported by Ssali *et al.* (2006), Zhou *et al.* (2012) and Takuva *et al.* (2012) with a focus on adult HIV-infected patients in SSA.

### 2.8.3 Opportunistic infections

As HIV multiplies and CD4 cells become depleted, the manifestation of infections, malignancies and organ damage becomes more prominent. The pathogenic tendency of infections that are caused by opportunistic organisms, normally not prone to cause life-threatening diseases, is frequently reported in HIV/AIDS patients (Beck, 2008). Opportunistic infections occur more frequently in HIV patients with severe immunosuppression (Sepkowitz, 2002) and as is noted by Beck (2008), by secondary pathways of infection notably marrow (stromal cells) and brain cells. Therefore, indirectly, OIs has a major effect on haematopoiesis by interfering with cytokine production (Beck, 2008).

All HIV-related OIs and other diseases increase in prevalence and morbidity as the CD4 cell count deteriorates and these patients are at higher risk to pathogenic organisms than the normal population (CDC, 2013). Bacterial pneumonia and candidiasis are some of the most common OIs that can easily progress to life-threatening status and are considered associated risk factors for anaemia in HIV-infected individuals (Takuva *et al.*, 2013; Volberding *et al.*, 2004). Tuberculosis, being the most common OIs, is a leading cause of death in the HIV population worldwide (NDoH, 2014). Besides the morbidity and mortality caused by OIs, they also increase the progression of HIV/AIDS. OIs are used in the classification of the WHO clinical stage.

#### 2.8.3.1 Tuberculosis-HIV co-infection

Concern regarding the influence of HIV on TB and vice versa was already thoroughly documented in the 90's. Coker and Miller (1997) did an editorial piece on HIV-associated TB at the time. Now a perilous similarity between HIV and the global multi-drug resistant TB (MDR-TB) epidemic is emerging with overwhelming concern about the epicentre of the TB co-epidemic in the south of the SSA region. South Africa contributes to more than one quarter of all reported TB cases in the world (Lawn & Churchyard, 2009). Kanabus (2016) indicates that the number of TB–HIV co-infected patients who died at the end of 2014 on the African continent reached a staggering 310 000, with South Africa contributing to 72 000 of those deaths.

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The HIV epidemic, as explained by Swaminathan *et al.* (2010), has led to a substantial upsurge in the incidence of TB globally, particularly in SSA, with the WHO claiming that the risk of getting TB when infected with HIV is 20-37 times higher in resource-limited settings (Hall *et al.*, 2011). Co-infection with HIV leads to complications in both the diagnosis and management of TB, however HAART significantly reduced new HIV/AIDS-related OIs and death in co-infected patients (Jones *et al.*, 2000; Dheda *et al.*, 2004), mainly due to the strong bifacial interaction between HIV and the acceleration of TB (Hall *et al.*, 2011). In 2010, based on what is known about TB-HIV co-infection, Harries *et al.* (2010) argued for a more forceful approach to the early diagnosis of HIV and earlier initiation of HAART in communities stongly affected by the epidemic, resulting in both short and long-term regression in TB cases with regard to immune reconstitution of affected individuals and decreased transmission of HIV.

Tuberculosis treatment is therefore frequently initiated in HIV-infected patients already started on ART (McIlleron *et al.*, 2007). However, intricate pharmacokinetic drug-drug interactions between the major antituberculer drugs, rifamycins and two general ARV drug classes, PIs and NNRTIs, lead to decreased plasma concentrations of ARVs, influencing efficacy and opening the possibility to resistant mutations to develop (Dumon *et al.*, 2000; CDC, 2002). These interactions are of the utmost importance as NNRTIs form an essential part of initial ART regimens in countries heavily burdened by HIV. Plasma concentrations of some ARVs are severely reduced due to CYP2B6 and CYP3A4 induction by rifamycins, especially rifampin (WHO, 2004). Gulick *et al.* (2004) and Losso *et al.* (2004) reported that although ARV regimens comprising triple NRTIs do not significantly interact with rifamycins, they are associated with substandard VL suppression and are contraindicated. More specifically, slight reductions in AZT levels occur, though individual patient variation may influence the clinical significance of this interaction (Burger *et al.*, 1993; CDC, 2002).

### 2.8.4 Serum creatinine and creatinine clearance in HIV patients

Creatinine has been found to be a fairly reliable indicator of kidney function. An elevated creatinine level signifies impaired kidney function. Creatinine clearance (CrCl, in millilitre/minute) is determined after analysing serum creatinine (SCr) in samples (Stevens *et al.*, 2007). Although many formulas exist for determining CrCl, the NDoH uses an adapted Cockcroft-Gault formula, adjusting for gender (NDoH, 2014). South African guidelines also stipulate that TDF should be avoided in adult patients with a CrCl of < 50 ml/min as indicated by the eGFR provided by laboratory results (NDoH, 2014). According to Peters *et al.* (2008), renal dysfunction manifests as a severe complication of advanced HIV disease and chronic anaemia may also contribute to diminished renal function in susceptible patients.

### 2.9 Synopsis

HIV and the burden it places on South Africa has forced a task-shifting approach to expand access to ART in healthcare settings with poor resources (Sanne *et al.*, 2010). Although South Africa has shown tremendous progress in ART roll-out (Nyasulu *et al.*, 2013), the introduction and follow-up of patients on ART is severely inhibited by the limited number of doctors (Colvin *et al.*, 2010) in the public sector.

Available evidence shows that NIMART is not only accessible, but also effective and sustainable in assisting the country to lessen the burden on health care systems and achieving future therapy goals (AVERT, 2016). Despite the negative influence of poor resources and limited healthcare professionals, toxicity of ART increases the need for more intense patient monitoring. Zidovudine, although primarily used as second line therapy, is often still part of first line regimens (NDoH, 2014). Meidani *et al.* (2012) clearly states that anaemia is not only a clinical manifestation of HIV itself, but the use of AZT-based regimens also increases the risk for developing the haematological disorders, making the monitoring of Hb and MCV levels in patients on AZT-based regimens crucial.

This study aimed to compare the clinical health outcomes of two treatment approaches, NIMART and physician-managed ART. This was accomplished by comparing major disease progression markers, diagnostic haematological parameters and possible associated risk factors in adults on AZT-based ART in a hospital clinic and NIMART cohort..

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CDC **see** Centre for disease control

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WHO **see** World Health Organization

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## CHAPTER 3: RESEARCH METHODOLOGY

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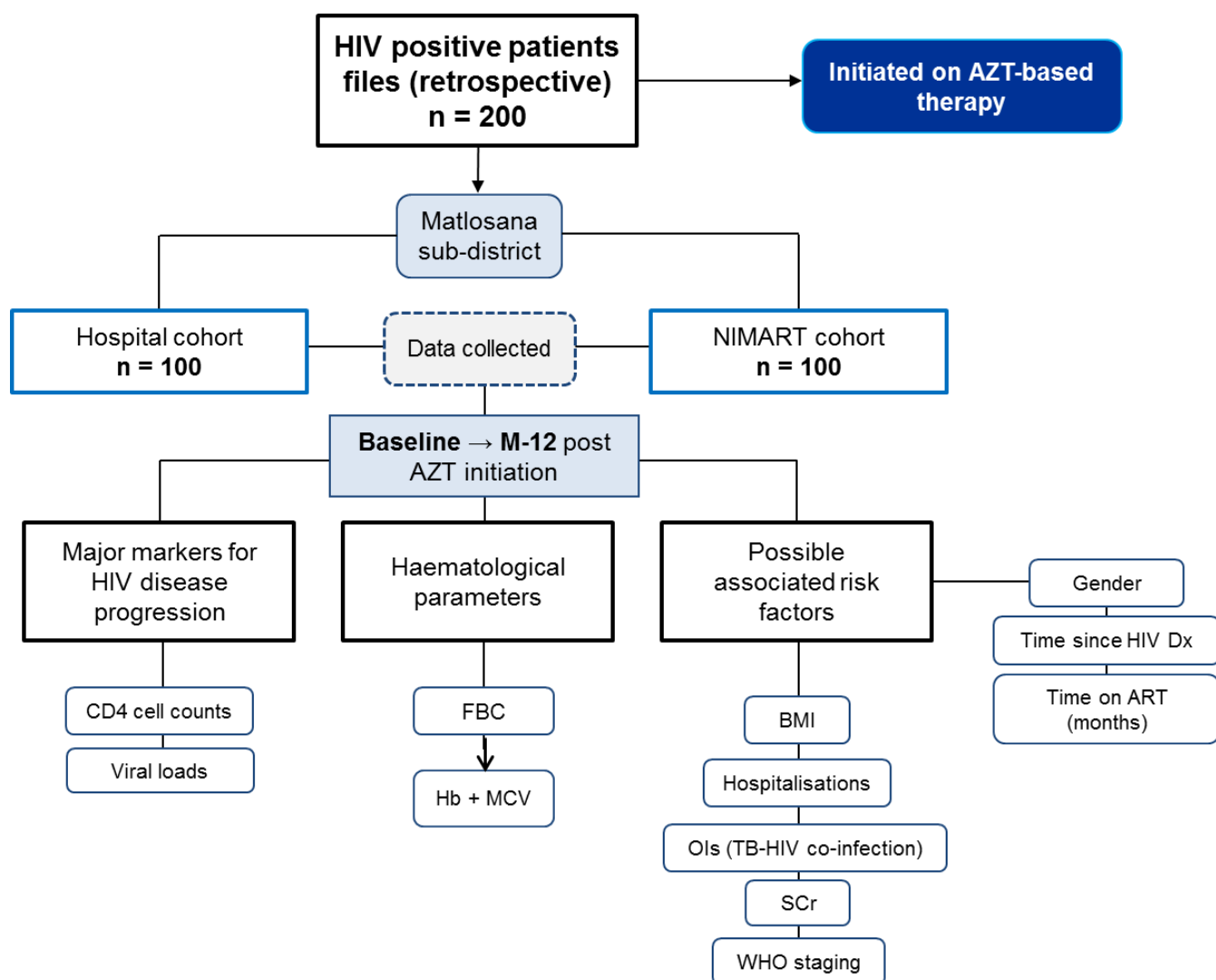
### 3.1 Introduction

This chapter addresses the study design, ethics approval, study population, inclusion and exclusion criteria, random sampling of patients, the process of data collection and the statistical methods.

### 3.2 Study design

The study design is schematically depicted in Figure 3-1. This study was a descriptive (occurrence of outcome) retrospective investigation of a hospital clinic and NIMART cohort of adult HIV-infected patients respectively that compared:

- major clinical markers (CD4 cell counts and VLs) of disease progression to assess the efficacy of patient health outcomes;
- the haematological parameters (Hb and MCV) of AZT-based ART which is an indication of the tolerability of the treatment and
- associated risk factors that may be indicative of the rate of disease progression, i.e. BMI, hospital admissions, OIs (especially TB), SCr and WHO staging.



**Figure 3-4:** Retrospective study design in sub-district of Matlosana City for adult patients 12 months post AZT-based regimen initiation. **HIV**, human immunodeficiency virus; **AZT**, zidovudine; **ART**, antiretroviral therapy; **NIMART**, nurse-initiated management of ART; **FBC**, full blood count; **Hb**, haemoglobin; **MCV**, mean corpuscular volume; **OIs**, opportunistic infections; **BMI**, body mass index; **SCr**, serum creatinine; **WHO**, World Health Organization; **TB**, tuberculosis; **Dx**, diagnosis; **M-12**, month 12.

All these variables were incorporated to investigate treatment outcomes (efficacy and tolerability over time on disease progression) of HIV-infected patients receiving AZT-based regimens between the cohorts (NIMART vs hospital clinic level (outpatient based)) in the City of Matlosana sub-district in the DKKD. Among the advantages of this retrospective study was the ability to produce results from carefully, although sometimes limited, collected data. This retrospective

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study design was also chosen as it did not entail any physical contact with patients. The study was population-based with regard to HIV-infected cases.

### 3.2.1 Ethics approval

- Approval for this study (data at baseline, month 1, 3 and 6) was obtained from the Human Research Ethics Committee (HREC) of the North-West University (HREC), Potchefstroom (NWU 00362-15-S1) on 4 April 2016. The HREC approved an amendment to extend the collection of data to month 12 after AZT initiation on 16 May 2016.
- The Human Research Ethics Committee (Medical) of the University of the Witwatersrand also provided unconditional approval on 30 March 2016 as two of the collaborators (Dr N Martinson and Prof E Variava) are affiliated to Wits and the study sites are also affiliated to the University of Witwatersrand, Johannesburg.
- The North West Department of Health (Policy, Planning, Research, Monitoring and Evaluation – PPRM&E), Mahikeng, provided permission on 11 February 2016 to continue with this investigation and to access study participants' related health information from their public healthcare records.
- Permission from the CEO of the Klerksdorp-Tshepong Tertiary Hospital (Mr P.E. Mokatsane) and the acting PHC manager in the Matlosana sub-district (Me C Lebeko) was obtained on 19 February 2016 and 23 March 2016 respectively (*refer to all the relevant approval and permission letters enclosed under ADDENDUM A*).

### 3.2.2 Study sites

The City of Matlosana municipality includes thirteen PHCs, four community health centres (CHCs), five mobile clinics and one regional hospital complex (Tshepong Hospital and Klerksdorp Hospital) adding up to 23 health facilities (NDoH, 2012). The main study sites (all accredited ART initiation and on-going treatment sites) were based on their large patient numbers already on ART which included:

- Tshepong Hospital
- Stilfontein PHC clinic
- The two largest clinics in the Matlosana sub-district;
  - Jouberton CHC

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- Park Street Clinic

### 3.2.2.1 Justification for chosen study sites

The following arguments were used during the selection of the respective study sites:

- Before 2010, ART was initiated by physicians only at hospitals or in hospital clinics and AZT formed part of first and second line ART.
- Since beginning 2011, according to South African guidelines (Long *et al.*, 2011), first line ART regimens were also slowly being initiated at clinic level by registered nurses if they had the required training.
- Since beginning 2011, NIMART in South Africa allowed patients to be switched from first line to AZT-based second line therapy at primary healthcare clinics and community health centres if virological failure was detected on first line therapy. These clinics are primarily managed by registered nurses.
- Patients are only referred back to hospital level in case of severe complications or when adjustments are needed when failing on second line based regimens at clinic level.
- The researcher was required to travel to the Tshepong Hospital and the clinics in this large, but topographical similar area to gather data for the study. It was also assumed that by choosing the two largest clinics in Matlosana, the largest number of adult patients on AZT-based regimens would be found at these two clinics.
- Collaborating experts, Dr E Variava (Head of Internal Medicine, Klerksdorp Tshepong Hospital Complex, North West Department of Health) and Dr N Martinson (PHRU, University of the Witwatersrand Johannesburg) are working and conducting research in the City of Matlosana sub-district.
- It was not possible in the limited time frame (January 2016 - July 2016) to investigate all of the available sites in all four sub-districts in the DKKD.
- The City of Matlosana municipality was convenient since it is nearby and this significantly decreased the travelling distance. The NWU Potchefstroom Campus is situated in the Tlokwe sub-district and travelling was relatively cost-effective.

### 3.2.3 Study population, sample and effect size

The overall sample size was determined using a G\* power approach, i.e.

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$$n = \left[ \frac{z \left( \frac{\alpha}{2} \right) \sigma}{M} \right]$$

Where:

- $n$  = overall sample size
- $M$  = power
- $\sigma$  = population standard deviation
- $z \left( \frac{\alpha}{2} \right)$  = quantile values under the standard normal distribution.

Effect size ( $d$ ) is the magnitude of the differences between groups and indicates substantive significance (Sullivan & Feinn, 2012). Effect size estimation is crucial in the understanding and explanation of study results (Ellis, 2010). The following standard interpretation of effect size was used:

- 0.8 = large (80% of a standard deviation unit)
- 0.5 = moderate (50% of a standard deviation unit)
- 0.2 = small (20% of a standard deviation unit)

The mean (SD) Hb values of  $12 \pm 2$  g/dL in HIV-infected adult patients on ARVs from a large ( $n=322$ ) South African study was incorporated (Takuva *et al.*, 2013) to calculate the above effect size and study size justification, unfortunately this study did not record MCV values.

**Table 3-1:** Sample size determination with t- tests using means (difference between two independent variables / groups) for different effect sizes ( $d$ ).

	Effect size ( $d$ )	$d = 0.2$	$d = 0.25$	$d = 0.3$	$d = 0.35$	$d = 0.4$
#	Power ( $1 - \beta$ err prob)	Total sample size				
1	0.800	619.613	397.043	276.143	203.246	155.953
2	0.900	577.411	549.444	381.976	280.999	215.463

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A *priori* power analysis was conducted using the software package G\*Power. According to the sample size determination, a sample size of between 78 and 108 (per cohort) was sufficient to detect an effect size between 0.35 and 0.4 (see highlighted area in Table 3-1) with a power of 80% and an alpha of 0.05. The final sample size depended on the availability and completeness of the patient files (as per figure study design) and was determined at **n = 100** HIV-infected adults (patient files) on AZT-based ART **per cohort**.

### 3.2.4 General inclusion and exclusion criteria

Patient files were assessed by the following inclusion and exclusion criteria to select n = 100 files for patients initiated on ART in a hospital clinic (outpatient based) and NIMART cohort respectively. The general criteria included all adult HIV-positive patients on an AZT-based regimen at Tshepong Hospital, Stilfontein PHC, Jouberton CHC and Park Street Clinic in the Matlosana sub-district.

#### 3.2.4.1 Inclusion criteria

- Adult patients initiated on AZT-containing ART regimens at the Tshepong hospital (outpatients);
- Adult patients receiving AZT-containing ART through the NIMART initiative at Stilfontein PHC, Jouberton CHC **and** Park Street Clinic in the Matlosana sub-district;
- Similar WHO staging for both cohorts at baseline (AZT-based initiation).

Patients included only those who were initiated on AZT-based regimens within the following time frame:

- January 2011 – October 2015 for hospital clinic patients;
- January 2013 – October 2015 for NIMART patients.

The time period allocated for hospital clinic and NIMART patients differ due to revised guidelines only authorising NIMART from April 2010 and the implementation of NIMART from beginning 2011. By January 2013, the NIMART system was sure to have been successfully implemented in all clinic settings.

#### 3.2.4.2 Exclusion criteria

- Adult HIV-patients initiated on non-AZT based ART regimens;
- HIV-positive children under 18 years;

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- Study subjects initiated on ART at hospital clinic level that were referred down to clinic level (stabilised) for the continuation of ARV therapy;
- Study subjects initiated on ART at clinic level via NIMART and then referred to hospital level for the continuation of ARV therapy;
- Pregnant women, as they are more prone to experience certain degrees of anaemia during pregnancy;
- Incomplete patient files with regard to crucial variables such as Hb, MCV, CD4 count and VLs available at baseline and 12 months after AZT-based initiation.

### 3.3 Data collection for parameters

#### 3.3.1 Random sampling of patient files with Rx Solutions software

The Rx Solutions software system from Management Sciences for Health (MSH) was developed to improve the efficiency with which HIV treatment and ART services are monitored in order to capture the minimum data components and subsequent indicators for effective patient care. Reports on HIV and ART data are generated on a monthly and quarterly basis and quick and easy selective searches can be made on this system. The system is operational in most healthcare settings providing ART, with patient numbers of a 100 and more.

An independent person employed by NWDoH (responsible pharmacist) was appointed by the manager of the hospital pharmacy at Tshepong Hospital to access the patient database to draw a list of all possible adult patients on AZT-based ART within the respective research sites. The responsible pharmacist based at the Wellness Clinic at the Tshepong Hospital supervise all the clinics in the Matlosana City sub-district. The set inclusion criteria were used and there was special focus on patients who had already completed at least fifteen months of AZT-based ART on the patient database to ensure that clinical and laboratory results at twelve months post AZT-based ART should already be available in patient files or on the electronic database of National Health Laboratory Service (NHLS) at the time of this investigation. From this selected list, depending on the number of patients, every third patient on the list was selected (an initial patient number was randomly generated by using the RANDBETWEEN built-in function in Microsoft Office Excel 2010). Relevant patient files were assessed to establish if information in the respective files, with regard to crucial variables, were complete. The actual retrieving of patient files from the hospital or respective clinics was handled according to the standard operating procedure within each of these facilities in question. There was no *carte blanche* access to any of the patient files at any of the respective study sites. A list of patient names with their dates of birth, was provided to the administration clerk (as per the facility protocol), who gave the

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researcher access to the patient health files in question to gather the retrospective data needed. All files in the hospital clinic cohort were returned to the administration clerk (as per hospital protocol) to be re-filed. With regard to the clinic cohorts, files were returned to the data capturer (as per clinic protocol) to be re-filed. If a file was found to be lacking with regard to research variables, the fourth name on the list was chosen and checked against the inclusion and exclusion criteria. All data were collected onto a case report form (CRF) (see ADDENDUM D) on site at either the hospital or PHC and transferred electronically to a Microsoft Office Excel spreadsheet after data collection on the hard copies had been completed. No patient files were taken from Tshepong hospital or any of the PHCs in question.

### 3.3.2 Baseline to month 12 clinical data collection

The following information was recorded for each patient from the selected patient files for the first 12 months of ART (in the hospital clinic and NIMART cohort) onto a retrospective CRF (see section 3.3.2):

- Demographic data (age, gender, HIV diagnosis date and duration of ART)
- CD4 cell counts
- VLs
- FBC (specifically Hb and MCV)
- Weight and length to calculate BMI
- Medication history, hospitalisation and OIs (HIV/TB co-infection),
- WHO clinical staging where available
- SCr used to calculate CrCl where available

The SCr value as taken from the laboratory results or from the patient files was used to calculate the creatinine clearance (CrCl) according to guidelines stipulated by NDoH (2014) as adapted from the Cockcroft-Gault equation **for women** as follows:

$$\frac{(140 - \text{age}) \times \text{weight in kg} \times 1.04}{\text{pCr in uMol/ml}}$$

**and men,**

$$\frac{(140 - \text{age}) \times \text{weight in kg} \times 1.23}{\text{pCr in uMol/ml}}$$

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where kg is the weight of the patient in kilograms and pCr is plasma / serum creatinine in micromole (uMol) per ml.

Due to the limited and incomplete nature of the data related to WHO staging, SCr, hospitalisation and OIs observed during the data collection process, data on these variables were only reported as descriptive statistics. In case of CrCl, either SCr, weight or length were missing resulting in the inability to calculate CrCl although it was compulsory to be recorded on each patient visit. The use of SCr was not intended to monitor renal function due to AZT-based regimen as is stated in the guideline to follow for TDF-based regimens. HIV itself and anaemia can impact on renal function and was therefore initially included in the study proposal.

### 3.3.3 Data collection tool: Case report form

Data were transferred from the CRF to a Microsoft Office Excel spreadsheet to ensure hard and electronic copies of recorded data. No personal information (identification numbers; addresses or contact numbers) was recorded for this study and patient anonymity was ensured throughout the entire data collection process by excluding all personal identifiers. A unique number was allocated to each patient that was linked to the same identical number on the CRFs and Microsoft Office Excel spreadsheet to ensure that no duplication of patient data occurred that may have been referred down from hospital level for the continuation of ART at clinic level (see exclusion criteria, section 3.2.4.2). After the possibility of duplicating patient data had been eliminated, the unique number allocated to each patient was removed from all data sheets. Data collection first commenced at Tshepong Hospital (hospital clinic cohort) from April–May and concluded at the mentioned clinics (NIMART cohort) during June–July 2016.

## 3.4 Statistical analysis

For the purpose of this dissertation, statistical analysis was done with IBM SPSS statistics (version 23) and graphical illustration with GraphPad Prism 6.

### 3.4.1 Descriptive statistics

The following descriptive statistics was used in the analysis of all variables during the study:

- Frequencies to indicate the occurrence of a value represented in the data, either reported as percentages or in absolute numbers as categorical data.
- Sample mean that indicates the average of all observations made (Sarantakos, 2012);

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- Standard deviation (SD) that calculates the variability of the population from which the sample was drawn. For data with a normal distribution, about 95% of individuals will have values within two standard deviations of the mean, the other 5% being equally scattered above and below these limits (Altman & Bland, 2005).
- The median is the number separating the higher half of a data sample or population from the lower half, while also giving a measure that is more robust in the presence of outlier values than is the mean (Sheskin, 2004).
- The interquartile range (IQR) measures variability based on dividing a data set into four quartiles. Quartiles divide a rank-ordered data set into four equal parts (Sheskin, 2004). The 25<sup>th</sup> and 75<sup>th</sup> quartile was reflected in the results section.

### 3.4.2 Inferential statistics

#### 3.4.2.1 Data distribution

Shapiro-Wilk's test for normality was used to determine if data were normally or abnormally distributed. Data distribution influences any further tests that have to be performed on study data. The null hypothesis of this test assumes normality and  $p < 0.05$  will reject the hypothesis, indicating abnormal distribution in the population. Normally, distributed data are further analysed by parametric tests and abnormally distributed data with non-parametric tests.

#### 3.4.2.2 Parametric tests

Parametric tests accept that collected data from a certain study population follow a probability distribution (Geisser and Johnson, 2006). Therefore, parametric tests are primarily used for underlying source populations with normally distributed data and include the independent (two-sample)  $t$  – test that tests if the null hypothesis of two unrelated groups have the same mean (Dawson et al., 2004) (also see section 3.4.2.4).

##### 3.4.2.2.1 *Assumption of homogeneity of variance*

The independent  $t$  – test assumes that the **variances** in two unrelated groups are equal and Levene's test for equality of variances tests the assumption of homogeneity, or rather the hypothesis that the average deviation is the same in two groups (Dawson et al., 2004) when the independent  $t$  – test is performed (Laerd Statistics, 2016a). This test provides an  $F$  – statistic and a significance value ( $p$  – value) that is seen as equal group variances when  $p > 0.05$ . If  $p < 0.05$ , equal variances are not assumed.

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### 3.4.2.3 Non-parametric tests

Non-parametric tests make no assumptions about the distribution of data from the study population (Dawson et al., 2004) and is used in general when data are not normally distributed.

#### 3.4.2.3.1 *The Mann-Whitney U test*

The Mann-Whitney U test is performed to compare differences between two unrelated groups when the dependent variable is constant, but not normally distributed (Laerd Statistics, 2016c, Dawson et al., 2004). This test allows different conclusions to be drawn from data depending on the assumptions made about the data's distribution (Laerd Statistics, 2016b, Long et al., 2011).

### 3.4.2.4 Linear mixed models: Analysis of covariance (ANCOVA)

Repeated measures refer to data with multiple observations taken over a period of time on the same sampling unit. Analysis of covariance (ANCOVA) statistically controls or removes the (linear) effect of those variables one does not want to examine in a study. These extraneous variables are called covariates, or control variables. It is a general linear model that blends ANOVA and regression (Littell *et al.*, 2000). Variables were accounted for by means of statistical analysis (more specifically ANCOVA) after the data gathering process,

ANCOVA both assumes:

- i. that a linear relationship exists within the data; and
- ii. the homogeneity of regression slopes (regression lines for the two individual groups are parallel) or parallelism (Horn, 2008).

Statistical linear mixed models declare that data that are observed usually consist of two parts; fixed (defining expected values from observations, specifically time and treatment) and random (defining variance and covariance of observations) effects. Random effects occur with variation between and within subjects (Littell *et al.*, 2000). For this study, linear mixed models tested for significance (significance level  $p < 0.05$ ) with regard to:

1. Time\*cohort interaction effect – tests if cohorts changed similarly over time
2. Cohort – meaningful difference between the cohorts
3. Time – meaningful difference between the cohorts over time

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### 3.4.2.4.1 Covariance matrix

Five covariance structures were compared: Identity, compound symmetry, autoregressive order 1 (AR-1), diagonal and unstructured (US); while the covariance structure that yielded the lowest Akaike's Information Criterion (AIC) for each variable was used in the model. The AR -1 covariance matrix was used for Hb, CD4 and BMI, while the US covariance matrix was used for MCV.

### 3.4.2.4.2 Post-hoc test: Bonferroni method

The Bonferroni method is used to compare means within analysis of variance (Dawson et al., 2004) and is considered to have a significant advantage over Tukey's method as it does not require equal sample sizes in each treatment group (Rice, 2006). This test is an indication of precisely where differences in the means of two independent groups occurred and Bonferroni post-hoc tests were subsequently used to determine if significant differences occurred on **specific time points** (baseline, month 6 and 12) between the two study cohorts within linear mixed models for CD4 cell counts, BMI, Hb and MCV.

*Statistics were based on all cases with valid data for all variables in the model. Therefore, only subjects on AZT therapy with repeated data at baseline, month 6 and 12 were included in the model.*

### 3.4.3 Kaplan-Meier Survival Analysis – Time to an event as an endpoint

Time-to-event analysis (also known to be a non-parametric test), as described by Lang and Secic (1997), estimates the possibility that an event will transpire at different time points, recording the period between the starting time and a certain event in the study. The Kaplan-Meier survival analysis gives meticulous survival proportions due to the fact that it uses exact survival times (Dawson et al., 2004), also incorporating the percentage of each patient reaching the "event" at the end for each of the specified time periods (Lang and Secic, 1997). Survival curves also indicate censored data or censored patients, assuming that all study subjects who exit during an interval (or specified time period) are known to be event free (Lachin, 2009). Primarily, these subjects are removed from the analysis not knowing what their outcomes were. Dawson et al. (2004) state that as the time from entry into the study increases, the number of patients (remaining in the study) decreases, indicating that the probability of reaching or experiencing a certain pre-defined event becomes increasingly less probable. In this research study, **undetectable (LDL) VL** was defined as the event, with study intervals indicated in months over a period of 12 months

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**(time to event)**. This is justified by the fact that the study only investigated diagnostic and treatment progressing markers for the first 12 months after AZT initiation.

Data from patients that had not yet achieved undetectable VLs at the time when data collection came to an end (month 12), were included as censored data at study end. Tests of equality of survival distributions for the different levels of treatment cohorts or overall comparison for factor levels (statistical significant difference between the hospital clinic and NIMART cohort) was determined by the Log rank (Mantel-Cox) test, Breslow (Generalised Wilcoxon) test and the Tarone-Ware test.

### 3.4.4 Statistical significance, practical significance and the relevance to effect size

Statistical significance “indicates the probability of finding a relationship in the sample when there is none in the population.” The term “significant” is usually assumed to imply that a so-called null hypothesis, affirming that there is no difference between the means, is rejected at a predetermined level of significance (usually 5%). Ultimately, this indicates that the so-called p-value is  $\leq 0.05$ . This significance shows that the probability of the null hypothesis being incorrectly rejected is small (for example  $\leq 0.05$ ). Practical significance is concerned with whether the result is useful or has an effect in a practical setting or in the “real world” and is contingent on the size of the effect (Ellis, 2010). Significant differences (rejecting the null hypothesis) mean that differences in group means are not likely due to a sampling error (Kirk, 1996) and is a contingent from the precision of the estimate of the effect size (Ellis, 2010). For the independent t-test the test statistic is:

$$T^* = \sqrt{n} \cdot \frac{\bar{X} - \mu}{S},$$

Where,

- $n$  is the sample size
- $X$  is the sample mean
- $\mu$  is the population mean
- $s$  is the sample standard deviation
- $\bar{X}$  is the unbiased estimator for  $\mu$

Practical significance implies that in above formula, the size of  $n$  influences whether the null hypothesis,  $H_0$  is rejected or not.

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$$H_0: \mu = \mu_0 \text{ vs } H_A: \mu \neq \mu_0$$

Null hypothesis for the two sample (independent)  $t$  –test is that the population means from two unrelated groups (hospital clinic and NIMART patients) are equal, but the aim is to reject the null hypothesis and accept that the two groups are not equal (Rice, 2006). This test was used to compare the Hospital and NIMART cohort to possibly determine crucial differences between the cohorts in terms of patient monitoring.

*P-values and Cohen's  $d$  values (practical significance) were interpreted accordingly for results in this study. Only medium and large practical significance were reported (small,  $0.2 \leq d < 0.5$ ; medium,  $0.5 \leq d < 0.8$ ; large,  $0.8 \leq d < 1.3$ ; very large,  $d \geq 1.3$ ).*

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# CHAPTER 4: RESULTS

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## 4.1 Introduction

The main purpose of this study was to investigate if any potential differences in disease and treatment markers, between this NIMART and hospital clinic cohorts on AZT-based regimen could be identified. This study entailed the retrospective recording of demographic data, disease progression markers (CD4 cell counts and VL), haematological parameters (Hb and MCV) and possible associated factors of disease progression (BMI, hospital admissions and OIs) of adult HIV-infected (n = 200) patients during the first 12 months post AZT-based regimen initiation from January 2011 to October 2015 for the hospital clinic and January 2013 to October 2015 for the NIMART cohort.

This chapter reports the results from the primary (1.3.1) and secondary (1.3.2) objectives as well as some of the primary outcomes (1.3.3) as listed in Chapter 1.

Primarily, descriptive statistics was used to describe categorical and continuous variables. Unfortunately, limited and incomplete data with regard to SrCr (and thus CrCl) and WHO staging data were reported in both cohorts and these variables were excluded in the final linear mixed model analysis.

Inferential statistics such as two-tailed parametric tests (independent  $t$ -test and Levene's test for equality of variances) and non-parametric tests (Mann-Whitney U) were used to compare differences in the means of male vs female within both cohorts respectively and in the hospital clinic vs NIMART cohorts for each variable on all the specific time points (repeated data). However, these parametric and non-parametric tests were unable to adjust for confounding variables.

Thus, linear mixed models with an autoregressive order 1 (AR-1) (for Hb, CD4 & BMI) and an US co-variance matrix (for MCV) was implemented to correct for three different co-variants (**age, duration since HIV positive diagnosis & total duration on ART**) between the two independent cohorts. For VL data, the fit of the linear mixed model was unsatisfactory and non-parametric test was reported instead. Kaplan-Meier survival analyses were also performed to determine the proportion of HIV patients achieving undetectable (below level of detection) VL over time (time to event).

### 4.2 Results

#### 4.2.1 Demographic results of the cohorts at baseline and over time

In Table 4-1 the demographics of the cohorts are presented and it includes categorical and continuous variables describing the study population.

In both cohorts, females comprised the largest proportion of patients (59% and 69% for the hospital clinic and NIMART cohort respectively). Only  $n = 6$  patients from the hospital cohort and  $n = 2$  patients from the NIMART cohort presented with TB-HIV co-infection. Patients receiving second line AZT therapy (HOSP:  $n = 94$ , NIMART:  $n = 56$ ) far outweighed those initiated on first line AZT (HOSP:  $n = 6$ , NIMART:  $n = 44$ ) in both study cohorts, although considerably more NIMART patients received AZT as first line when compared to hospital clinic patients. The WHO staging at baseline for the hospital and the NIMART cohort are presented and this staging is not altered even after years on treatment. The WHO staging data in the hospital cohort was not very complete and 65% of the data was not available compared to 16% for the NIMART cohort (Table 4-1).

The hospital clinic cohort had significantly more opportunistic infections (OIs) (2.75 vs 1.24,  $p < 0.0001$ ) and hospital admissions (1.64 vs 1.13,  $p = 0.1227$ ) on average but also more OIs (2.20 vs 0.31) and hospital admissions (0.59 vs 0.09) *per patient* than the NIMART cohort.

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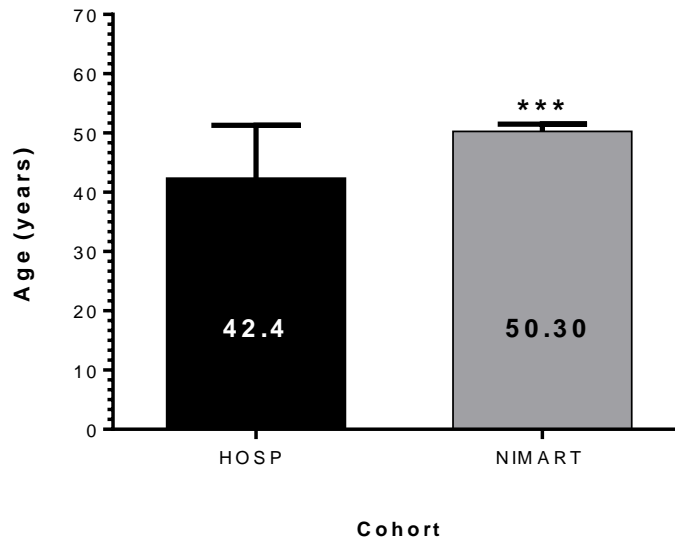
**Table 4-1:** Patient demographics for the hospital clinic and NIMART cohorts respectively.

Variable	Hospital Cohort (n = 100)				NIMART Cohort (n = 100)			
<b>Categorical variables (frequencies)</b>								
<b>Gender</b>	Male = 41		Female = 59		Male = 31		Female = 69	
<b>Clinical WHO staging (at baseline)</b>	Stage I	Stage II	Stage III	Stage IV	Stage I	Stage II	Stage III	Stage IV
	5	8	20	2	31	36	15	2
	Total (n) (%) reported = 35				Total (n) (%) reported = 84			
<b>TB-HIV co-infected (while on AZT therapy)</b>	<b>Yes = 6</b>		<b>No = 94</b>		<b>Yes = 2</b>		<b>No = 98</b>	
<b>n of patients receiving 1<sup>st</sup> line AZT</b>	6				44			
<b>n of patients receiving 2<sup>nd</sup> line AZT</b>	94				56			
<b>n of deaths <i>per cohort</i></b>	1				1			
<b>n of OIs; mean; OIs <i>per patient</i> *</b>	220; 2.75; 2.20				31; 1.24; 0.31			
<b>n of hospital admissions; mean; admissions <i>per patient</i> *</b>	59; 1.64; 0.59				9; 1.13; 0.09			
<b>Comparative patient demographics (continuous variables)</b>								
<b>Mean (± SD)</b>	<b>Hospital Cohort</b>			<b>NIMART cohort</b>		<b>Independent <i>t</i> - test: <i>p</i>-value (HOSP vs NIMART)</b>		
Age (years) at AZT initiation	42.40 ± 8.92			50.30 ± 1.18		<0.0001		
Duration (years) of positive RVD	7.60 ± 2.72			7.75 ± 2.26		0.7546		
Total duration (years) of all ART	6.44 ± 2.31			5.34 ± 3.21		0.0067		
Duration (years) of AZT-based regimens	2.90 ± 2.88			2.50 ± 1.15		0.0235		

\*OIs and hospital admissions per patient calculated only as an overall indicator to make a comparison between the two cohorts from baseline to month 12 (total OIs or hospital admissions per cohort divided by 100 patients = per patient; mean = total number of OIs or hospital admissions per cohort divided by the actual number of patients that experienced an OI or was admitted to hospital).

## CHAPTER 4: RESULTS

Figure 4-1 illustrates the mean (SD) age at AZT initiation and Figure 4-2 the mean time since HIV infection, total time spent on ART and AZT for the hospital clinic and NIMART cohorts respectively. Unpaired t-tests indicated a significant difference ( $p < 0.0001$ ) in age for hospital clinic ( $42.40 \pm 8.92$  years) vs NIMART ( $50.30 \pm 1.18$  years) patients, where the NIMART group were on average almost eight years older.

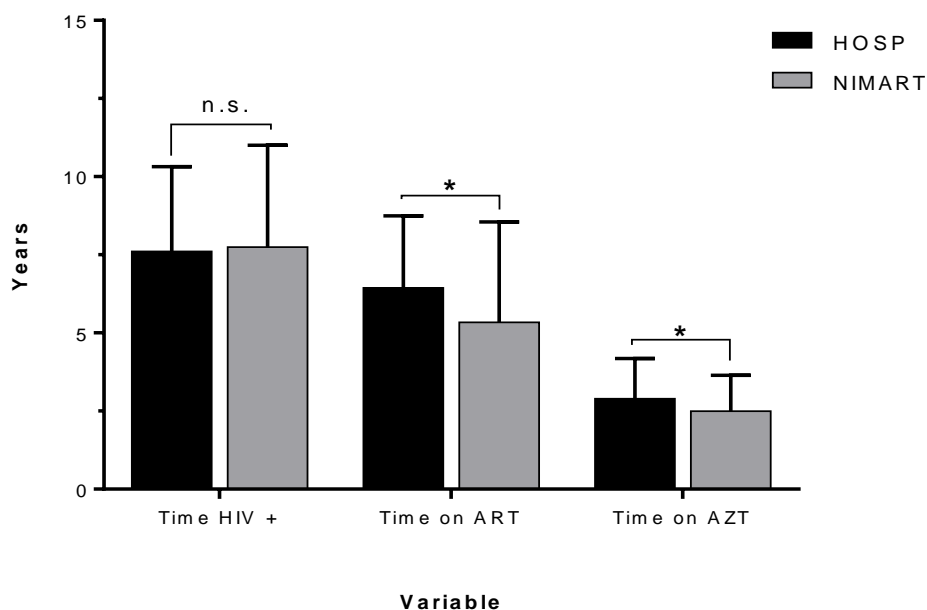


**Figure 4-1:** Mean (SD) age for the hospital clinic and NIMART cohorts when AZT-based regimen was initiated.

\*\*\*  $p < 0.0001$  ( $n = 100$  per cohort)

There was also statistical significant difference between the cohorts regarding duration on all ART ( $p = 0.0067$ ) and duration on AZT-based only regimen ( $p = 0.0235$ ). In both instances the time spent on ART regimens were shorter in the NIMART cohort. The duration of HIV infection showed no statistical significant difference ( $p = 0.7546$ ) when comparing hospital clinic ( $7.61 \pm 2.72$  years) vs NIMART ( $7.75 \pm 2.26$  years).

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**Figure 4-2:** Mean (SD) time duration since HIV diagnosis, total time on ART and AZT-based regimens.

\* $p < 0.05$  (n = 100 per cohort)

### 4.2.2 Descriptive results for clinical markers without correcting for confounding factors

Tables 4-2 and 4-3 present descriptive results for the major clinical markers used to compare these cohorts without correcting for confounding factors. Mean (SD) was used for haematological markers (Hb & MCV), risk factors associated with disease progression (BMI and CrCl), while median (IQR) was used for disease progression markers (CD4 cell counts and VLs) due to the wide individual variation in these variables for each patient. The number of viral copies in the blood portrays the degree of immune suppression and recovery. In total, n = 52 patients had VLs < 20 copies/ml at baseline (hospital clinic, n = 8; NIMART, n = 44). Twelve months after the initiation of AZT, those with VLs < 20 copies/ml increased to n = 116 (hospital clinic, n = 36; NIMART, n = 80) (Table 4-3 & Table 4-6). This is further elaborated upon in the Kaplan-Meier survival estimate under section 4.2.4.

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**Table 4-2:** Patient mean variables for hospital clinic and NIMART from baseline to 12 months post AZT-based initiation

		Continuous variables			
		Hospital Clinic Cohort		NIMART Cohort	
Variable		Mean ± SD	n (per time point)	Mean ± SD	n (per time point)
<b>Hb (g/ dL)</b>	Baseline	12.31 ± 1.98	95	12.78 ± 1.67	83
	Month 1	11.40 ± 1.82	18	11.27 ± 1.40	3
	Month 3	11.73 ± 1.96	20	13.11 ± 1.41	8
	Month 6	12.26 ± 1.93	37	13.21 ± 1.58	26
	Month 12	12.70 ± 2.10	57	13.38 ± 1.46	42
<b>MCV (fL)</b>	Baseline	93.55 ± 7.90	91	104.53 ± 14.39	74
	Month 1	100.77 ± 10.80	12	92.13 ± 9.15	3
	Month 3	101.06 ± 7.97	21	111.40 ± 6.84	6
	Month 6	99.59 ± 17.29	35	112.00 ± 12.74	22
	Month 12	100.75 ± 16.28	55	108.00 ± 18.45	41
<b>BMI (kg/ m<sup>2</sup>)</b>	Baseline	24.77 ± 7.69	79	26.46 ± 7.89	14
	Month 1	24.10 ± 6.89	28	27.01 ± 8.51	7
	Month 3	23.01 ± 5.43	49	26.34 ± 7.78	9
	Month 6	26.00 ± 7.24	43	26.21 ± 6.61	12
	Month 12	23.99 ± 6.02	45	26.37 ± 6.92	10
<b>CrCl (ml/ min)</b>	Baseline	123 ± 0.53	76	No data specified for this time period	0
	Month 1	133 ± 0.20	2	132 ± 0.39	5
	Month 3	118 ± 0.56	4	115 ± 0.31	4
	Month 6	133 ± 0.47	16	102 ± 0.39	20
	Month 12	106 ± 0.37	36	115 ± 0.30	37

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**Table 4-3:** Patient median variables for hospital clinic and NIMART from baseline to 12 months post-AZT initiation.

		Continuous variables			
		Hospital Clinic Cohort		NIMART Cohort	
Variable		Median (IQR*)	n (per time point)	Median (IQR*)	n (per time point)
<b>CD4 (cells/ <math>\mu</math>l)</b>	Baseline	173 (50; 333)	93	312 (184; 455)	93
	Month 1	152 (67; 406)	9	375 (61; 732)	5
	Month 3	228 (55; 387)	11	448 (358; 728)	10
	Month 6	287 (146; 532)	30	364 (252; 654)	40
	Month 12	286 (191; 463)	57	492 (361; 748)	60
<b>VL (copies/ ml)</b>	Baseline	58109 (11366; 245711)	94	20 (20; 3778)	82
	Month 1	106 (20; 92981)	9	27203 (20; 164355)	8
	Month 3	20 (20; 74)	15	20 (20; 20)	11
	Month 6	187 (20; 13619)	34	20 (20; 39)	44
	Month 12	175 (20; 25376)	58	20 (20; 38)	70

\*IQR, interquartile range

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### 4.2.2.1 Differences between clinical variables of the cohorts at different time points

The following data analyses were performed:

- CD4 cell counts and VLs (Mann-Whitney test) for HOSP vs NIMART at baseline, 3, 6 and 12 months post-AZT initiation;
- Hb (independent *t* -test) and MCV (Mann-Whitney test) for HOSP vs NIMART at baseline, 3,6 and 12 months post-AZT initiation
- BMI (Mann-Whitney test) for HOSP vs NIMART at baseline, 3, 6 and 12 months post-AZT initiation.

Other associated risk factors for disease progression such as WHO staging, CrCl, hospitalisation and opportunistic infections were incomplete as the data was either not reported, missing or not relevant at the different time points to be included in the inferential analyses. Some of these aspects were only reflected as part of demographic results (Table 4-1).

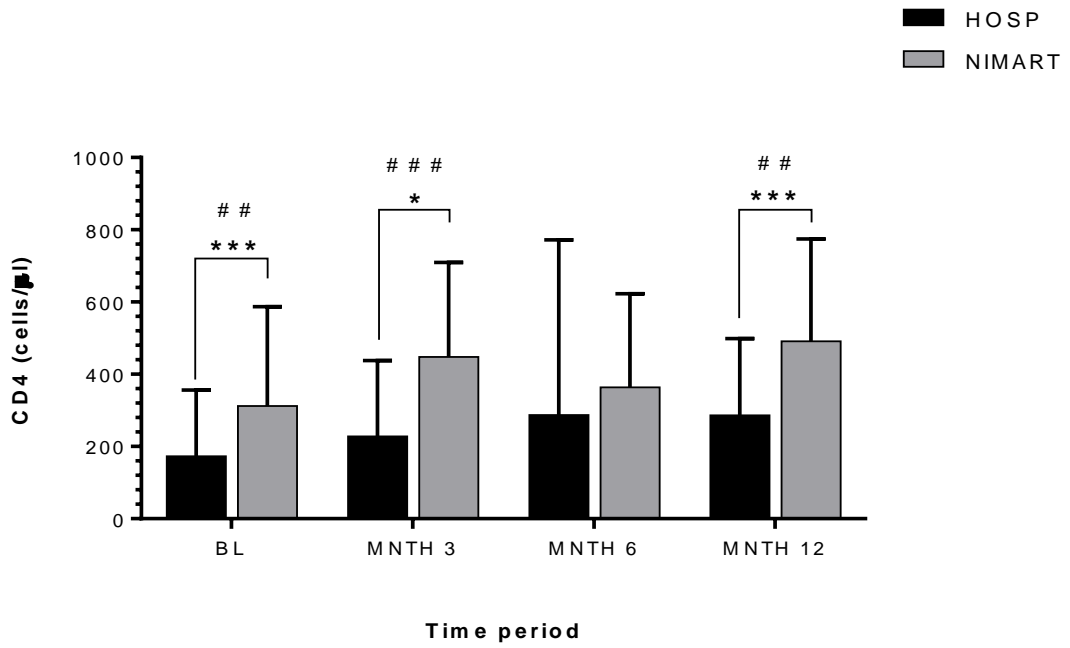
ADDENDUM E1 provides the complete summary table of differences between clinical variables of the cohorts at different time points.

The clinical markers indicative of the clinical progression of the HIV disease are presented in Figures 4-3 and 4-4. CD4 cell counts (immunological improvement) are interpreted in combination with VLs (virological suppression) to provide clinical insight into the progression of HIV disease and to monitor the efficacy of the treatment.

Median (IQR) CD4 cell counts over time are illustrated in Figure 4-3. CD4 cell counts for NIMART patients at baseline ( $p < 0.0001$ ;  $d = 0.605$ ), 3 months ( $p = 0.016$ ,  $F = 0.293$ ) and 12 months ( $p < 0.0001$ ;  $d = 0.765$ ), showed considerable significant increases and practical differences when compared to hospital clinic patients. At 3 months', significant differences ( $p = 0.016$ ,  $F = 0.293$ ) in CD4 cell counts between hospital clinic (228 cells/ $\mu$ l) and NIMART (448 cells/ $\mu$ l) groups were evident. The largest practical significance ( $d = 1.036$ ) were found at 3 months.

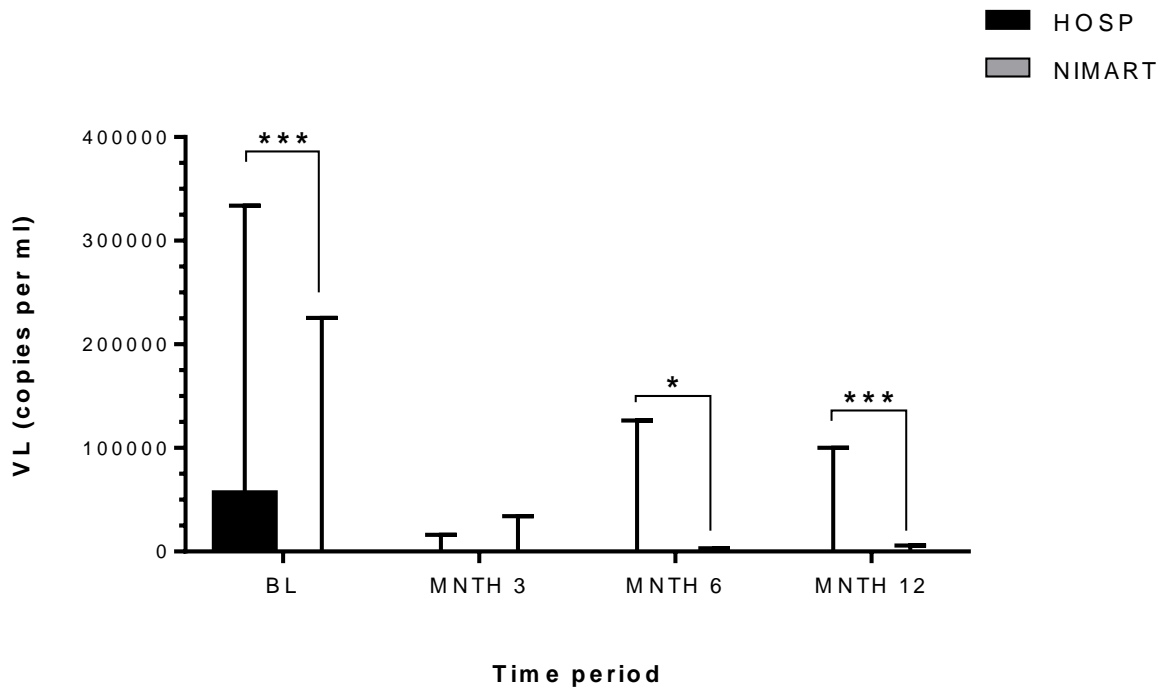
Figure 4-4 indicates a considerable decrease in median (IQR) viral load copies for both study cohorts over the first-year post-AZT initiation. Baseline ( $p < 0.0001$ ), month 6 ( $p = 0.001$ ) and 12 ( $p < 0.0001$ ) showed that VL was significantly higher in the hospital clinic cohort compared to the NIMART cohort. Overall, no practical significance was observed.

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**Figure 4-3:** Median (IQR) CD4 cell counts (cells/mm3) for the hospital clinic vs NIMART cohorts during 12 months post-AZT initiation.

\*  $p < 0.05$  \*\*\*  $p < 0.0001$  ##  $0.5 \leq d < 0.8$  ###  $0.8 \geq d < 1.3$



**Figure 4-4:** Median (IQR) VLs for hospital clinic vs NIMART cohorts during 12 months post-AZT initiation.

\*  $p < 0.05$  \*\*\*  $p < 0.0001$

## CHAPTER 4: RESULTS

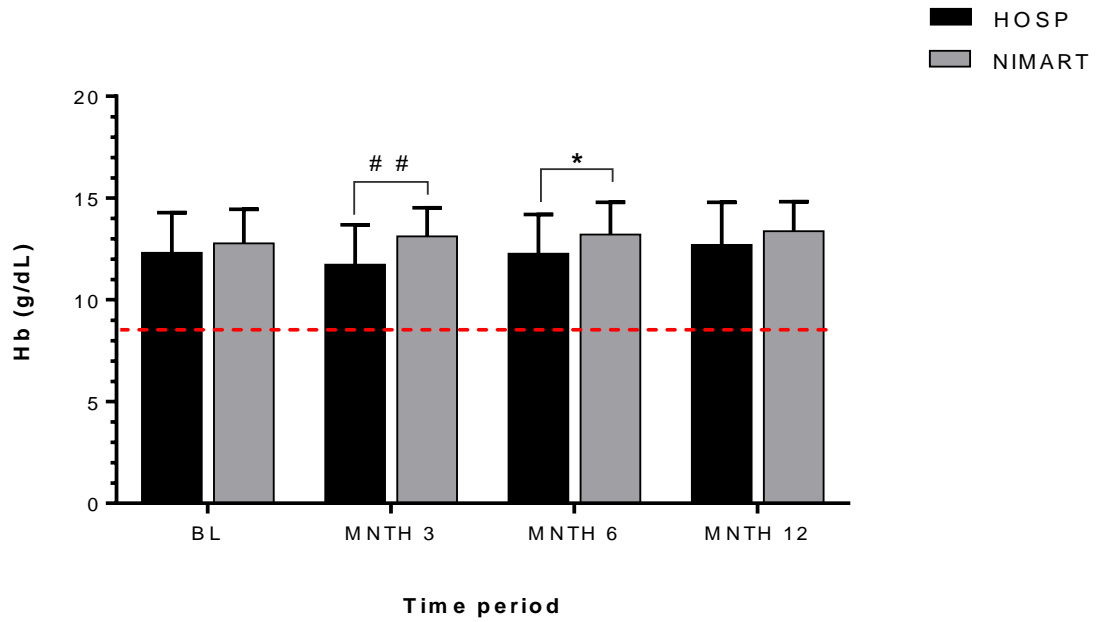
The haematological markers (Hb and MCV) are presented in Figure 4-5 and 4-6. Figure 4-5 illustrates the mean (SD) Hb values for hospital clinic vs NIMART subjects at baseline, month 3, 6 and 12 post-AZT initiation.

Overall, only month 6 showed significant difference ( $p = 0.043$ ,  $F = 1.549$ ) between the hospital clinic ( $12.26 \pm 1.93$ ) and NIMART cohorts ( $13.21 \pm 1.58$ ). Month 3 indicated medium practical significance ( $d = 0.708$ ) with  $11.73 \pm 1.96$  and  $13.11 \pm 1.41$  respectively. When compared, month 3 and 6 showed slight fluctuations in Hb while baseline and month 12 indicated a slight decrease in Hb, overall not reaching anaemic status for the first 12 months based on the mean values of the two cohorts. In total, 1 % ( $n = 1$ ) of patients in the hospital clinic cohort had a Hb level  $< 8$  g/dL, 12 months post-AZT initiation compared to zero percent in the NIMART cohort.

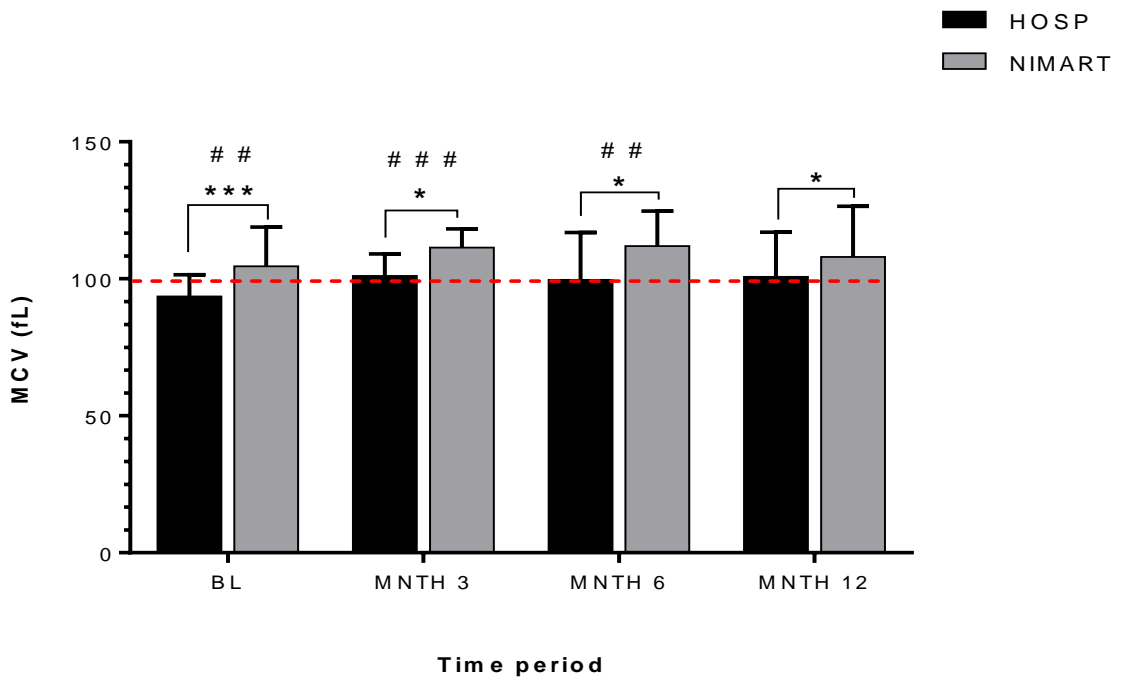
Figure 4-6 reflects mean (SD) MCV values for the hospital clinic vs NIMART cohorts over time. MCV is the most important parameter indicative of the diagnosis of macrocytosis. The mean MCV values for NIMART patients were higher compared to hospital clinic patients for all time points. Significant differences ( $p < 0.0001$ ) and medium practical significance ( $d = 0.763$ ) occurred between hospital clinic ( $93.55 \pm 7.90$ ) and NIMART ( $104.53 \pm 14.39$ ) subjects at baseline.

Furthermore, month 3 ( $p = 0.008$ ,  $F = 0.128$ ), 6 ( $p = 0.001$ ) ( $d = 0.717$ ) and 12 ( $p = 0.001$ ) also showed significant differences with large practical significance at month 3 ( $d = 1.296$ ). The mean MCV values for the NIMART cohort increased from baseline to 12 months and was always above the cut off value ( $\geq 100$  fL), indicating macrocytosis. However, the within-cohort (hospital clinic) increased from 93.55 to just above 100 at 3 months and remained constant for the 12-month period in question.

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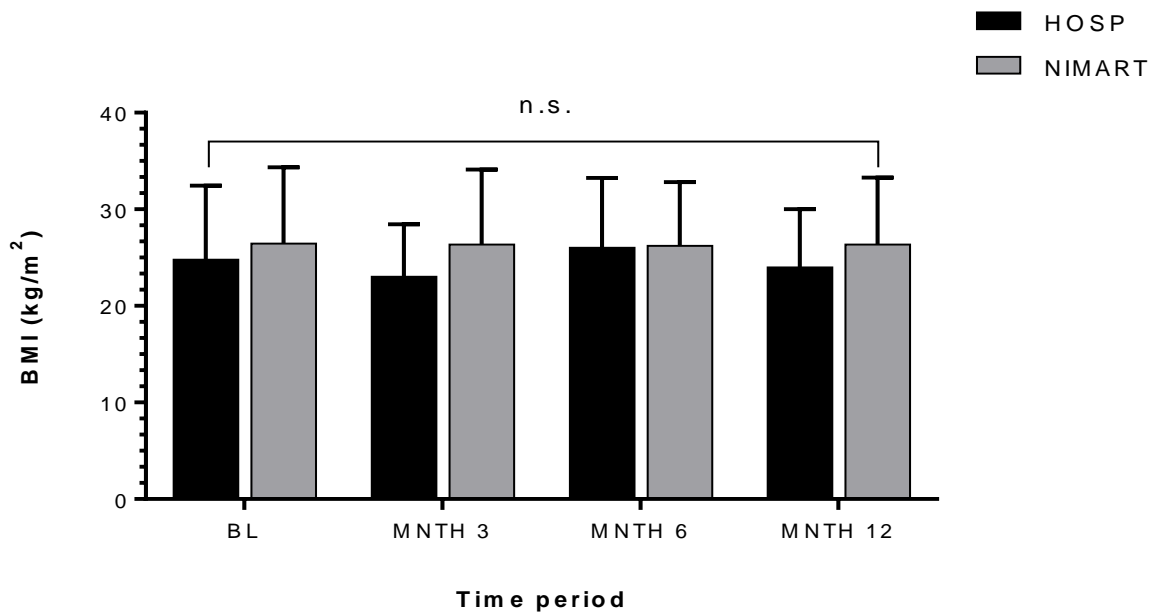


**Figure 4-5:** Mean (SD) Hb values at baseline to 12 months post-AZT initiation for hospital clinic vs NIMART cohorts. Red dotted line (----) indicates moderate to severe anaemia ( $\leq 8$  g/dL). \*  $p < 0.05$  ##  $0.5 \leq d < 0.8$



**Figure 4-6:** Mean (SD) MCV values at baseline to 12 months post-AZT initiation for hospital clinic vs NIMART cohorts. Red dotted line (----) indicates macrocytosis ( $\geq 100$  fL). \*  $p < 0.05$  \*\*\*  $p < 0.0001$  ##  $0.5 \leq d < 0.8$  ###  $0.8 \leq d < 1.3$

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**Figure 4-7:** Mean (SD) BMI for hospital clinic vs NIMART cohorts at baseline to 12 months post-AZT initiation.  
n.s., not significant ( $p > 0.05$ )

Overall, mean BMI values (Figure 4-7) showed no significant differences ( $p > 0.05$ ) between the two cohorts over the 12-month period post AZT-based regimen initiation. Hospital clinic patients showed small fluctuations between baseline ( $24.77 \pm 7.69$ ), month 3 ( $23.01 \pm 5.43$ ), 6 ( $26.00 \pm 7.24$ ) and 12 ( $23.99 \pm 6.02$ ) when compared to NIMART patients (baseline:  $26.46 \pm 7.89$ ; month 3:  $26.34 \pm 7.78$ ; month 6:  $26.21 \pm 6.61$  and month 12:  $26.37 \pm 6.92$ ) showing very little variation on average over the time period in question.

### 4.2.2.2 Gender differences within the respective cohorts at baseline and 12 months post - AZT initiation

Here follows a brief recap of how the gender differences were analysed. These results are presented in ADDENDUM E2 as gender differences within the same cohort was not part of the initial objectives set out to investigate.

The following data analyses were performed:

- CD4 cell counts and VLs (Mann-Whitney for male vs female at baseline and 12 months post-AZT initiation);
- Hb (independent  $t$ -test) and MCV (Mann-Whitney) for male vs female at baseline and 12 months post-AZT initiation;
- BMI (Mann-Whitney) for male vs female at baseline 12 months post-AZT initiation.

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Briefly, the results indicated that females had significantly higher CD4 cell counts ( $p < 0.001$ ), MCV levels ( $p < 0.05$ ) and BMI ( $p < 0.05$ ) at baseline in the **hospital clinic** cohort but significantly lower Hb levels ( $p < 0.05$ ) at baseline and month 12 post-AZT initiation in the **NIMART** cohort when compared to men.

### 4.2.3 Comparative clinical markers over time after adjusting for confounding variables

Due to the inability of parametric and non-parametric tests to adjust for age, duration since HIV diagnosis and total duration on ART regimen(s), linear mixed models with an autoregressive order-1 (AR-1) co-variance matrix for CD4, Hb and BMI and an unstructured (US) co-variance matrix for MCV, was used to investigate if fixed effects such as significant differences either occurring over time or between the cohorts were present.

Testing for significance in itself *does not* take into account factors that may bias results and thus, even though significant differences are not found for variables between two treatment groups, it is still necessary to confound for all possible biases to diminish the possibility of their impact (Skelly *et al.*, 2012).

Table 4-1 and Figure 4-2 shows no significance for time since positive HIV diagnosis but it was still included as a confounding variable. Time spent on AZT was not adjusted for as **all** patients were assessed for **the first 12 months** after they have been initiated on AZT-based therapy. The interaction effect of time\*cohort was also assessed to test if the two study groups acted similarly according to a certain trend over time. Significance level was set at  $p < 0.05$ . The fit of the model with regard to VL data did not converge and resulted in the reporting of Mann-Whitney U tests instead (Figure 4-4).

Table 4-4 provides a summary of adjusted means, 95% confidence intervals (CI) and fixed effects for CD4, Hb, MCV and BMI for baseline, 6 and 12 months as determined by linear mixed models. Month 1 and 3 were left out of the analysis due to lack of data observations and the desired fit of the mixed model.

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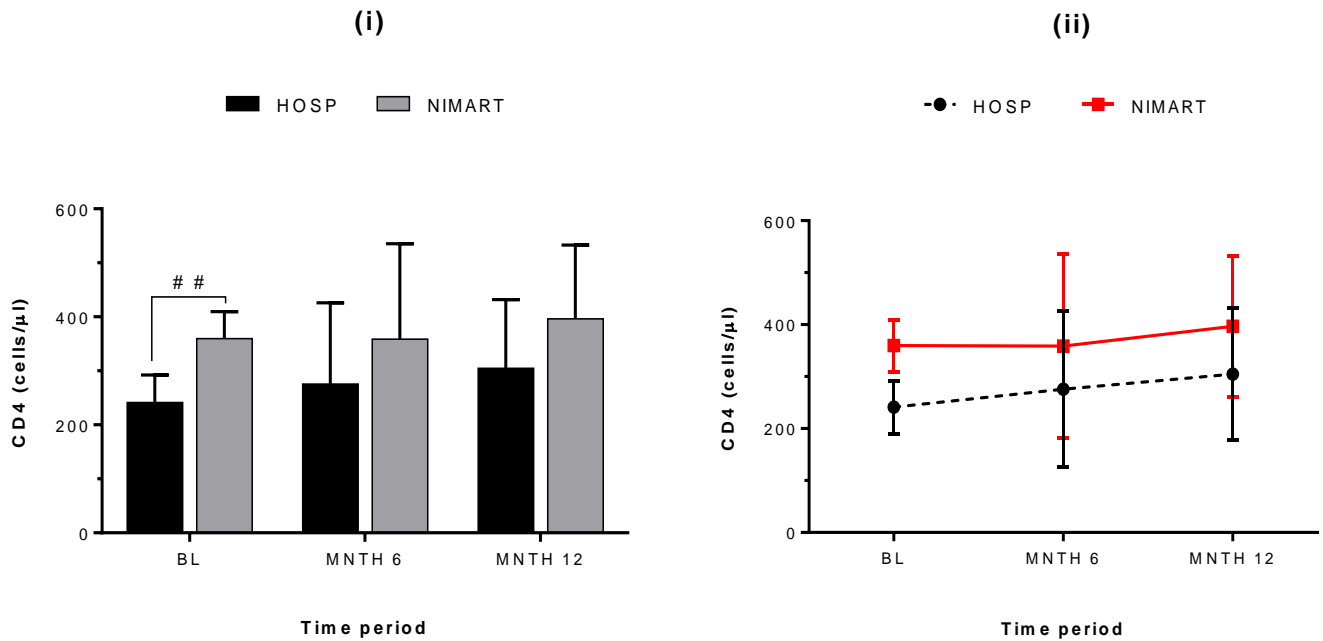
**Table 4-4:** Adjusted means at baseline, 6 and 12 months with estimate of intervention effect for CD4, Hb, MCV and BMI in the two study cohorts.

		Time*Cohort		Fixed effects (Significance: p - value)			
		Hospital clinic	NIMART	Co-variance matrix	Interaction (Time* Cohort)	Cohort	Time
<b>CD4 (cells/ <math>\mu</math>l)</b> Baseline	<b>Mean</b>	241	360	AR-1	0.835	0.323	0.647
	<b>95% CI</b>	190.18, 292.15	309.64, 409.90				
Month 6	<b>Mean</b>	276	359				
	<b>95% CI</b>	125.11, 426.13	182.33, 535.41				
Month 12	<b>Mean</b>	305	397				
	<b>95% CI</b>	177.55, 432.14	260.27, 532.79				
<b>Hb (g/dL)</b> Baseline	<b>Mean</b>	12.47	12.84	AR-1	0.835	0.323	0.647
	<b>95% CI</b>	12.07, 12.88	12.41, 13.27				
Month 6	<b>Mean</b>	11.90	12.29				
	<b>95% CI</b>	10.99, 12.80	9.98, 14.60				
Month 12	<b>Mean</b>	12.16	12.94				
	<b>95% CI</b>	11.39, 12.92	11.78, 14.11				
<b>MCV (fL)</b> Baseline	<b>Mean</b>	94.19	103.19	US	0.128	0.227	<b>0.009</b>
	<b>95% CI</b>	91.83, 96.54	100.52, 105.87				
Month 6	<b>Mean</b>	102.72	99.58				
	<b>95% CI</b>	97.05, 108.39	87.46, 111.69				
Month 12	<b>Mean</b>	102.64	107.47				
	<b>95% CI</b>	98.61, 106.66	100.39, 114.55				
<b>BMI (kg/m<sup>2</sup>)</b> Baseline	<b>Mean</b>	25.10	26.36	AR-1	0.389	0.703	0.952
	<b>95% CI</b>	23.05, 27.15	21.34, 31.39				
Month 6	<b>Mean</b>	24.94	26.34				
	<b>95% CI</b>	22.85, 27.02	21.27, 31.42				
Month 12	<b>Mean</b>	25.41	26.04				
	<b>95% CI</b>	23.35, 27.47	20.98, 31.09				

**Hb**, haemoglobin; **MCV**, mean corpuscular volume; **BMI**, body mass index; **AR-1**, autoregressive order-1; **CI**, confidence interval (upper and lower limit); **US**, unstructured; **Adjusted for confounding variables:** Age, time since positive HIV diagnosis (duration of infection) and total time on ART.

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Figures 4-8, 4-9, 4-10 and 4-11 graphically illustrate the data presented in Table 4-4 to visually communicate and show the general trend over time. The figures indicate patterns or relationships for the respective variables.

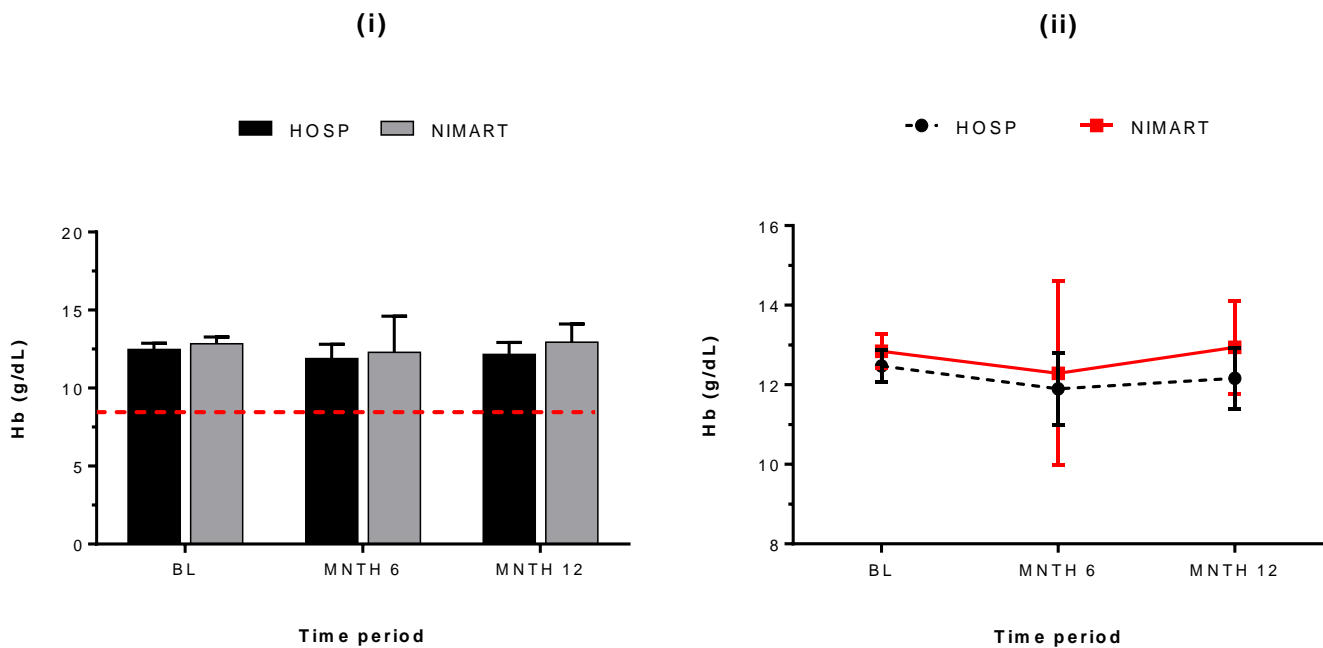


**Figure 4-8:** Adjusted mean (SD) CD4 cell counts (i) and 95% CI (ii) at baseline, 6 and 12 months post-AZT initiation for hospital clinic vs NIMART cohorts.

##  $0.5 \leq d < 0.8$ .

CD4 cell count (Figure 4-8 i & ii), increased overall but indicated no significant differences ( $p > 0.05$ ) over time ( $p = 0.562$ ,  $F = 0.582$ ), between the two cohorts ( $p = 0.091$ ,  $F = 2.899$ ) or for the time\*cohort interaction ( $p = 0.927$ ,  $F = 0.076$ ) (Table 4-4).

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**Figure 4-9:** Adjusted mean (SD) Hb levels (i) and 95% CI (ii) for baseline, month 6 and 12 post-AZT initiation for hospital clinic vs NIMART cohorts.

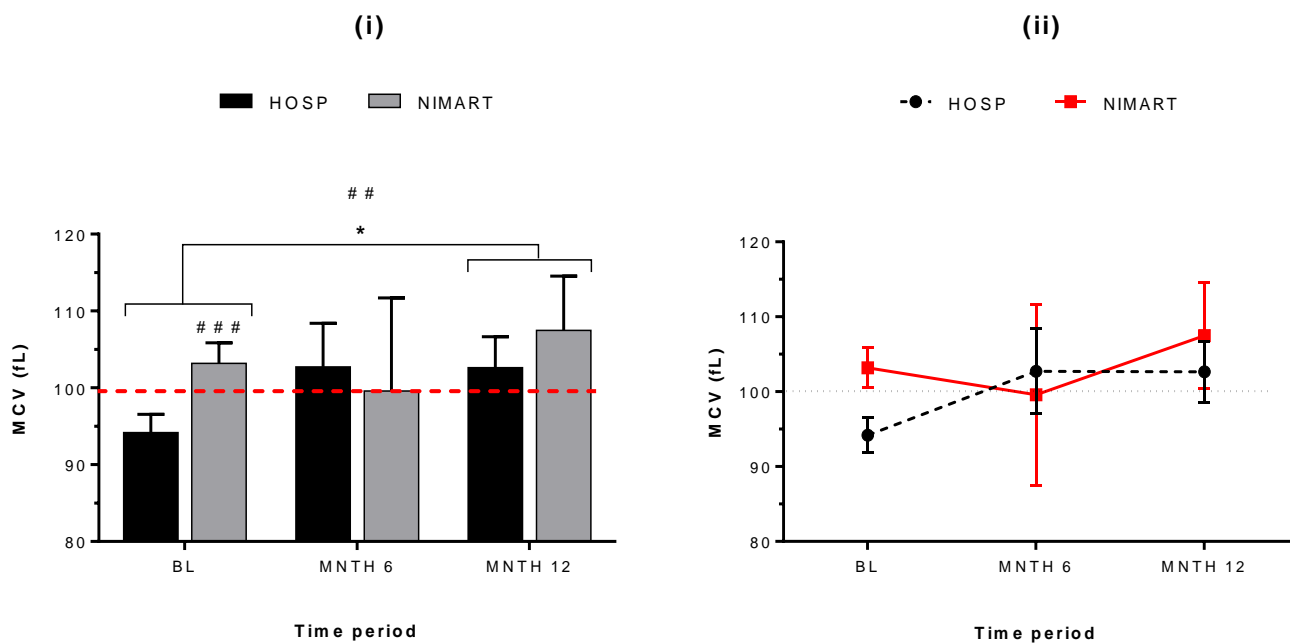
Red dotted line (----) indicates moderate to severe anaemia ( $\leq 8$  g/dL)

Figure 4-9 depicts means (SD) (i) and 95% CI (ii) Hb values at baseline, 6 and 12 months post AZT-based regimen initiation. Hb levels varied slightly without significance over time ( $p = 0.647$ ,  $F = 0.438$ ) or between the cohorts ( $p = 0.323$ ,  $F = 0.984$ ). The trend for the two groups reacted similarly over time with no interaction (time\*cohort;  $p = 0.835$ ,  $F = 0.180$ ) to report, and did not reflect anaemic status ( $Hb \leq 8$  g/dL) after 12 months on AZT-based regimen.

The MCV values however, increased significantly over time ( $p = 0.009$ ,  $F = 5.255$ ), more specifically between baseline and month 12 ( $p = 0.008$ ) but showed no differences between the cohorts ( $p = 0.227$ ,  $F = 1.476$ ) or indicated an interaction between time\*cohort ( $p = 0.128$ ,  $F = 2.152$ ).

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Macrocytic values (> 100 fL) were already evident at baseline, 6 and 12 months in the NIMART cohort but only evident after 6 and 12 months post AZT initiation in the hospital clinic cohort. According to the interpretation of Cohen's *d*-values, medium practical significant MCV values were detected between baseline and month 12 ( $d = 0.607$ ) and large practical significance between the two groups at baseline ( $d = 0.85$ ) (Figure 4-10).



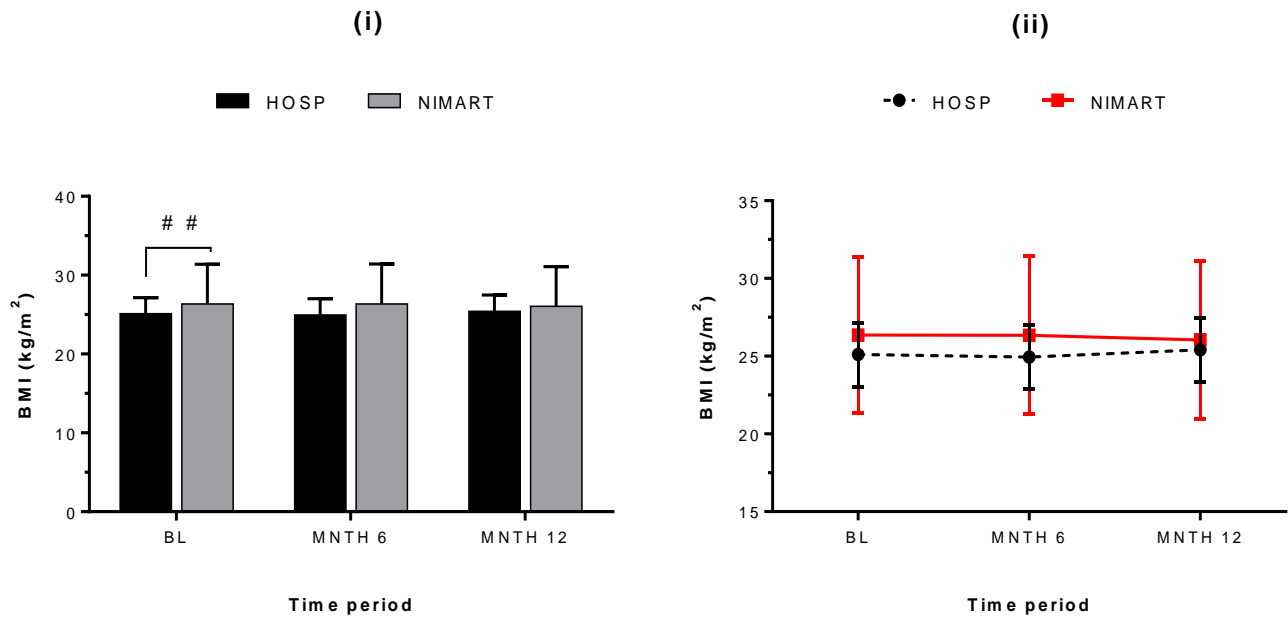
**Figure 4-10:** Adjusted mean (SD) MCV levels (i) and 95% CI (ii) for baseline, month 6 and 12 post-AZT initiation for hospital clinic vs NIMART cohorts.

Red dotted line (---) indicates macrocytosis ( $\geq 100$  fL)

\*  $p < 0.05$  ##  $0.5 \leq d < 0.8$  ###  $0.8 \leq d < 1.3$

The BMI values were almost unchanged over the first year on AZT-based therapy (Figure 4-11, i & ii) supported by no time\*cohort interaction ( $p = 0.389$ ,  $F = 0.955$ ) and no noteworthy changes between hospital clinic and NIMART subjects ( $p = 0.703$ ,  $F = 0.146$ ) or over time ( $p = 0.952$ ,  $F = 0.049$ ). A medium practical difference occurred between the cohorts on initiation of AZT ( $d = 0.52$ ).

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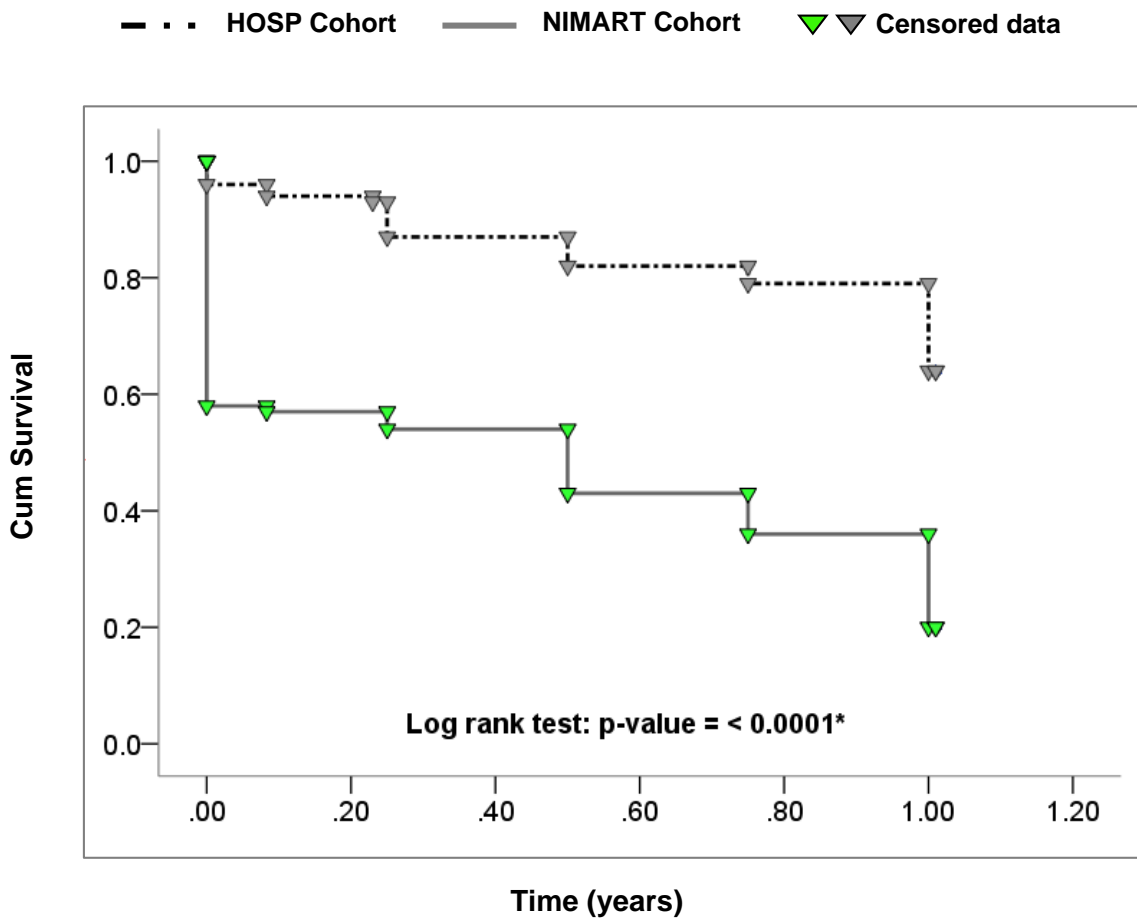


**Figure 4-11:** Adjusted mean (SD) BMI (i) and 95% CI (ii) at baseline, 6 and 12 months post-AZT initiation for hospital clinic vs NIMART cohorts.

### 4.2.4 Comparative viral load suppression over time (Kaplan-Meier analyses)

The Kaplan-Meier (also a non-parametric) estimator is used to determine how rapidly or how slow patients reach a certain event, in this case undetectable VL suppression ( $\leq 20$  viral copies/ml). It determined the effect of time on all of the HIV positive adult patients ( $n = 200$ ) for VL suppression by generating two pictorial curves, one for each of the cohorts illustrated by Figure 4-12 over the first 12 months post-AZT initiation.

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**Figure 4-12: Kaplan-Meier survival analysis.** Time until undetectable VL for n = 200 HIV adult patients in a hospital clinic vs NIMART cohorts.

**Censored data:** Cohort’s data was censored at study end – censored data includes patients that did not yet achieve undetectable VL at the time when data collection came to an end and it is not known if these patients achieved VL suppression after month 12 post-AZT initiation.

The pictorial curves (factor levels) were compared by means of three types of Chi-Square statistical tests to test for equivalence or differences; Log-rank test (emphasising patients achieving VL suppression later in the study tending to compare curves more profoundly as time passes), Breslow (generalised) Wilcoxon test (tends to look at differences more strongly early on in time) and the Tarone Ware test (emphasising a stronger comparison during the middle portion of the time course). The null hypothesis assumed equivalence and significance was set at  $p < 0.05$ . All three tests rejected the null hypothesis ( $p < 0.0001$ , Table 4-5 indicating that the curves were not equivalent). This correlates with Figure 4-4.

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Censored data points are defined as patients that did not yet achieve undetectable VL at the time when data collection came to an end and it is not known if these patients achieved VL suppression after month 12 post-AZT initiation. Kaplan-Meier did not assume adjustment for the previous three confounding variables as none of the variables were applicable to this analysis. Survival analysis only depended on whether a patient achieved suppression of viral copies (< 20 viral copies/ml).

NIMART patients (n = 80, 80%) were considerably more successful in achieving VL suppression compared to hospital clinic patients (n = 36, 36%). When combined, 58% (n = 116) of all patients (n = 200) reached undetectable VL suppression after  $8.04 \pm 3.6$  months on AZT-based regimens. After 6 months, an estimated 81% of hospital clinic and 41% of NIMART patients (out of n = 100 per cohort) had not yet reached VL suppression. A total of 42% (n = 84) of patients were censored (HOSP: n = 64, NIMART: n = 20).

Figure 4-12 indicates that more than half of all study subjects succeeded at inhibiting viral copies through successful ART. The estimate mean time (months) for NIMART subjects to have reached VL suppression ( $5.74 \pm 5.4$  months) were almost twice as fast compared to hospital clinic subjects ( $10.36 \pm 3.6$  months).

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**Table 4-5:** Kaplan-Meier graph (time to event analysis – undetectable VL)

Case processing summary (patients reaching undetectable VLs)				
Cohort	<sup>b</sup> Event (n) BL	<sup>b</sup> Event (n) M-6	<sup>b</sup> Event (n) M-12	<sup>a</sup> Censored (n)
Hospital clinic cohort	n = 8	n = 19	n = 36	n = 64
NIMART cohort	n = 44	n = 59	n = 80	n = 20
<b>TOTAL</b>	n = 52	n = 78	n = 116	n = 84
Estimate mean $\pm$ SD time to undetectable VLs				
			95% CI	
Cohort	Years	Months	Upper bound	Lower bound
Hospital clinic cohort	0.86 $\pm$ 0.30	10.36 $\pm$ 3.60	0.80	0.92
NIMART cohort	0.48 $\pm$ 0.45	5.74 $\pm$ 5.40	0.39	0.57
Overall	0.67 $\pm$ 0.30	8.04 $\pm$ 3.60	0.61	0.73
<sup>c</sup> Overall comparisons for factor levels (Chi-Square tests)				
Test		Significance level (p-value)		
Log rank (Mantel-Cox)		< 0.0001		
Breslow (Generalised Wilcoxon)		< 0.0001		
Tarone-Ware		< 0.0001		

**BL**, baseline; **M-12**, month 12 post-AZT initiation. <sup>a</sup> **Censored data**: Cohort's data was censored at study end – censored data includes patients that did not yet achieve undetectable VL at the time when data collection came to an end and it is not known if these patients achieved VL suppression after month 12 post-AZT initiation. <sup>b</sup> **Event**: Undetectable VL suppression reached <sup>c</sup> **Test of equality of survival distributions** for the different levels of treatment cohort.

## CHAPTER 5: DISCUSSION OF RESULTS

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### 5.1 Introduction

The fast-tracking of ART in South Africa has reinforced the need to develop task-shifting models for delivery at both clinical and primary health system levels and to merge inclusive HIV/AIDS care into primary healthcare (Crowley & Mayers, 2015). In April 2010, South Africa authorised revised treatment guidelines enabling a task shifting model from physician-based and managed, hospital-centric ART to decentralised nurse-initiated management of antiretroviral therapy (NIMART) (Davies *et al.*, 2013).

To our knowledge, this study is the first retrospective investigation in the Matlosana City sub-district whereby physician (hospital clinic) vs NIMART managed ART were compared with regard to major markers for HIV disease progression (CD4 cell counts & VLs), haematological parameters (Hb & MCV) and possible associated risk factors for disease progression for patients on AZT-based ART regimens.

### 5.2 Demographical comparison of the hospital clinic and NIMART cohort

It was evident in both study settings that females comprised the biggest proportion (59% and 69%) of patients. These results correspond with most HIV research results reported in the literature from the United States (Wilson *et al.*, 2005) and South Africa. Kerkhoff *et al.* (2014) reported the female incidence at 73 % and Omole and Semanya (2016) at 69%. Muula *et al.* (2007) stated that even though the greater HIV infection prevalence in females is accounted for, in most Southern African countries, comparably more females are on ART than men. This reflects, to an extent, the gender proportions of the adult inhabitants infected with HIV in southern Africa. Biber *et al.* (1999) also stated that during the early stages of the ART era, men were more prone to seek treatment for HIV only in later stages of the disease compared to women. Long *et al.* (2011) conducted a South African study on treatment outcomes for stable ART patients shifted to the NIMART approach and confirmed that in both groups of patients care at hospital level and those shifted to nurse-managed ART, females made up over three quarters of the sample size.

Although the NIMART patients (50.3 years) were significantly older compared to physician (42.4 years) managed patients, a specific age range was not specified as part of an inclusion criteria.

## CHAPTER 5: DISCUSSION

Study patients were randomly selected, as previously stated. However, when the data was further analysed, age was confounded for to remove the effect on remaining data.

Clinical WHO-staging is only reported at the time of positive HIV diagnosis or initiation of ART (baseline) and is not altered even after years of therapy. The majority of patients initiated on AZT-based treatment by physicians (n = 20 patients, n = 35 WHO stages reported; 57%) were at WHO-stage III compared to majority of NIMART patients being at stage I (n = 31 patients, n = 84 WHO stages reported; 36.9%) and II (n = 36 patients, 84 WHO stages reported 42.9%).

In 2014, Green and colleagues (n = 149) found similar results during a cross-sectional quality care assessment study done in a sub-district of Cape Town where a greater proportion (40.6%) of physician-managed patients had WHO stage III and NIMART patients (52.5%) dominantly stage I.

The majority of patients in both of our cohorts received AZT as part of second line regimen (94% and 56%), although those initiated on first line AZT-based regimens in the NIMART cohort represented 44%. This could possibly be contributed to patients presenting with contraindications or side effects to more popular first line medication. Only two patients died, one patient in each of the cohorts. The hospital clinic patient died after the 12-month study period. No further explanation for the NIMART patients' death was documented in the file. However, a major confounder of this study is that these effects could not be attributed to AZT alone, as all of the patients were receiving a multitude of ARV combinations as part of their therapy.

Opportunistic infections (OIs) and hospital admissions per patient (during the first year after AZT initiation) for the hospital clinic cohort (2.2 and 0.59 (n = 59) respectively) ( $p < 0.0001$ ) were much higher when compared to the NIMART cohort (0.31 and 0.09 (n = 9)) ( $p > 0.05$ ) keeping in mind that no adjustment was made yet for age, time spent on ART or time since positive HIV diagnosis. In their retrospective investigation into the clinical health outcomes of HIV patients in a rural clinic in South Africa, Omole and Semanya (2016) (n = 124) found, with no reference to correction for confounding variables, that only nine (7.2%) patients were admitted to hospital (0.07 admissions per patient) for the duration of the study. At baseline, the prevalence of OIs in their cohort was 58.4%. They also included AZT as part of a second line regimen.

Although the mean age was significantly different for patients in the cohorts, the time since positive HIV diagnosis (7.6 and 7.7 years) showed no statistical difference and these patients had similar period of time since HIV diagnosis, irrespective of the clinical setting in which AZT-based regimen was initiated. Time spent on total ART (6.44 vs 5.34 years) and more specifically on AZT therapy (2.9 vs 2.5 years) was longer in the hospital clinic cohort compared to the NIMART cohort. Previous research at Themba Lethu Clinic in Johannesburg reported time spent on ART for the

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majority of patients were between 0.83-1.5 years for the NIMART (n = 148, 21.5%) and hospital (n = 1025, 34.5%) group with 30.1% (n = 209) and 33.6% (n = 997) of NIMART and hospital patients respectively receiving AZT-based regimens. Only 1.4% (NIMART) and 1% (hospital based) of patients received ART  $\geq$  4 years (Brennan *et al.*, 2011).

### 5.3 Comparing patient variables from baseline to 12 months post - AZT initiation between the cohorts

Testing for statistical significance in itself does not take into account factors which may bias results and thus, even though significant differences are not found for variables between two treatment groups, it is still necessary to account for all possible biases to diminish the possibility of their impact (Skelly *et al.*, 2012). In this section, the discussion of results is presented before and after confounding variables were adjusted for, to illustrate the importance of bias. Viral load was not included in the linear mixed model results (after adjusting for confounding variables) due to the wide variation that occurred between patients within the hospital clinic cohort and the limited variation between individuals in the NIMART cohort that can be attributed to the high prevalence of patients achieving undetectable VL suppression. Thus, VL was subjected to the Kaplan-Meier analysis instead.

#### 5.3.1 CD4 cell count and viral load before adjustment for confounding variables

Viral load is one of the major factors of disease progression alongside CD4 cell count and its measurement at 2-3 months after ART initiation must indicate a significant reduction in viral copies, according to Spencer (2005).

In our study a notable trend in increased median CD4 cell counts for both study cohorts were reported from one to twelve months (Table 4-3 and Figure 4-3). More specifically, the NIMART cohort showed not only statistically significant ( $p > 0.05$ ) higher increase) (180 cells/ $\mu$ l) in CD4 cells when compared to the hospital clinic cohort (113 cells/ $\mu$ l) from baseline to twelve months, but were also practically higher on all time points except month 6. The hospital clinic cohort experienced the largest increase (114 vs 52 cells/  $\mu$ l) during the first 6 months. Sanne and colleagues (2010) published supporting results in the prospective randomised *CIPRA-SA* trial conducted in two separate PHC clinics. Two treatment monitoring strategies (physician-based, n = 408 and NIMART, n = 404) were compared for adult HIV patients on ART, investigating composite endpoints of treatment limiting events (virological failure, mortality and limiting toxic effects). They noted that median baseline CD4 cell counts were slightly higher for their nurse group compared to the doctor group. We found that month 6 post-AZT initiation shows a slight drop in CD4 cells when compared to month 3 but recovered and increased to higher values than

## CHAPTER 5: DISCUSSION

reported earlier. The majority of patients' baseline CD4 counts ( $n = 93$  for both cohorts) were available for analysis with month 6 and 12 showing similar but lower number of reported values for both clinical settings. This is congruent with current guidelines implemented by the NDoH (2014), stating that CD4 cell counts are a required to be measured at initiation and 12-monthly thereafter if not clinically indicated otherwise. Subsequently, findings by Green *et al.* (2014) indicated that baseline CD4 cell counts were recorded for all their NIMART patients as evidence to effective task-shifting approaches.

Viral loads decreased remarkably over time in the majority of hospital clinic and NIMART patients resulting in VL levels below the detected limit ( $< 20$  copies/ml) already at 3 months post-AZT initiation (Table 4-3). Some hospital clinic patients experienced viral rebound ( $> 1000$  copies/ml) at month six ( $n = 2$ ) and NIMART patients at month six ( $n = 1$ ) and twelve ( $n = 2$ ) respectively, but recovered well. Greub *et al.* (2002) explained that in most cases of virological rebound, the next VL is again below level of detection (LDL) as is evident with NIMART patients (month three). An extensive study done by Brennan *et al.* (2011) found that more "not-down-referred patients" (hospital based therapy) experienced viral rebound compared to "down-referred patients" (NIMART). In this case, Brennan *et al.* (2011) states that viral rebound was possibly attributed to poor patient adherence as drug resistance would lead to substantial elevation in VLs and a decrease in CD4 counts. A substantial proportion of the hospital group ( $n = 94$ ) were already on second line treatment which includes a PI, usually LPV/r which is known to cause diarrhoea and vomiting (NDoH, 2014) and often requires a change to a different PI to improve tolerability and adherence and could thus contribute to this outcome. In addition, it was evident from our study that median CD4 cell counts continued to increase even when the concurrent VL was still detectable. In this study, out of the  $n = 34$  and  $n = 58$  hospital clinic patients on month 6 and month 12 respectively,  $n = 13$  (month 6) and  $n = 19$  (month 12) hospital clinic patients had a VL  $< 20$  copies/ml.

In this study, there were significant differences in VL between the cohorts. The median VL was statistically higher in the hospital clinic cohort at baseline ( $p < 0.0001$ ), month 6 ( $p = 0.001$ ) and 12 ( $p < 0.0001$ ). Median VLs provide evidence that VLs for NIMART patients (20 copies/ml at baseline and 12 months) on decreased (Table 5-3) significantly compared to hospital clinic patients (58 109 copies/ml at baseline and 175 copies/ml at 12 months). However, no practical significance was observed (Figure 4-4). This may support the fact that NIMART patients are likely more stabilised on ART and hospital clinic patients are prone to closer observation by physicians. Patients may also have been changed to AZT due to toxicity on TDF and not due to virological failure. The trend for both CD4 and VL over time provides strong clinical evidence for improved immunological function (continued increase in CD4) and viral suppression (undetectable VL) over the first year after initiation on AZT-based therapy in both of these cohorts and supporting the

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NDoH HIV guidelines. In an observational study, Mocroft *et al.* (2007) summarised that normalisation of CD4 counts for all infected patients might be attainable if viral suppression with combination ART can be sustained for an adequate period of time.

### 5.3.2 CD4 cell count after adjustment for confounding variables

In this study the effect of age, total time spent on ART and time since positive HIV diagnosis were removed from the analysed data, fundamentally, the trend of increased CD4 and viral suppression remained the same between the cohorts (NIMART constantly showing higher CD4 values), but the differences observed became less significant with only a significant and practical difference at baseline (Figure 4-8). This emphasises the role that time has on HIV/AIDS progression. This also included a non-significant ( $p = 0.927$ ) time\*cohort interaction result during the linear mixed model analysis, ultimately concluding that hospital clinic and NIMART patients followed the same trend and reacted similarly over time (Table 4-4). It is important to take note of the fact that significant differences were no longer relevant after correcting for confounding variables. This is a major basis for supporting task shifting and includes research done by Long and colleagues (2011) that stated that stable patients managed at PHC clinics had comparable or even improved 12-month outcomes when compared to hospital clinic-based ART. Previous collaborative quality improvement research, conducted by Wilson *et al.* (2005), found that professional nurses (NIMART based care) and physician assistants had higher performance rates compared to non-HIV experts ( $p < 0.05$ ) with regard to HIV disease progression marker outcomes (HAART use and CD4 cell counts) and were similar to generalist HIV experts.

### 5.3.3 Hb and MCV before adjustment for confounding variables

Despite anaemia being the most common haematological manifestation of HIV disease, the extensive toxic effects of AZT on blood parameters, more specifically macrocytosis, are also well known (Aslinia *et al.*, 2006; Agarwal *et al.*, 2010; Kerkhoff *et al.*, 2014). Haemoglobin and especially MCV is thus thoroughly monitored when AZT is initiated (NDoH, 2014). Over the first 12 months, Hb levels showed only slight increases, but no statistical differences between the cohorts have been shown over time except for NIMART patients showing statistically higher levels 6 months post-AZT initiation compared to hospital patients ( $p < 0.05$ ). NIMART patients also consistently presented with slightly higher Hb levels from baseline to study end. In addition, the slightly higher, but not significant, Hb levels observed for NIMART patients at month 3 also presented practical significance. Overall, anaemic status could not be attributed to any of the two groups due to only one percent of all patients presenting with AZT-induced anaemia with Hb levels  $< 8$  g/dL at 12 months post-AZT initiation in both cohorts. This was to be expected if the

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NDoH guidelines were followed in both cohorts, as regimen changes would have been made if anaemia was diagnosed when tested at 3, 6 and then 12 monthly intervals. Therefore, macrocytic anaemia was only present in 1% of patients, irrespective of the average MCV values. Also, this could not be attributed to AZT alone. Too many other factors including HIV infection itself, smoking, alcohol abuse and malnutrition could have contributed to this and was not part of the scope of this investigation. These findings however correlated with a South African study (prospective generalised clinical cohort study for AZT-based regimens, n = 853) by Hoffman *et al.* (2008) and a study in Uganda and Zimbabwe by Ssali *et al.* (2006) (DART trial for AZT based regimens *comparing two strategies* for HIV management, n = 3314) where Hb levels either remained normal or increased after a year and a year and a half on ART respectively. Median Hb levels from the previous mentioned study done by Green *et al.* (2014), were also within normal range (physician-based therapy, 11 g/dL; NIMART based, 12 g/dL) although it was unclear if they received AZT-based therapy. In addition, Kerkhoff *et al.* (2014) concluded with their retrospective analysis of prospective clinical and haematological data that the median Hb levels for all patients (n = 814) improved over the first 12 months of ART, irrespective of AZT use, gender, TB status, baseline CD4 cell count or VL and that three-quarters of patients had normal Hb levels after one year on ART. In contrast with these studies, 14.7% (n = 178) of patients in (n = 1221), developed AZT-induced anaemia when Hb levels were measured after three to six months (Dash *et al.*, 2015).

Despite little change in Hb values over 12 months in both of our study cohorts the MCV values increased considerably post AZT-based initiation in the NIMART compared to hospital clinic cohort study (Figure 4-6). The NIMART cohort presented with consistent mean MCV above 100 fL which was indicative of macrocytosis at baseline, 3, 6 and 12 months compared to hospital clinic cohort. These results were also indicative of medium and large practical significance. This is in congruence with other studies claiming that AZT is prone to macrocytosis although not specifically causing anaemia (Kiragga *et al.*, 2010). The hospital clinic cohort also presented with MCV value > 100fL and seemed to have stabilised at this level for the duration of this investigation. As already stated above only 1% of the total population presented with macrocytic anaemia.

### 5.3.4 Hb and MCV after adjustment for confounding variables

The adjusted mean Hb values over the first year on AZT-based therapy, remained relatively unchanged and no indication of anaemia during this period in both cohorts and the significant difference between the cohorts at month 6, as described above, were annihilated. The Hb results indicated similar change for both cohorts and showed no significance ( $p = 0.647$ ) between the hospital clinic and NIMART patients over time (Table 5-4). It is also important to mention that the

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lower limit of the 95% confidence intervals (CI) for the Hb data in this study was far above anaemic values ( $\leq 8$  g/dL) for both cohorts.

The results of this study confirmed results from an observational study by Kiragga *et al.* (2010) in an urban clinic in SSA ( $n = 5494$ ), after they adjusted for age and gender, found that AZT was not associated with early anaemia in men and women and that the most important risk factors for anaemia could be correlated to low MCV levels and pre-existing TB-HIV co-infection, both of these factors were not prominent in our study results. Kiragga *et al.* (2010) further concluded that elevated MCV and low BMI may be more useful in predicting anaemia compared to Hb as a determinant due to most patients showing Hb recovery after ART initiation, even in those on AZT-based regimens. Their research collectively suggested, after looking at two Hb values within a period of 6 months, that AZT was still not predictive of early severe anaemia (OR; 1.4%, CI; 0.92-2.21).

Mean corpuscular volume was the only variable with mentionable changes after adjusting for the three confounding variables. The MCV values increased dramatically between baseline and month 12 (over time,  $p = 0.009$ , Table 4-4). Macrocytic values ( $> 100$  fL) were already evident at 6 months post-AZT initiation for both cohorts. Medium practical significant MCV values were detected between baseline and month 12 ( $d = 0.61$ ) and large practical significance between the two groups at baseline ( $d = 0.85$ ). Although no time\*cohort interaction (groups changed according to the same trend over time) and no statistical difference between the cohorts was observed, MCV levels changed significantly over time and macrocytic values, was subsequently confirmed for the majority of patients in both cohorts. A prior African study by Musiime *et al.* (2011) ( $n = 710$ ), investigated the efficacy and toxicity of ART in women and found a median increase of 16 fL in MCV from baseline (88 fL) to month 12 (104 fL) post-HAART (AZT or d4t based) initiation although Hb was not investigated. Although this study was performed in women only, other research documented the correlation between AZT and macrocytosis as a basis for ARV adherence, claiming that increased MCV levels over time is an indication of successful AZT therapy (Romanelli *et al.*, 2002 and Muqisha *et al.*, 2012).

### 5.3.5 BMI before adjustment for confounding variables

Body mass index for NIMART patients remained almost unchanged over the first year after the initiation of AZT-based regimen. Except for fluctuations in BMI for hospital clinic patients between month 3 and 12, differences between the two groups were non-significant. These findings were consistent with Koethe *et al.* (2011), an observational retrospective cohort study ( $n = 915$ ), that stated for BMI values in the range of 25-30 kg/m<sup>2</sup> may be related with optimal immune reconstitution in the first year of ART. The mean BMI values of our study in both cohorts varied

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from 24-27 kg/m<sup>2</sup> over the first 12-month period post AZT-based initiation. Additionally, Masaisa *et al.* (2011) found that lower BMI (odds ratio (OR), 3.4; 95% CI, 2.4-4.1) was a risk factor for anaemia alongside the use of AZT (OR, 1.14; 95% CI, 1.01-1.29). Results from the DART trial, comparing two strategies for HIV/AIDS management (Ssali *et al.*, 2006), showed that patients with BMI < 18 kg/m<sup>2</sup>, had a higher risk for presenting with anaemia. Due to the fact that our study results show evidence of normal BMI levels, this possibly could have contributed to a general non-anaemic status within this study population that showed normal Hb levels and elevated MCV levels.

### 5.3.6 BMI after adjustment for confounding variables

Results for BMI before and after the adjustment for confounding variables are almost identical in both cohorts. The adjusted mean BMI values from patients in the respective cohorts reacted similarly (following the same trend) for the first year after the initiation of AZT. Although we didn't investigate correlation between variables, the lower 95% CI limit (> 20 kg/m<sup>2</sup>) can indicate that BMI can possibly be excluded as risk factor for anaemia in this study as determined by Ssali *et al.* (2006) and Kiragga *et al.* (2010). Additionally, Mangili *et al.* 2006 stated that weight loss and subsequent lower BMI is associated with low CD4 cell counts even after the initiation of ART. In response, results from our study delivered contrasting evidence with mean BMI values within normal range and progressive elevated CD4 counts for the first year after AZT-based initiation suggesting that normal BMI ranges are most beneficial for immune recovery on ART.

### 5.3.7 Kaplan-Meier survival analysis

We investigated the pattern in achieving VL suppression and recorded statistical significant differences for each time interval between the two study cohorts for VL suppression. While it is simple to visualise the difference between the two pictorial survival curves, the differences must be quantified. The vertical gap (no overlapping) between the curves over the 12 months post AZT-based initiation provides evidence that the NIMART group had a greater fraction of subjects achieving VL suppression at month 6 (59% vs 19%) and study end (80% vs 36%). Also, patients in the NIMART cohort that achieved suppression, attained it nearly twice (5.7 vs 10.4 months respectively) as fast compared to hospital clinic patients. It also shows that the curves were not equivalent although they followed the same trend. Fairall *et al.* (2012) found in their pragmatic, cluster-randomised trial, comparing control groups (physician-based care) and intervention groups (NIMART based, *STRETCH* programme), that 71% from the intervention group and 70% of the controlled group achieved viral suppression, after ≥ 6 months on ART. Omole and Semanya (2016) subsequently confirmed in their study that 84% of clinic patients achieved viral suppression

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6 months after ART initiation. However, in this study we found that the NIMART cohort already had more subjects (44%) with VL suppression at baseline, an occurrence not observed for the hospital cohort (only 8% viral suppression). These results can be related to research done with regard to HIV VL response to ART by Phillips *et al.* (2001) within three European based clinic cohorts (of which one was a hospital clinic), capturing clinical and laboratory marker status (n = 3226). They found that patients with a lower CD4 count had a slight lesser tendency to achieve viral suppression.

At baseline, NIMART patients presented with more undetectable viral loads (n = 44 were already virally suppressed). This can possibly be attributed to:

1. Patients in the NIMART cohort were switched to second line regimens (AZT-based) not due to virological failure but rather because of side effects or intolerability of ARVs;
2. Patients in the NIMART cohort presented dominantly with WHO stage I (n = 31) and II (n = 36) compared to the hospital cohort (WHO stage III; n = 20, stage IV; n = 2). AIDS progression in patients from the hospital clinic group were thus more severe (although data regarding clinical WHO staging were limited);
3. The frequency of OIs and hospitalisations were higher for the hospital clinic patients compared to NIMART patients;
4. The total time spent on ART were also significantly longer for hospital clinic patients compared to NIMART patients.

In our study, hospital clinic patients had higher median VLs (58109 vs 20 copies/ml) and lower CD4 cell counts (173 vs 312 cells/ $\mu$ l) at baseline and after 12 months (175 vs 20 copies/ml; 286 vs 492 cells/ $\mu$ l) before correcting for confounding variables. This can possibly also explain the tendency for the lower percentage hospital patients achieving undetectable VL suppression. The patients who did not achieve VL suppression at study end, were censored but we assumed at any time that patients who were censored, had the same prospects in achieving viral suppression than those who already reached undetectable VL levels.

Many more hospital clinic patients were censored (Table 4-5) compared to NIMART patients indicating that the time it took for these patients to achieve viral suppression could not be accurately determined as their outcome was unsure. This emphasises the importance of illustrating censored patients as it is an indication of treatment outcomes. Studies using a Kaplan-Meier analysis to determine time until undetectable VL suppression for physician vs NIMART managed cohorts, comparing the efficiency between to treatment approaches are extremely limited.

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### 5.4 References

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## CHAPTER 5: DISCUSSION

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# CHAPTER 6: CONCLUSIONS, LIMITATIONS & RECOMMENDATIONS

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## 6.1 Conclusions

The number of studies designed to compare the efficiency of ART initiation and management by physicians in a hospital clinic setting and NIMART in a clinic setting is increasing. Large task shifting (physician to NIMART) was officially implemented in South Africa after revised guidelines was approved (April 2010) on the general implementation of NIMART in South Africa in beginning 2011. This approach of task shifting is the way forward to address the profound impact of the HIV epidemic in South Africa to dramatically enhance access to HIV care and improvement in patients' life quality. The recent UNAIDS Global Update Report stated that 3.4 million people were on ART in South Africa by the end of 2015, making it the country with the largest number of patients on ART worldwide (UNAIDS, 2016). As the number of research studies investigating the impact of NIMART grows, studies need to become more focussed and region specific, looking at more crucial elements in HIV care. Davies *et al.* (2013) recommended the need for follow-up studies after the establishment of NIMART in more PHC facilities in South Africa.

The following objectives were investigated and concluded upon:

- **Primary objectives**

- 1) To compare disease progression markers (CD4 and VL) following AZT-based regimens between hospital clinic and NIMART cohorts and
- 2) To compare haematolytic related parameters (Hb and MCV values) following AZT-based therapy between the hospital clinic and NIMART cohorts.

- **Secondary objective:**

- 1) To investigate possible associated risk factors (BMI, hospital admissions, and frequency of OIs especially TB, CrCl and WHO staging) with disease progression

The associated risk factors for disease progression such as WHO staging, CrCl, hospitalisation and opportunistic infections were incomplete as the data was either not reported, missing or not relevant at the different time points to be included in the inferential analyses. Some of these aspects were only reflected as part of the descriptive results.

## CHAPTER 6: CONCLUSIONS, LIMITATIONS & RECOMMENDATIONS

This study was conducted in the City of Matlosana in the North West province and included only adults on AZT-based regimens. Retrospective health file data was used to compare Tshepong Hospital clinic adult patients (n = 100) and adult patients (n = 100) who attended three clinics (Stilfontein PHC, Jouberton CHC and Park Street Clinic) implementing NIMART. The patients included in this study were randomly selected from the Rx Solutions software system from MSH and they all had to be on AZT-based regimens for 12 months.

A significant CD4 cell increase (positive immunological recovery) and VL decrease (effective viral suppression), two important disease progression markers, were observed in both cohorts. The majority of patients had normal Hb levels after 12 months on AZT-based regimens in both cohorts. An increase in MCV values irrespective of duration since HIV diagnosis, total duration on ART or age were observed in both cohorts. The ever increasing MCV values over time were expected for patients on AZT-based regimens as it had been described in published literature on AZT-induced macrocytosis post ART initiation (Meidani *et al.*, 2012). Mean corpuscular volume showed macrocytosis in both cohorts during the 12 months post AZT-based initiation. The BMI remained constant throughout the study period, irrespective of the study setting. Despite the significant higher VLs of the hospital clinic cohort at baseline both cohorts reflected the same tendency in viral suppression over the 12-month period after AZT-based initiation. The NIMART cohort achieved an 80% viral suppression compared to the 36% of the hospital clinic cohort and also reached VL suppression almost two times faster (5.7 vs 10.4 months).

In conclusion, before we adjusted for confounding variables, differences between the patients from the hospital clinic and NIMART cohort were quite significant and it seemed that the treatment outcomes for those receiving AZT-based ART by means of NIMART were more successful when compared to the hospital clinic cohort. However, after the effect of age, total time spent on ART and time since HIV positive diagnosis had been removed from analysed data, it became evident that the treatment outcomes were in fact similar between the cohorts. This small-scale, retrospective observational study supports that NIMART were non-inferior to physician-initiated and managed ART in this public healthcare ART programme as both study cohorts clinically improved in a similar trend with no significant differences between them when the effects of the confounding variables were removed. Clinical health outcomes supported the feasibility of NIMART for this region and this shows support for future plans that these two treatment approaches can be successfully implemented and managed in other SSA countries that are also burdened with crumbling health systems and drastically in need of an influx in human research capacity (Zachariah *et al.*, 2009). This study sheds some light on not only the clinical health outcomes (CD4 cell count, VLs, Hb, MCV and BMI) of HIV adult patients on AZT-based regimens for a one year time period, but also places focus on adaptation of local regions to changing roles of healthcare providers. This was widely supported by studies in SSA countries (Iwu & Holzemer,

## CHAPTER 6: CONCLUSIONS, LIMITATIONS & RECOMMENDATIONS

2014), more specifically Namibia (O'Malley *et al.*, 2014) and South Africa (Sanne *et al.*, 2010; Long *et al.*, 2011; Georgeu *et al.*, 2012; Green *et al.*, 2014). The results of this study support the expanded access to treatment using models of task shifting in clinics and were also previously encouraged by Sanne and colleagues (2010). Additionally, task shifting should be seen as part of an overall strategy to improve healthcare in South Africa especially in regions where the HIV burden and related opportunistic infections places large demands on health services. Although task shifting from physicians to nurses alone will not resolve all limited human resource problems for HIV treatment, it remains one of the most feasible answers to HIV care (Brennan *et al.*, 2011). It will not only free up time and resources for physicians to manage complicated (non-responding) HIV cases, but also intensify available treatment coverage to ultimately provide and meet the large need for ART (Long *et al.*, 2011) and the newly implemented treatment guidelines that provide easier access to treatment for all HIV-infected people.

Study strengths include conducting this research in a public hospital clinic and three clinics of the Matlosana City community which is an area with a high HIV burden and very typical of the South African healthcare system and facilities. The statistical power of this study was 80% and despite missing data on certain variables and at time points it was still possible to incorporate sophisticated statistical tools and models to analyse the complex data between these cohorts.

### 6.2 Limitations and future research

**Several limitations should be considered alongside the findings of this study and in interpreting results:**

- Retrospective nature of the observational study:
  - Results may be limited by the fact that the data were collected retrospectively from patient files. The unavailability of especially WHO-staging and serum creatinine data from the participants' medical records for certain time points excluded these variables from any other statistical analysis except descriptive demographical data;
  - Improper record keeping: a large number of patient health records was incomplete or poorly reported and missing results a regular occurrence within both study settings with regard to clinical findings, diagnostic test results and progress reports;
  - Retrieving hard copy patient files proved a challenge as public filing systems are disarrayed and still rely heavily on employed personnel for organisation and data capturing. Also, file selection was constrained if the selected patient had a scheduled hospital or clinic visit on the day of data collection resulting in the respective files to be disregarded for data analysis due to unavailability.

## CHAPTER 6: CONCLUSIONS, LIMITATIONS & RECOMMENDATIONS

- Possibility of essentialism bias:
  - Categorising NIMART patients as healthier individuals, despite individual variations, compared to HIV patients receiving ART at hospital clinic level due to the essential assumption that patients at hospital level needed more intense monitoring by physicians.
- The study was also only restricted to adult patients initiated on AZT-based ART regimens in a single government hospital and three clinics in the Matlosana sub-district. Thus, results may not be generalisable to the overall South African HIV population or to other PHC settings that lack necessities for successful HIV monitoring and care. Having said that these settings were very typical of other public hospitals and clinics in South Africa.

### 6.3 Recommendations

Suitability of this approach to countries where access to physicians is even more restricted than in South Africa or is non-existent can be assessed in separate studies. Further research may be beneficial to investigate the impact of NIMART on time-saving aspects of physician-managed ART.

Taking into consideration the most recent change in guidelines, enabling the initiation of all HIV positive patients on ART irrespective of their CD4 cell count or clinical WHO- stage, the immense burden placed on the public healthcare system, including physicians and nurses, intensifies the need for comparative research studies.

Further analyses can be considered within the same region and other provinces for different ARV-based regimens with a longer duration of follow-up, both retrospectively and prospectively, in order to aggressively evaluate these two approaches. This could contribute to ultimately gaining a bigger picture on the efficacy of NIMART and its implementation in South Africa.

For future comparative studies, study designs with specific inclusion criteria for baseline characteristics can be incorporated to ensure patients from both cohorts are equal with regard to age, CD4 cell counts, clinical WHO-staging and BMI, eliminating the possibility of essentialism bias. This research model can be adapted as needed to suit any context when assessing two treatment approaches with regard to ARVs, study region, time period and clinical variables.

## CHAPTER 6: CONCLUSIONS, LIMITATIONS & RECOMMENDATIONS

### 6.4 References

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## CHAPTER 6: CONCLUSIONS, LIMITATIONS & RECOMMENDATIONS

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UNAIDS **see** Joint united nations programme on HIV/AIDS

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# **ADDENDUM A: ETHICS CLEARANCE CERTIFICATES AND LETTERS**

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ADDENDUM A contains the following ethics clearance certificates and letters

- Ethics clearance certificate from the NWU HREC (Potchefstroom Campus)
- Ethics clearance certificate from WITS HREC (medical)
- Letter of approval from the PPRM&E directorate (North West Department of Health)
- Letter of approval from the CEO of the Klerksdorp-Tshepong Hospital Complex
- Letter of approval from the acting PHC manager (Matlosana sub-district)



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**Institutional Research Ethics Regulatory Committee**

Tel: +27 18 299 4849

Email : [Ethics@nwu.ac.za](mailto:Ethics@nwu.ac.za)

2016-05-17

**ETHICS APPROVAL CERTIFICATE OF PROJECT**

Based on approval by **Health Research Ethics Committee (HREC)** at the meeting held on **19/11/2015**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

<b>Project title:</b> Descriptive and retrospective investigation into the clinical health outcomes of HIV patients on AZT-based regimens in a NIMART and hospital cohort respectively															
<b>Project Leader/Supervisor:</b> Dr M Viljoen															
<b>Student:</b> R van Graan															
<b>Ethics number:</b>	N	W	U	-	0	0	3	6	2	-	1	5	-	A	1
	Institution				Project Number				Year		Status				
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation															
<b>Application Type:</b> Full Single Application															
<b>Commencement date:</b> 2016-04-01					<b>Expiry date:</b> 2017-03-31					<b>Risk:</b>		Medium			

**Special conditions of the approval (if applicable):**

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

**General conditions:**

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
  - annually (or as otherwise requested) on the progress of the project, and upon completion of the project
  - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
  - Annually a number of projects may be randomly selected for an external audit.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the HREC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC via HREC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
  - request access to any information or data at any time during the course or after completion of the project;
  - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
    - any unethical principles or practices of the project are revealed or suspected,
    - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
    - the required annual report and reporting of adverse events was not done timely and accurately,
    - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for any report templates [Ethics-Monitoring@nwu.ac.za](mailto:Ethics-Monitoring@nwu.ac.za) or further assistance via [Ethics-HRECApply@nwu.ac.za](mailto:Ethics-HRECApply@nwu.ac.za); 018 299 1206.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Prof LA  
Du Plessis

Digitally signed by Prof LA Du Plessis  
DN: cn=Prof LA Du Plessis, o=North-West  
University, ou=Campus Rector,  
email=Linda.DuPlessis@nwu.ac.za, c=ZA  
Date: 2016.05.17 13:28:29 +02'00'

Prof Linda du Plessis



R14/49 Miss Rentia van Graan et al

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**  
**CLEARANCE CERTIFICATE NO. M160267**

**NAME:** Miss Rentia van Graan et al  
**(Principal Investigator)**

**DEPARTMENT:** Pharmacy and Pharmacology  
Tshepong Hospital, Klerksdorp, Botshabelo, Jouberton  
Park Street Community Health Clinic

**PROJECT TITLE:** Descriptive and Retrospective investigation into Clinical Health Outcomes of HIV Patients on AZT-Based Regimens in a NIMART and Hospital Clinic Cohort Respectively

**DATE CONSIDERED:** Adhoc

**DECISION:** Approved unconditionally

**CONDITIONS:**

**SUPERVISOR:**

**APPROVED BY:**

Professor P. Cleaton-Jones, Chairperson, HREC (Medical)

**DATE OF APPROVAL:** 30/03/2016

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

---

**DECLARATION OF INVESTIGATORS**

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/2nd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report.

Principal Investigator Signature

Date: 30/03/2016

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES



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Health  
North West Province  
REPUBLIC OF SOUTH AFRICA

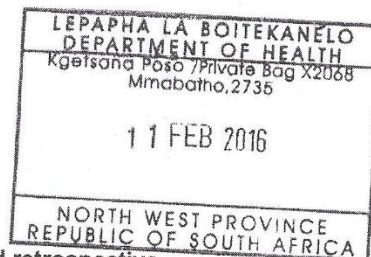
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**POLICY, PLANNING, RESEARCH, MONITORING AND EVALUATION**

Name of researcher : Me Rentia van Graan  
North West University

Physical Address (Work/ Institution) Department of Pharmacology  
Pharmacen, Building G23  
Potchefstroom Campus



Subject : **Research Approval Letter- Descriptive and retrospective investigation into the clinical health outcomes of HIV patients on AZT-based regimens in a NIMART and hospital Clinic cohort respectively.**

This letter serves to inform the Researcher that permission to undertake the above mentioned study has been granted by the North West Department of Health. The Researcher is expected to arrange in advance with the chosen facilities, and issue this letter as proof that permission has been granted by the Provincial office.

This letter of permission should be signed and a copy returned to the department. By signing, the Researcher agrees, binds him/herself and undertakes to furnish the Department with an electronic copy of the final research report. Alternatively, the Researcher can also provide the Department with electronic summary highlighting recommendations that will assist the department in its planning to improve some of its services where possible. Through this the Researcher will not only contribute to the academic body of knowledge but also contributes towards the bettering of health care services and thus the overall health of citizens in the North West Province.

Kindest regards

\_\_\_\_\_  
Dr. FRM Reichel  
Director: PPRM&E

10/2/2016  
\_\_\_\_\_  
Date

\_\_\_\_\_  
Researcher

11/02/2016  
\_\_\_\_\_  
Date



Healthy Living for All



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North West Province  
REPUBLIC OF SOUTH AFRICA

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**KLERKSDORP/TSHEPONG HOSPITAL COMPLEX - OFFICE OF THE CEO**

**TO: DR MICHELLE VILJOEN  
(B.PHARM; M.SC; PHD PHARMACOLOGY)  
CENTRE OF EXCELLENCE FOR PHARMACEUTICAL SERVICES  
FACULTY OF HEALTH SCIENCES  
SCHOOL OF PHARMACY, DEPARTMENT OF PHARMACOLOGY  
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**FROM: OFFICE OF THE CEO  
K/T HOSPITAL COMPLEX**

**DATE: 18<sup>TH</sup> FEBRUARY 2016**

**SUBJECT: NWD<sub>o</sub>H APPROVAL (RESEARCH PROPOSAL)**

The above-mentioned matter refers.

This letter serves to confirm that NWD<sub>o</sub>H (PPRM&E) is responsible for all research that are taking place in the facilities of NWD<sub>o</sub>H.

All requests that are submitted directly to the facilities of health including Klerksdorp / Tshepong Hospital Complex are redirected to PPRM&E for approval.

Therefore, the approval that you have received suffices.

Hope you find this in order.

Regards,

**MR. P.E. MOKATSANE  
CHIEF EXECUTIVE OFFICER  
KLERKSDORP / TSHEPONG HOSPITAL**

**DATE: 19/2/2016**



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1

**MATLOSANA SUB DISTRICT- OFFICE OF THE PHC MANAGER**

**TO:** DR MICHELLE VILJOEN  
 (B.PHARM; M.SC; PHD PHARMACOLOGY)  
 CENTRE OF EXCELLENCE FOR PHARMACOLOGY  
 NORTH WEST UNIVERSITY  
 POTCHEFSROOM CAMPUS, SOUTH AFRICA

**CC:** JOUBERTON AREA MANAGER-ME MAHOKO-0731982995  
 (JOUBERTON CHC-SR MOTSEMME-0184653157)  
 STILFONTEIN AREA MANAGER- ME MVUNDLE-0828070986  
 (STILFONTEIN CLINIC- SR METSWAMERE-0781526730)  
 TIGANE AREA MANAGER- ME TLOBORO-0783753024  
 (PARK STEET CLINIC-SR LEDIMO-0732497961)

**FROM:** ME C LEBEKO  
 ACTING PHC MANAGER  
 MATLOSANA SUB DISTRICT

**DATE:** 22/03/2016

**SUBJECT: NWD<sub>o</sub>H APPROVAL (RESEARCH PROPOSAL)**


The above subject has reference.

This letter serves to confirm that NWD<sub>o</sub>H (PPRM&E) is responsible for all research that will be taking place in the above mentioned facilities of NWD<sub>o</sub>H.

Approval is hereby granted for Dr Viljoen to conduct the research at the identified facilities. Kindly afford her all the necessary support she needs during the research.

Hope you find this in order.

Kind regards

  
**ME C LEBEKO**  
 ACTING PHC MANAGER  
 MATLOSANA SUB DISTRICT



**DATE**



Healthy Living for All

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# ADDENDUM B1: ABSTRACT- DKK RESEARCH

## DAY: 24 AUGUST 2016

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### A retrospective analyses of adverse drug reactions of antiretrovirals in the Tlokwe District (Jan 2010 – Dec 2014)

R van Graan<sup>1</sup>, M Viljoen<sup>1</sup>, M Rheeders<sup>1</sup>, F Motara<sup>2</sup>

1. Centre of Excellence for Pharmaceutical Sciences (Pharmacem), Division of Pharmacology, North-West University, Potchefstroom, South Africa.
2. Potchefstroom Hospital Pharmacy, Chris Hani & Kruis Street, Potchefstroom. Department of Health North West, South Africa.

Corresponding author: Tel: +27 18 299 2232; Fax: +27 18 299 2225; e-mail: [michelle.viljoen@nwu.ac.za](mailto:michelle.viljoen@nwu.ac.za)

**Background:** Adverse drug reaction (ADR) reporting are often limited in developing countries and pharmacovigilance (PV) systems are poor, insufficient and intermittent. In the South African context, antiretroviral medicines consume the largest part of the PV system that is coordinated by the National Pharmacovigilance Centre (NPC) and limited feedback to the respective sub-district clusters such as the Tlokwe sub-district are experienced. The objectives of this investigation were to investigate and analyse ADRs and to report ADR frequencies that occurred in the Tlokwe sub-district from January 2010-December 2014.

**Methods:** This was a retrospective, observational, quantitative and clinical analysis of already completed ADR forms in various healthcare facilities in the Tlokwe sub-district in the North West province of South Africa from 1 January 2010 to 31 December 2014. Descriptive statistics and frequency table analysis were performed on 11 predefined ADR categories.

**Results:** A total number of 1110 credible ADR forms which included 1196 ADR cases for this 5-year time period were incorporated. Four hundred and seventeen (417) forms were not related to any type of ADR. The median (IQR) age of the patients was 39 (1-73) years and the majority (71.5%) of the ADRs were reported within the hospital setting compared to the clinics (28.5%). The majority (71%) of the cases were from female patients. Abnormal fat distribution was reported the most frequently (60%), peripheral neuropathy (21.6%) mainly due to stavudine (d4t) and thirdly renal dysfunction (6.6%) due to tenofovir use.

**Conclusions:** It was evident that nearly a third of the reported ADR forms were used to indicate regimen changes, down referrals or treatment failure and not as actual ADRs. It was not surprising that abnormal fat distribution was associated with d4t use as it formed part of the first line regimen

in SA since 2005 and it was phased out gradually from 2010 and replaced with tenofovir. Training and better communication among healthcare professionals will improve accurate ADR reporting, curb widespread underreporting and address gaps in the PV clusters and system.

**Key words:** ADRs; Pharmacovigilance, ARVs

# ADDENDUM B2: ABSTRACT- ALL AFRICA CONGRESS ON BASIC AND CLINICAL PHARMACOLOGY AND PHARMACY: 5-8 OCTOBER 2016

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## Retrospective clinical analysis of adult HIV-infected patients on AZT-based regimen

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**Purpose:** Anaemia affects 60-80% of patients presenting with advanced HIV. Highly active antiretroviral therapy (HAART) has reduced the prevalence of severe anaemia; however, mild-to-moderate anaemia continues to be prominent after 12 months on HAART. The use of HAART is associated with increased haemoglobin (Hb) concentrations and a decrease in the prevalence of HIV induced anaemia. Zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, is widely used to treat HIV/AIDS; however, due to macrocytic anemia frequently occurring when AZT therapy is ceased, we investigated such anaemia-related factors with AZT in a hospital cohort in the sub-district of Matlosana, North West.

**Methods:** Hb, MCV (mean corpuscular volume) and markers of HIV disease progression (CD4 counts, viral load (VL) and BMI (body mass index)) were retrospectively abstracted over a 12-month period from first AZT-based HAART initiation (baseline) in adult patients (n = 100) in the hospital cohort. Hb values were subjected to Wilcoxon matched-pairs signed rank and Welch's correction parametric tests. Other parameters (MCV, BMI, CD4 and VL) were analysed by the Mann-Whitney (non-parametric) test. Statistical significance ( $p < 0.05$ ) was determined through unpaired t-test by comparing males and females at baseline (BL) and month 12 respectively, and paired t-test for comparing all patients for the same time period.

**Results:** The mean (SD) age was 42.4 ( $\pm 8.919$ ) years (males, n = 41 and females n = 59). VL decreased and CD4 count increased ( $p < 0.0001$ ) in men and women at baseline compared to month 12 post AZT-based initiation. There was no significant difference in Hb over the first 12 months of therapy. Males (101.59 fL) and females (100.19 fL) at month 12 had abnormal higher mean MCV ( $p = 0.7321$ ) compared to BL therapy (91.84 and 94.83 fL) ( $p = 0.0209$ ). For both male and female patients, MCV and CD4 mean values increased from 93.55 to 100.75 fL and 208.77 to 337.83 cells/ $\mu\text{l}$  respectively ( $p < 0.0001$ ) over 12 months. BMI decreased slightly from 24.77 to 23.99  $\text{kg}/\text{m}^2$  ( $p = 0.018$ ) over the first year of AZT therapy.

**Conclusions:** The first year of AZT-based therapy for this hospital clinic cohort was associated with macrocytosis but no reduction in Hb. Understanding the role of anaemia in HIV/AIDS is necessary to reduce mortality and morbidity and frequent monitoring of anaemia-related factors is essential to prevent AZT-induced anaemia in HIV-infected patients.

# ADDENDUM B3: ABSTRACT- SOMCHAT CONFERENCE: 18 NOVEMBER 2016

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## COMPARING CLINICAL HEALTH OUTCOMES OF ADULT HIV PATIENTS: HOSPITAL CLINIC VS NIMART COHORT

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**Background:** Initially ART programmes in South Africa followed a physician-initiated and managed ART model. However, the limited number of physicians in the public sector forced a task-shifting approach from physicians to nurses to respond to the challenge to deliver ART programmes to a greater number of people. Zidovudine (AZT) is widely used to treat HIV/AIDS; however, due to macrocytic anaemia frequently occurring 4-12 weeks after AZT therapy is initiated, we investigated such anaemia-related factors with AZT and other clinical disease progression markers between a hospital clinic and NIMART cohort in the sub-district of Matlosana, North West.

**Methods:** HIV disease progression markers (CD4 counts, viral load (VL) & BMI (body mass index)) and diagnostic anaemia markers (Hb & MCV (mean corpuscular volume)) were retrospectively abstracted over a 12-month period from AZT-based HAART initiation in adult patients in a hospital clinic (n = 100) and NIMART cohort (n = 100) in order to compare these two approaches. Kaplan-Meier survival analysis was used to determine the effect of time on undetectable VL suppression. Other parameters (CD4, Hb, MCV & BMI) were analysed with linear mixed models after adjusting for age, time on ART and time since HIV diagnosis. Statistical significance ( $p < 0.05$ ) was tested for either as a time\*cohort interaction, over time or between cohorts.

**Results:** The mean (SD) age for hospital clinic and NIMART patients was 42.4 ( $\pm 8.92$ ) and 50.30 ( $\pm 1.82$ ) years respectively. CD4 cell count increased overall but indicated no significant

differences ( $p > 0.05$ ) over time ( $p = 0.562$ ,  $F = 0.582$ ), between cohorts ( $p = 0.091$ ,  $F = 2.899$ ) or for the time\*cohort interaction ( $p = 0.927$ ,  $F = 0.076$ ). BMI values were almost unchanged over the first year on AZT therapy for both cohorts. Hb for both groups reacted similarly over time with no interaction (time\*cohort;  $p = 0.835$ ,  $F = 0.180$ ) to report, thus not achieving anaemic status after 12 months on AZT. MCV however, increased dramatically over time ( $p = 0.009$ ,  $F = 5.255$ ), more specifically between baseline and month 12 ( $p = 0.008$ ) but showed no statistical differences between the groups ( $p = 0.227$ ,  $F = 1.476$ ) or indicated an interaction between time\*cohort ( $p = 0.128$ ,  $F = 2.152$ ). NIMART patients ( $n = 80$ ; 80%) were considerably more successful in achieving VL suppression (hospital,  $n = 36$ ; 36%). The estimate (mean) time for NIMART subjects to have reached VL suppression ( $5.736 \pm 5.4$  months) were almost two times faster compared to hospital clinic subjects ( $10.356 \pm 3.6$  months).

**Conclusions:** The first year of AZT-based therapy for both cohorts were associated with increased CD4 counts, decreased VLs and macrocytosis (to be expected from AZT) but no reduction in Hb and BMI. NIMART is associated with improved patient outcomes and is found to be non-inferior to physician-managed ART in this study setting.

**Key words:** NIMART, disease progression markers, AZT.

# **ADDENDUM C1: CHANGES IN THE NATIONAL CONSOLIDATED GUIDELINES FOR ART FROM 2004-2015**

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ADDENDUM C1 contains a brief summary on the changes in the national consolidated guidelines for ART (adolescents & adults) that occurred from 2004 to 2015 in all state facilities South Africa.

The summary includes changes on the following:

- CD4 cell counts;
- WHO clinical staging;
- first line regimens;
- second line regimens and
- third line regimens.

Year	CD4 cell count (cells/mm <sup>3</sup> )	WHO clinical stage	1 <sup>st</sup> line regime	2 <sup>nd</sup> line regime	3 <sup>rd</sup> line regime
2004	All HIV positive patients with a <b>CD4 &lt; 200 cells/mm<sup>3</sup></b> irrespective of WHO clinical stage, should start ART	HIV patients with <b>clinical stage IV</b> should start ART irrespective of CD4 count	<p><u>Woman <b>able</b> to guarantee reliable contraception:</u></p> <p>d4T + 3TC + <b>EFV</b></p> <p><u>Woman <b>not able</b> to guarantee reliable contraception:</u></p> <p>d4T + 3TC + <b>NVP</b></p>	<b>AZT + 3TC + LPV/r</b>	No options indicated for 3 <sup>rd</sup> line treatment
2010	All HIV positive patients with a <b>CD4 &lt; 200 cells/mm<sup>3</sup></b> irrespective of WHO clinical stage, should start ART	HIV patients with <b>clinical stage IV</b> should start ART irrespective of CD4 count	<p><u>All new patients:</u></p> <p><b>d4T + 3TC/ FTC + EFV</b></p> <p><b>or</b></p> <p><b>TDF + 3TC/ FTC + NVP</b></p>	<p><u>Failing: d4T based regimen:</u></p> <p><b>TDF + 3TC/ FTC + LPV/r</b></p> <p><u>Failing: TDF based regimen:</u></p> <p><b>AZT + 3TC + LPV/r</b></p>	Specialist referral where possible but maintain patient on failing regimen
2013 (FDC introduced)	All HIV positive patients with a <b>CD4 &lt; 350 cells/mm<sup>3</sup></b> irrespective of WHO clinical stage, should start ART	HIV patients with <b>clinical stage III or IV</b> should start ART irrespective of CD4 count	<p><b>TDF + FTC/3TC +EFV</b> (FDC preferred)</p> <p><u>Contraindication: EFV:</u> TDF + FTC/ 3TC + <b>NVP</b></p> <p><u>Contraindication: TDF:</u> <b>AZT + 3TC + EFV/ NVP</b></p> <p><u>Contraindication: TDF &amp; AZT:</u> <b>d4T + 3TC+ EFV/ NVP</b></p> <p><u>Contraindication: TDF, AZT &amp; d4T:</u> <b>ABC + 3TC + EFV/ NVP</b></p> <p><u>Currently on d4T-based regimen:</u> <b>TDF + FTC/ 3TC + EFV</b> (FDC preferred)</p>	<p><u>Failing: TDF-based 1st line regimen:</u></p> <p><b>AZT + 3TC + LPV/r</b></p> <p><u>Failing: d4T-based 1st line regimen:</u></p> <p><b>TDF + 3TC/ FTC and LPV/r</b></p>	Specialist referral and genotype resistance testing with supervised care

<p><b>2014 (Dec)</b></p>	<p>All HIV positive patients with a <b>CD4 &lt; 500 cells/mm<sup>3</sup></b> irrespective of WHO clinical stage (prioritise those with CD4 350 cells/mm<sup>3</sup>), should start ART</p>	<p>HIV patients with <b>clinical stage III or IV</b> should start ART irrespective of CD4 count</p>	<p><b>TDF + 3TC/ FTC + EFV (FDC)</b></p> <p><u>Adults and adolescents on d4T</u>  <b>Change d4T to TDF:</b>  <b>No patient must be on d4T (phased out)</b></p> <p><u>Adolescents &lt;15 years or weight &lt;40kg:</u>  ABC + 3TC + EFV</p> <p><u>Contraindication to EFV:</u>  TDF + FTC/ 3TC + NVP <b>or</b> LPV/r</p> <p><u>TDF contraindication:</u>  Creatinine clearance of &lt;50 mL/min  ABC+ 3TC + EFV (or NVP)</p>	<p><u>Failing: TDF-based first-line regimen:</u>  <b>AZT</b> + 3TC + LPV/r AZT</p> <p><b>Or</b></p> <p>TDF + 3TC + LPV/r (If HBV co-infected)</p> <p><u>Failing: d4T or AZT-based first line regimen:</u>  <b>TDF</b> + 3TC/ FTC + LPV/r</p> <p><b>Anaemia and renal failure Switch to ABC</b></p>	<p>Expert consultation and genotype resistance testing with supervised care</p>
<p><b>2015</b></p>	<p>Guidelines were revised in Aril 2015. The only changes made were ART regarding pregnant and breastfeeding woman (Revised in April 2015).</p>				

## ADDENDUM C2: WHO CLINICAL STAGING OF HIV/AIDS FOR ADOLESCENTS AND ADULTS

Clinical Stage	Clinical condition or symptom
<b>Primary HIV infection</b>	<ul style="list-style-type: none"> <li>• Asymptomatic</li> <li>• Acute retroviral syndrome</li> </ul>
<b>WHO clinical stage I</b>	<ul style="list-style-type: none"> <li>• Asymptomatic</li> <li>• Persistent generalised lymphadenopathy</li> </ul>
<b>WHO clinical stage II</b>	<ul style="list-style-type: none"> <li>• Moderate unexplained weight loss (&lt; 10 % of presumed or measured body weight)</li> <li>• Recurrent respiratory infections (sinusitis, tonsillitis, otitis media and pharyngitis)</li> <li>• Herpes zoster</li> <li>• Angular cheilitis</li> <li>• Recurrent oral ulceration</li> <li>• Papular pruritic eruptions</li> <li>• Seborrheic dermatitis</li> <li>• Fungal nail infections</li> </ul>
<b>WHO clinical stage III</b>	<ul style="list-style-type: none"> <li>• Unexplained severe weight loss (&gt; 10 % of presumed or measured body weight)</li> <li>• Unexplained chronic diarrhoea for &gt; 1 month</li> <li>• Unexplained persistent fever for &gt; 1 month (&gt; 37.6°C, intermittent or constant)</li> <li>• Persistent oral candidiasis (thrush)</li> <li>• Oral hairy leukoplakia</li> <li>• Pulmonary tuberculosis (current)</li> <li>• Severe presumed bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)</li> <li>• Acute necrotising ulcerative stomatitis, gingivitis or periodontitis</li> <li>• Unexplained anaemia (Hb &lt;8 g/Dl)</li> </ul>

	<ul style="list-style-type: none"> <li>• Neutropenia (neutrophils &lt; 500 cells/<math>\mu</math>L)</li> <li>• Chronic thrombocytopenia (platelets &lt; 50 000 cells/<math>\mu</math>L)</li> </ul>
<p><b>WHO clinical stage IV</b></p>	<ul style="list-style-type: none"> <li>• HIV wasting syndrome (as defined by the CDC)</li> <li>• Pneumocystis pneumonia</li> <li>• Recurrent severe bacterial pneumonia</li> <li>• Chronic herpes simplex infection (orolabial, genital or anorectal site for &gt; 1 month, orovisceral herpes at any site)</li> <li>• Oesophageal candidiasis (or candidiasis of trachea, bronchi, or lungs) • Extra pulmonary tuberculosis</li> <li>• Kaposi sarcoma</li> <li>• Cytomegalovirus infection (retinitis or infection of other organs)</li> <li>• Central nervous system toxoplasmosis</li> <li>• HIV encephalopathy</li> <li>• Cryptococcosis, extra pulmonary (including meningitis)</li> <li>• Disseminated non-Tuberculosis mycobacteria infection</li> <li>• Progressive multifocal leukoencephalopathy</li> <li>• Candida of the trachea, bronchi, or lungs</li> <li>• Chronic cryptosporidiosis (with diarrhoea)</li> <li>• Chronic isosporiasis</li> <li>• Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis)</li> <li>• Recurrent non-typhoidal Salmonella bacteraemia</li> <li>• Lymphoma (cerebral or B-cell non-Hodgkin)</li> <li>• Invasive cervical carcinoma</li> <li>• Atypical disseminated leishmaniasis</li> <li>• Symptomatic HIV-associated nephropathy</li> <li>• Symptomatic HIV-associated cardiomyopathy</li> <li>• Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)</li> </ul>

# **ADDENDUM D: CASE REPORT FORM (CRF)**

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ADDENDUM D contains the case report form (CRF) used during clinical data collection in the sub-district of Matlosana, North West.

Pt CODE: #

RETROSPECTIVE (CRF): NMARTHOSPcohort-HIV&AZT

<b>Date of data collection:</b> Ex. DD/YY/MM	<b>Site (encircle):</b> Hospital/ clinic
<b>Date of HIV diagnosis:</b>	<b>Date of ART initiation:</b>
<b>Period of treatment:</b>	

DEMOGRAPHIC INFORMATION	
<b>Gender (M/F)</b>	
<b>Patient code</b>	Ex. N/H - 24 - 09 - 27 - F - 1
<b>Weight (kg):</b>	
<b>Length (meter):</b>	
<b>Does patient satisfy inclusion/exclusion criteria?</b>	YES/NO

MAJOR MARKERS FOR HIV DISEASE PROGRESSION					
HAEMATOLOGY					
LABORATORY DRUG MONITORING	VALUE				
<b>1. Full Blood Count (FBC)</b>					
	<b>M-0</b>	<b>M-1</b>	<b>M-3</b>	<b>M-6</b>	<b>M-12</b>
A. Hb					
B. MCV					
2. CD4 cell count					
3. Viral load (VL)					
POSSIBLE ASSOCIATED RISK FACTORS WITH DISEASE PROGRESSION					
BMI (own calculation)					
WHO Clinical stage <i>(if applicable)</i>					
OTHER					
Creatinine clearance					

Pt CODE: #

RETROSPECTIVE (CRF): NMARTHOSPcohort-HIV&AZT

TB/HIV CO-INFECTION STATUS (indicate with X)							
History of TB infection				Current TB infection			
<b>Received treatment?</b>				<b>Receiving treatment?</b>			
YES		NO		YES		NO	
PARAMETER ABNORMAL (indicate with X)							
	Hb	MCV	CD4	VL	BMI	CrCl	WHO CS
0							
1							
3							
6							
12							
TREATMENT FAILURE (YES/NO)							
Virological failure on 1 <sup>st</sup> line regime				YES/NO			
Virological failure on 2 <sup>nd</sup> line regime				YES/NO			
ART							
Received: Fixed dose combination (FDC)?				YES/NO			
Zidovudine (AZT) containing regime)?				YES/NO			
1 <sup>st</sup> line regimen (if applicable)							
<b>Drug combination:</b>							
<b>Regimen change?</b>							
2 <sup>nd</sup> line regimen (if applicable)							
<b>Drug combination:</b>							

HOSPITALISATION OF PATIENTS		
Number of hospital admissions (while on AZT & why)?		
TREATMENT OUTCOME (indicate with X)		
Dead before 6 months?	Alive at 6 months	Still receiving Rx
MEDICATION (RX) HISTORY (any current/ previously used drugs either for chronic diseases or opportunistic infections)		
Drug name	Type of disease/ opportunistic infection	Dosage and treatment period
1.		
2.		
3.		
4.		
5.		
6.		

# **ADDENDUM E1: DIFFERENCES BETWEEN CLINICAL VARIABLES FOR THE COHORTS AT DIFFERENT TIME POINTS - BEFORE ADJUSTING FOR CONFOUNDING VARIABLES**

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Table 1 represents all analysed data for clinical variables as described under section 4.2.2.1 and 4.2.2.2 in Chapter 4.

The following additional information (not disclosed in Chapter 4) is presented for all clinical variables at baseline, month 1, 3, 6 and 12:

- Outcome for Shapiro-Wilk test for normality (distribution of data)
- Test done based on data distribution: Parametric or non-parametric (Mann-Whitney)
- For statistical significance ( $p < 0.05$ ):
  - Outcome for Levene's test for equality of variances in case of parametric tests
  - Wilcoxon W in case of non-parametric (Mann-Whitney) tests
- Cohen's  $d$  values for practical significance

**Table 1:** Clinical variables for cohorts on different time points before adjusting for confounding variables

Variable & Time period (month)*	Mean ± SD		n		Shapiro-Wilk Test (Data distribution)	Test done based on data distribution	Levene's test for equality of variances / Wilcoxon W test	p-value	Cohen's <i>d</i>
	Hospital clinic	NIMART	Hospital clinic	NIMART					
<b>Hb BL</b> (g/dL)	12.31 ± 1.98	12.78 ± 1.70	95	83	Normal	Parametric independent t-test	Equal variances assumed	0.90	0.24
Hb 1	11.41 ± 1.82	11.27 ± 1.40	18	3	Normal	Parametric independent t-test	Equal variances assumed	0.90	0.08
Hb 3	11.73 ± 13.11	13.11 ± 1.41	20	8	Normal	Parametric independent t-test	Equal variances assumed	0.08	0.71
Hb 6	12.26 ± 1.93	13.21 ± 1.59	37	26	Normal	Parametric independent t-test	Equal variances assumed	0.04	0.49
Hb 12	12.70 ± 2.09	13.37 ± 1.45	57	42	Normal	Parametric independent t-test	Equal variances <b>not</b> assumed	0.06	0.32
<b>MCV BL</b> (fL)	93.55 ± 7.90	104.53 ± 14.39	91	74	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	< 0.0001	0.76
MCV 1	100.77 ± 10.80	92.13 ± 9.15	12	3	Normal	Parametric independent t-test	Equal variances assumed	0.228	0.80
MCV 3	101.06 ± 7.97	111.40 ± 11.18	21	6	Normal	Parametric independent t-test	Equal variances assumed	0.008	1.30
MCV 6	99.59 ± 17.29	112 ± 12.74	35	22	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.001	0.72
MCV 12	100.75 ± 16.28	107.99 ± 18.45	55	41	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.001	0.39
<b>BMI BL</b> (kg/m <sup>2</sup> )	24.77 ± 7.69	26.46 ± 7.89	79	14	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.41	0.21

BMI 1	24.09 ± 6.90	27.011 ± 8.51	28	7	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.248	0.34
BMI 3	2.01 ± 5.43	26.34 ± 7.78	49	9	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.209	0.43
BMI 6	26.00 ± 7.24	26.21 ± 6.61	43	12	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.783	0.03
BMI 12	23.99 ± 6.02	26.37 ± 6.92	45	10	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.156	0.34
<b>CD4 BL</b> (cells/μl)	208.80 ± 183.02	375.60 ± 275.35	93	93	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	< 0.0001	0.61
CD4 1	217.90 ± 173.05	392.20 ± 366.45	9	5	Normal	Parametric independent t-test	Equal variances assumed	0.24	0.48
CD4 3	239.09 ± 210.00	510.20 ± 261.53	11	10	Normal	Parametric independent t-test	Equal variances assumed	0.016	1.04
CD4 6	402.10 ± 450.39	485.12 ± 259.40	30	40	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.08	0.10
CD4 12	337.83 ± 212.50	554.64 ± 283.20	57	60	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	< 0.0001	0.77
<b>VL BL</b> (copies/ml)	176709± 275877	62278 ± 225623	94	82	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	< 0.0001	0.41
VL 1	46854 ± 90098	9197 ± 133145	9	8	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.658	0.34
VL 3	6194 ± 16123	11240 ± 34213	15	11	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.37	0.15
VL 6	40843 ± 126317	904 ± 3161	34	44	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	0.001	0.32
VL 12	34838 ± 99943	1022 ± 5875	58	70	Not normal	Non-parametric Mann-Whitney U-test	Wilcoxon W	< 0.0001	0.34

\***BL**, baseline; **Hb**, haemoglobin (g/dL); **MCV**, mean corpuscular volume (fL); **CD4** (cells/μl); VL, viral load (copies/ml).

# ADDENDUM E2: GENDER DIFFERENCES WITHIN THE RESPECTIVE COHORTS AT BASELINE AND 12 MONTHS POST-AZT INITIATION

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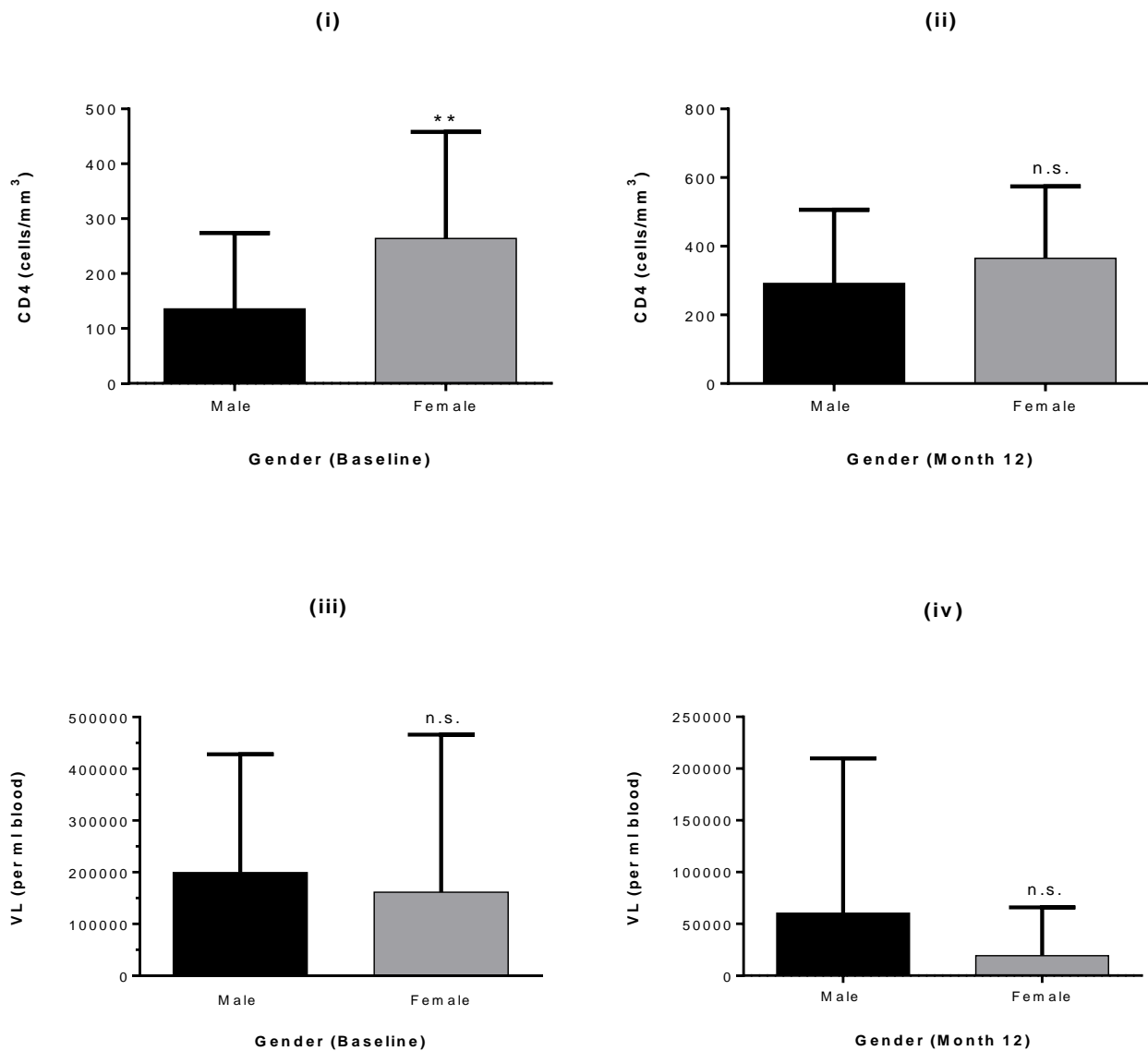
The following data analysis was performed:

- CD4 cell counts and VLs (Mann-Whitney for male vs female at baseline and 12 months post-AZT initiation);
- Hb (independent  $t$  -test) and MCV (Mann-Whitney) for male vs female at baseline and 12 months post-AZT initiation;
- BMI (Mann-Whitney) for male vs female at baseline 12 months post-AZT initiation.

## **CD4 cell counts and viral loads for male vs female at baseline and at 12 months post-AZT initiation in both cohorts**

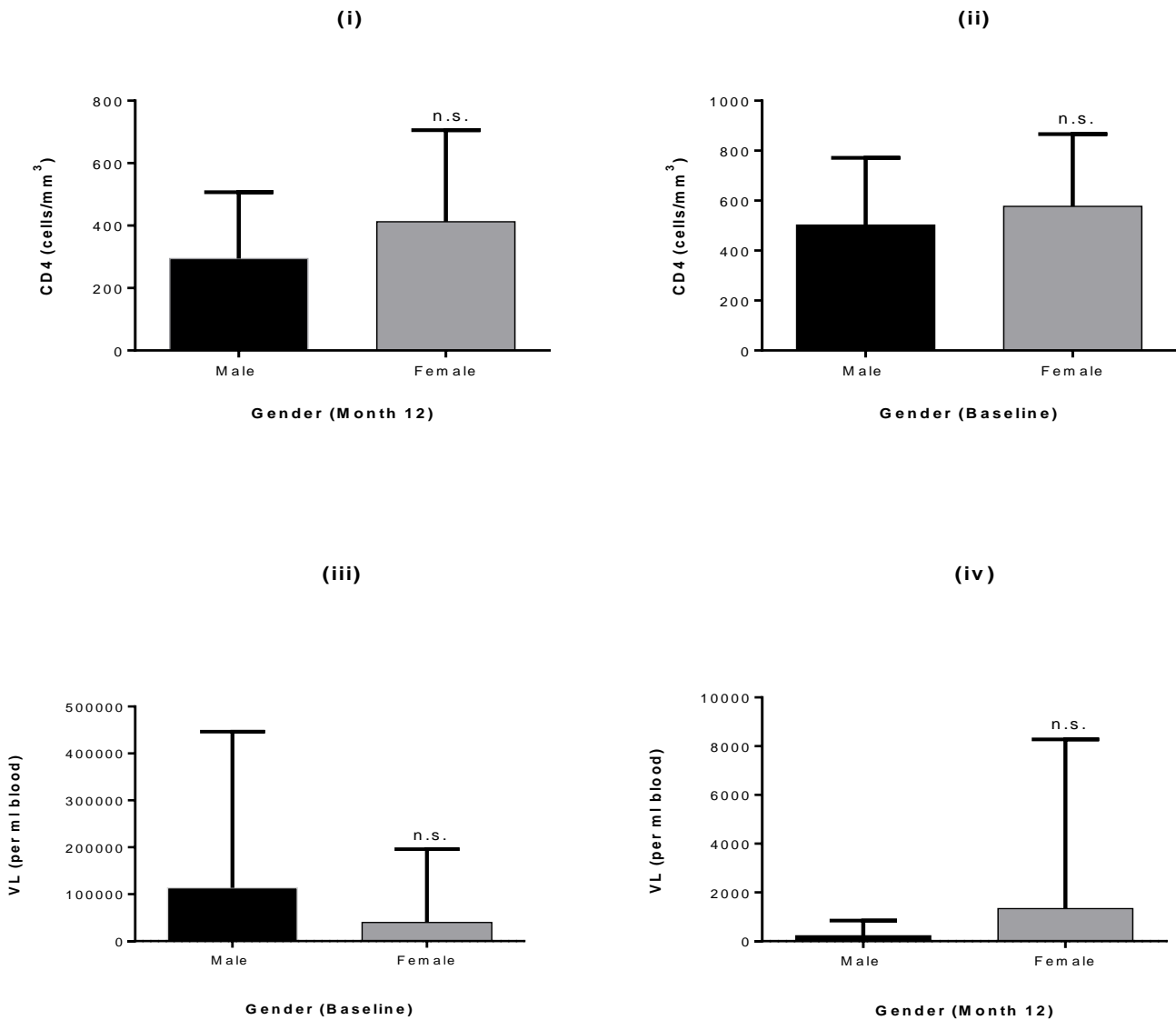
### *a) Hospital clinic*

At baseline, females (median, 206 cells/mm<sup>3</sup>) had significantly higher ( $p = 0.0004$ ) CD4 cell counts compare to men (124 cells/mm<sup>3</sup>). CD4 increased for both genders over time, although not significantly between males (256 cells/mm<sup>3</sup>) and females (330 cells/mm<sup>3</sup>) at month 12 post-AZT initiation (Figure A, i & ii). Figure A (iii) and (iv) depicts median VLs according to gender over time and shows males (105 699 copies per ml blood) presenting with higher viral copies than women (48 238 copies) at baseline. Viral loads decreased vastly over the first 12 months on AZT with no significant difference ( $p > 0.05$ ) in gender (men, 162 viral copies; women, 194).



**Figure A:** Unpaired non-parametric comparison for male vs female within the hospital clinic cohort at baseline (i & iii) and month 12 (ii & iv) post-AZT initiation w.r.t. CD4 cell counts (i & ii) and viral copies per ml blood (iii & iv). **n.s., not significant (p > 0.05), \*\* p < 0.001.**

a) NIMART cohort



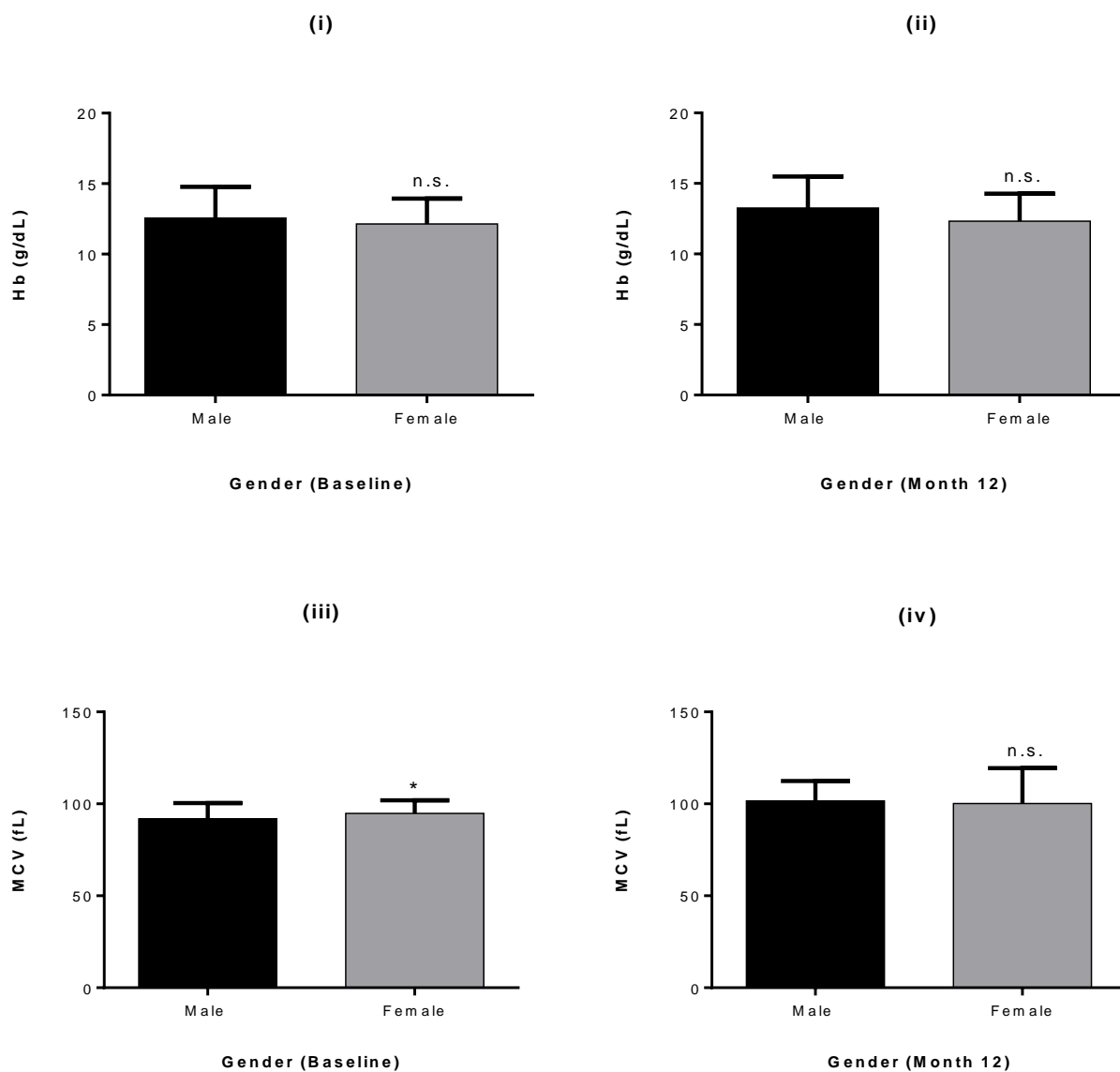
**Figure B:** Unpaired non-parametric comparison for male vs female within the NIMART cohort at baseline and month 12 post-AZT initiation w.r.t. CD4 cell counts (i & ii) and viral copies per ml blood (iii & iv). **n.s., not significant ( $p > 0.05$ ).**

In comparison with the hospital clinical cohort, no significant differences were found between genders in the NIMART cohort ( $p < 0.05$ ). Figure B illustrates median CD4 cell counts and VLs for men and women within the NIMART cohort over time. The disease progression (i & iii) marker increased for both men (290 to 491.50 cells/mm<sup>3</sup>) and women (344.50 to 487 cells/mm<sup>3</sup>) from baseline to month 12 with no significance differences found. Viral loads (iii & iv) were higher for

men at baseline but lower at month 12 compared to women who also showed a slight increase in median VL copies 12 months after AZT initiation.

### Hb and MCV for male vs female at baseline and at 12 months post-AZT initiation in both cohorts

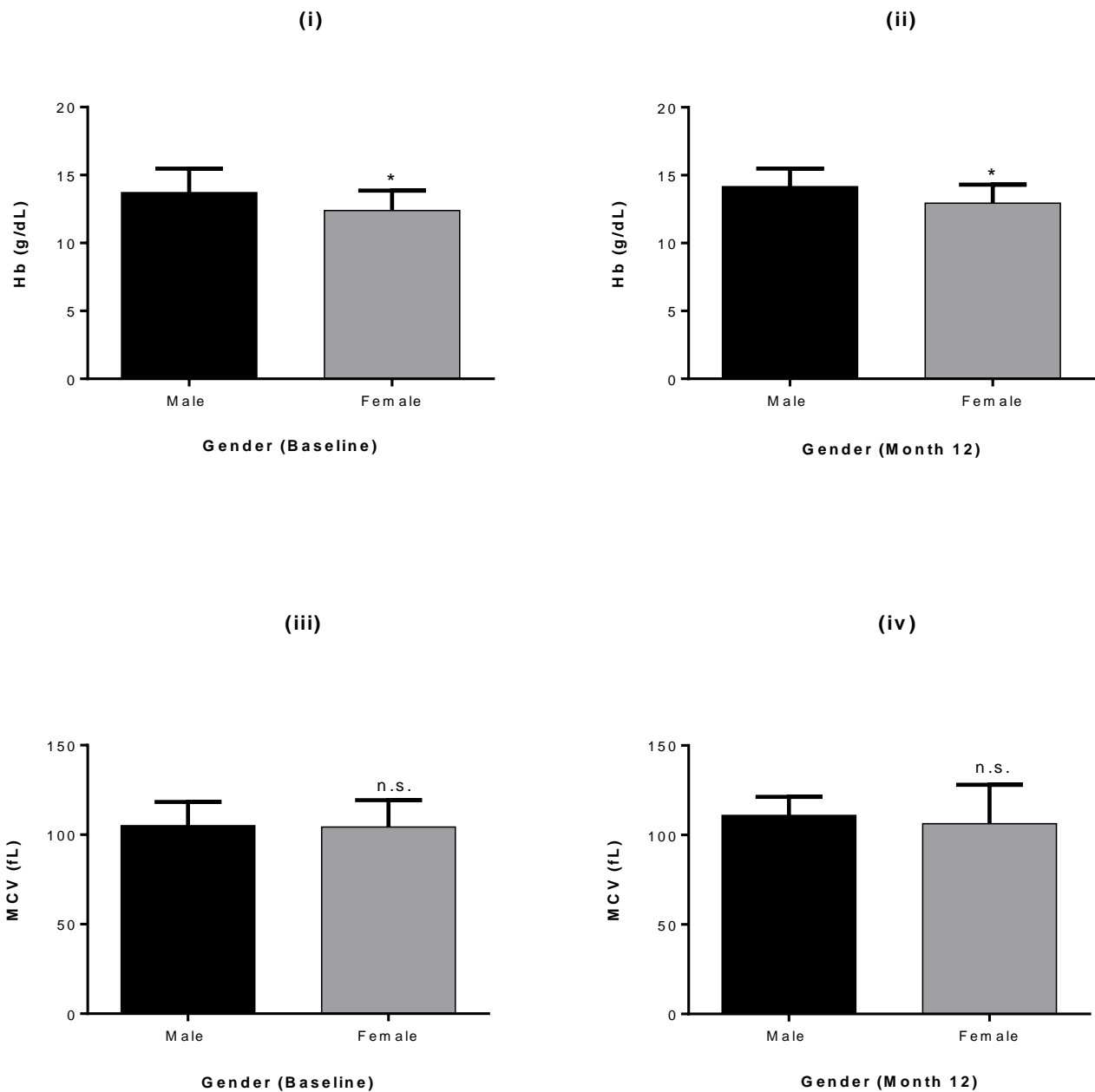
a) Hospital clinic



**Figure C:** Unpaired comparison for male vs female within the hospital clinic cohort at baseline and month 12 post-AZT initiation w.r.t. Hb levels (parametric - independent t-test) (i & ii) and MCV levels (iii & iv) (Mann-Whitney test). **n.s., not significant ( $p > 0.05$ ); \*  $p < 0.05$ .**

Figure C indicates mean Hb and median MCV levels over time for male and female patients within the hospital clinic cohort. Hb levels, although relatively similar for both genders, were slightly higher for the male study subjects (12.56 g/dL) at baseline (females, 12.14 g/dL). At month 12, levels between men (13.26 g/dL) and women (12.33 g/dL) assumed a larger, although not significant difference (Figure C, ii) but increased none the less. With regard to MCV at baseline (iii), woman (94.60 fL) showed significantly higher ( $p = 0.021$ ) levels than men (91.80 fL). After a year on AZT, MCV levels increased dramatically for both genders, with the male population (102.90 fL) showing higher, non-significant values when compared to females (101 fL).

b) NIMART cohort



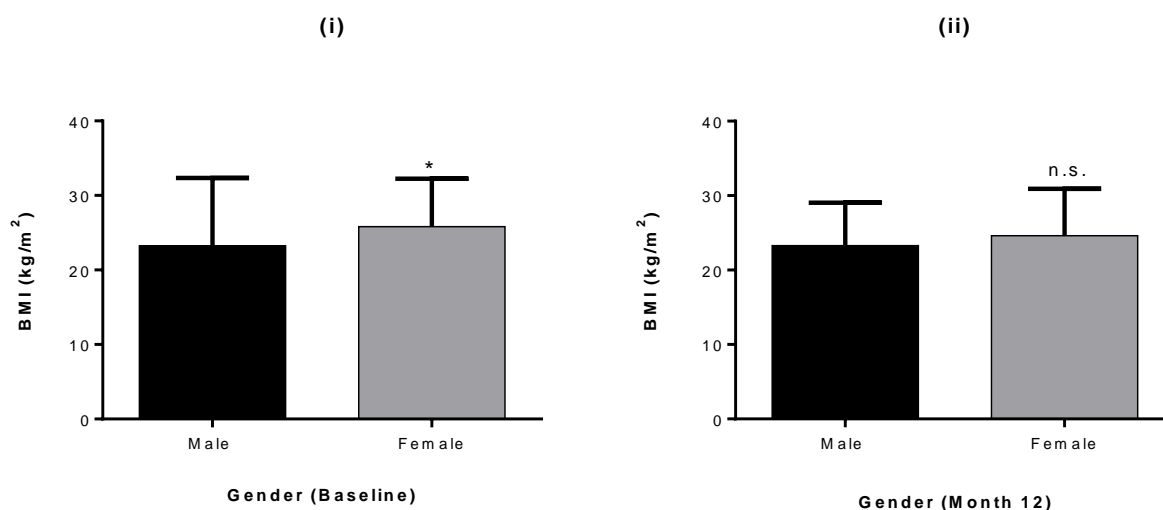
**Figure D:** Unpaired comparison for male vs female within the NIMART cohort at baseline and month 12 post-AZT initiation w.r.t. Hb levels (parametric - independent t-test) (i & ii) and MCV levels (iii & iv) (Mann-Whitney test). **n.s.**, not significant ( $p > 0.05$ ); \*  $p < 0.05$ .

Baseline and month 12 in Figure D (i) and (ii) illustrates higher mean Hb values for men (13.71; 14.14 g/dL) compared to woman (12.39; 12.94 g/dL) although levels increased over 12 months on AZT. Woman showed significantly lower levels compared to men for both time points ( $F = 1.448$ ,  $p = 0.0022$  for baseline and  $F = 1.042$ ,  $p = 0.0089$  for month 12 respectively). This is in line with human physiology and most publications. In terms of MCV (Figure D, iii & iv), median levels were almost similar for both men (106.3; 113.8 fL) and woman (108.3; 111.6 fL) at both time periods with no significant differences. Men showed lower median MCV values at baseline but higher values at month 12 compared to woman.

### **BMI for male vs female at baseline and at 12 months post-AZT initiation in both cohorts**

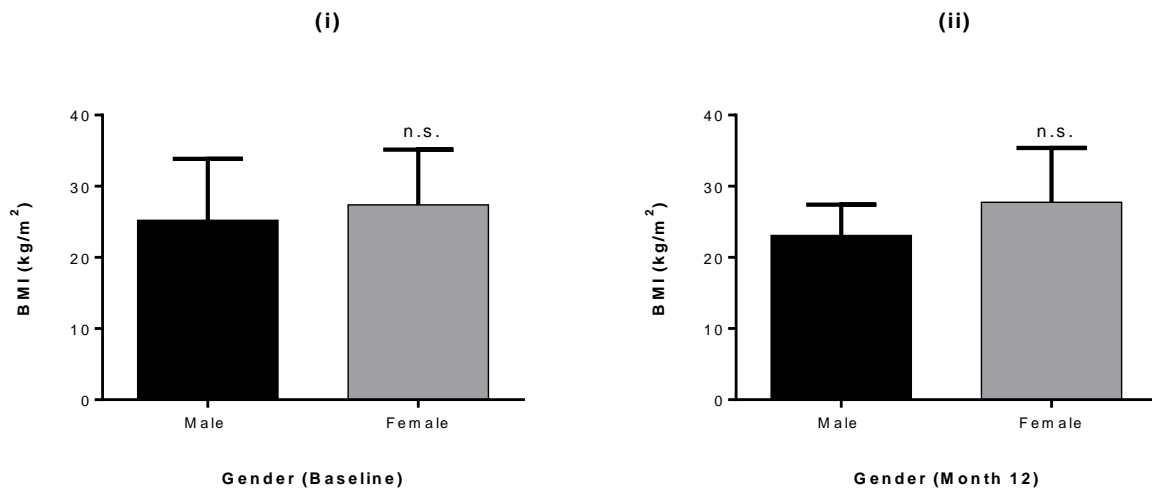
Mean BMI (Figure E) increased for males (20.66 to 21.77 kg/m<sup>2</sup>) but decreased for females (25.60 to 22.38 kg/m<sup>2</sup>) from baseline to month 12. However, women showed BMI values significantly higher ( $p = 0.017$ ) than men on AZT initiation, a characteristic not seen at month 12 ( $p > 0.05$ ).

#### a) Hospital clinic



**Figure E:** Unpaired non-parametric comparison for male vs female within the hospital clinic cohort at baseline and month 12 post-AZT initiation w.r.t. BMI (i & ii). **n.s., not significant ( $p > 0.05$ );** \*  **$p < 0.05$ .**

b) NIMART cohort



**Figure F:** Unpaired non-parametric comparison for male vs female within the NIMART cohort at baseline and month 12 post-AZT initiation w.r.t. BMI (i & ii). **n.s., not significant ( $p > 0.05$ );** \*  **$p < 0.05$ .**

Within the NIMART cohort, Figure F indicates that woman maintain a higher mean BMI over time although not significantly ( $p > 0.05$ ) different compared to men. For male patients, mean BMI values decreased (24.06 to 22.70 kg/m<sup>2</sup>) over the one-year period on AZT while female patients appeared to show increased mean BMI values (24.68 to 25.95 kg/m<sup>2</sup>).

# ADDENDUM F1: MANUSCRIPT A (ADDITIONAL RESEARCH PROJECT AS PART OF A QIP)

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In this addendum, a manuscript titled:

## **“Retrospective clinical analysis of adverse drug reactions associated with antiretroviral therapy in Tlokwe sub-district in South Africa”**

is presented. The manuscript was submitted to *African Journal of AIDS Research* (IF: 0.716) (publishing articles that makes an original contribution to the understanding of social dimensions of HIV/AIDS in African contexts) as a full-length research paper. The complete feedback report (regarding **all** reported ADRs) was submitted to Potchefstroom Hospital as part of the quality improvement project on 19 October 2016 and is not included herewith.

This manuscript is prepared and presented according to the *Instructions to the Authors* outlined on the journal website:

<http://www.nisc.co.za/products/1/journals/african-journal-of-aids-research>

This manuscript will include the title page and contributing authors with their respective affiliations followed by the abstract. This will be followed by keywords, the main body of the manuscript including the background (introduction), methods, data and results, discussion, conclusions, declarations, references, figures and tables. As per the journal submission format, all figures and tables are separate and placed at the end of the manuscript.

## Retrospective clinical analysis of adverse drug reactions associated with antiretroviral therapy in Tlokwe sub-district in South Africa

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### Abstract

**Background:** South Africa had 3.4 million people on antiretroviral therapy (ART) at the end of 2015, making it the country with the highest number of people on ART globally. Limited numbers of trained healthcare professionals and inadequate knowledge on how adverse drug reaction (ADRs) should be identified and reported, were some of the obstacles identified in effective reporting of ADRs in resource limited countries. This investigation was part of an internal quality improvement project aimed to investigate, analyze and classify the prevalence of reported ADRs.

**Methods:** This observational study utilized a quantitative descriptive design to investigate retrospective ADR forms completed by healthcare professionals in various healthcare facilities in the Tlokwe sub-district, South Africa (January 2010 to December 2014). Descriptive and inferential analyses were incorporated to describe the variables.

**Results:** A total of 1133 forms were analysed of which 770 comprised ART related ADRs. The mean age was 40.1 ( $\pm$  10.1%) years with significantly higher ADRs reported by females (70.8%, n = 551). Physicians reported 99% compared to only 1% of ADRs reported by registered nurses. The majority of these were more likely to have been reported in the hospital (46.6%) setting compared to clinic settings (15.6%). Abnormal fat distribution (58%), peripheral neuropathy (21.6%) and renal dysfunction (6.6%) were most frequently reported. Females presented at significantly younger age with abnormal

fat distribution ( $38.1 \pm 4.6$  vs.  $43.4 \pm 5.7$  years,  $p < 0.0001$ ) and peripheral neuropathy ( $39.7 \pm 1.1$  vs.  $45.1 \pm 9.2$  years,  $p < 0.001$ ) compared to males. Gender was statistically and practically significant ( $p < 0.001$  and Cramer's  $V = 0.3$ ) for all three of the major reported ADRs. Stavudine was the most reported causative drug (74.2%) responsible for ADRs.

**Conclusion:** Efforts should be made to train and create more awareness among registered nurses in this sub-district. Gender was highly dependent between the major reported ADR categories and females presented with abnormal fat distribution and peripheral neuropathy at a significantly earlier age compared to their male counterparts. This investigation corroborates the policy change during 2010 to omit stavudine from ART regimens by the National Department of Health. This retrospective analysis can now also serve as a platform for future ADR studies within the sub-district.

**Keywords:** Antiretroviral therapy, Adverse drug reporting, Adverse drug reactions, Drug safety, Pharmacovigilance, Developing countries, North West South Africa

## Introduction

Pharmacovigilance relates to the science involved in the demodulation, management, assessment and ultimately minimization of risks and prevention of ADRs (WHO, 2002). It can be described as a dynamic umbrella term that holds the key to effective ADR monitoring (Jeetu & Anusha, 2010).

In South Africa, the Medicines Control Council (MCC) and the National *Pharmacovigilance Centre (NPC)*, as part of the *National Department of Health (NDoH)*, facilitate healthcare professionals in the reporting of ADRs associated with the use of registered medicine, the management of safety data which arise during clinical trials, spontaneous reporting and post-marketing surveillance (MCC, 2013). Public health pharmacovigilance promotes risk minimizing of preventable ADRs whereas institutional or clinical pharmacovigilance targets hospitals and clinics aiming to reduce mortality and morbidity associated with ADRs (Mehta et al., 2014).

Inadequate knowledge and training by healthcare professionals on how ADRs should be identified and reported were identified as obstacles in effective reporting of ADRs in some

of the limited studies performed in South Africa. These studies found that newer drugs may be reported as safe out of insufficient experience and knowledge from clinicians (Suleman, 2010), limited number of doctors, nurses and pharmacists in charge of pharmacovigilance systems (WHO, 2007) and on-going routine monitoring of ADRs were also neglected (Mehta, 2011).

Surveys conducted on the status of pharmacovigilance in South Africa confirmed that the communication and analysis of safety data can be improved by collective strengthening efforts in all current and future programs (Mehta, 2014). The problematic occurrence of widespread underreporting of ADRs and the importance of addressing gaps effectively in pharmacovigilance activities in the public healthcare setting needs to be appropriately addressed (Ruud, Srinivas, Toverud, 2010).

Furthermore, knowledge regarding antiretroviral (ARV) toxicity is limited in developing countries and the coverage poor, insufficient and intermittent. These toxicities can result in unknown long term effects and damage patient confidence and adherence (WHO, 2007; Kenny, Musiime, Judd & Gibb, 2012). A study conducted in the Cape Town metropolitan area at the New Somerset Hospital in South Africa indicated that the pharmacovigilance system has evolved since the introduction of combination of antiretroviral therapy (ART) but the focus should be shifted to implementing the programs on a more practical and clinical basis to ensure enhanced efforts for the optimization of patient benefits (Mehta, 2007).

With the highest prevalence of HIV-infections in the world, South Africa's ART program consumes the biggest part of the South African pharmacovigilance system (Ruud, Srinivas, Toverud, 2010). The morbidity and mortality caused by this epidemic are globally well known (Dheda et al., 2013). The recent Global AIDS update indicated that 3.4 million people were on ART in South Africa making it the country with the highest number of people on ART compared to the 17 million globally at the end of 2015 (UNAIDS, 2016).

Patients with epidemic diseases like tuberculosis (TB) and HIV have an increased risk of presenting with ADRs that can be attributed to the safety profile of complex ARV and anti-TB regimens and the compromised immune system of HIV-infected patients. The out roll of the ART program in South Africa highlighted the significance of targeted monitoring of the safety of the medicines used in patients and to develop targeted pharmacovigilance

systems to address and solve the particular problems with the drugs commonly used in ARV regimens (WHO, 2007).

The public and private healthcare systems in South Africa have a pharmacovigilance system for the reporting of ADRs and are obligated to report these to the MCC and /or NPC. Various initiatives have been established to link and integrate programmatic, regulatory and clinical pharmacovigilance activities and it became clear that a national consolidated plan was needed (Mehta et al., 2014). Currently in the South African public sector the majority of ADRs are reported spontaneously. This data is then collectively used in the national pharmacovigilance database to enable change in national health policies if and when required. All reported ADRs from the Tlokwe sub-district are sent to Potchefstroom Hospital from where the pharmacy manager or designated pharmacist sends it off to MCC or NPC. The Tlokwe sub-district identified the need to self-analyse and summarize the prevalence of already reported ADRs within the sub-district for future reference. The NPC also acknowledged that feedback was sometimes substandard and not timeous (Dheda, 2013).

Collaboration between Department of Pharmacology (North-West University) and Potchefstroom Hospital pharmacy was initiated to perform an internal quality improvement project (QIP) to retrospectively investigate, analyse and classify all the reported ADRs from the Tlokwe sub-district from 1 January 2010 to 31 December 2014. The main focus however for this paper was on the prevalence of antiretroviral ADRs.

## **Methods**

### ***Study design and setting***

This observational study utilized a quantitative design to describe retrospective data from ADR forms completed by healthcare professionals in various healthcare facilities (hospitals and primary healthcare facilities) from 1 January 2010 to 31 December 2014. The Tlokwe sub-district is one of four local municipalities within the Dr Kenneth Kaunda District (DKKD) in the North West. Potchefstroom Hospital is the main site for the distribution of medicines to the respective Tlokwe areas which include nine primary healthcare (PHC) clinics. The inclusion criteria for this analysis were: reporting from 1 January 2010 to 31 December 2014, date of birth, date on which treatment was discontinued if the ADR so required it and signature of a registered healthcare

professional. Forms were excluded from this analysis if they were used to indicate regimen changes due to virological failure or pregnancy, regarded as incorrect reporting due to out-phasing of stavudine, down referral to clinics or due to patient non-adherence.

### ***Data***

The following information was captured from completed ADR forms onto an electronic database: age, gender, hospital or clinic setting, date and reporter (doctor, pharmacist or registered nurse) of the ADR, date of onset of the ADR, name of suspected drug/s responsible for the ADR and the type of ADR reported (divided into eleven broad predefined categories, see Table 2). Data were recorded using only one electronic data collection tool in the form of a set designed Excel<sup>®</sup> spread sheet. To add significant integrity and validity to the handling process of the data recorded, 20% of randomly sampled data were re-entered and all data double checked. This reduced the possibility of ADR duplication from the same patient and assisted in excluding errors of possible omissions. Patient records were anonymised by removing any personal identifiers prior to capturing onto the electronic data base.

### ***Statistical analysis***

Descriptive statistics were used to describe and summarize the clinical and demographic characteristic of ADRs. Mean (standard deviation ( $\pm$ SD)) was incorporated for variables that were normally distributed and median (interquartile range (IQR)) were calculated for variables not normally distributed. Statistical significance was set at  $p < 0.05$ .

Frequencies and percentages were used for categorical variables. The categorical variables of the three most frequent ADR categories, fat distribution, peripheral neuropathy and renal dysfunction, were compared using a non-parametric Mann-Whitney test. Pearson's Chi-squared test was applied to compare categorical variables between groups. Data analysis was performed using Statistica<sup>®</sup> version 13.0 with the consultative guidance of a statistician from North-West University Potchefstroom Campus.

## Results

### *Patient demographics*

A total of 1295 ADR forms from 1 January 2010 to 31 December 2014 were evaluated. After applying the set inclusion criteria only 1195 ADR cases from 1110 patients (eligible ADR forms) remained. However, a further cleaning up of the reported ADR forms resulted in 5.2% (n = 62) excluded due to incomplete data, regimen changes due to virological failure (14.4%, n = 172), pregnancy (2.6%, n = 31) and incorrect reporting (12.7%, n = 152) which failed to meet the inclusion criteria due to missing data.

Figure 1 illustrates the number of collected ADR forms and how they were assessed according to the inclusion and exclusion criteria to derive to the total number of correctly reported ADRs (n = 770) associated only with ART which comprised the largest portion (99%). Only 1% (n = 8) of the reported ADRs were not related to ART during this 5-year observation period.

The majority of ADRs (71.5%, n = 556) were reported during 2011 and the least number (0.6%, n = 5) were reported during 2010. During 2012 (n = 112, 14.4%), 2013 (n = 64, 8.2%) and 2014 (n = 41, 5.3%), the frequency of reported ADRs decreased significantly. Surprisingly only 0.7% of patients who reported ADRs were TB-HIV co-infected and thus concurrently received ART and TB treatment.

Table 1 presents the patient demographic data for the reported period 2010 to 2014, indicating that 70.8% of ADRs were reported by females with the mean total population age 40.1 ( $\pm 10.1\%$ ) years.

### *ADRs reported and gender*

The ADRs were classified into eleven categories of which the frequencies of ADRs per category, according to gender are reflected (Table 2). The three most reported ADRs were abnormal fat distribution (58%, n = 451), peripheral neuropathy (21.6%, n = 168) and renal dysfunction (6.6%, n = 51). Abnormal fat distribution comprised the highest frequency of ADRs in females (46%, n = 358) and men (9.9%, n = 77), followed by peripheral neuropathy in women (12.5%, n = 97) and in men (7.8%, n = 61) and thirdly renal dysfunction with a slight tendency to occur more in men (3.2%, n = 25) compared

to women (3.0%, n = 23). Females had the highest reported frequencies in eight of the eleven categories and renal dysfunction was slightly higher (3.2% vs. 3%) in men.

### ***ADRs reported and healthcare professional stratification***

Table 3 reports on the frequencies of ADRs stratified by healthcare professionals (doctors or professional nurses) in either a hospital or clinic facility. Significantly more doctors (98.8%) reported ADRs compared to only 1.0% of the professional nurses. During this period, no pharmacists were responsible for reporting any ADRs. The majority of ADRs reported were from the hospital (46%, n = 363) setting compared to the clinics (15.6%, n = 121) although a large number (37.8%, n = 294) did not specify the actual facility.

### ***ADRs and associated ARVs***

The frequencies of the respective ARVs responsible for the reported ADRs are summarised (Figure 2). Stavudine (d4t) was clearly the ARV with the most (74.2%, n = 577) reported ADRs. It was followed by a combination of three or more ARV drugs (5.7%, n = 44), TDF most commonly known to cause renal toxicity (5.0%, n = 39), AZT the major cause of anaemia and peripheral neuropathy in susceptible patients (4.4%, n = 34), the FDCs (3.2%, n = 25), NVP responsible for inducing liver abnormalities (3.1%, n = 24) and EFV responsible for central nervous system associated side effects (2.2%, n = 17). Adverse drug reactions that could not be attributed to a causative drug, reported as 5.7% (n = 44), are not reflected in figure 2 because they were not specified.

### ***ADRs and stratification according to age and gender***

Additional stratification of the results according to mean ( $\pm$ SD) age and gender are depicted (Figure 3) for the three major reported ADR categories (abnormal fat distribution, peripheral neuropathy and renal dysfunction). Age was highly significantly ( $p < 0.0001$ ) different between female (38.1 years  $\pm$  4.95) and male (43.4 years  $\pm$  5.66) for abnormal fat distribution and statistically significant for peripheral neuropathy (females at 39.7 years  $\pm$  1.41 and males at 45.1 years  $\pm$  9.19,  $p = 0.0011$ ). However, no significant difference was found between the age of the genders for the reporting of renal dysfunction ( $p = 0.66$ ).

Additional analysis with Pearson's Chi-squared test indicated statistically significant ( $p < 0.0001$ ) dependence between gender and for all three reported categories (renal

dysfunction, abnormal fat distribution and peripheral neuropathy) as well as medium practical significance with a Cramer's V-value of 0.28.

## **Discussion**

Adverse drug reactions with primary focus on ART had not been specifically studied in the Tlokwe sub-district before. This study aimed at describing the frequencies of reported ADRs related to ART and more specifically characterizing the clinical impact on patients as South Africa had the largest number of people on ART in the world at the end of 2015 (UNAIDS, 2015).

Only 65% (n = 778 out of 1195 cases) of all the reported ADR cases during Jan 2010 to Dec 2014 were used in this retrospective statistical analysis. It was evident that ARV related ADRs were predominant as only a small percentage (1%) of non-ARV related ADRs were reported. The mean age (SD) of these patients was 40.1 ( $\pm$ 10.1) years. There was a higher rate of ADR occurrences (n = 634, 81.5%) in middle aged adults (31-59 years) compared to 30 years and younger which was also corroborated by other recent studies in South Africa (Masenyetse et al., 2015; Birbal, Dheda, Ojewole & Oosthuizen, 2016). Gender differences were also found to play a profound role as more than two thirds (70.8%) of the females reported ADRs compared to men (24.6%). This gender variance in reporting ARV-related ADRs was also observed in other African settings where ADR reporting were investigated in public healthcare settings with regards to gender differences as well as the incidence, type and risk factors for ADRs in Nigeria (Ofotokun & Pomeroy, 2003; Eluwa, Badru & Akpoigbe, 2012; Luma et al., 2012). These gender demographics findings were supported by baseline characteristic data in a structured ARV pharmacovigilance centre which included patients (n = 590) from Gauteng, Limpopo and Mpumalanga during January 2007 - August 2011 (Masenyetse et al., 2015) and another South African study performed in Mpumalanga (Dheda et al., 2007). Women account for a large proportion (estimated 47%) of the world's HIV population with 18.6 million currently infected (The United Nation's Children Fund, 2016) and women are an emergent concern due to several observational studies pointing towards greater and recurrent toxic effects of ARVs in female populations (Ofotokun, 2005).

Despite efforts made to improve awareness through training during 2011 it was alarming to note the extensive incorrect use (35%) of the ADR forms to report virological failure,

regimen changes due to various reasons not related to ADRs and down referral to clinics. Pharmacovigilance activities within this local hospital setting before 2010 were inattentive and insufficient and local clusters were absent. This may explain the very small number (0.6%) of ADRs reported during 2010 compared to over two-thirds (71.5%) of ADRs that were reported in 2011 in this investigation. Focused and dedicated training sessions as well as modified systems were set in place in this sub-district during 2011. According to Hanafi et al. (2014), improved ADR reporting will lead to an improvement in drug assessment and can result in earlier discovery of severe reactions. This was supported by findings showing that multiple interventions, including constant training of healthcare professionals ultimately improved ADR reporting (Gonzalez-Gonzalez, Lopez-Gonzalez, Herdeiro & Figueiras, 2013). The same case can be made for the phasing out of d4t initiated in 2012 by the South African NDoH in order to reduce d4t associated ADRs, correlating with the drastic decrease in ADRs from 2011 to 2012 which was also evident from our study.

Physicians reported 98.8% of the ADRs compared to only 0.9% by professional registered nurses. Various other investigations proved that educational interventions can improve physician awareness and reporting of ADRs and that these physicians were able to use knowledge gained from close-up training in daily practices (Tabali et al., 2009). Except for the great need that is suggested by Gupta and Udupa (2011) to enhance the correct reporting of ADRs, they also emphasized that closer relationships between healthcare professionals and local pharmacovigilance centres as well as response on pharmacovigilance activities in hospitals were beneficial in long term ADR reporting. The largest percentage (46.6%) of all the ADRs were reported from the hospital setting compared to only 15.6% from clinic settings.

The most prevalent ADRs reported in this retrospective study were abnormal fat distribution (58%), peripheral neuropathy (21.6%) and renal dysfunction (6.6%). The high frequency of abnormal fat distribution was similar to the early stavudine ARV era in other studies (Carr & Cooper, 2000; Ys & Smita, 2005; Leclerq et al., 2013) but decreased over time in correlation with the phasing out of d4t. Peripheral neuropathy (19%), lipodystrophy (18.9%) and renal failure (5.6%) associated with ARVs were reported by a study conducted over similar period (Birbal, Dheda, Ojewalo & Oosthuizen, 2016) which were more representative of most of South Africa. It is interesting to note that a much lower incidence rate for abnormal fat distribution (6%) during 2007-2012 was reported by

MEDUNSA National ARV Pharmacovigilance centre when d4t was still frequently used as part of first line (1a & 1b) regimens (Masenyetse et al., 2015).

Similar peripheral neuropathy frequencies to this study were reported by Kampira et al. (2013) (25%), Luma et al. (2012) (21.2%) and Masenyetse et al. (2015) (20%) after patients were initiated on d4t containing regimens. Dheda et al. (2013), also strongly suggested that peripheral neuropathy and renal dysfunction were most commonly reported in the Mpumalanga region.

Maja, 2014 found renal dysfunction related ADRs to be fewer (2.3%) in Lesotho HIV-infected patients compared to this study (6.6%). Higher CNS related ADRs (9.9%) by Luma et al. (2012) and even more significant higher incidence of CNS symptoms (27.4%) and anaemia (16.1%) were reported by Tadesse et al. (2014) in a self-reported cross sectional study (n = 384) at a university hospital in Gondar, Ethiopia on adult patients receiving ART on a follow-up basis.

Prior to 2010, the ADR forms used in South Africa were product complaint forms with no separate allocated space for the reporting of ARV related ADRs. After 2010, the forms were specifically designed for the indication of ADRs caused by ARV and anti-TB treatment which explains the high percentage of reported ADRs due to ART (98.9%) but not the low frequency (0.7%) of reported clinical manifestations in TB-HIV co-infected patients in this study. The most frequent ARV causative drug in this study was d4t (74.2%), responsible for the dissolute majority of abnormal fat distribution manifestations, particularly during 2011. Stavudine was previously identified and implicated as the cause of abnormal fat distribution, peripheral neuropathy, hyperlactatemia and lactic acidosis in various investigations in South Africa (Masenyetse et al., 2015; Birbal, Dheda, Ojewalo & Oosthuizen, 2016; Maskew, Westreich, Fox, Maotoe & Sanne, 2012).

Patients infected with HIV and on ART are at greater risk for age-associated toxicities (High et al., 2008). Females presented with earlier reported abnormal fat distribution and peripheral neuropathy compared to the males. It is further evident that 75% of the patients in this retrospective study, who experienced the three major ADRs, were  $\geq 30$  years of age which was also supported by other studies (Masenyetse et al., 2015; Mugomeri, Olivier & van den Heever, 2014; Yu et al., 2015).

In this study gender was not only statistically significant but also practically significant ( $V = 0.28$ ) in patients who reported the three most frequent ADRs (abnormal fat distribution, peripheral neuropathy and renal dysfunction). Among these HIV-infected adults, women were more likely to present with abnormal fat distribution compared to the men. These results are in line with reports by Ofotokun & Pomeroy (2003) and Ofotokun (2005). The gender differences with regard to the frequency of renal dysfunction were not significant although men who did report renal related ADR's seemed to experience it at a slightly younger mean age (46.6 vs. 47.6 years) compared to the females. However, other studies have shown that females are predisposed to experiencing ADRs more frequently when receiving NRTIs such as AZT and d4t (Ofotokun & Pomeroy, 2003; Luma et al., 2012; Singh, Dulhani, Tiwari, Singh & Sinha, 2009).

Tenofovir was the major causative drug of renal dysfunction with focus on renal tubule toxicity as the other combination drugs were not prone to adverse renal effects. This was reported by Poizot-Martin et al. (2013) and similarly by Agu and Oparah (2013) in a spontaneous reported system in Nigeria. Zidovudine and d4t (Agu & Oparah, 2013; Phanuphak et al., 2012) was reported to be the major cause of peripheral neuropathy and d4t as the major cause of abnormal fat distribution in various studies by Domingo et al. (2012) and Tadesse et al. (2014) which is in congruence with the findings of this retrospective study.

In conclusion, the most reported ADRs were abnormal fat distribution, peripheral neuropathy and renal dysfunction; these incidences were higher in females and in older patients (31-59 years). The importance of this study is that the findings correlate with both national (Dheda et al., 2013; Birbal, Dheda, Ojewalo & Oosthuizen, 2016, Mehta et al., 2008) and international studies (Carr & Cooper, 2000; Agu & Oparah, 2013) contributing to information on a demographic area not specifically focused on previously. The reporting of d4t, predominantly responsible for the abnormal fat distribution, retrospectively supports the phasing out of d4t as first-line regimen as was published in the South African guidelines in 2010-2012.

## **Conclusions**

Adverse drug reactions associated with ART are common and therefore should be a main concern of all healthcare providers to improve the quality of life of HIV-patients on ART. Although pharmacovigilance forms an essential part in the public healthcare system and has transformed the way healthcare professionals approach the administration and monitoring of essential drugs, it still faces many challenges. The collection ADR data will be inconsequential if not clinically analysed and findings effectively reported back to responsible reporting healthcare staff.

This retrospective observational study found that ADRs due to ARVs were very frequent and dominant in this study cohort. Our results highlight the high frequency of ADRs in HIV-infected adult patients receiving ART and emphasize the importance of maintained ART durability and comprehensive ADR monitoring. Over this five-year time period where the frequency of ARV ADRs within the Tlokwe sub-district was investigated, women experienced significantly more abnormal fat distribution and peripheral neuropathy and also at an earlier age compared to men.

Intensive pharmacovigilance activities are needed to identify patients pre-disposed to risk factors for ADRs so that treatment, with regard to dosage and regimen adjustments, can be personalized for each patient where relevant. This can assist to early identify shortcomings in the South African pharmacovigilance system as the toxicities of antiretroviral drugs can easily overshadow the success of HAART. The use of homogeneous methodologies would contribute significantly towards a better control of the nature and magnitude of ADRs in general and certain measures to prevent frequent occurrences and reactions.

### ***Limitations of the study***

The study was subject to mostly spontaneous ADR reporting, which is prone to a lack of information, inaccurate data and under-reporting of ADRs. Due to the retrospective nature of this study, data were restricted to what have been already reported or available while the accuracy of reported data could not be confirmed. The data was only representative of ADR reports from public healthcare facilities in the Tlokwe sub-district that were sent to the central point for storage and did not include ADRs reported in private healthcare settings.

The initial sample size of the number of reported ADRs appeared extensive before the exclusion criteria were applied as the ADR paper form was also used to indicate reasons or motivations for changing a specific regimen.

No further investigations were performed into laboratory results or other diagnostic tests of patients and no differentiation between the different grades of ADRs or medication adherence was made.

### ***Recommendations***

The current system in this sub-district needs to be monitored and re-evaluated on a regular basis to ensure optimum reporting of future ADRs. This is paramount due to the high prevalence of underreporting of ADRs that were predominantly reported by doctors, a few by nurses and none by pharmacists. It is suggested that at least one monthly meeting takes place where all relevant pharmacovigilance cluster role players are involved. More awareness needs to be created among healthcare professionals on the importance of accurate ADR reporting as the impact can lead to a change in policy and drug regimens. Continuous training of clinicians, registered nurses and pharmacists should form part of the regular pharmacovigilance activities within this sub-district and the respective clusters. Specific training and education should be provided with regard to the crucial information that needs to be accurately reported as requested on the forms.

HIV-patient education should also play an integral part of ARV associated ADRs as patients should also be made more aware of sharing and reporting their medication related problems to expedite pharmacovigilance processes with regard to ADR management and reporting. This will ensure that ADRs are correctly reported as a true and direct side effect of specific drugs and not due to other aspects related to the patients (etc. pregnancy, virological failure, down referral to local clinics or regimens changes).

This retrospective investigation can form a reference platform from which reported ADRs post December 2014 can now be compared with as certain shortcomings were identified in the local pharmacovigilance system. This platform can also be used to compare the frequency of reported and less prevalent ADRs from the currently fixed dose combinations to the period before it was introduced within the Tlokwe sub-district.

It would be ideal to initiate patients on ART and concurrently enter them into pharmacovigilance studies to detect ADRs at an early stage and improve the risk-benefit profile and promote better patient adherence as was also suggested by Dube and co-workers (2012).

This Tlokwe sub-district in collaboration with the NPC should decisively decide on a system on how to handle the specific reporting of regimen changes due to various motivations. Currently the same ADR forms are also used to indicate regimen changes and it may create a false impression of high numbers of ADRs reported.

### **Ethics approval**

The Human Research Ethics Committee from the NWU Potchefstroom Campus approved this study (NWU-00031-15-A1) on 3 November 2015. The Department of Health (DoH) of the North West province (PPRM&E directorate) also approved this study on 17 November 2015. All researchers involved in this retrospective analysis signed a non-disclosure agreement to ensure confidentiality with regard to personal patient information.

### **Competing interests**

The authors declare no competing interests.

### **Authors' contributions**

RvG performed the data gathering, data management, statistical analysis and assisted with the first draft of the initial manuscript. MV conceptualized the study, assisted with quality control and monitoring of the data, ethics application, review and interpretation of the statistical analysis and assisted with the drafting of the manuscript, MR assisted with the review of the statistical analysis and revision of the manuscript, FM helped with the logistical aspects of the investigation and revision of the manuscript. All authors have read and approved the final manuscript.

### **Acknowledgement**

We acknowledge the guidance of Ms Marike Cockeran, a biostatistician from the Faculty of Health Sciences, North-West University Potchefstroom Campus. We would like to

thank the Potchefstroom Hospital and pharmacists who have gathered and stored the completed ADR forms as part of their duty over the period in question.

## **Funding**

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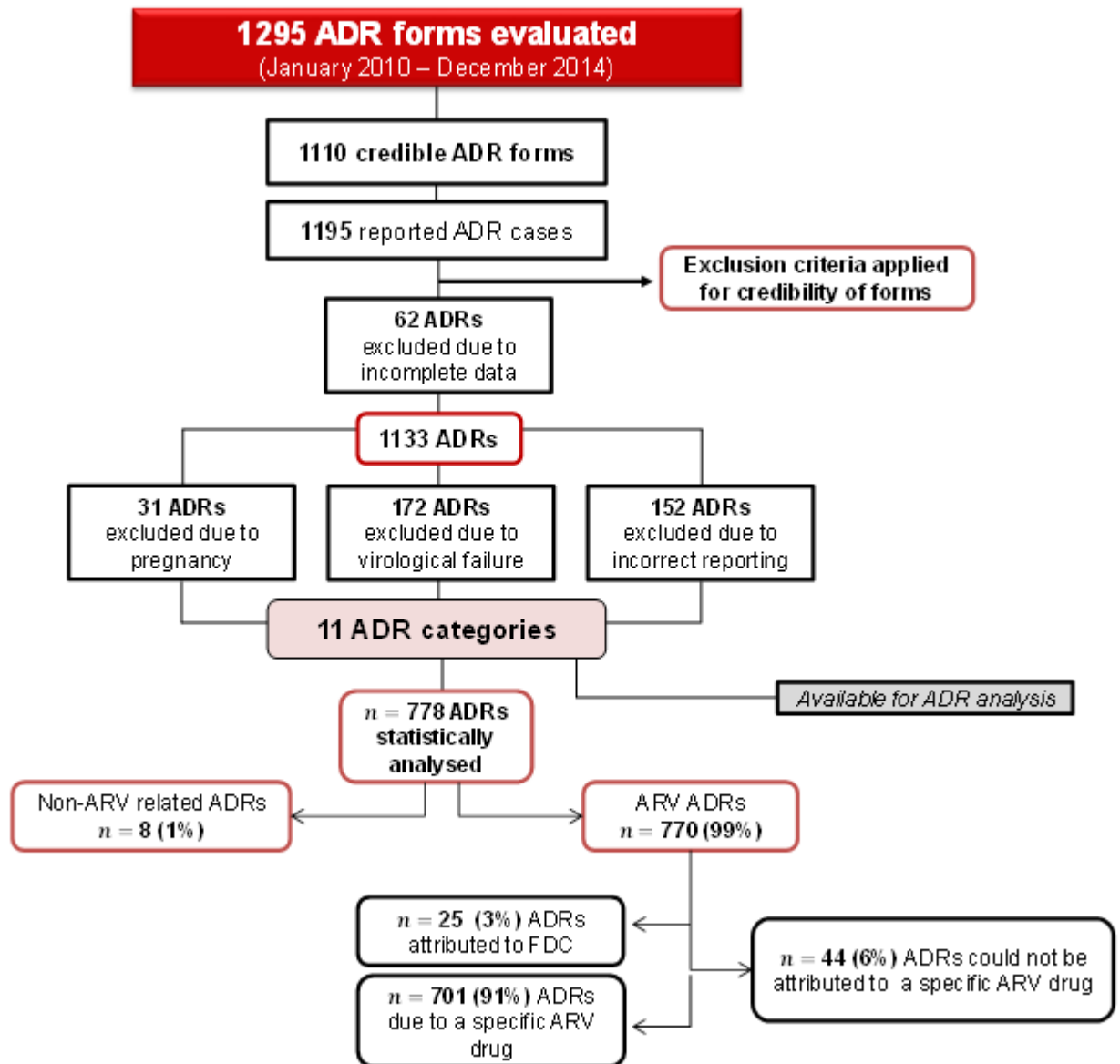
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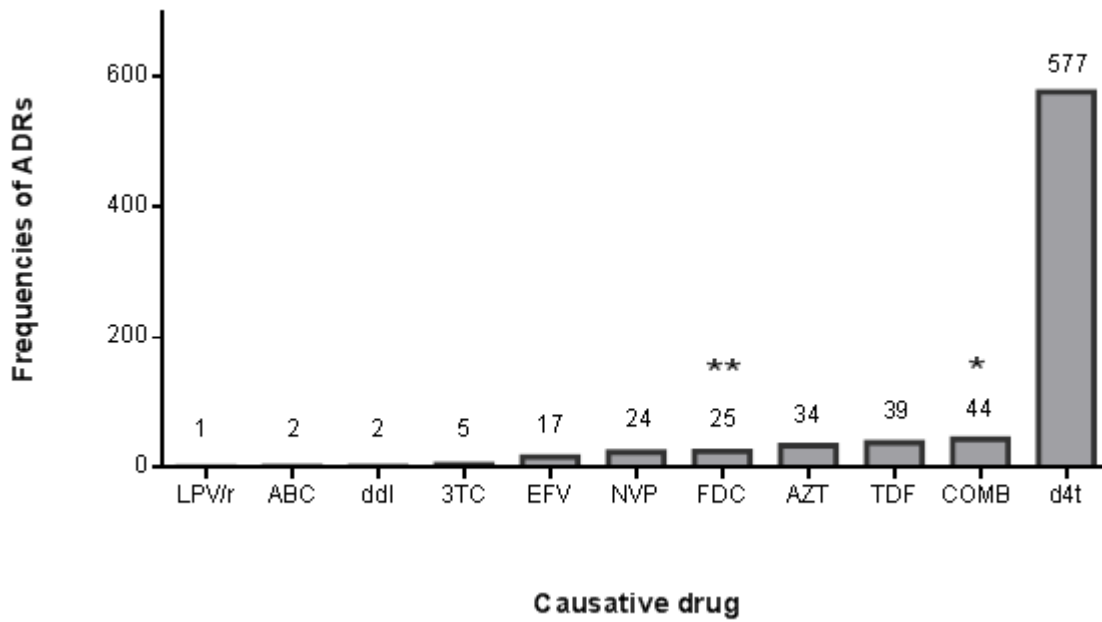
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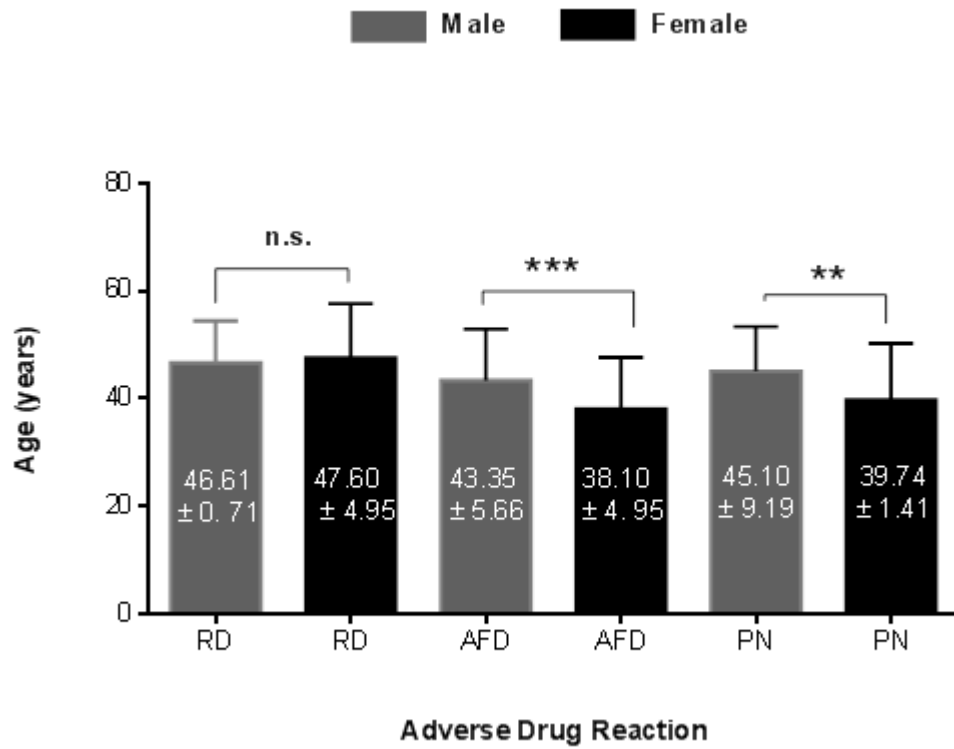
**Figure 1: Flow diagram of data collected from already reported ADR forms.**

ADR: adverse drug reaction; ARV; antiretroviral, FDC; fixed dose combination



**Figure 2: Frequency of adverse drug reactions according to causative drugs.**

LPV/r: lopinavir/ ritonavir combination; ABC: abacavir; ddl: didanosine, 3TC: lamivudine; EFV: efavirenz; NVP: nevirapine; FDC: fixed dose combination; AZT: zidovudine; TDF: tenofovir disoproxil fumarate; d4t: stavudine). \*COMB: Any combination of three or more ARV drugs; \*\* FDC: TDF+ 3TC+EFV



**Figure 3: Gender comparison of the mean ( $\pm$ SD) age of the highest reported ADRs that were experienced.**

RD: renal dysfunction; AFD: abnormal fat distribution; PN: peripheral neuropathy. n.s: not significant, \*\*  $p < 0.001$ , \*\*\*  $p < 0.0001$

**Table 1: Patient demographics recorded for reported ADRs during 2010-2014**

<b>Characteristic</b>	<b>Result</b>
Number of ADRs related to ARVs, <i>n</i> (%)	770 (99.0)
Males, <i>n</i> (%)	191 (24.6)
Females, <i>n</i> (%)	551 (70.8)
Gender not specified, <i>n</i> (%)	36 (4.6)
Age (years), mean (SD)	40.1 ±10.1
Age (years), median (min; max)	40 (5.0; 73.0)
Age categories, <i>n</i> (%)	
Children: 0-18 years	16 (2.1)
Young adults 19-30 years	109 (14.0)
Middle aged adults: 31-59 years	634 (81.5)
Elderly: ≥ 60 years	18 (2.3)
Age not specified, <i>n</i> (%)	1 (0.1)
Number of reported ADRs per facility, <i>n</i> (%)	
Hospital	363 (46.6)
Clinic	121 (15.6)
Facility not specified	294 (37.8)
Number of reported ADRs per HCP, <i>n</i> (%)	
Doctor	769 (98.8)
Registered professional nurse	8 (1.0)
HCP not specified	1 (0.1)

ADR: adverse drug reaction

ARV: antiretroviral

HCP: Healthcare Professional

**Table 2: Frequencies of ADR per category reported according to gender**

Type of ADRs	Male: n (%)	Female: n (%)	NS*: n (%)	Total: n (%)
Abnormal fat distribution	77 (9.9)	358 (46)	16 (2.1)	451 (58)
Peripheral neuropathy	61 (7.8)	97 (12.5)	10 (1.3)	168 (21.6)
Renal dysfunction	25 (3.2)	23 (3.0)	3 (0.4)	51 (6.6)
Blood abnormalities	10 (1.3)	12 (1.5)	2 (0.3)	24 (3.1)
Gynecomastia	8 (1.0)	2 (0.3) **	2 (0.3)	12 (1.5)
Hyperlactatemia	8 (1.0)	0	0	8 (1.0)
CNS abnormalities	5 (0.6)	12 (1.5)	2 (0.3)	19 (2.4)
Skin abnormalities	2 (0.3)	12 (1.5)	1 (0.13)	15 (1.9)
GI abnormalities	1 (0.1)	6 (0.8)	0	7 (0.9)
Lactic acidosis	1 (0.1)	15 (1.9)	0	16 (2.0)
Diverse ADRs	1 (0.1)	6 (0.8)	0	7 (0.9)

NS\*: gender not specified \*\* breast hypertrophy

**Table 3: Frequencies of clinical manifestation related to ADRs reported by healthcare professionals in the hospital or clinics**

Healthcare professional			Type of ADR	Facility			TOTAL n (%)
RPN n (%)	Doctor n (%)	NS n (%)		Hospital n (%)	Clinics n (%)	NS n (%)	
5 (0.6)	446 (57.3)	0	<b>AFD</b>	215 (27.6)	55 (7.1)	181 (23.3)	451 (58)
0	168 (21.6)	0	<b>PN</b>	65 (8.4)	26 (3.3)	77 (9.9)	168 (21.6)
1 (0.1)	50 (6.4)	0	<b>RD</b>	29 (3.7)	14 (1.8)	8 (1.0)	51 (6.6)
0	24 (3.1)	0	<b>BA</b>	13 (1.7)	6 (0.8)	5 (0.6)	24 (3.1)
0	19 (2.4)	0	<b>CNS</b>	12 (1.5)	4 (0.5)	3 (0.4)	19 (2.4)
0	16 (2.1)	0	<b>LA</b>	9 (1.2)	4 (0.5)	3 (0.4)	16 (2.1)
1 (0.1)	13 (1.7)	1 (0.1)	<b>SKIN</b>	8 (1.0)	4 (0.5)	3 (0.4)	15 (1.9)
0	12 (1.5)	0	<b>GCM</b>	4 (0.5)	5 (0.6)	3 (0.4)	12 (1.5)
0	8 (1.0)	0	<b>HL</b>	0	0	8 (1.0)	8 (1.0)
1 (0.1)	6 (0.8)	0	<b>Diverse</b>	3 (0.4)	2 (0.3)	2 (0.3)	7 (0.9)
0	7 (0.9)	0	<b>GIA</b>	5 (0.6)	1 (0.1)	1 (0.1)	7 (0.9)
8 (0.9)	769 (98.8)	1 (0.1)	<b>TOTAL n (%)</b>	363 (46.6)	121 (15.6)	294 (37.8)	778 (100)

AFD: abnormal fat distribution; PN: peripheral neuropathy; RD: renal dysfunction; BA: blood abnormalities; CNS: central nervous system abnormalities; LA: lactic acidosis; SKIN: skin abnormalities; GCM: gynecomastia in men and breast hypertrophy in women; HL: hyperlactatemia; GIA: gastrointestinal abnormalities; NS: Healthcare professional or facility not specified; RPN: registered professional nurse.

# **ADDENDUM F2: ETHICS CLEARANCE CERTIFICATES AND LETTERS (ADDITIONAL RESEARCH PROJECT AS PART OF A QUALITY IMPROVEMENT PROJECT**

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ADDENDUM F2 contains the following ethics clearance certificates and letters:

- Ethics clearance certificate from the NWU HREC (Potchefstroom Campus)
- Letter of approval from the PPRM&E directorate (North West Department of Health)
- Letter of approval from the CEO of Potchefstroom Hospital
- Proof of attendance: The Basics of Health Research Ethics



**health**

Department of  
Health  
North West Province  
REPUBLIC OF SOUTH AFRICA

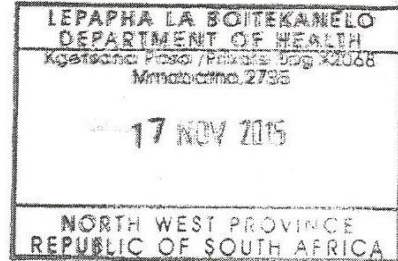
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**POLICY, PLANNING, RESEARCH, MONITORING AND EVALUATION**

Name of researcher : Dr M Viljoen  
North West University

Physical Address  
(Work/ Institution) Department Pharmacology  
Building G23 Room 319  
Potchefstroom Campus



Subject : **Research Approval Letter- A retrospective clinical analysis of  
pharmacovigilance reports in the Tlokwe Sub-District  
(Ethics Number: NWU-00031-15-A1).**

This letter serves to inform the Researcher that permission to undertake the above mentioned study has been granted by the North West Department of Health. The Researcher is expected to arrange in advance with the chosen facilities, and issue this letter as proof that permission has been granted by the Provincial office.

This letter of permission should be signed and a copy returned to the department. By signing, the Researcher agrees, binds him/herself and undertakes to furnish the Department with an electronic copy of the final research report. Alternatively, the Researcher can also provide the Department with electronic summary highlighting recommendations that will assist the department in its planning to improve some of its services where possible. Through this the Researcher will not only contribute to the academic body of knowledge but also contributes towards the bettering of health care services and thus the overall health of citizens in the North West Province.

Kindest regards

\_\_\_\_\_  
Dr. FRM Reichel  
Director: PPRM&E

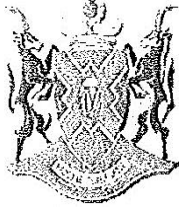
16/11/2015  
Date

\_\_\_\_\_  
Researcher

24/11/2015  
Date



Healthy Living for All



health

Department of  
Health  
North West Province  
REPUBLIC OF SOUTH AFRICA

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**POTCHEFSTROOM HOSPITAL-OFFICE OF THE CEO**

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TO: Dr M Viljoen  
North West University

FROM: Dr M Shakung  
Acting Chief Executive Officer

Date: 09 December 2015

Subject: REQUEST FOR RESEARCH APPROVAL IN POTCHEFSTROOM  
HOSPITAL

---

This communiqué serves to inform you that your request to conduct a retrospective clinical analysis of pharmacovigilance study in Potchefstroom hospital has been approved and will commence from 07 December 2015.

Hope you will find this in order.

Sincerely,

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**Dr M Shakung**  
Acting Chief Executive Officer  
Potchefstroom Hospital

POTCHEFSTROOM  
HOSPITAL

2015 -12- 09

PRIVATE BAG X938  
POTCHEFSTROOM 2520



2016-09-20

## ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Health Research Ethics Committee (HREC)** on **03/11/2015** after being review at the meeting held on **10/09/2015**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

**Study title:** Evaluation of the pharmacovigilance system in the Dr Kenneth Kaunda district in the North West Province.

**Sub-study title:** A retrospective clinical analysis of pharmacovigilance reports in the Tlokwe sub-district.

**Study Leader/Supervisor:** Dr M Viljoen

**Student:** R van Graan

**Ethics number:**

N	W	U	-	0	0	0	0	3	-	1	5	-	A	1
				Institution				Study Number				Year		Status

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

**Application Type:** Sub-study

**Commencement date:** 2015-11-03

**Risk:**

**Minimal**

**Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years.**

### Special conditions of the approval (if applicable):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

### General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
  - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
  - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
  - request access to any information or data at any time during the course or after completion of the study;
  - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
    - any unethical principles or practices of the study are revealed or suspected,
    - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
    - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
    - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for further information or any report templates via [Ethics-HRECAppl@nwu.ac.za](mailto:Ethics-HRECAppl@nwu.ac.za) or 018 299 1206.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

**Prof LA Du Plessis**  
Digitally signed by  
Prof LA Du Plessis  
Date: 2016.09.27  
14:33:46 +02'00'

**Prof Linda du Plessis**

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)



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5 October 2015

Dear Rentia van Graan

## **PROOF OF ATTENDANCE**

This letter certifies that you have attended and successfully completed the 2 day ethics training, entitled:

### **The Basics of Health Research Ethics**

Presented by Prof Minrie Greeff (Head of the Health Sciences Ethics Office for Research, training and Support) on the 21 and 22<sup>nd</sup> of September 2015.

This proof of attendance letter is valid for 3 years and expires on the 22<sup>nd</sup> of September 2018.

Yours sincerely

A handwritten signature in black ink, appearing to read 'Minrie Greeff', written over a horizontal line.

Prof Minrie Greeff  
Head of Health Sciences Ethics  
Office for Research, Training and Support

A handwritten signature in black ink, appearing to read 'Awie Kotze', written over a horizontal line.

Prof Awie Kotze  
Dean of Faculty of Health Sciences