Monitoring lipid and haematological abnormalities in paediatric patients on antiretroviral therapy at a Community Health Centre in the Cape Metropole

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A thesis submitted in fulfilment of the requirements for the degree of Magister Pharmaceuticiae in the School of Pharmacy, University of the Western Cape.

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ABSTRACT

Monitoring lipid and haematological abnormalities in paediatric patients on antiretroviral therapy at a Community Health Centre in the Cape Metropole

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South Africa faces a huge Human Immunodeficiency Virus (HIV) burden with more than 400,000 children currently on antiretroviral therapy (ART). Studies on lipid and haematological profile changes in paediatrics are of particular interest since these children are exposed to ART in the course of a developmentally significant period and will possibly have longer collective exposure to ART. As such, monitoring for adverse effects, including lipid and haematological abnormalities, is essential for curtailing morbidity and mortality rates of children on ART. There is a dearth of studies assessing lipid and haematological abnormalities in the South African paediatric population on ART where genetic differences, co-morbidities, malnutrition and use of traditional medicines, all influence the safety profile of a drug. The goal of this study was two-fold: Firstly to identify a suitable parameter for assessing lipid and haematological abnormalities in paediatrics on Antiretroviral (ARV) treatment using available secondary data and secondly, to assess prescriber adherence to routine monitoring tests in the ART guidelines.

This study was a retrospective review of secondary data obtained from 168 patient clinical records at a Community Health Centre in the Cape Metropole, Western Cape and corresponding laboratory data from the National Health Laboratory Service (NHLS) database. Appropriate cholesterol, triglyceride, haemoglobin and neutrophil test results were compared against the standard reference ranges/values. The Chi-Squared test identified associations between total

cholesterol (TC) /triglycerides and haemoglobin (Hb)/neutrophil and other independent variables. Evaluation of health care provider adherence to routine monitoring tests was assessed against relevant national ARV management guidelines.

There was a paucity of baseline data for all laboratory markers and infrequent follow-up tests were ordered by healthcare providers. This precluded the measurement of changing lipid and haematological levels and an alternative parameter, viz., the highest available laboratory test value for each marker per patient, was assessed against reference values/ranges. Only nine out of the 36 (25%) patients on an AZT regimen had any Hb or neutrophil laboratory tests performed and 23 and two out of 97 (24% and 2%) patients, respectively, on a protease inhibitor (PI) had a TC and triglyceride laboratory test performed. Anaemia was detected in 45.5 % of children below five years of age, in 21.7% between ages of six and 11 and in 65.5 % between 12 and 14 years of age. Neutropenia was detected in 25.6% of children below five years of age and in 50% aged between six and 11. Hypercholesterolemia was found in 13.1% of patients. The only statistically statistical associations were found between the TC and CD4 count in children aged six to 14 years (χ^2=5.000; p=0.025) and between neutrophil counts and viral load in children aged six to 14 years (χ^2=6.4532; p=0.0240). A significant association was also found between Hb levels and viral load (χ^2=7.000; p=0.008).

In the absence of baseline test results and routine monitoring of haematological and lipid profiles, this study presents a potential alternative marker for assessing lipid and haematological abnormalities using the highest level of neutrophil, Hb, TC and triglycerides recorded for each patient.

DECLARATION

I declare that *Monitoring lipid and haematological abnormalities in paediatric patients on antiretroviral therapy at a Community Health Centre in the Cape Metropole*, has not been submitted before for any degree or examination at any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Winnie Nambatya

November 2014

Signed…….

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ABC	Abacavir
ADR	Adverse Drug Reaction
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Zidovudine
CCMT	Comprehensive, Care, Management and Treatment
CHC	Community Health Centre
DDI	Didanosine
DNA	Deoxyribonucleic acid
DOH	Department of Health
D4T	Stavudine
DHIS	District Health Information Systems
EMRs	Electronic Medical Records
EFV	Efavirez
FBC	Full blood count
Hb	Haemoglobin
HIS	Health information systems
HIV	Human Immunodeficiencyvirus
IRIS	Immune Reconstitution Inflammatory Syndrome
LPV/r	Lopinovir/ritonavir
M&E	Monitoring and evaluation
NDOH	National Department of Health
NHLS	National Health Laboratory Services
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor

NSP	National Strategic Plan
NVP	Nevirapine
PI	Protease inhibitor
PCV	Packed cell volume
PMTCT	Prevention of Mother to Child transmission
SOPs	Standard Operating Procedures
STIs	Sexually transmitted infections
TB	Tuberculosis
TC	Total Cholesterol
UNMP	United Nations Millennium Development Goals
WHO	World Health Organisation
3TC	Lamivudine

Chapter 1 Introduction

1.1 Global and national HIV burden

The Human Immunodeficiency Virus (HIV) is one of the leading causes of death worldwide. Globally, an estimated 35.3 million people were living with HIV in 2012 (UNAIDS, 2013), with 97% residing in low and middle-income countries. By 2011, 330,000 children were infected with HIV and more than 90% of them lived in sub-Saharan Africa (UNAIDS, 2012). South Africa's population is estimated to be 52.83 million and 10% of this population is estimated to be living with HIV infection (Statistics South Africa, 2013).

The Western Cape Province situated in the South West of the Republic of South Africa is home to approximately 11.4% of the national population (Statistics South Africa 2013, South African National AIDS Council, 2012), with an HIV prevalence of approximately 16.5%. Children in the age group from zero to 15 years constitute more than 9% of the total population of the Western Cape and trends in mortality indicators show that HIV/Acquired Immunodeficiency Syndrome (AIDS) is one of the major causes of death in this age group (Statistics South Africa, 2013; Roman & Hall, 2011).

The Cape Metropole district in the Western Cape has an HIV prevalence of 18.5%. It is divided into eight sub-districts; Khayelitsha having the highest HIV prevalence of 33.4% followed by Klipfontein (23.4%), Northern (21.4%), Eastern (18.9%), Western (16.6%), Mitchell's Plain (13.9%), Tygerberg (11.3%) and Southern (9.9%) (Provincial Department of Health, 2010).

While new infection rates among younger children (aged zero-14 years) have halved in the last decade as a result of the prevention of mother to child transmission programme (PMTCT), children in this age group remain vulnerable to infection and it has been estimated that 438,000 children under the age of 15 years were HIV positive, with an estimated 43,000 new infections occurring every year (Roman& Hall, 2011).

1.2 Policies in the management of HIV infection

The Comprehensive, Care, Management and Treatment programme, together with the national and provincial policies on quality in health care, share the vision of improving the quality of healthcare provided by the health care providers (National Department of Health, 2009). The South African National Strategic Plan (NSP) on HIV, Sexually Transmitted Infections (STIs) and Tuberculosis (TB) 2012– 2016 (NSP), has aimed to initiate at least 80% of eligible patients on antiretroviral therapy (ART) (National Department of Health, 2012). With regard to the management of HIV infection in the paediatric population, the South African guidelines on the management of HIV in children recommend the start of ART in children as early as less than one year, irrespective of the CD4 count (National Department of Health, 2010). This has led to the vastly increased use of double and triple combination ART regimens.

1.3 Monitoring and evaluation

Monitoring and Evaluation (M&E) is a vital yet often neglected aspect of HIV management (Kumalo, 2006). Both data management and record-keeping are essential aspects of M&E of HIV care within which special consideration should be placed on the use of national guidelines and standard operating procedures (SOPs) (Hoskins et al., 2012;WHO, 2006a). Data from M&E

can be used by decision-makers and policy makers to track progress, report on results, allocate resources, plan and improve service delivery. This requires complete, accurate and the timely flow of data between primary health care facilities, hospitals and a central information hub (Kumalo, 2006). For both the clinic staff and health system managers, having access to reliable data that reflects the processes of care and clinical outcomes, is the first step to ensuring effective delivery of high quality HIV treatment (Kumalo, 2006).

As countries report their progress towards the achievement of the United Nations Millennium Development Goals (UNMDP) for 2015 in the management of HIV and reduction of child mortality (United Nations, 2001), the need for quality data has never been greater (Rugg et al., 2009). Studies from resource poor countries on M&E of HIV management, have documented problems such as inaccuracy and incomplete data (Mate et al., 2009; Makombe et al., 2008).

1.4 ART in the management of HIV infection

Management of HIV infection requires the use of a combination of at least three antiretroviral (ARV) drugs to maximally suppress the HIV and stem the progression of disease. The nucleoside reverse transcriptase inhibitors (NRTIs) commonly form the "backbone" of therapy, together with a non-nucleoside reverse transcriptase inhibitor (NNRTI) and a protease inhibitor (PI).

According to the South African guidelines for the management of HIV (2010), the preferred option when choosing a first-line regimen for infants and children is two NRTIs and either a NNRTI or a PI(WHO, 2013a; NDoH, 2010). These same guidelines recommend the use of

ART in children and all infants under one year of age who are found to have the HIV infection (National Department of Health, 2010).

1.5 Magnitude of ADRs of ARV drugs

The complete safety of the drug can never be guaranteed and an ADR can either be actual or potential. The World Health Organisation (WHO) (WHO, 2002:40) defines an ADR as a "response to a drug which is noxious and unintended, which occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for the modifications of physiological function".

Studies conducted in sub-Saharan Africa have demonstrated the link between mortality and ART regimens and identified some risk factors for mortality like the immune reconstitution syndrome (IRIS), opportunistic infections and ART toxicities (Johannessen et al., 2008; Lawn et al., 2005). In a study by Puthanakitet al (2007), an increased risk of mortality rates in children on ART due to ART toxicities and IRIS was reported at 23.4% and 6.3%, respectively.

Literature has associated ART with ADRs, and these can be regarded as early, medium and late ADRs with mechanisms grouped into four categories, viz., mitochondrial toxicity, metabolic abnormalities, hematologic abnormalities and allergic reactions (NDoH, 2009). The presence of ADRs is an important cause of hospitalizations, particularly among HIV positive patients on ARVs (Mehta et al., 2008) and ADRs have been one of the limiting factors in the success of ART as they may result in decreased adherence to treatment which may consequently lead to virological failure and poor prognosis. This, in the long run, may undermine the confidence in any national ART programme (WHO, 2007) bearing in mind that African children constantly

endure the burden of various infections such as bacterial, parasitic and viral infections as well as substantial nutritional deficiencies (Domingo& Lozano 2011; Taha et al., 2002). These ADRs may be caused by the drug itself or by an interaction of the ARVs with food, herbal medicines, or traditional medicines and other medications used in the treatment of co-morbidities (Domingo& Lozano 2011). A review of 40 publications by WHO on ARV-related ADRs found that anaemia and dyslipidemias were the top identified haematological and lipid abnormalities in Africa (WHO, 2008).

1.6 Monitoring of ART

Clinical assessment and laboratory tests play a key role in assessing individuals before ART is initiated and when monitoring their treatment responses and possible toxicities (WHO, 2013a). The ability to provide and sustain safe and effective ART requires patient monitoring systems in which spontaneous reports from health care professionals or patients about one or more ADRs is required (HELA, 2006). In 2006, WHO (WHO, 2006 a; WHO, 2006b) published guidelines for monitoring patients on ART, including templates of standardized tools to collect and report such data. Proper patient monitoring enables effective clinical management of the patients and also generates data used for programme monitoring and management, contributing to standardized indicators at the provincial and national levels. With the increase in the number of patients receiving ART, the growing monitoring, evaluation and reporting requirements have also increased, hence the need to link paper records with the electronic medical records (EMRs) in order to maintain the quality of data available.

1.7 Lipid and haematological abnormalities of ART

Management of HIV infection with ART has been associated with lipid (Jacobson et al., 2011; Gonzalez et al., 2008; Farley et al., 2005) and haematological (Njuguna et al., 2013; Abebe & Alemseged, 2009; Shet et al., 2005; European collaborative study, 2004) abnormalities. Haematological abnormalities and dyslipidemias are among the most common clinical-pathological manifestations in the management of HIV infection and these abnormalities increase the risk of morbidity and mortality in HIV infected individuals (Tindyebwa et al., 2011; Moyle et al., 2004)

The prevalence of haematological abnormalities associated with HIV management range from 8% to 94% and is attributed to multiple factors which include HIV infection, drug toxicities and nutritional deficiencies among others (Opie, 2012; Moh et al. 2005). Abnormalities may include impaired haematopoiesis and immune mediated cytopenias which may lead to a decrease in haemoglobin and neutrophils (Opie, 2012; Adetifa et al., 2006). Adeifa et al (2006) found a statistically significant decrease in neutrophils and haemoglobin (Hb) in HIV infected patients. Similarly, Bunders and colleagues found deficits in neutrophils that persisted through eight years of age in children on ART (European Collaborative Study, 2004).

Dyslipidemias have been described in 50-70% of children receiving ART (Rhoads et al., 2011; Jacobson et al., 2011; Chantry et al., 2008). Among studies of children on ART, there have been several small cross-sectional and cohort studies that have examined the relationship between factors associated with dyslipidemias such as age, nutritional status, ART and the infection its-self and dyslipidemias (Kevin et al., 2011; Rhoads et al., 2011; Kim et al 2009; Gortmaker et al.,

2001). Regimens including NNRTIs (Fontas et al., 2004), NRTIs (Kumar et al. 2003, Staszewski et al., 2003) and PIs (Kim, Rutstein 2010; Kim et al. 2009), have been associated with elevated total cholesterol (TC) and triglycerides.

1.8 Problem statement

The safety of drug prescribing has become a highly visible topic in adult medicine but less attention is paid to the paediatric population (Eley et al., 2006). Paediatric patients constitute a vulnerable group with regard to rational drug prescribing since many new drugs are released onto the market without the benefit of experience in this age group. These deficiencies cause paediatricians to often prescribe adult formulations. Adequate controlled clinical trials in children are lacking and the few clinical trials that have been performed involving children focused on the efficacy of drugs and rarely monitored their safety (Eley et al., 2006; O'Brien et al., 2006).

Treatment in children is complicated by changing drug pharmacokinetics with age caused by the continuing development and maturation of the organs involved in drug metabolism. Drug pharmacokinetics is further complicated by malnutrition, drugs required for treatment of co-infections, and inherited factors that affect the kinetics and dynamics of the drugs. Therefore, it is important to monitor this population when on ART and to maintain appropriate records as missing clinical information is associated with harmful medical errors (Elderet al., 2004).

As international and national efforts to increase access to health care and supportive services for people living with HIV/AIDS are strengthened, the need for information on M&E of HIV

treatment and its toxicities also grows (DOH, 2009). The consequence of improper monitoring of ARV lipid and haematological abnormalities would be an increase in morbidity and mortality rates in children. The extent to which monitoring occurs in South African health facilities is unknown. Literature on ARV haematological and lipid abnormalities in the South African population is generally limited, more especially in the paediatric population. As such, there is a need to investigate the actual monitoring of lipid and haematological abnormalities at facilities and the extent to which records can be utilized to evaluate lipid and haematological abnormalities in paediatrics.

1.9 Research questions

i) What data is available for monitoring lipid and haematological abnormalities?

ii) What is the quality of this data?

iii) Are laboratory testing guidelines for haematological and lipid abnormalities adhered to at primary health care facility level?

iv) To what extent could current clinical and laboratory data be utilized to evaluate trends in haematological and lipid abnormalities?

v) What is the prevalence of lipid and haematological abnormalities in paediatric patients on ARVs and which risk factors are associated with these abnormalities?

1.10 Aim of the study

This study aimed to monitor lipid and haematological abnormalities in paediatric patients on antiretroviral therapy at a Community Health Centre in the Cape Metropole

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1.11 Specific study objectives

The specific objectives of this study were**:**

i) To determine completeness of data from the patient medical folders;

ii) To assess for lipid and haematological abnormalities using a suitable parameter;

iii) To identify the risk factors associated with the lipid and haematological abnormalities and

iv) To assess adherence to the HIV management guidelines with regards to the recommended haematological and lipid laboratory testing.

The primary objective of this thesis was to identify lipid and haematological abnormalities from secondary data on paediatric patients on ART in a CHC in the Cape Metropole. To achieve this, patient information was retrieved from medical records used at the CHC and laboratory test results were requested from the NHLS.

The second chapter of this thesis summarizes and critiques relevant literature on monitoring and evaluating the safety profile of medicine and elaborates on the link between various factors associated with haematological and lipid abnormalities in the paediatric population of ARV users. This information is sourced from local and international government policies, published research study findings and reviews. The third chapter presents the research design and the methods used in executing the study, analysing the data and achieving an ethical study. Results are summarised mainly as descriptive and inferential statistics in chapter four. The main findings are discussed in chapter five and chapter six contains concluding remarks and recommendations for further study and for optimising practices at facility level.

Chapter 2 Literature review

This literature review analyses published research study findings and reviews, with a view to determine completeness of patient medical folders, assess for lipid and haematological abnormalities from secondary data in paediatrics on antiretroviral therapy, and the factors associated with lipid and haematological profiles.

2.1 M&E of ART in HIV infected persons

Monitoring HIV treatment and providing reliable information is an important component of HIV management. The data generated is transferred to national programmes, which in turn helps in guiding the delivery of high quality care and treatment to HIV infected individuals. Both data management and record-keeping are essential components for M&E of HIV management. This makes it easy to identify early on which patient level data is needed to manage individual facilities as well as monitor and report on ADRs (Hoskins et al., 2012;WHO, 2006). A publication by WHO(2002:15) stated that the "possibility of harm due to ADRs is immense, especially if they are not monitored by a strategy aimed at good reporting and early detection, review and management".

Young et al (2010) carried out a study to assess the completeness of patient medical records in Mozambique in which poor documentation of CD4 count, weight, height, WHO clinical staging at baseline and follow-up were observed. Completeness across all data elements was 72% at baseline and 65% for follow-up visits. Many data elements critical to high quality care were not recorded completely at baseline or follow-up visits, these included weight, clinical disease stage,

ART regimen and CD4 cell count. Similarly a study on the West African database by the West African paediatric collaboration showed that baseline laboratory data was recorded in only 59.4% of the patients. According to Smith et al. (2005), in one of seven patient visits in the United States of America (USA), important clinical information from laboratory reports, radiology results, history and physical examination, and medications may be missing. This would result in a delay of care or at the least, a duplicate of medical services (Smith et al., 2005).

Over the past 10 years, South Africa has been focusing on developing and strengthening the Health Information System (HIS). However, one of the key challenges remains getting health facilities to improve the accuracy and completeness of data at the various levels of the health system so the collected data can be used optimally during decision making at the national level.

Gimbel (2011) argued that effective monitoring and supervision of health care programmes depended on complete, accurate, and timely flow of data between primary health care facilities, hospitals, and a central information hub. He further posited that the quality of M&E is based and dependent on the HIS. According to Impicciatore et al (2001), the incompleteness and inaccuracy of data in prescriptions as well as clinical records makes it difficult for health practitioners to implement evidence based preventive strategies.

The WHO (WHO, 2006: 92-95), published guidelines for monitoring patients on ART which include examples of standardized tools to collect and report such data. Patient monitoring is defined by WHO (2006:11) as "the routine collection, compilation and analysis of data on patients over time and across service delivery points, using information either directly from paper forms or entered into a computer".

In a study by Hoskins et al (2012), whose main aim was to evaluate the systems used to monitor HIV populations accessing therapy and care in low and middle -income countries, it was noted that there was a lack of a common use of recommended data in the national tools utilized to monitor the clinical progress of the patients. From the limited studies that have evaluated data collected during the routine delivery of HIV care, weaknesses such as missing, incomplete or inaccurate data, have been documented (Blaya et al., 2011; Han et al. 2011; Dabis et al. 2005; Doyle, Glynn & Groseclose, 2002; Phillips et al., 1999).

In a study by Mate et al (2009) to assess the completeness and accuracy of key PMTCT data elements routinely collected and reported through the District Health Information System (DHIS) of all clinics and hospitals in KwaZulu-Natal (KZN) province in South Africa, it was noted that the data analysed were complete half of the time (50.3%).A survey of a data system that tracks the public sector ART programme in Malawi found that most clinics had complete case registration and clinical outcomes data complete in only 40% of the sites (Makombe et al., 2008)

2.2 ADRs in HIV infected persons on ART

Determining the number of ADRs is not always possible given the difficulties in identifying the causality and the low number of ADRs that are reported. Data on ADRs is studied using severity scales which are based on estimates of their effect on the individual and also by use of laboratory events on the degree of perturbation relative to reference ranges which are not always reported for each ADR and for each individual (Carr, 2002).

A study on adherence and interrelated factors of AIDS patients receiving ARV drugs in Henan Province, China, showed that the main reason (66.95%) for the patients' non-compliance to ART is ADRs (Li et al., 2005). All ARV drugs can have both short-term and long-term ADRs. The risk of specific ADRs varies from drug to drug, from drug class to drug class, and from patient to patient (Hoffman, 2005).

In a systematic review of studies on ADRs in hospitalised children by Impicciatore et al (2001), whose aim was to evaluate ADRs causing paediatric hospital admissions, 17 prospective studies were included. In hospitalised children, the overall incidence of ADRs was 29.5% (95% confidence interval [CI], 6.8, 12.3) and severe reactions accounted for 12.3% (95% CI, 8.4, and 16.17) of the total. The overall rate of paediatric hospital admissions due to ADRs was 2.09% (95%CI, 1.02, 3.8) and 39.3% (95%CI, 30.7, 47.9) of the ADRs, causing hospital admissions with life threatening reactions. For outpatient children the overall incidence of ADRs was 11.46% (95% CI, 0.7, and 3.03). These results showed that ADRs in children are a significant public health issue.

In a Swiss HIV Cohort Study to assess ADRs associated with potent ARV treatment, 47% of patients presented with clinical and 27% with laboratory ADRs. Among these, 9% (47 of 545) and 16% (30 of 194), respectively, were graded as serious or severe. Single PI, Zidovudine (AZT) use and three class ARV treatment, were associated with higher prevalence of ADRs (odds ratio [OR] 2.0 [95% CI 1.0–4.0], and 3.9 [1.2–12.9] (Fellay et al., 2001).

2.3 ART: The South African perspective

The demand for ART in developing countries like South Africa has increased with the advent of the HIV/AIDS epidemic. South Africa as a developing country is faced with the immense task of increasing the coverage of the population with mass treatment using new medicines with better safety profiles. The large populations covered and the use of new medicines provides both the potential for benefit and harm.

ARVs may be used alone or in a combination form to manage HIV and AIDS but it does not cure the illness. ARVs retard the growth of HIV enabling the immune system to function optimally and defend the body against opportunistic infections (Odunukwe et al., 2005). The use of ARVs enables HIV patients to live longer and it offers improved quality of life (Carr & Cooper, 2000). Patients are provided with a combination of at least three ARVs in the form of a regimen as the different ARVs work at different stages of HIV (National Department of Health, 2010). ART usually consists of a combination of PIs, NRTIs, and/or NNRTIs. Many studies, including that of Sension (2007), have shown that ART regimens are effective in reducing viral load and boosting CD4 cell counts. Each ART agent has its own unique ADR profile and it is therefore, important, to select ART agents with limited ADRs when developing a multi-drug regimen as numerous safety concerns have emerged regarding the use of ART (Sension, 2007).

The first South African HIV management guidelines were published in 2004 (National Department of Health, 2004). These were later replaced by the HIV management guidelines in 2010 (Department of Health, 2010) and 2013 (National Department of Health, 2013). A guideline has been defined as a "document that may contain recommendations about health

interventions and may also contain processes and procedures which help in guiding health service delivery and management" (Orem et al., 2012: 2). Guidelines are developed for various reasons including: to bridge the gap between evidence and practice; to minimise variations in practice; to improve health outcomes; to improve quality of care and to reduce costs (Schünemann et al., 2006). Guidelines are a source of knowledge which translates evidence from research into practice (Shiffman et al. 2004).

In South Africa, treatment initiation is currently guided by the level of immune suppression of the patient, which is measured by the number of circulating CD4 cells in the blood. A typical healthy individual will have about 1100 CD4 (95% CI: 610 to 2100) cells per microlitre of blood (cells/µl) (Williams et al., 2006). In the past 10 years, guidelines for both adults and children specified that treatment should be initiated when the CD4 cell count drops below200 cells/µl. However, in 2009 the WHO changed its guidelines to provide treatment for all HIV infected paediatric patients regardless of the CD4 cell count because of evidence that this would improve patient clinical outcomes (WHO, 2010).

In 2004, the majority of children in South Africa were diagnosed on the basis of symptomatic HIV disease. For children aged between six months and three years of age, the first line regimen consisted of stavudine (D4T), lamivudine (3TC), lopinavir/ritonavir (LPV/r) and the second regimen consisted of AZT, DDI and nevirapine (NVP). For children older than three years and having a weight greater than 10 kg, the first line regimen consisted of D4T, 3TC, efavirenz (EFV) and the second line regimen consisted of AZT, DDI and LPV/r. These guidelines were then changed to the 2010 HIV management guidelines in which all HIV infected children were

to be started on ART. For children under three years, weighing less than 10 kilograms, the first - line ART consisted of abacavir (ABC), 3TC and LPV/r and for children above three years weighing more than 10 kilograms, the first line ART regimen consisted of ABC, 3TC and EFV (NDoH, 2010).

When the 2010 guidelines were updated to the current 2013 HIV management guidelines, ART was recommended to all HIV infected children less than five years of age, irrespective of CD4 cell count and children from five to 15 years of age with WHO clinical stage three or four or CD4 less than 350 cells/µl. For infants and children under three years (or weight less than 10kg), the first line ART regimen consists of ABC + 3TC + LPV/r and for children older than three years of age and having a weight greater than10kg, the first line regimen consists of ABC + 3TC + EFV. With regards to the second line regimen, ABC +3TC + EFV (or NVP) changed to AZT + 3TC + LPV/r and a regimen that consists of D4T +3TC + EFV (or NVP) changed to AZT + ABC + LPV/r (National Department of Health, 2013).

Changing from one ART regimen to the other is a decision which should only be undertaken after careful consideration by the health care professionals. Second-line treatment is generally used subsequent to treatment failure, as reflected by a viral load greater than 1000 copies/ml despite good adherence (National Department of Health, 2013; NDoH, 2010).

2.3.1 Monitoring of outcomes on ART using laboratory parameters in South Africa

Some of the baseline clinical and laboratory information that should be clearly documented in the patient medical folder include:

• Weight and height;

• WHO Clinical Staging;

• CD4 count and percentage;

• Viral load and

• Recent Full Blood Count (or Haemoglobin) (National Department of Health, 2010: 30)

After the start of ART, monitoring should include the following laboratory tests:

- CD 4 count and percentage performed at initiation of ART, after six months, one year and then annually;
- Viral load test performed at initiation of therapy, after six months, one year and then annually;
- FBC if the child is on AZT and the test performed at one month, two months, three months and then annually and
- Total cholesterol (TC) and triglycerides performed annually when children have been treated with LPV/r (National Department of Health, 2010: 31).

2.4 Pharmacology of ARV drugs

2.4.1 NRTIs and nucleotide reverse transcriptase inhibitors (NtRTIs)

NRTIs are analogues of endogenous 2-deoxy-nucleosides and nucleotides. They are inactive in their parent forms and require phosphorylation by host cell kinases and phosphor transferases to form deoxynucleoside tri-phosphate (dNTPs) analogues, capable of viral inhibition. In their respective tri-phosphate forms, NRTIs compete with their matching endogenous dNTPs for incorporation by HIV reverse transcriptase. Once incorporated, they serve as chain terminators of viral reverse transcripts, thus, acting early in the viral replication cycle by inhibiting a critical

step of pro-viral DNA synthesis prior to integration into the host cell genome (Dienstag, 2012 Dudley, 1995).

The major toxicities of NRTI and NtRTI therapy, particularly over the medium to long term, are thought to be secondary to inhibition of mitochondrial DNA polymerase, resulting in impaired synthesis of mitochondrial enzymes that generate adenosine tri-phosphate by oxidative phosphorylation (Sanne et al., 2005). These include haematological abnormalities leading to anaemia which are commonly associated with NRTIs, myopathy by AZT, neuropathy by D4T, didanosine (DDI), zalcitabine, hepatic steatosis and hyperlactatemia due to DDI, D4T, AZT and possibly all the NRTIs, predominantly D4T, cause peripheral lipoatrophy and pancreatitis (Carr & Cooper, 2000).

2.4.2 NNRTIs

The NNRTIs directly inhibit the HIV-1 reverse transcriptase by binding in a reversible and non-competitive manner to the enzyme. The NNRTIs commonly used are NVP, whose common side effects are rash, Steven-Johnson syndrome, hepatitis, including fatal hepatic necrosis, systemic hypersensitivity syndrome with potential for multisystem organ involvement and shock. EFV is associated with rash, central nervous system symptoms such as dizziness, somnolence, insomnia, abnormal dreams, impaired concentration, psychosis, seizures, and increased transaminases (Leith et al., 2005; Carr& Cooper, 2000; Kakunda, 2000).

2.4.3 PIs

PIs competitively inhibit the cleavage of the gag-pol polyprotein which is a crucial step in the viral maturation process there by resulting in the production of immature and non-infectious virions (Huisman et al., 2000). PIs appear to bind to Low density lipo protein receptor-related protein (LRP), reducing the cleavage of fatty acids from circulating triglycerides by the LRP-lipoprotein lipase complex on vascular endothelium and impairing the uptake of remnant hepatic chylomicrons (Souza et al., 2013; Kim & Rutstein, 2010). The most commonly used PIs are the combination of LPV/r which are associated with gastrointestinal intolerance i.e. nausea, vomiting, diarrhoea, taste alteration; asthenia; hyperlipidemia, especially hypertriglyceridemia, elevated transaminases, hyperglycaemia, abnormal fat distribution, possible increased bleeding in patients with haemophilia, PR interval prolongation, QT interval prolongation and Torsade de Pointes, and risk of toxicity including life-threatening cardiotoxicity (OARAC, 2014).

2.5 Factors associated with haematological abnormalities

In paediatrics both ARV treated and untreated, haematological abnormalities are common and they increase the risk of morbidity and mortality (Enawgaw et al., 2014; Ezeonwu et al., 2014; Abebe & Alemseged, 2009; Eley et al., 2002). The haematological abnormalities have been associated with HIV infection itself, HIV related co-morbidities, nutrient deficiencies, use of antibiotics (cotrimoxazole, fluconozale, dapsone) demographic characteristics (Lubomirov et al. 2011), ART regimen effects, the number of months that patients have been on ART, previous exposure to ART and patient gender (Dryden-Peterson et al., 2013, Strehlau et al. 2012; Renner et al.,2012; Aurpibul, 2008; Moyle et al. 2004; Moore et al. 2001; Meynard et al. 1997).

In Ethiopia, Enawgaw enrolled 290 HIV infected patients in a comparative cross-sectional study to determine haematological and immunological parameters among HIV positive patients taking ART. In this study the prevalence of anaemia and neutropenia was 11.7% and 28.3% respectively. In India, a retrospective study conducted by Shet et al (2005) revealed the prevalence of anaemia to be 66% in children on ART. In a prospective European collaborative study conducted by Bunders et al (2004), data on 156 HIV-infected and 1533 uninfected children was followed from birth until eight years of age. After approximately four months of age, neutrophil counts were consistently and substantially lower in children on ART. In both groups, black children had significantly lower counts than white children across the whole age range. Male gender and ARV exposure were associated with reduced neutrophil count until at least eight years of age.

2.5.1 Association between ART and haematological abnormalities

Haematological abnormalities due to ART have been associated with anaemia and neutropenia in children. A longer term haematological impact of in utero exposure to ART has been reported by European studies (PEP study Group, 2005; European Collaborative study, 2004; Le Chenadec et al., 2003). The mechanism underlying this toxicity is still unclear although it has been linked to mitochondrial toxicity (Lewis et al., 2003). Studies have also shown that ART is associated with haematological abnormalities in pregnant mothers as well as in their newborns, because these drugs can cross the placental barrier and negatively affect foetal erythropoiesis (Wongnoi et al., 2013;Baroncelli et al., 2011; Feiternaet al., 2007).

In Kenya, Njuguna el al (2013) conducted a study in which 104 infants were enrolled. In this study hematologic profiles of infants born to mothers receiving ART were assessed. All mothers had CD4+ cell counts of >350 mm^{-3}; 93% received AZT-containing ART; infants received NVP up to six weeks and cotrimoxazole after six weeks. Among 84 infants at 19 weeks, 67% had hematologic toxicity; 44% had neutropenia and 23% had anaemia. Breastfeeding was associated with a 3.8-fold higher risk of neutropenia (RR 3.8, 95% CI 1.03–14.1, p = 0.008). In a study by Le Chenadec et al (2003) conducted in Europe, the Hb and neutrophil levels were rapidly reduced in new-borns exposed to AZT. The multivariate analysis indicated that the effect of treatment was significant: treated infants had significantly lower levels of neutrophils and Hb (-0.277, p less than 0.0001; Hb, -0.164, p less than 0.0001).

In a retrospective study by Shet et al (2009), data from 248 HIV-infected children aged one to 12 years attending three outpatient clinics in South India was collected between 2004 and 2006 and analysed using statistical analyses i.e. Chi Squared, t - tests, univariate and multivariate logistic regression analyses. The overall prevalence of anaemia (defined as Hb less than 11 gm/dl) was 66%, and 8% for severe anaemia (Hb less than 7 gm/dl). Independent risk factors of anaemia by multivariate analysis were ages younger than six years (OR: 2.87; 95% CI: 1.45, 5.70; p less than 0.01);rural residence (OR: 12.0; 95% CI: 5.6, 26.0; p below 0.01); advanced HIV disease stage (OR: 6.9; 95% CI: 3.1, 15.6; p less than 0.01) and presence of stunting (Height-for-age Z Scoreless than -2) (OR: 3.2; 95% CI: 1.7, 6.4; p less than 0.01). No statistical significant association was found between anaemia and ART type (AZT vs.D4T) and duration on therapy. However, in a multicentre cohort study of HIV- infected children in Asia, Bunupuradah et al (2013:808) noted that the potential risk factors associated with anaemia assessed by logistic

regression were: previous or current use of AZT and median duration of six months on therapy (p less than 0.0001 and p equal to 0.013, respectively).

Aurpibul et al (2008) carried out a retrospective cohort study in which HIV-infected children two to 15 years of age at the time of ART initiation had never had haematological effects and had switched from D4T to AZT at least 48 weeks previously. Seventy eight children were included in the study of which 36(46%) were male. The mean age was 10.3 years. The switch had been made a median time of 65 weeks (range 48–97 weeks) previously. There was no significant change in CD4 lymphocyte count or percentage, or HIV RNA level, after the switch. There was a statistically significant decrease in haemoglobin level (12.6 vs.12.1 g/dl; p less than 0.001), total white blood cell (WBC) count (8088 vs. 6910 cells/μl; p less than 0.001) and absolute neutrophil count (ANC) (4320 vs. 3448 cells/μl; p less than 0.001). In 2013, Wongnoi et al (2013) carried out a study to investigate the effects of ARV drugs on haematological parameters and thymic function in HIV infected new-borns of HIV infected mothers. Cord blood samples of new-borns from HIV uninfected and HIV-infected mothers were collected. Haematological parameters were measured using automatic blood cell count. T-cell receptor excision circles levels in cord blood mononuclear cells, CD4 and CD8T cells were quantified using real-time PCR. Results showed that new-borns of HIV-infected mothers tended to have lower mean levels of Hb than those of HIV-uninfected mothers (137 ± 22 vs.146 ± 17 g/L, p equal to 0.05). The mean of red blood cell counts and haematocrit and the median of TRECs in CD4 T-cells in the new-borns of the former, were significantly lower than those of the latter (3.6 ± 0.7 vs. 4.8 ± 0.6 x 1012 cells/l, p less than 0.001; 0.40 ± 0.07 vs. 0.46 ± 0.05 L/L, p less than 0.001 and 0.5 (IQR:0.03-5.8) vs. 13.2 (IQR: 2.8 -27.5) x 10^{-3} pg/ μl, p equal to 0.02, respectively).

2.5.2 Association between demographic characteristics and haematological abnormalities

Demographic characteristics such as age, race and gender have been associated with haematological abnormalities in children. In a study to determine the levels and patterns of neutrophil cell counts over the first eight years of life in children of HIV infected mothers that formed part of the European collaboration, it was noted that after approximately four months of age, neutrophil counts were consistently and substantially lower in HIV-infected children than in uninfected children; in both groups, black children had significantly (p equal to 0.006) lower counts than white children across the whole age range. In uninfected children, male gender and ARV exposure were associated with reduced neutrophil count until at least eight years of age, with an average difference in z-score of –0.15 (p equal to 0.04) after adjusting for child's sex, ethnicity and prematurity (European collaboration, 2004).

In a study conducted by Gedefaw et al (2013: 4), a high prevalence of haematological abnormalities in children less than 15 years of age was reported. However, between 2004 and 2006, Shet et al (2009) enrolled 248 HIV infected children aged between one to 12 years in a retrospective study in South India to evaluate anaemia and growth failure among HIV infected children. Independent risk factors of anaemia by multivariate analysis included pre-school age (age younger than six years) (OR: 2.87; 95% CI: 1.45, 5.70; p less than 0.01) and rural residence (OR: 12.04; 95% CI: 5.64, 26.00; p less than 0.01). No statistically significant association was found between anaemia and gender (p equal to 0.145).

2.5.3 Association between medications used to treat co-morbidities in HIV infection and haematological abnormalities

HIV infected children are at risk of opportunistic infections irrespective of their CD4 percentage (Calis et al., 2008). Several medications given to treat co-morbidities have been associated with haematological abnormalities in children and these include: antiviral agents (England et al., 2006; Brandy et al., 2002); antibiotics (Ahmed et al., 2001), Tuberculosis (TB) medications (Knox-Macaulay et al., 1992) and prophylactic regimens against P. jiroveci pneumonia (anti-folate agents) (WHO, 2006; Hughes et al., 2005; McIntosh-Kenneth et al., 1999).

A prospective study of HIV infected children on treatment with ART in combination with cotrimoxazole was carried out to determine the effects of these drug combinations on the Hb profile of infected children three monthly. The Packed Cell Volume (PCV) level was carried out using a haematocrit centrifuge and reader. A total of 173 patients were started on ART during the first year recruitment period of which 90 (52.0%) were males and 83 (48.0%) were females. One hundred and seventeen (67.7%) patients were on AZT - containing ART, while 56 (32.3%) were on D4T- containing combination. The mean PCV of patients on AZT- containing a combination with cotrimoxazole decreased from 30.2 ± 5.5% to 29.0 ± 2.3%, with a net decrease of 1.2% after one year of treatment. Those on D4T- containing a combination with cotrimoxazole instead showed an increase from initial PCV of 28.3 ± 4.2% to 34.2 ± 3.0% with a net increase of 5.9% after the same duration of treatment, which was not statistically significant (p greater than 0.05). Patients on an AZT combination alone and without cotrimoxazole prophylaxis, had a minimal decrease of 0.9% in their PCV level after one year of treatment while those on D4T combination alone without any prophylaxis instead showed an increase of 6.8% in

their PCV after the same duration of treatment. Any ART regimen containing AZT plus cotrimoxazole carries a greater risk of anaemia than that of D4T containing combination with cotrimoxazole (Okechukwu, 2010). However, in a study conducted by Dryden-Peterson et al (2013) in which 1705 children were included, anaemia occurred in 87 (5.1%) infants, and neutropenia in 164 (9.6%) infants. Analysis stratified by the infant feeding method showed no significant differences in the risk of anaemia by prophylactic cotrimoxazole exposure (risk difference −0.69%, 95% confidence interval [CI] −2.1 to 0.76%). Similarly, there were no significant differences observed for severe neutropenia by cotrimoxazole exposure (risk difference 2.0%, 95% CI −1.3 to 5.2% and OR 0.80, 95% CI 0.33 to 1.93).

2.5.4 Association between HIV infection and haematological abnormalities

Haematological abnormalities are common complications of HIV infection in children and these abnormalities increase as the disease advances (Eley et al., 2002). HIV infection suppresses the bone marrow and leads to decreased levels of granulocyte colony stimulating factor; the factor that stimulates production of white blood cells in the bone marrow and affects the granulocyte macrophage lineage, resulting in leucopenia and neutropenia (Alem et al., 2013). Although HIV associated anaemia is multi-factorial, the principal factors are infiltration of the bone marrow by neoplasm or infection (Alem et al., 2013).

Anaemia is a common haematological manifestation in HIV infected paediatrics. Iron deficiency is widespread in HIV infected children in developing countries (Eley et al., 2002). In a study conducted in Cape Town by Eley et al (2002) in HIV infected children, anaemia was present in 73% of children. Compared to mild HIV infection, Hb was lower in children with moderate infection (104g/l vs. 112g/l, p=0.04) and severe clinical infection (96g/l vs. 112g/l, p equal to

0.006). There was a statistical significant relationship between immunological status and Hb (p equal to 0.008).

2.5.5 Association between nutritional status and haematological abnormalities

Childhood malnutrition occurs in HIV infected children due to decreased food intake, increased nutrient loss from malabsorption, diarrhoea and increased metabolic rate. Deficiencies of micronutrients such as iron, vitamin B complex, and folic acid are factors that have been associated with anaemia (Tindyebwa et al., 2006).

In Uganda, Bachou et al (2006) enrolled 315 children in a study to describe the clinical features, haematological findingsand CD4 and CD8 cell counts of children in relation to HIV. 123 children were infected with HIV and 192 were not infected with HIV. Of the 123 children infected with HIV, 43% had oedematous malnutrition. The median Hb concentration of these children was below 9g/dl and the total neutrophil counts was significantly lower in the HIV-positive children (8.9 × 106; IQR 5.4–11.3) than in the HIV-negative children (9.1 × 106; 7.2-3.5) (p equal to 0.028). A nutritional survey conducted by the South African Vitamin A consultative group (SAVACG) showed that approximately 20% of South African children were anaemic and 10% were iron deficient. Another study conducted on Italian children documented iron deficiency caused by intestinal malabsorption in a large proportion of HIV infected children (Labadarios et al., 1996).

Ezeonwu et al (2014) designed a cross−sectional descriptive study in Abuja, Nigeria with the aim to determine the prevalence of haematological abnormalities and malnutrition in HIV- positive

children aged between 18 and 59 months. The study consisted of 67 HIV infected children. Fifteen of the 67 children were malnourished and were not on ART. Of the 15 malnourished children, two were anaemic and there was no statistical association between nutritional status and Hb levels (p equal to 0.121). However, between 2004 and 2006, Shet et al (2009) enrolled 248 HIV-infected children aged between and 12 years in South India to evaluate anaemia and growth failure among HIV-infected children. Anaemia was significantly associated with poor growth (p less than 0.005) in a multivariate analysis.

2.6 Factors associated with dyslipidemias

According to literature, factors that have been associated with lipid profile abnormalities include demographic characteristics such as age, gender, weight and height (body mass index[a]), CD4 count, CD4 percentage, viral load, ART regimen (PI containing or PI naïve), nutritional status, previous exposure to PMTCT and duration of therapy (Souza et al., 2013; Kim et al., 2009; Fauvel et al., 2001). The pathogenesis of dyslipidemias is still unknown. One hypothesis suggests that it might be due to lipogenesis via altered retinoid acid signalling and the inhibition of lipid and a dipocyte regulatory protein that have partial homology to the catalytic site of HIV protease, to which PIs all bind (Carr & Cooper, 2000).

2.6.1 Association between ART and dyslipidemias

Cross-sectional studies with HIV-infected children and adolescents receiving ART have shown high frequency of dyslipidemias and a prevalence of 50 to 70 % (Jacobson et al., 2011; Gonzalez

[a]Body mass index is defined as the individual's body weight divided by the height squared.

et al., 2008; Chantry, 2008; Lainkaetal, 2002). The characteristic pattern of dyslipidemias includes elevated TC (10 to 50%), and triglyceride (40 to 80%) (Rhoads et al., 2011; Lapphraet al., 2005; Farley et al., 2005). These lipid abnormalities were first described in adult patients who used ARV regimens containing PIs, but were later observed in patients who received regimens consisting of NRTI and NNRTI (Rhoads et al., 2006; Fauvel et al., 2001).

The Paediatric AIDS Clinical Trials Group 219C was the first large prospective cohort study to examine the effect of PIs and other ARVs on the incidence of hypercholesterolemia among HIV-infected children and adolescents. This group indicated that the use of PIs leads to a marked increase in TC levels (Tassiopoulos et al., 2008).

In South Africa, a study done by Strehlau et al (2012) to assess lipid profiles in young HIV infected children who were initiated on and changing ART, 155 HIV-infected children who initiated LPV/r based therapy were enrolled.TC, low-density lipoprotein, and triglycerides increased from pre-treatment (p equal to 0.0001). Through 31 months relative to remaining on the LPV/r-based regimen this increase in TC and triglycerides persisted in 16% and 36% of the study population respectively. There were higher changes in lipid profiles in males than the females and in the younger children, although this was not significant (p equal to 0.361).

In a retrospective cohort study involving HIV-infected children with ART vs. HIV-infected children without ART, it was noted that those using the NRTI/PI regimen presented with significantly higher TC levels than NRTI and NRTI/NNRTI. Of the 178 HIV-1 infected children eligible for study, 72.4% had TC greater than 180 mg/dl, 53.4% had TC greater than 200 mg/dl.

For TC greater than 200, the multivariate analysis showed increased risk with NRTI/NNRTI (HR: 1.86, 95%CI: 1.34–2.19) and NRTI/PI (HR: 3.45, 95 % CI: 2.65–4.51) although compared to NRTI/NNRTI, NRTI/PI increased the risk for TC greater than 200 mg/dl (HR: 1.86, 95%CI: 1.45–2.39) in the multivariate model (Kim et al., 2009).

Souza et al (2013) carried out a study to review HIV/AIDS patients in relation to use of ART. Distinct ART regimens appear to promote different changes in lipid metabolism. PIs, particularly indinavir and LPV, were commonly associated with hypercholesterolemia and hypertriglyceridemia after a duration of 15 months. Some NRTIs (DDI, D4T, and AZT), induced lipoatrophy and hypertriglyceridemia, ABC increased the risk of cardiovascular diseases even in the absence of apparent lipid disorders, and tenofovir resulted in lower levels of TC and triglycerides. Although NNRTIs are predisposed to hypertriglyceridemia and hypercholesterolemia, NVP was particularly associated with high HDL levels, a protective factor against cardiovascular diseases (Souza et al., 2013).

2.6.2 Association between demographic characteristics and dyslipidemias

Adolescents present with more dyslipidemias while pre-pubertal children express a milder clinical presentation and this could be brought about due to the hormonal changes during puberty (Taylor, 2004). Some studies have reported an association between gender and TC levels like the European paediatric lipodystrophy group which concluded that there is a higher risk of girls developing dyslipidemias than boys (European Paediatric Lipodystrophy Group, 2004) and in a study conducted by Kim et al., (2009) males were at a higher risk of developing dyslipidemia.

However, in Werner's study there were no differences in prevalence between males and females (Werner, 2005).

Tassiopoulos et al (2008) conducted a study aimed at examining the incidence of hypercholesterolemia in a prospectively followed cohort of 2576 prenatally HIV-infected children while adjusting for potentially confounding factors. 13% of the children had hypercholesterolemia. A younger age (less than six years) was statistically associated with high TC levels (p equal to 0.04). Farley et al (2005) enrolled 1812 HIV infected children aged between four and 19 years in a paediatric AIDS clinical trials group 219C (PACTG 219C) prospective cohort study whose aim was to determine the prevalence of elevated TC and associated risk factors among prenatally HIV- infected children. Of the 1812 children enrolled, 229 (13%) had hypercholesterolemia. In ages between four and six (OR=2.9, 95% CI: 1.7-4.9), the viral load was less than 400 copies/ml (OR=5.3, 95% CI: 1.7-3.2,), ages between six and 12 years (OR=1.9, 95% CI: 1.3-2.9), were independently associated with the presence of hypercholesterolemia.

2.6.3 Association between nutritional status and dyslipidemia

In HIV infected persons low serum concentrations of vitamins and minerals termed as micro nutrients, are associated with progression of HIV infection and mortality. In a systemic review of articles by Drain et al (2007) with the aim of evaluating micronutrients in HIV positive persons receiving ART, five cross sectional studies were analysed. Micronutrients were regarded as essential nutrients necessary for maintaining proper immunologic function in HIV infected patients. Conclusions from this review were made and these indicated that vitamin A

deficiency reduces lymphocyte response; vitamin C deficiency depresses cell mediated immune response; vitamin E deficiency impairs T cell mediated function and lymphocyte proliferation; riboflavin deficiency impairs the generation of humoral antibody response; vitamin B-6 deficiency reduces lymphocyte maturation and diminishes antibody production and vitamin B-12 deficiency impairs neutrophil function. With regard to certain minerals, folic acid deficiency depresses the cell-mediated immunity response; zinc deficiency decreases lymphocyte concentrations; copper deficiency reduces the cytokine response, and selenium is needed for proper functioning of neutrophils and T lymphocytes.

Beraldo-Battistini et al (2010) enrolled 30 HIV infected children and 30 uninfected healthy children to a cross sectional study whose aim was to evaluate lipodystrophy, lipid profile changes and low serum retinol and carotinoid levels. Dyslipidemia was detected in 60% and 23% of subjects with HIV and control subjects respectively (p equal to 0.004). A significantly high frequency of low serum retinol (60% vs. 26.7%, p equal to 0.009) and β-carotene (23.3% vs. 3.3%, p = 0.026) was found in the AIDS group compared with the control group.

2.6.4 Association between HIV infection and dyslipidemia

Retinol and carotenoid deficiencies are a common occurrence in HIV-infected subjects and are associated with worse clinical and immunologic courses and higher mortality (Drain et al., 2007). There seems to be a synergistic effect of retinol/carotenoid deficiency, HIV infection, and ART in the exacerbation of the cell oxidative stress. Dyslipidemias in HIV-infected patients, results from a combination of causes such as HIV infection, antiretroviral therapy and genetic factors among others (Drain et al., 2007).

Between 2005 and 2006 in Yaoundé, Cameroon, Nguemaïm et al (2010) enrolled 376 HIV infected adults to evaluate Serum lipid profile in HIV-infected patients. Compared with control subjects, patients with CD4 counts less than 50 cells/ml had significantly lower TC (p equal to 0.0001) and triglycerides (p equal to 0.0001). Patients with CD4 counts between 50 and 199 cells/ml had significantly lower TC (p equal to 0.001) and significantly higher TG values (p equal to 0.001); patients with CD4 counts between 200 and 350 cells/ml (p equal to 0.003) and higher than 350 cells/ml (p equal to 0.0001) had significantly high TG.

2.7 Lessons learnt from literature

This chapter focused on the published literature pertaining to the challenges faced by M & E of HIV treatment, parameters used to monitor trends in lipid and haematological abnormalities and factors associated with these abnormalities.

Although HIV management guidelines are widely available for improving the management of the disease in many countries, accuracy and completeness of patient medical folders has been noted in literature as a major challenge in the health facilities that provide ART in developing countries. In most of the studies reviewed, dyslipidemias have been assessed by comparing TC and triglyceride levels at baseline and after ART initiation. Similarly, haematological abnormalities have been assessed by comparing Hb at baseline and after ART initiation. This carries the advantage of excluding existing abnormalities prior to ART initiation. Most literature has linked ART regimen, duration of ART, demographic characteristics, nutritional status and the HIV infection to lipid and haematological abnormalities. However, in some longitudinal studies, the main factor that is significantly associated with either lipid or haematological

abnormalities is the type of ART regimen and duration of ART therapy. Any ART regimen containing AZT has been significantly associated with haematological abnormalities after an average of six months on therapy. Similarly any ART regimen containing a PI has been associated with dyslipidemia in children after an average of 12 months on therapy.

3.0 Chapter 3 Methodology

This chapter describes the research design, study population, sampling strategy, selection criteria, strategies that were adopted to improve validity and reliability as well as the ethical considerations.

3.1 Study design

This was a descriptive, retrospective review of patient medical records from a CHC and laboratory data from the NHLS to: i) assess the completeness of data from the patient medical folders; ii) assess for lipid and haematological abnormalities using a suitable parameter; iii) identify the risk factors associated with the lipid and haematological abnormalities and iv) assess prescriber adherence to the HIV management guidelines with regards to the recommended haematological and lipid laboratory testing.

Descriptive studies collect information without changing the environment and are used to describe the distribution of existing variables without regard to causality or hypothesis testing. Descriptive studies document the health of populations which later prompts more rigorous studies (Grimes& Schulz 2002). Despite the fact that in a retrospective study some data may not be available, it is a study that can be done on a smaller scale, requires less time to complete and is better for analysing multiple outcomes (Hess, 2004).

3.2 Data sources

Data were collected from two sources, viz., patient medical records and the NHLS database. We sourced relevant laboratory data from the NHLS which would provide data on markers for lipid and haematological abnormalities. Patient medical records remained a key source of demographic and some clinical data which would be combined with laboratory data

The NHLS is the sole provider of diagnostic pathology services to the public sector in South Africa. It provides these services across the country at all levels of health service delivery, covering over 80% of the population. NHLS diagnostic laboratories serve provincial and district hospitals in large metropolitan centres and remote rural areas. They play a major role in the national HIV and AIDS treatment programme through CD4 testing, viral load and HIV treatment monitoring by carrying out laboratory tests that are linked to ART (National Department of Health, 2014).

3.3 Study setting

This study was carried out in the Cape Metropole, a district in the city of Cape Town in the Western Cape of South Africa (refer to Figure 1). The Cape Metropole accommodates approximately 66% of the total population in South Africa and is one of six districts in the province (NDoH, 2010). It is geographically divided into eight sub-districts, namely: Northern, Klipfontein, Southern, Eastern, Western, Mitchells Plain, Tygerberg and Southern (Statistics South Africa, 2011).

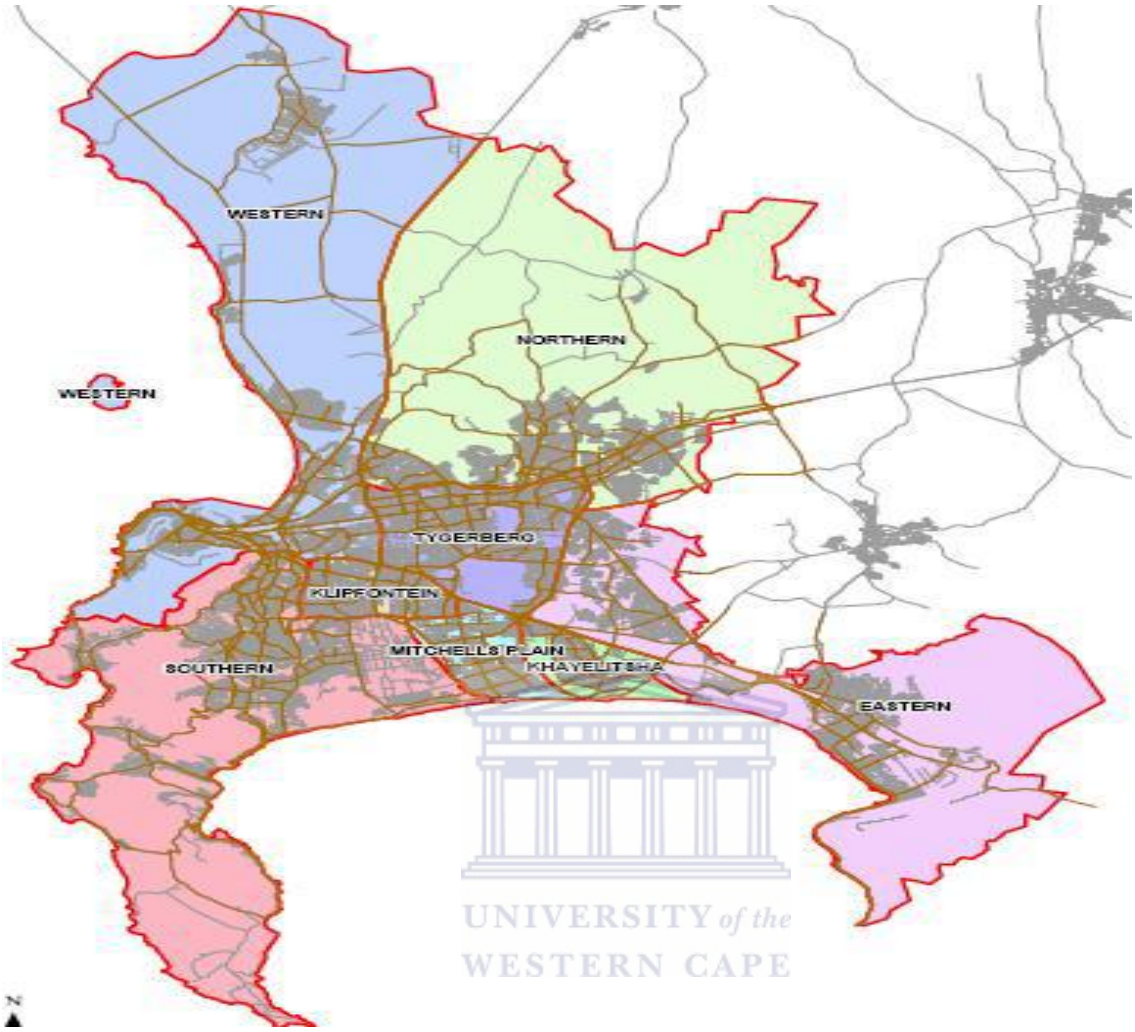

Figure 1: Geographical location of the sub-districts in the Cape Metropole

(Provincial Department of Health, 2010)

The area selected for our study is a suburb in the northern part of the Cape Metropole, with a population of approximately 362,980 and113,787 households having an average size of 3.19 family members. This area has a diverse spread of races comprising so-called "Black Africans", "Coloureds", "Asians", "Whites", and "other" as seen in table 1(SDI&GIS 2013). The study area has approximately 79,151 children with 39% under five years of age and 61% between the

ages of five and 14. The majority (34.9%) of children under five are classified as "Blacks" followed by "Whites" (32.9%), "Coloureds" (29.6%) and "Asians" (0.87%). Children between the ages of five and 14 were mainly classified as "White" (38.2%) followed by "Coloureds" (31.7%), "Black Africans" (27.7%) and "Asians" (1.1%) (refer to table 1).

Table 1 Racial composition of children under 14 years in the study area

Age(Yrs)	"Black Africans"	"Coloureds"	"Asians"	"Whites"	"Others"
Less than 5	34.9%	29.6%	0.87%	32.9%	1.77%
5-14	27.7%	31.7%	1.1%	38.2%	1.3%

(SDI&GIS, 2013)

The public sector primary health care facilities in this district are administered under either the Western Cape Provincial Government (PGWC) or the Cape Town City Health (CTCH). For this study, the facility selected was administered by the PGWC.

The CHC has an HIV clinic which provides services such as TB, HIV/AIDS programmes and a pharmacy that stocks ARVs and other medication required for the management of HIV and TB. The facility has general practitioners, nurses as well as pharmacists. Laboratory tests that need to be performed, such as the absolute CD4 cell counts, CD4 percentages, viral load, full blood counts and other blood chemistry investigations, are sent either directly to the NHLS from the CHC or patients are referred to a tertiary hospital which takes responsibility for ordering tests from the NHLS. Results from the NHLS are sent back to the relevant sites.

3.4 Study population

The study population comprised HIV positive children between the ages of zero and 14 years enrolled in the ART programme at the study site. The paediatric population is considered to be between zero and 14 years (WHO, 2013b).

3.5 Sampling

3.5.1 Sampling strategy

The facility was selected based on its accreditation as an ARV site and the researcher's awareness of the relatively diverse racial profile of the area. Most other facilities in the Cape Metropole serve a distinct racial group. Furthermore, this site has been earmarked by the research team for future longitudinal studies on paediatric HIV patients. This study serves to provide some baseline information as well as insights on feasibility of pursuing specific research questions.

3.5.2 Selection criteria

Inclusion criteria

The study included all HIV positive paediatric patients at the study site who are currently (as of 14 June2013) on ART, regardless of where therapy was initiated.

Exclusion criteria

Those who were transferred out, were lost to follow up or died more than six months prior to the starting date of data collection, were excluded.

3.5.3 Sample size

The complete list of HIV positive children (N=280) on ART at the CHC was obtained from the electronic patient register. The paediatric patients who were lost to follow up, transferred out of the CHC or who died six months before the start of the study were identified and removed from the list (refer to figure 2). The final sample size was, therefore, 168 paediatric patients who were currently receiving ART.

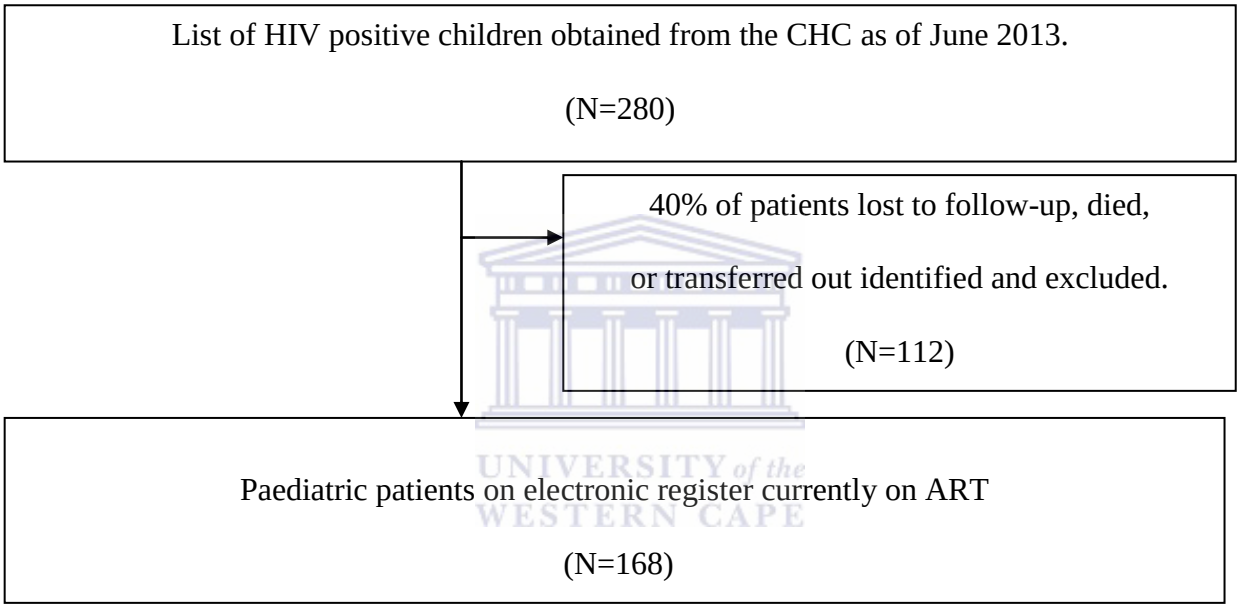

Figure 2: Sampling of the patient medical records

3.6 Data collection

3.6.1 Preparation for data collection

Prior to the start of data collection, the researcher contacted the facility manager and the sister in charge to schedule appointments. The patient medical record form used at the CHC formed the basis for developing our data collection tool which was used to extract patient demographic characteristics and clinical information. The data collection tool was used to collect data for a

larger study on adherence, ARV dosages and adverse effects. Similarly a tool for extracting data from the NHLS database was created (refer to appendix I).

3.6.2 Content of the medical record tool

The tool consisted of the following fields:

- Demographic data: date of birth and gender,
- Clinical data:
  - Year the patient was diagnosed with HIV, the child exposure to ART through Prevention of Mother to Child Transmission (PMTCT).
  - Baseline data: baseline CD4 count, baseline CD4 percentage, baseline viral load, baseline WHO clinical staging, baseline weight and height, baseline haematological and lipid test results and tuberculosis screening and treatment
- Data after baseline on each visit: ART regimens prescribed, months on ART, additional medications, absolute CD4 count, CD4 percentage, viral load, weight and height as well as identified opportunistic infections and description of adverse events (AEs), the AE grade, records on whether it was a new or recurring AE.

3.6.3 Content of the NHLS tool

The tool consisted of the following fields:

- Viral load;
- Absolute CD4;
- CD4 percentage;
- Haematological test results (Hb and Neutrophils);

- Lipid test results (TC, triglycerides) and
- Dates for all the corresponding laboratory results

3.7 Training of data collectors

Twelve final year pharmacy students were selected and trained for the data collection and data capturing. One day of training was conducted at the School of Pharmacy. Training encompassed the following:

- Understanding the structure and content fields of the patient folder;
- Completing the data collection tool in a systematic and rational manner and
- Capturing data using a mock folder.

3.8 Pilot study

A pilot study was conducted to pretest the data collection tool and the data collection process. The aim of the pilot study was to develop, adapt or check the feasibility of techniques / methods to be used in the main study when collecting the data and also to determine the general completeness of data for key variables. The pilot study was carried out in two phases.

3. 8.1 Phase one

A total of 15 patient medical folders were selected and used to pretest the data collection tool and the procedures. Patient medical records are known to have limitations in terms of completeness. The goals of the first phase of the pilot study were: i) to determine the fields that were routinely omitted and therefore not suitable for inclusion in the tool and ii) to familiarise fieldworkers with

the data collection tool and to construct a feasible work plan that did not impinge on the daily routine of staff at the CHC.

Table 2 Phase one of the pilot study

	Lessons learnt
Step one: Identified which demographic and baseline clinical data are routinely recorded	Routinely recorded: age, gender, baseline CD4%, viral load, baseline absolute CD4 count, WHO clinical staging, ART regimens and ART start date Not routinely recorded: Nutritional status, height and weight at baseline and after start of ART, description of the AEs, AE grades, records of new or recurring AEs, records on PMTCT exposure, baseline and after start of ART haematological and lipid test results, TB treatment and the description of the opportunistic infections.
Step two: Identified the different categories of ART regimens prescribed	The combinations were categorised as : 1. AZT+DDI+ kaletra(LPV/r) 2. D4T+ 3TC+EFV 3. ABC+ 3TC+LPV/r 4. D4T+ 3TC+ LPV/r 5. ABC+ 3TC+ EFC 6. AZT+ 3TC+EFV 7. AZT+ 3TC+ LPV/r

	8. AZT+ 3TC+ NVP 9. ABC+ AZT+ LPV/r
Step three: Identified other medications prescribed	Apart from ARVs, the CHC HIV clinic provides other medication for HIV and TB management. The medications of interest in this study were those shown in literature to be associated with haematological and lipid profile abnormalities viz., cotrimoxazole, fluconazole, isoniazid and dapsone.
Step four: Identified whether any regimen changes were due to AEs.	There was no explanation given in patient folder as to the reason for a change in regimen.
Step five: Assessed the simplicity of the data collection tool	Data collection tool was easy to understand and structure and sequencing of fields facilitated efficient data extraction.
Step six: Identified the best way to minimise interference with the health care worker's daily routine and to manage time optimally.	Patient folders were retrieved during the less busy times of the day, i.e. in the afternoons (in preparation for the next day).

3. 8.2 Phase two

During this phase, the methods of managing and analysing data were pretested. Fifteen patient medical folders were linked to the NHLS laboratory database by the folder number (refer to table 3).

Table 3 Phase two of the pilot study

	Lessons learnt from pilot
Step one : Identified and extracted relevant immunological, haematological and lipid test results and the corresponding dates when the tests were done from the NHLS database	Able to extract the absolute CD4 count, CD4%, viral load, Hb, neutrophils, TC, triglycerides and the dates for each of these tests
Step Two: Ran frequency distribution curves for each of the extracted variables to identify anomalies	No anomalies found
Step three: Identified the date of ART initiation from the patient medical folder for each patient with a view to check the corresponding (baseline) test results in the NHLS database.	Very few test results corresponding to the date of ART initiation were available in the NHLS database (see frequencies of availability below): 1. CD4 count (20%); 2. CD4% (20%); 3. Viral load (27%); 4. Hb (13%); 5. Neutrophils (0%); 6. TC (7%) and

	7. Triglycerides (7%) Based on the poor availability of baseline data, a decision was taken to compare haematological and lipid levels to their respective normal reference ranges or values for TC, triglycerides, Hb and neutrophils. Patient medical folders were used as the source for the following baseline information: 1. CD4 count; 2. CD4% and 3. Viral load.
Step four: The NHLS database provides laboratory data as distinct row entries for each set of laboratory tests ordered. Therefore multiple visits generated more than one row of data for a particular patient.	This data was transformed from the long format to the wide format in SPSS(IBM SPSS, 2013) so that all laboratory data for a particular patient is presented in the same row (refer to appendix II)
Step five: Identified a suitable parameter for assessing haematological and lipid abnormalities	The required annual tests for Hb, neutrophils, TC and triglycerides were not performed in the majority of patients and a decision was taken to identify at least one reading for each test per patient – decided by researchers to be the maximum or highest value.

Step six: Identified viral load, CD4% and absolute CD4 count recorded on the date when maximum haematological and lipid levels were noted from NHLS database	For the majority of the patients, these tests were not taken on the same date as the maximum haematological and lipid level. Therefore, a decision was taken to record any CD4 count, CD4% and viral load performed within six months before or after the said date.
Step seven: Combined relevant variables from the NHLS and the patient medical folder data to compute: the age and months on ART at the time of maximum haematological and lipid levels.	Age [2]= date of test minus date of birth Months on ART = date of test minus date of ART initiation
Step eight: Assessed the adherence to HIV management guidelines with regards to laboratory testing.	None of the 15 patients had their lipid and haematological laboratory tests conducted as per the relevant HIV management guidelines and some had no tests performed at all. A decision was taken to assess the number of patients on a PI or on AZT who had at least one of each of the relevant tests performed.
Step nine: Validity and reliability of the data collection and data entry process	

[2] Age and months on ART were computed using SPSS software version 22 (IBM SPSS, 2013)

Identified and trained fourth year students for data collection and data capturing	The same set of students indentified for data collection was used for the entire data collection process and the same applied to the data capturers.
Assessed the sections included in the data collection tool.	Mock patients were used at the School of Pharmacy to assess the suitability of the sections included in the data collection tool. All the sections were relevant for the main study. Each section answered the questions on the variables to be measured. Data fields like the social information were not necessary, hence were not to be completed in the main study.
Randomly selected 11 patient folders at the CHC and assessed the simplicity of the data collection tool.	The data collectors found the tool to be clear and easy to comprehend.
Replaced patient folder numbers with unique patient identifiers.	Patient information was not easily identified with the unique patient identifiers.
Consulted a statistician on ways to analyse the randomly selected patient folders.	Due to the small sample size, data was to be categorised and analysed by Chi-squared analysis in the main study.
Identified ways of reducing error rates in the data collection and capturing process.	Data was to be entered onto a Microsoft Excel spreadsheet by data capturers the same day the data collectors returned from the facility.

Created manuals for transforming variables, recoding variables, merging datasets and analysis.	Manuals were accessible and all the processes which included merging data sets, creating the new variables by transforming variables, recoding variables, and analysis, were to be done as per the manuals.

3.9 Data collection procedure

After the pilot study was conducted, the actual data collection was carried out from 17July 2013to 3July 2014. At the facility, the principal researcher searched for the folders from the electronic system at the facility and this indicated whether the patient was within the CHC, lost to follow-up, died or was transferred out. For the patients registered under the CHC, the principal researcher retrieved folders from shelves and handed them over to the data collectors, who then extracted the necessary information onto the data collection form. Each completed data collection tool was then assigned a unique ID to replace the patient folder number, filed and kept in the principal investigator's office at the university. The last visit date was different for each patient depending on when the data was collected but no clinical data entered after 17 July 2013 was collected.

Two datasets were created, one for data from the patient medical folders and the second for data from the NHLS. The immunological, haematological and lipid test results were extracted from the NHLS database (refer to table3). The highest laboratory values for the Hb, neutrophils, TC and triglycerides laboratory tests with the corresponding dates were computed (refer to appendix III). From the patient medical folders the following information was obtained: the gender; date

of birth; year the patient started their ART; WHO clinical staging; baseline viral load; baseline CD4 count; baseline CD4 percentage; and the age was computed from date of birth using Microsoft Excel ™. The finalised datasets were then merged.

3.10 Research variables

3.10.1 Dependent variables

3.10.1.1 Main dependent variables

The highest Hb, neutrophil, TC and triglyceride levels for each patient were the main dependent variables in the study. All dependent variables, the units of measurement, categories and sources of data are outlined in table 4.

Table 4: Units, categories and sources of data for main dependent variables

Dependent variables	**Units**	**Categories**	**Ideal source (as determined by pilot study)**
Hb levels (highest)	g/dl	Refer to table 5	NHLS data set
Neutrophils levels (highest)	$x10^9$/L	Refer to table 5	NHLS data set
TC levels (highest)	mmol/L	Refer to table 5	NHLS data set
Triglyceride levels (highest)	mmol/L	Refer to table 5	NHLS data set

These levels were compared to the age related reference ranges (refer to table 5) to identify anaemia, neutropenia, hypercholesterolemia and hypertriglyceridemia.

Table 5: Lipid and age - related haematological profile reference ranges or values

	Reference ranges or values for age groups		
	0-5 years	**6-11 years**	**12-14 years**
Haematological profile			
Hb (g/dl)	10.0-14.1	11.5-14.0	11.5-15.5
Neutrophil count (x 10^9/L)	1.0-6.0 x 10^9	1.0-7.0 x 10^9	2.0-7.0 x 10^9
Lipid profile			
TC (mmol/L)	5.2	5.2	5.2
Triglyceride (mmol/L)	1.7	1.7	1.7

(Merck, 2013; Strehlau et al., 2012, Department of Health, 2010, WHO, 2008; Alton, 2005)

3.10.1.2 Other dependent variables

3.10.1.2.1 Completeness of records for baseline viral load

The baseline viral load was obtained from the patient medical folders. The number of patients with a recorded baseline viral load value was determined.

3.10.1.2.2 Completeness of records for baseline CD4 count

The baseline CD4 count was obtained from the patient medical folders. The number of patients with a recorded baseline CD4 count value was determined**.**

3.10.1.2.3 Completeness of records for baseline CD4%

The baseline CD4 % was obtained from the patient medical folders. The number of patients with a recorded baseline CD4 count value was determined**.**

3.10.1.2.4 Adherence to HIV management guidelines

The National HIV Management Guidelines (2004) required that laboratory testing for the full blood count be carried out at baseline then monthly for three months thereafter every six months for children on an AZT-containing regimen. For TC and triglycerides the guidelines required that the tests be carried out at baseline, six month intervals up to one year, then every 12 months for children on a PI-based regimen (Department of Health, 2004). The 2010 guidelines required a baseline full blood count or Hb test for all the patients before start of ART and thereafter for patients on an AZT-based ART regimen, an Hb test was to be performed every month for three months then annually. For patients on ART with a PI, triglyceride and TC tests were to be performed annually (National Department of Health, 2010).

The ART regimens which were later categorised into AZT-containing and without AZT, PI-containing and without PI as indicated in table 6, were sourced from the patient medical folders. Based on the findings in the pilot study, a decision was taken to assess the number of patients on a PI or on AZT who had at least one of each of the relevant tests performed (refer to table 3).

3.10.2 Independent variables

According to literature the factors that have been associated with haematological abnormalities include: age; sex; weight; CD4 count; CD4 percentage; viral load; ART regimen (AZT-containing or without AZT); the nutritional status; antibiotics such as cotrimoxazole and

fluconazole; previous exposure to PMTCT and duration of therapy (Meynard et al., 1997; Moore et al., 2001; Moyle et al. 2004; Aurpibul, 2008; Strehlau et al., 2012; Renner et al.,2012; Dryden et al., 2013,). Literature cites factors associated with lipid profile abnormalities which include: age; sex; weight and height used to determine the BMI; CD4count; CD4 percentage; viral load; ART regimen (PI- containing or without PI); the nutritional status; previous exposure to PMTCT and duration of therapy (Fauvel et al., 2001; Kim et al., 2009; Souza et al., 2013). Since the nutritional status, height and weight (body mass index) were not routinely collected (refer to table 3), they were not taken into consideration for this study.

The independent variables included in the study together with the source of data, units and categories for these variables are outlined in table 6.

Table 6: Units, categories and sources of data for the independent variables

Independent variables	**Units**	**Categories**	**Ideal source (as determined by pilot study)**
Absolute CD4 count at time x[c]	Cells/mm^3	Refer to table 7	Patient medical folder/NHLS data set
Age at time x	Years	1. 0-5 2. 6-11 3. ≥12	Patient medical folder + NHLS data set

[c] Time X is the date on which the maximum laboratory value was noted

ART regimen		Refer to table 3 (Later categorised to AZT-containing or PI-containing)	Patient medical folder
Baseline absolute CD4	Cells/mm^3	Refer to table 7	Patient medical folders
Baseline CD4 percentage	Percentage	Refer to table 7	Patient medical folders
Baseline viral load	Copies/ml	Refer to table 7	Patient medical folders
Baseline WHO clinical staging		1. I 2. II 3. III 4. IV	Patient medical folders
CD4 percentage at time x	Percentage	Refer to table 7	Patient medical folder/NHLS data set
Gender		1. Male 2. Female	Patient medical folders
Months on ART (months) at time x	Months	Haematological profiles: 1. 0-6 2. 7-12 3. >12	Patient medical folder + NHLS data set

		Lipid profiles: 1. 0-12 2. >12	
Other medications		Refer to table 3	Patient medical folder
Viral load at time x	Copies/ml	Refer to table 7	Patient medical folder/NHLS data set

The immunological status markers viz., CD4 count, CD4% and viral load were categorized using the WHO categories as indicated in table 7 (WHO, 2005)

Table 7: Immunological classification according to age categories

	Less than 5 years	6-12 years	Above 12 years
CD4 count (Cells/mm^3)			
Severe immune suppression	<750	<200	<200
Moderate immune suppression	750-1500	200-500	200-500
No immune suppression	>1500	>500	>500
CD4 %			
Severe immune suppression	<15	<15	<15
Moderate immune suppression	15-25	15-25	15-25
No immune suppression	>25	>25	>25
Viral load (Copies/ml)			
Lower than detectable	<40	<40	<40
Moderate detection	40-1000	40-1000	40-1000
Detectable	>1000	>1000	>1000

3.11 Data analysis

The merged dataset was analysed using IBM SPSS statistical software version 22 (IBM SPSS, 2013). Descriptive analysis included only frequencies. Cross-tabulation was performed to assess the association between the variables using Chi-Squared test. Significance was set at less than 0.05.

3.11.1 Completeness of clinical records

The frequency of patients whose baseline clinical data were routinely recorded in the patient medical folders was calculated.

3.11.2 Lipid and haematological abnormalities

The frequency of patients with the maximum Hb and neutrophil test value below, within or above the upper limit of the reference range, respectively, was determined. In relation to TC and triglyceride laboratory tests, the frequency of patients with the maximum TC and triglyceride test value at and below borderline as well as above the reference value was determined. The lipid and haematological abnormalities were further categorised as follows:

- Anaemia was defined as the value below the lower limit of the haemoglobin reference range
- Neutropenia was defined as the value below the lower limit of the neutrophil count reference range
- Hypercholesterolemia was defined as the value above the upper limit of the total cholesterol reference value

- Hypertriglyceridemia was defined as the value above the upper limit of the triglyceride reference value

3.11.3 Factors associated with lipid and haematological abnormalities

The age, ART regimen, months on ART, CD4 percentage, absolute CD4 count and viral load count within six months before or after ART initiation and the additional medications prescribed at the time of the maximum Hb and maximum neutrophil levels, were taken into consideration when analysing factors associated with haematological abnormalities. With regards to factors associated with lipid abnormalities, the age, ART regimen, months on ART, CD4 percentage, absolute CD4 count and viral load count within six months before or after ART initiation at the time of the maximum TC and maximum triglyceride levels, were taken into consideration. The frequency of patients with lipid or haematological abnormalities in relation to each factor mentioned above was calculated. In setting the hypothesis (see below) for the study, the ART regimen was considered to be the main factor associated with haematological and lipid abnormalities.

3.11.3.1 Hypotheses

3.11.3.1.1 Null hypotheses

The main null hypotheses state:

i) There is no association between the ART regimen (PI-containing or without PI) and the lipid levels.

ii) There is no association between the ART regimen (AZT containing or without AZT) and the haematological levels.

3.11.3.1.2 Alternative hypotheses

The main alternative hypotheses state:

i) There is an association between the ART regimen (PI-containing or without PI) and the lipid levels.

ii) There is an association between the ART regimen (AZT-containing or without AZT) and the haematological levels.

The first null hypothesis was supported when the Chi-Squared test produced a p-value greater than 0.05 indicating no association between independent variables and TC or triglyceride levels. Similarly, the second null hypothesis was supported when the Chi-Squared test produced a p-value greater than 0.05 indicating no association between the independent variables and Hb or neutrophil levels. Conversely, the null hypotheses were rejected in favour of the alternative hypotheses when the Chi-Squared test produced a p-value of less than 0.05.

3.11.4 Adherence to the HIV management guidelines

The frequency of patients with at least one Hb, neutrophil, TC and triglyceride laboratory test performed, respectively, was determined.

3.12 Strategies to ensure validity

To ensure data validity the following strategies were implemented:

- The data collection tool was pretested in a pilot study;
- Data entry by data capturers was performed on the same day as data collection by the data collectors and both had prior training on each activity respectively and

- Before and after the two datasets were merged, frequencies were run for each variable to identify abnormal values.

3.13 Strategies to ensure reliability

To ensure reliability the following strategies were employed:

- The data collection tool was clear and understandable;
- The data collectors were trained to ensure uniformity in the data collection process;
- The data capturers were also trained to ensure uniformity in the data capturing process;
- Manuals for transforming variables, recoding variables, merging datasets and analysis were created and used for data analysis;
- The principal researcher was available at the site throughout data collection to answer any questions from the data collectors and
- Data collection was performed by the same set of students with each visit and the same applied to data capturers.

3.14 Ethical considerations

Ethical clearance was obtained from the University of the Western Cape Ethics Committee and approval for accessing the site was obtained from the Western Cape Provincial Health Research Committee.

The facility manager at the health facility selected for the study was contacted and informed about the aims and procedures of the study and reassured of minimal interference with the daily staff operations at the CHC. Data obtained from the health centre was not used for any other

purposes apart from this research and was also kept safely in an office where access was limited to the researchers.

Results from this study will be disseminated to the CHC where the research was conducted and the NHLS.

A unique ID was captured and this replaced the patient folder number to ensure anonymity and confidentiality.

Chapter 4 Results

This chapter presents the results of statistical analysis comprising descriptive and inferential analyses. All univariate descriptive statistics are summarized in text, tables and figures as percentages and frequencies. Results of associations between the categorical variables are also presented in the form of text and tables.

4.1 Demographics of the study population

Of the 168 patient medical folders included in the study 88(52.4%) were for male and 80 (47.6%) were for female patients. Most 81 (48.2%) patients were between six and 11 years of age, 51 (30.4%) were five years of age or under and 36(21.4%) were 12 years of age or older (refer to table 8).

Table 8: Demographic characteristics of patients on ARVs at the CHC (N=168)

	n	%
Gender		
Male	88	52.4
Female	80	47.6
Age (years)		
0-5	51	30.4
6-11	81	48.2
>12	36	21.4

4.2 Baseline clinical characteristics

Fifty-six (54.4%) and 24 (23.3%) patients had a WHO clinical staging of III and IV, respectively, while 18 (17.5%) and 5 (4.9%) had a staging of II and I, respectively. Nearly one third (30%) of patients had undetectable viral loads (under 40 copies/ml) while nearly two thirds (64%) had more than 1000 copies of the virus per millilitre (refer to table 7).

With regards to children aged five and under, 18(48.7%) had a CD4 cell count of less than 750 cells per cubic millimetre, 11(29.7%) had a CD4 cell count between 750 and 1500 cells per cubic millimetre and 8 (21.6%) had a CD4 cell count higher than 1500 cells per cubic millimetre (refer to table 7).

The majority (73.1%) of children older than five years had a CD4 cell count higher than 500 copies per millilitre, 16(20.5%) had a CD4 cell count between 200 and 500 cells per cubic millimetre and 5(6.4%) had a CD4 cell count of less than 200 cells per cubic millimetre (refer to table 9).

Table 9: Baseline clinical characteristics of patients at the CHC

	n	%
WHO clinical stages (N=103)		
Clinical stage I	5	4.9
Clinical stage II	18	17.5
Clinical stage III	56	54.4
Clinical stage IV	24	23.3
Viral load (Copies/ml) (N=93)		
Less than 40	26	30.1
40-1000	7	5.4
Higher than 1000	60	64.5
CD4 percentage (%) (N=113)		
<15	30	26.5
15-25	44	38.9
>25	39	34.5
Baseline CD4 count≤5years (N=37)		
CD4 count (cells/mm^3)	**n**	**%**
<750	18	48.7
750-1499	11	29.7
>1500	8	21.6
Baseline CD4count >5 (years) (N=78)		
Absolute CD4 count (cells/mm^3)	**n**	**%**
<200	5	6.4
200-500	16	20.5
>500	57	73.1

4.3 Prescribed ART regimens

4.3.1 Prescribed one ART regimen

All the patients (N=168) included in the study were on ART. The majority (31%) were prescribed D4T+3TC+ LPV/r followed by D4T+ 3TC+ EFV (21.4%) as indicated in figure 3.

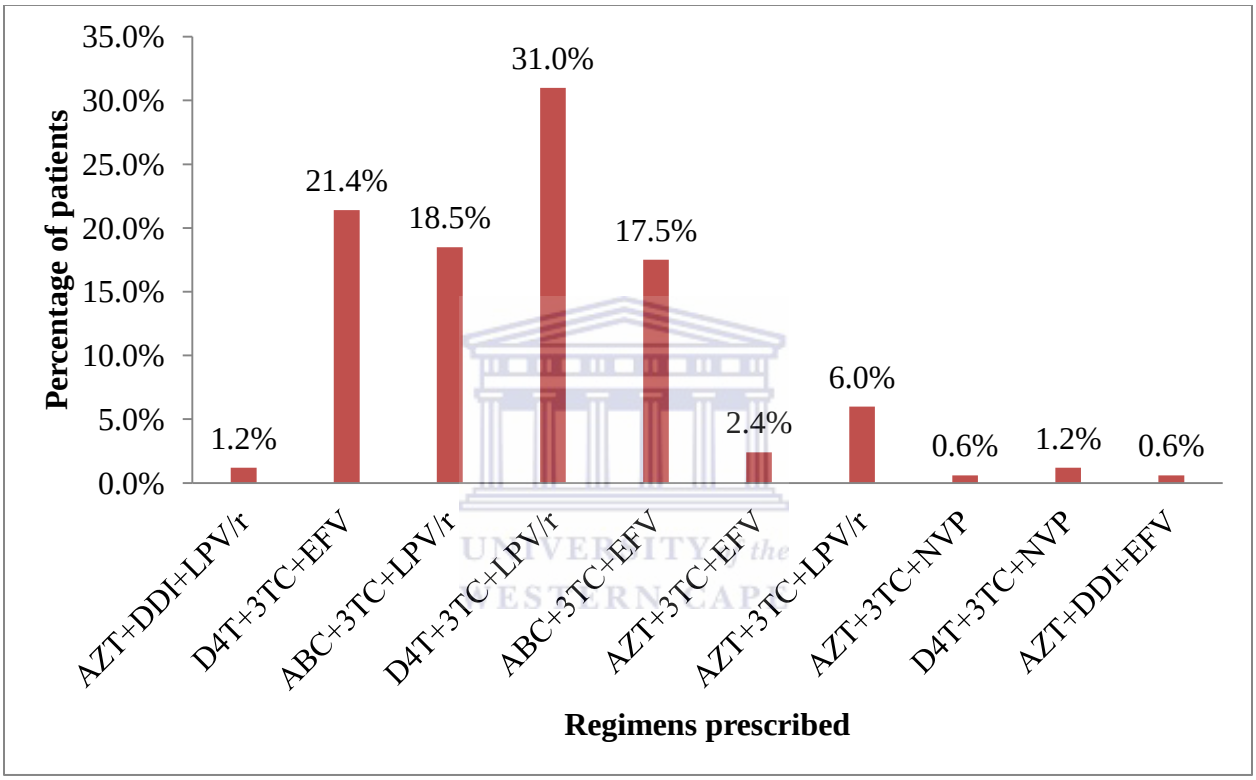

Figure 3: Prescribed one ART regimen (N=168)

4.3.2 Changes made to ART regimens

All patients (N=168) commenced on a first regimen, 40(19.6%) patients changed from a first regimen to a second regimen and 7(4.8%) patients changed to a third regimen (refer to figure 4).

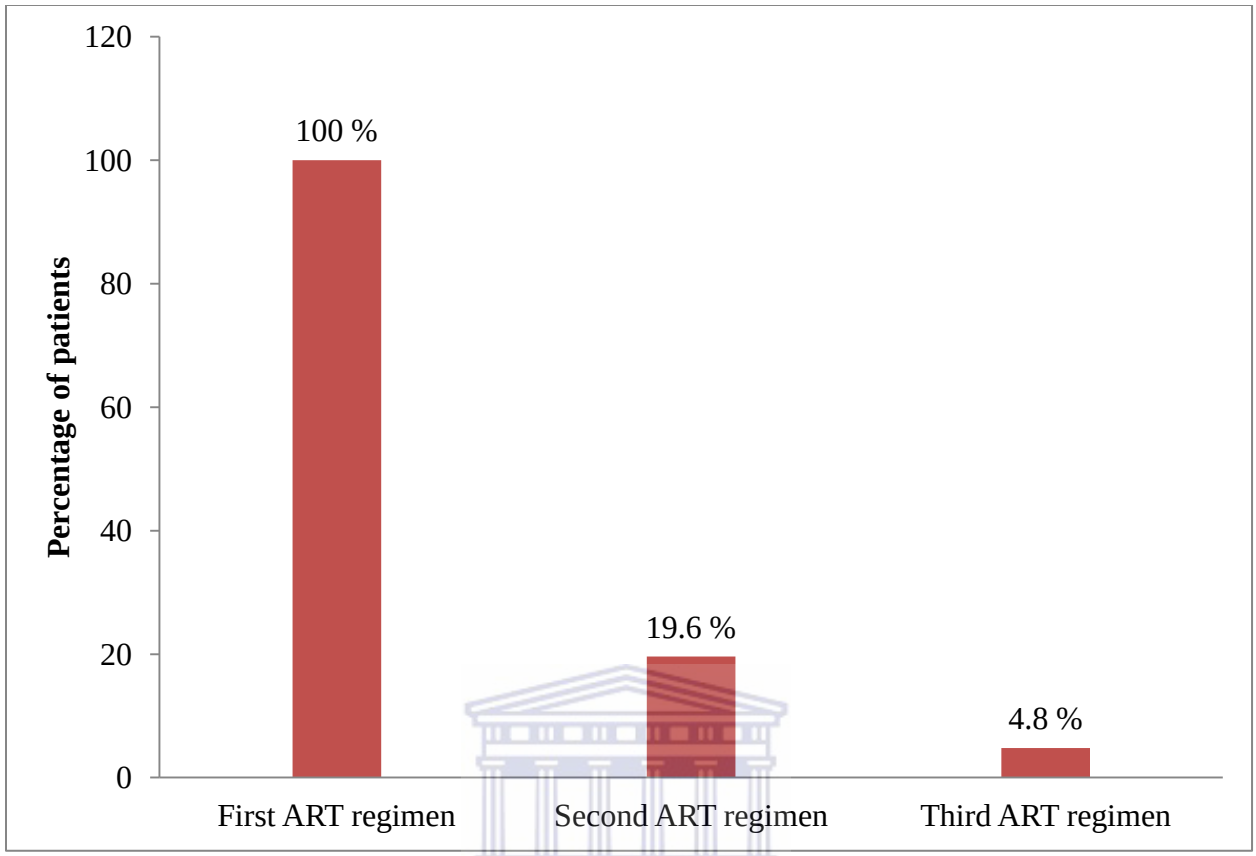

Figure 4: ART regimen changes (N=168)

4.3.4 Other medications prescribed along with ART

The patients included in the study may have been on other medications, but the HIV clinic only prescribes ART and TB medications. The most commonly used medication in addition to ART was cotrimoxazole (74.4 %) followed by cotrimoxazole in combination with isoniazid (7.7 %).Twenty one (12.5%)patients were not prescribed any other medications apart from ART (refer to figure 5).

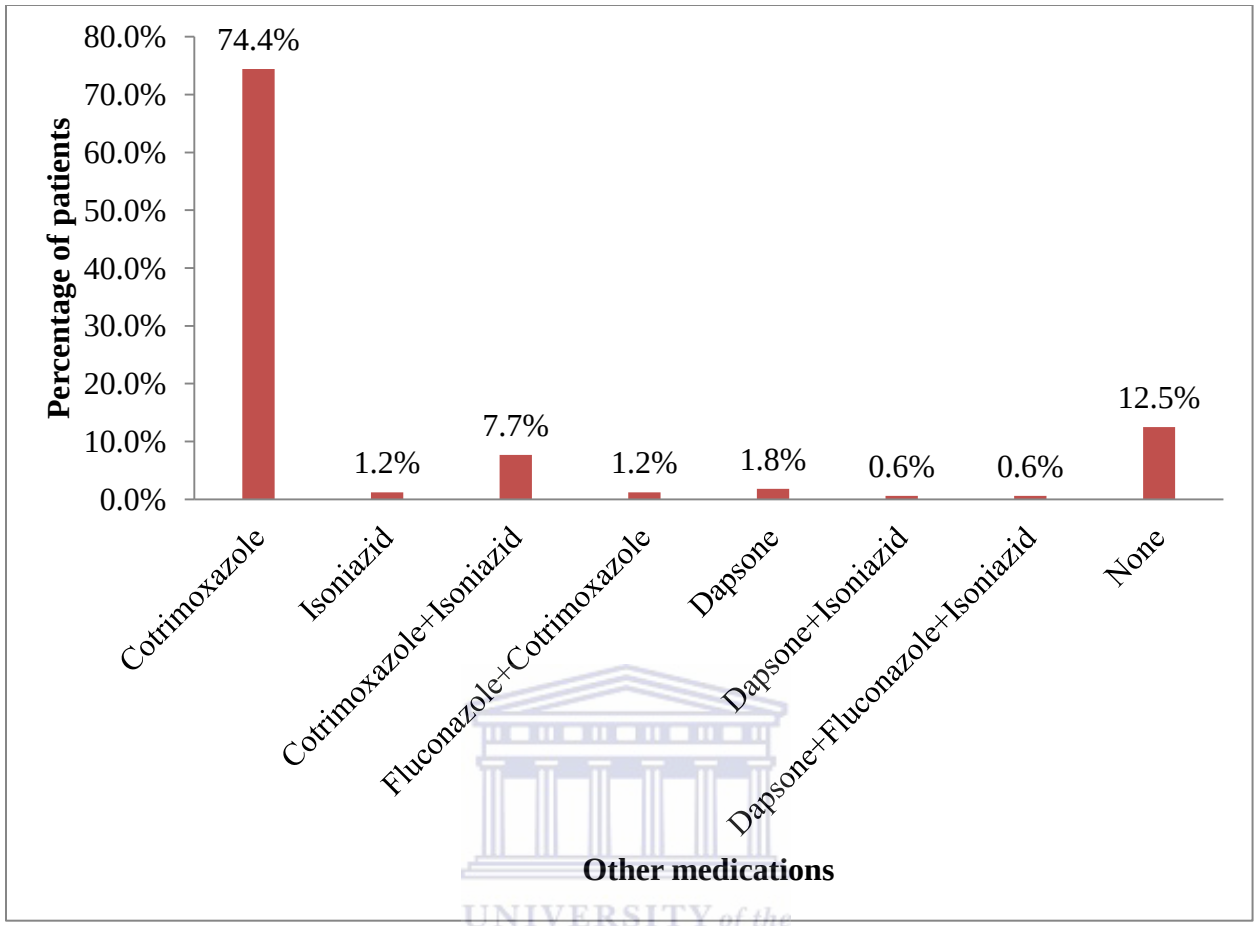

Figure 5: Other medications prescribed (N= 168)

4.3.5 Year of ART initiation

The majority of the patients (57.1%) started their ART regimens between the years 2003 and 2009, followed by 68 (40.5%) patients who started their ART regimen between 2010 and 2012 and 4 (2.4%) started in 2013 (refer to figure 6).

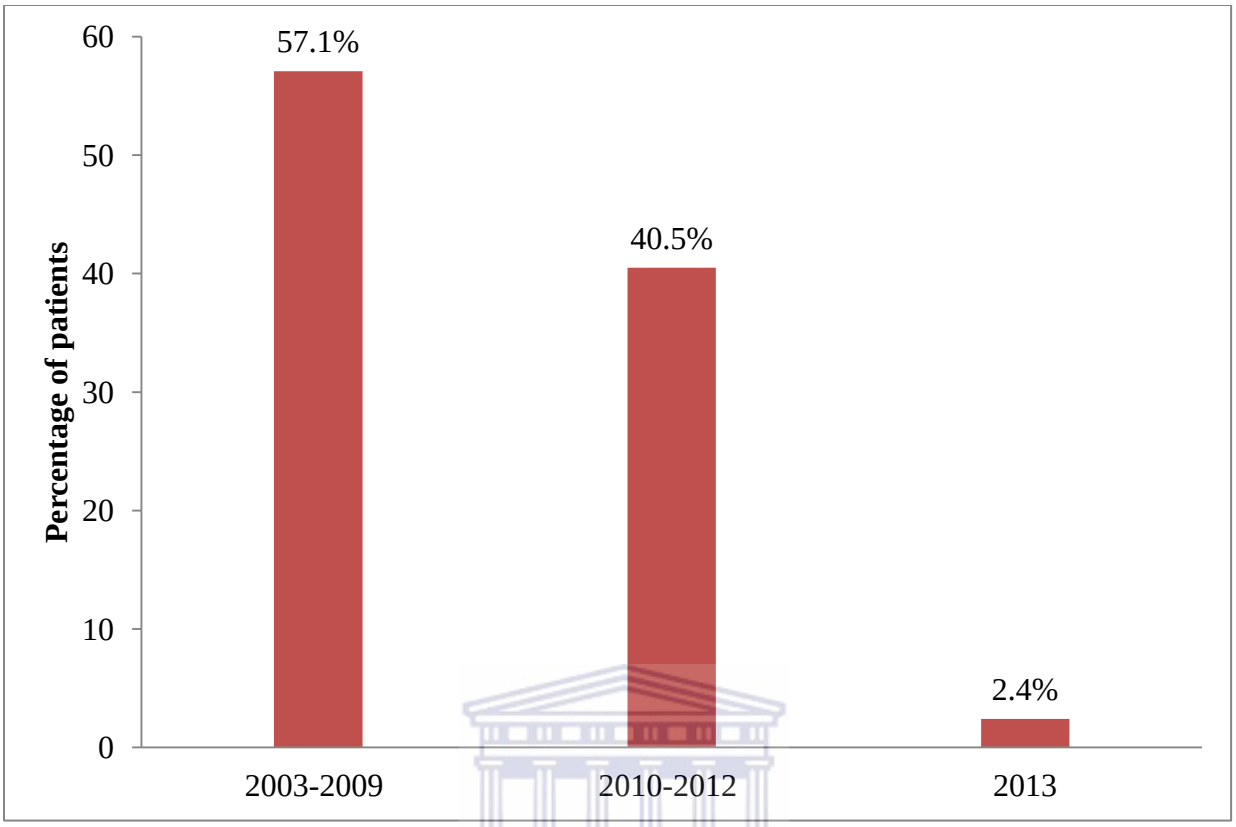

Figure 6: Years in which ART was started (N=168)

4.4 Completeness of patient medical records

The WHO staging at baseline was recorded for only 103(61.3%) patients. Ninety-three (55.4%) patients had a baseline viral load recorded in their folder. The absolute CD4 count and CD4 percentage fields were populated with data for only 53(31.5%) and 113 (67.3%), respectively (refer to table 10).

Table 10: Completeness of patient medical records (N=168)

Completeness of patient medical records	n	%
WHO clinical staging records	103	61.3
CD4% records	113	67.3
CD4 count records	93	54.4
Viral load records	53	31.5

4.5 Haematological abnormalities

4.5.1Neutrophil levels

4.5.1.1 Frequency of neutropenia in patients aged five and less than 5 years

Of the 94 patients with a maximum neutrophil value subsequent to ART initiation, 20(45.5%) had neutropenia and 22 (54.5%) had a maximum neutrophil count within the normal range (refer to table 11).

Table 11: Frequency of neutropenia in patients aged 5 years and under (N=44)

Neutrophil levels	n	%
Neutropenia (less than $1.0x10^9$/L	20	45.5
Normal range ($1.0x10^9$/L to $6.0x10^9$/L)	22	54.5

4.5.1.2 Frequency of neutropenia in patients aged between six and 11 years

Within this age group, only five (21.7%) patients had neutropenia and 18 (76.2%) had a neutrophil count within the normal range (refer to table 12).

Table 12: Frequency of neutropenia in patients aged between 6 and 11years (N=21)

Neutrophil levels	n	%
Neutropenia (less than $1.0x10^9$/L)	5	21.7
Normal range ($1.0x10^9$ /L to $7.0x10^9$/L)	16	76.2

4.5.1.3 Frequency of neutropenia in patients aged between 12 and14years

The majority of patients 19 (65.5%) had neutropenia while 10 (34.5%) had the neutrophil count within the normal range (refer to table 13).

Table 13: Frequency of neutropenia in patients aged between 12 and14 years (N=29)

Neutrophil levels	n	%
Neutropenia (less than 2.0 $x10^9$/L)	19	65.5
Normal range ($2.0x10^9$/Lto$7.0x10^9$/L)	10	34.5

4.5.1.4 Association between ART regimen and neutrophil levels

4.5.1.4.1 Effect of ART regimen in patients aged five and less than five years

The Chi-Squared test analysis supported the null hypothesis of no association between regimen type and neutrophil levels as indicated in table 14 (χ^2=0.0241, p = 0.877).

Table 14: Effect of ART regimen on neutrophil levels for ages five and under

Regimen	Neutropenia (less than $1.0X10^9$/L)		Normal range ($1.0X10^9$/L -$6.0X10^9$/L)		Total	
	n	%	n	%	n	%
AZT- containing	5	35.8	9	64.2	14	100
Without AZT	10	33.33	20	66.7	20	100
Chi-Squared test=0.0241 , p= 0.877						

4.5.1.4.2 Effect of ART regimen in patients aged between six and 11 years

The Chi-Squared test analysis supported the null hypothesis of no association between regimen type and neutrophil levels as indicated in table 15 (χ^2=0.5243, p=0.469).

Table 15: Effect of ART regimen on neutrophil levels for ages between six and 11

Regimen	Neutropenia (less than $1.0X10^9$)		Normal ranges ($1.0X10^9$/L -$7.0X10^9$/L)		Total	
	n	%	n	%	n	%
AZT- containing	7	46.7	8	53.3	15	100
Without AZT	5	62.5	3	37.5	8	100
Chi-Squared test=0.5243 , p= 0.469						

4.5.1.4.3 Effect of ART regimen in patients aged between 12 and 14 years

The Chi-Squared test analysis supported the null hypothesis of no association between regimen type and neutrophil levels (χ^2=0.0120, p=0.913) as indicated in table 16.

Table 16: Effect of ART regimen on neutrophil levels for ages between 12 and 14

Regimen	Neutropenia (less than $1.0X10^9$/L)		Normal ranges ($1.0X10^9$/L -$7.0X10^9$/L)		Total	
	n	%	n	%	n	%
AZT- containing	6	35.3	9	64.7	15	100
Without AZT	4	33.3	8	66.7	12	100
Chi-Squared test=0.0120 , p= 0.913						

4.5.1.5 Association between age and neutrophil levels

The Chi-Squared test analysis supported the null hypothesis of no association between age and neutrophil levels (χ^2=4.975, p=0.083) as indicated in table 17.

Table 17: Association between age and neutrophil levels

Age (years)	Neutropenia		Normal range		TOTAL	
	n	%	n	%	n	%
<5	39	72.2	15	27.8	54	100
6-12	17	51.5	16	48.5	33	100
>12	2	40.0	3	60.0	5	100
Chi-Squared test= 4.975, p= 0.083						

4.5.1.6 Association between months on ART and neutrophil levels

4.5.1.6.1 Association between months on ART and neutrophil count for ages five and under five years

The Chi-Squared test analysis supported the null hypothesis of no association between months on ART and neutrophil levels (χ^2=0.5911, p=0.744) as indicated in table 18.

Table 18: Association between months on ART and neutrophil levels for ages five and under

Months on ART	**Neutropenia (less than 1.0x10^9/L)**		**Normal range (1.0x10^9/L to 6.0x10^9/L)**		**Total**	
	n	%	n	%	n	%
≤6	5	41.7	7	58.3	12	100
7-12	7	43.8	9	56.2	16	100
>12	5	31.3	11	68.7	16	100
Chi-Squared test analysis=0.5911, p=0.744						

4.5.1.6.2 Association between months on ART and neutrophil count for ages between six and 11years

The Chi-Squared test analysis supported the null hypothesis of no association between months on ART and neutrophil levels (χ^2=4.3743, p=0.112) as indicated in table 19.

Table 19: Association between months on ART and neutrophil levels count for ages between six and 11years

Months on ART	Neutropenia (less than $1.0x10^9$/L)		Normal range ($1.0x10^9$/L to $6.0x10^9$/L)		Total	
	n	%	n	%	n	%
0-6	1	16.7	5	83.3	6	100
7-12	7	70	3	30	10	100
>12	3	42.9	4	57.1	7	100
Chi- Squared test analysis=4.3743, p=0.112						

4.5.1.6.3 Association between months on ART and neutrophil count for ages between 12 and 14years

The Chi-Squared test analysis supported the null hypothesis of no association between months on ART and neutrophil levels (χ^2=2.3750, p=0.305) as indicated in table 20.

Table 20: Association between months on ART and neutrophil levels for ages between 12 and 14 yeas

Months on ART	Neutropenia (less than $1.0x10^9$/L)		Normal range ($1.0x10^9$/L to $6.0x10^9$/L)		Total	
	n	%	n	%	n	%
0-6	5	45.5	6	54.5	11	100
7-12	3	30.0	7	70.0	10	100
>12	1	16.7	5	83.3	6	100
Chi-Squared test analysis=2.3750, p=0.305						

4.5.1.7 Association between CD4 count and neutrophil count

4.5.1.7.1 Association between CD4 count and neutrophil count for ages five and under five years

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 count and neutrophil (χ^2=0.4185, p=0.811) as indicated in table 21.

Table 21: Association between absolute CD4 count and neutrophil levels for ages five and under

CD4 count (cells/mm^3)	**Neutropenia (less than1.0x10^9/L)**		**Normal range (1.0x10^9/Lto 6.0x10^9/L)**		**Total**	
	n	%	n	%	n	%
< 750	10	62.5	6	37.5	16	100
750-1499	12	75.0	4	25.0	16	100
>1500	8	66.7	4	33.3	12	100
Chi-Squared test = 0.4183, p=0.811						

4.5.1.7.2 Association between CD4 count and neutrophil count for ages between six and 11 years

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 count and neutrophil levels (χ^2=0.0112, p=0.994) as indicated in table 22.

Table 22: Association between absolute CD4 count and neutrophil levels for ages between six and 11 years

CD4 count (cells/mm^3)	Neutropenia (less than 1.0x10^9/L)		Normal range (1.0x10^9/L to 7.0x10^9/L)		Total	
	n	%	n	%	n	%
<200	2	66.7	1	33.3	3	100
200-500	1	33.3	2	66.7	3	100
>500	9	52.9	8	47.1	17	100
Chi-Squared test = 0.0112, p=0.994						

4.5.1.7.3 Association between CD4 count and neutrophil levels for ages between 12 and 14 years

The Chi-Squared test analysis supported the null hypothesis of no association between CD4 count and neutrophil levels (χ^2= 2.000, p=0.157) as indicated in table 23.

Table 23: Association between CD4 count and neutrophil levels for ages between 12 and 14 years

CD4 count (cells/mm^3)	Neutropenia (less than 2.0x10^9/L)		Normal range (2.0x10^9/L to 6.0x10^9/L)		Total	
	n	%	n	%	n	%
<200	0	0.0	0	0.0	0	0.0
200-500	0	0.0	8	100.0	8	100
>500	10	52.6	9	47.4	19	100
Chi-Squared test= 2.000, p=0.157						

4.5.1.8 Association between CD4 percentage and neutrophil levels

4.5.1.8.1 Association between CD4 percentage and neutrophil count for ages five years and under five years

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 count and neutrophil levels (χ^2=2.8947, p=0.235) as indicated in table 24.

Table 24: Association between CD4 percentage count and neutrophil levels for ages five years and under

CD4 count (cells/mm^3)	Neutropenia (less than 1.0x10^9)		Normal range (1.0x10^{9}to 6.0x10^9)		Total	
	n	%	n	%	n	%
< 15	13	72.2	5	27.8	18	100
15-25	12	54.5	10	45.5	22	100
>25	0	0.0	4	100.0	4	100
Chi-Squared test= 2.8947, p=0.235						

4.5.1.8.2 Association between CD4 percentage and neutrophil count for ages between six and 11years

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 count and neutrophil levels for ages (χ^2=1.3838, p=0.501) as indicated in table 25.

Table 25: Association between CD4 percentage and neutrophil levels for ages between six and 11 years

CD4 percentage	Neutropenia (less $1.0x10^9$/L)		Normal range ($1.0x10^9$/L to $7.0x10^9$/L)		Total	
	n	%	n	%	n	%
<15	10	66.7	5	33.3	15	100
15-25	4	57.1	3	42.9	7	100
>25	0	0.0	1	100.0	1	100
Chi-Squared test= 1.3838, p=0.501						

4.5.1.8.3 Association between CD4 percentage and neutrophil count for ages between 12 and 14 years

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 count and neutrophil levels for ages five and under (χ^2=2.2250, p=0.3290) as indicated in table 26.

Table 26: Association between CD4 percentage and neutrophil levels for ages between 12 and 14 years

CD4 count (cells/mm^3)	Neutropenia (less than $2.0x10^9$/L)		Normal range ($2.0x10^9$/L to $6.0x10^9$/L)		Total	
	n	%	n	%	n	%
<15	5	33.3	10	66.7	15	100
15-25	7	63.6	4	36.4	11	100
>25	0	0.0	1	100.0	1	100
Chi-Squared test= 2.2250, p=0.3290						

4.5.1.9 Association between viral load and neutrophil count

4.5.1.9.1 Association between viral load and neutrophil count for ages five and under five years

The Chi-Squared test analysis supported the null hypothesis of no association between viral load and neutrophil levels (χ^2=0.2890, p=0.865) as indicated in table 27.

Table 27: Association between viral load and neutrophil levels for ages five and under

Viral load (Copies/ml)	**Neutropenia (less than 1.0X10^9/L)**		**Normal range (1.0X10^9/L to 6.0X10^9/L)**		**Total**	
	n	%	n	%	n	%
<40	10	62.5	6	37.5	16	100
40-1000	9	64.3	5	35.7	14	100
>1000	10	71.4	4	28.6	14	100
Chi-Squared test= 0.2890, p=0.865						

4.5.1.9.2 Association between viral load and neutrophil count for ages between six and11years

The Chi-Squared test analysis supported the null hypothesis of no association between viral load and neutrophil levels (χ^2=0.1888, p=0.910) as indicated in table 28.

Table 28: Association between viral load and neutrophil levels for ages between six and 11 years

Viral load (Copies/ml)	**Neutropenia (less than 1.0X10^9/L)**		**Normal range (1.0X10^9/Lto7.0X10^9/L)**		**Total**	
	n	%	n	%	n	%
<40	1	50	1	50	2	100
40-1000	5	62.5	3	37.5	8	100
>1000	7	53.8	6	46.2	13	100
Chi-Squared test= 0.1888, p=0.910						

4.5.1.9.3 Association between viral load and neutrophil count for ages between 12 and 14years

The Chi-Squared test analysis rejected the null hypothesis of no association between viral load and neutrophil levels (χ^2=6.4532, p=0.040) as indicated in table 29.

Table 29: Association between viral load and neutrophil levels for ages between 12 and 14 years

Viral load (Copies/ml)	**Neutropenia (less than 2.0X10^9/L)**		**Normal range (2.0X10^9/L to 7.0X10^9/L)**		**Total**	
	n	%	n	%	n	%
<40	8	88.9	1	11.1	9	100
40-1000	1	25.0	3	75.0	4	100
>1000	6	42.8	8	57.2	14	100
Chi-Squared test= 6.4532, p= 0.040						

4.5.1.10 Association between the gender and neutrophil levels

4.5.1.10.1 Association between the gender and neutrophil levels for ages five years and under five years

The Chi-Squared test analysis supported the null hypothesis of no association between gender (χ^2=0.5698, p=0.450) as indicated in table 30.

Table 30: Association between gender and neutrophil levels for ages five years and under

Gender	**Neutropenia (less than 1.0x10^9/L)**		**Normal range (1.0x10^9/L to 6.0x10^9/L)**		**Total**	
	n	%	n	%	n	%
Male	17	70.8	7	29.2	24	100
Female	12	60	8	40	20	100
Chi-Squared test=0.5698, p=0.450						

4.5.1.10.2 Association between gender and neutrophil levels for ages between six and 11years

The Chi-Squared test analysis supported the null hypothesis of no association between gender and neutrophil levels (χ^2=3.4862, p=0.062) as indicated in table 31.

Table 31: Association between gender and neutrophil levels for ages six to 11 years

Gender	**Neutropenia (less than 1.0X10^9/L)**		**Normal range (1.0X10^9/L to 7.0X10^9/L)**		**Total**	
	n	%	n	%	n	%
Male	4	30.8	9	69.2	13	100
Female	7	70	3	30	10	100
Chi-Squared test= 3.4862, p=0.062						

4.5.1.10.3 Association between the gender and neutrophil levels for ages between 12 and14 years

The Chi-Squared test analysis supported the null hypothesis of no association between gender and neutrophil levels (χ^2=2.1817, p=0.140) as indicated in table 32.

Table 32: Association between gender and neutrophil levels for ages between 12 and 14 years

Gender	**Neutropenia (less than 2.0X10^9/L)**		**Normal range (2.0X10^9/L to 7.0X10^9/L)**		**Total**	
	n	%	n	%	n	%
Male	6	50	6	50	12	100
Female	4	26.6	11	73.3	15	100
Chi-Squared test= 2.1817, p=0.140						

4.5.1.11 Association between other medications used and neutrophil levels

The Chi-Squared test analysis supported the null hypothesis of no association between the other medications used by the patients and neutrophil levels (χ^2=3.830, p=0.574) as indicated in table 33.

Table 33: Association between other medications used and neutrophil count

Other medication	neutropenia		Within reference ranges	
	n	%	n	%
Cotrimoxazole	47	63.5	27	36.5
Isoniazid	1	100	0	0.0
Cotrimoxazole+isoniazid	4	66.7	2	33.3
Dapsone	1	100	0	0.0
Fluconazole + isoniazid	0	0.0	1	100
Dapsone+isoniazid	6	50.0	6	50.0
Not on additional medications	59	62.1	36	37.9
Chi-Squared test= 3.830, p=0.574				

4.5.2 Haemoglobin

4.5.2.1 Frequency of anaemia for patients aged five and less than five years

Of the seven patients that had a maximum Hb value subsequent to ART initiation, two (28.6%) had anaemia and five (71.4%) had Hb levels within the normal range as indicated in table 34.

Table 34: Frequency of anaemia in patients aged five and less than five years (N=7)

Haemoglobin (g/dl)	n	%
Anaemia (less than 10.0)	2	28.6
Normal range (10.0 to 14.1)	5	71.4

4.5.2.2 Frequency of anaemia for patients with ages greater than five years

Of the two patients with a maximum Hb value within this age group, only one (50 %) patient had anaemia and one (50 %) had Hb levels within the normal range (refer to table 35).

Table 35: Frequency of anaemia for ages greater than five years (N=2)

Haemoglobin (g/dl)	n	%
Anaemia (less than 11.5)	1	50
Normal range (11.5 to 15.5)	1	50

4.5.2.3 Association between age and Hb levels

The Chi-Squared test analysis supported the null hypothesis of no association between the age and Hb levels ($\chi^2 = 3.830$, $p = 0.574$) as indicated in table 36.

Table 36: Association between age and Hb levels

Age (years)	Anaemia		Normal range		Total	
	n	%	n	%	n	%
Less than 5	4	57.1	3	42.9	7	100
6 to 14	1	50	1	50	2	100
Chi-Squared test= 1.000, p = 0.722						

4.5.2.4 Association between gender and Hb levels

4.5.2.4.1 Association between gender and Hb levels for ages five and less than five years

The Chi-Squared test analysis supported the null hypothesis of no association between gender and Hb levels (χ^2=0.0583, p=0.809) as indicated in table 37.

Table 37: Association between gender and Hb levels for ages five and under

Gender	Anaemia (less than 10g/dl)		Normal range (10.0 to 14.1 g/dl)		Total	
	n	%	n	%	n	%
Male	2	66.7	1	33.3	3	100
Female	3	75	1	25	4	100
Chi-Squared test= 0.0583, p=0.809						

4.5.2.4.2 Association between gender and Hb levels for ages greater than five years

The Chi-Squared test analysis supported the null hypothesis of no association between gender and Hb levels (χ^2=2.000, p=0.157) as indicated in table 38.

Table 38 Association between gender and Hb levels for ages greater than five

Gender	Anaemia (less than 11.5 g/dl)		Normal range (11.5 to 15.5 g/dl)		Total	
	n	%	n	%	n	%
Male	0	1	1	100	1	100
Female	1	0	1	100	1	100
Chi-Squared test= 2.000, p=0.157						

4.5.2.5 Effect of ART regimen on Hb levels

4.5.2.5.1 Effect of ART regimen on Hb count for ages five and less than five years

The Chi-Squared test analysis supported the null hypothesis of no association between regimen type and Hb levels for ages five and under (χ^2=0.0583, p=0.809) as indicated in table 39.

Table 39: Association between ART regimen and Hb levels for ages five and under

Regimen	Anaemia (less than 10.0 g/dl)		Normal range (10.0-14.1 g/dl)		Total	
	n	%	n	%	n	%
AZT -containing	1	50.0	1	50.0	2	100
Without AZT	3	60.0	2	40.0	5	100
Chi-Squared test= 0.0583, p=0.809						

4.5.2.5.2 Effect of ART regimen on haemoglobin count for ages greater than five years

The Chi-Squared test analysis supported the null hypothesis of no association between regimen type and Hb levels for ages greater than five years (χ^2=2.000, p=0.157) as indicated in table 40.

Table 40: Association between ART regimen and Hb levels for ages greater than five years

Regimen	Anaemia (less than 11.5 g/dl)		Normal range (11.5-15.5 g/dl)		Total	
	n	%	n	%	n	%
AZT- containing	1	100.0	0	0	1	100.0
Without AZT	0	100.0	1	0	1	100.0
Chi-Squared test= 2.000, p= 0.157						

4.5.2.6 Association between months on ART and Hb levels

4.5.2.6.1 Association between months on ART and Hb for ages five and less than five years

The Chi-Squared test analysis supported the null hypothesis of no association between months on ART and Hb levels (χ^2=2.000, p=0.157) as indicated in table 41

Table 41: Association between months on ART and Hb levels for ages five and under

Months on ART	Anaemia (less than 10.0g/dl)		Normal range (10.0 to 14.1 g/dl)		Total	
	n	%	n	%	n	%
0-6	5	100.0	0	0.0	5	100.0
7-12	0	0.0	2	100.0	2	100.0
>12	0	0.0	0	0		
Chi-Squared test=2.000 , p=0.157						

4.5.2.6.1 Association between months on ART and Hb for ages greater than five years

The Chi-Squared test analysis supported the null hypothesis of no association between regimen type and neutrophil levels for ages five and under (χ^2=2.000, p=0.157)as indicated in table 42.

Table 42: Association between months on ART and Hb levels for ages greater than five

months on ART	Anaemia (less than 10.0g/dl)		Normal range (10.0 to 14.1 g/dl)		Total	
	n	%	n	%	n	%
0-6	1	100	0	0	1	100.0
7-12	0	0	1	100	1	100.0
>12	0	0	0	0	0	0
Chi-Squared test=2.000 , p=0.157						

4.5.2.7 Association between other medications used and haemoglobin count

The Chi-Squared test analysis supported the null hypothesis of no association between medications used and Hb levels (χ^2=2.000, p=0.157) as indicated in table 43.

Table 43: Association between other medicines used and Hb levels

Other meds	Anaemia		Within the reference ranges		Total	
	n	%	n	%	n	%
Cotrimoxazole	2	50.0	2	50.0	4	100
Cotrimoxazole + Isoniazid	0	0.0	1	100.0	1	100
Chi-Squared test=1.914, p=0.384						

4.5.2.8Association between CD4 count and Hb count

4.5.2.8.1 Association between CD4 count and Hb levels for ages five and under

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 count and Hb levels (χ^2=2.000, p=0.157) as indicated in table 44.

Table 44: Association between CD4 count and Hb levels for ages five and under

CD4 count (cells/mm^3)	Anaemia (less than 10.0 g/dl)		Normal range (10.0-14.1 g/dl)		Total	
	n	%	n	%	n	%
<750	0	0.0	1	100.0	1	100.0
750-1500	1	33.3	2	66.7	3	100.0
>1500	2	50.0	2	50.0	4	100.0
Chi-Squared test= 2.000, p =0.157						

4.5.2.8.2Association between CD4 count and Hb levels for ages greater than five years

The Chi-Squared test analysis could not be applied to identify the association between absolute CD4 count and haemoglobin levels because the sample size was small (refer to table 45).

Table 45: Association between CD4 count and Hb levels for ages greater than five

CD4 count (cells/mm^3)	Anaemia (less than 11.5 g/dl)		Normal range (11.5-15.5 g/dl)		Total	
	n	%	n	%	n	5
<200	0	0	0	0	0	0.0
200-500	1	100	1	100	1	100.0
>500	0	0	0	0	0.0	0.0

4.5.2.8 Association between CD4 percentage and Hb levels

4.5.2.8.1 Association between CD4 percentage and Hb levels for ages five and less than five years

The Chi-Squared test analysis supported the null hypothesis of no association between CD4 percentage and Hb (χ^2=0.0583, p= 0.809) as indicated in table 46.

Table 46: Association between percentage and Hb levels for ages five and under

CD4 (%)	Anaemia (less than 10.0 g/dl)		Normal range (10.0-14.1 g/dl)		Total	
	n	%	n	%	n	%
< 15	0	0	0	0	0	100.0
15-25	2	66.7	1	33.3	3	100.0
>25	3	75	1	25	4	100.0
Chi- squared test= 0.0583, p=0.809						

4.5.2.8.2Association between CD4 count and Hb levels for ages greater than five years

The Chi-Squared test analysis supported the null hypothesis of no association between CD4 percentage and Hb levels (χ^2=2.000, p= 0.157) as indicated in table 47.

Table 47: Association between CD4 percentage and Hb levels for ages greater than five years

CD4 percentage (%)	Anaemia (less than 11.5 g/dl)		Normal range (11.5-15.5 g/dl)		Total	
	n	%	n	%	n	%
<15	1	100	0	0	1	100.0
15-25	0	0	0	0	0	0.0
>25	0	0	1	100	1	100.0
Chi- squared test= 2.000, p=0.157						

4.5.2.9Association between viral load and Hb levels

4.5.2.9.1Association between viral load and Hb levels for ages five and less than five years

The Chi-Squared test analysis rejected the null hypothesis of no association between viral load and haemoglobin levels for ages five and under (χ^2=7.000, p=0.008) as indicated in table 48.

Table 48: Association between viral load and Hb levels for ages five and under

Viral load (Copies/ml)	**Anaemia (less than 10.0 g/dl)**		**Normal range (10.0-14.1 g/dl)**		**Total**	
	n	%	n	%	n	%
< 40	6	100	0	0	6	100.0
40-1000	0	0	1	100	1	100.0
>1000	0	0	0	0	0	100.0
Chi- squared test= 7.000, p=0.008						

4.5.2.9.2Association between viral load and Hb levels for ages greater than five years

The Chi-Squared test analysis supported the null hypothesis of no association between CD4 percentage and Hb levels (χ^2=2.000, p= 0.157) as indicated in table 49.

Table 49: Association between viral load and Hb levels for ages greater than five

Viral load (Copies/ml)	**Anaemia (less than 11.5 g/dl)**		**Normal range (11.5-15.5 g/dl)**		**Total**	
	n	%	n	%	n	%
<40	1	100	0	0	1	100.0
40-1000	0	0	0	0	0	0.0
>1000	0	0	1	100	1	100.0
Chi- squared test= 2.000, p=0.157						

4.6 Dyslipidemia

4.6.1 TC levels

4.6.1.1 Frequency of hypercholesterolemia

Of the 23 patients that had at least one total cholesterol test conducted subsequent to ART initiation, 3(13.1%) had hypercholesterolemia and 20 (86.9%) were at borderline or had less than 5.2milimole per litre (refer to table 50).

Table 50: Frequency of hypercholesterolemia (N=23)

Cholesterol (mmol/L)	n	%
≤5.2	20	86.9
Above 5.2	3	13.1
Total	23	100

4.6.1.2 Association between gender and TC levels

The Chi-Squared test analysis supported the null hypothesis of no association between gender and TC levels (χ^2=0.735, p=0.385) as indicated in table 51.

Table 51: Association between the gender and TC levels

Gender	Borderline and below 5.2 mmol/L		Above 5.2 mmol/L		Total	
	n	%	n	%	n	%
Male	8	80.0	2	20.0	10	100.0
Female	12	92.3	1	7.7	13	100.0
Chi-Squared test=0.735, p= 0.385						

4.6.1.3 Association between age and TC levels

The Chi-Squared test analysis supported the null hypothesis of no association between age and TC levels (χ^2=0.388, p=0.824) as indicated in table 52.

Table 52: Association between age and hypercholesterolemia

Age	Borderline and below 5.2 mmol/L		Above 5.2 mmol/L		Total	
	n	%	n	%	n	%
0-5	15	88.2	2	11.8	17	100.0
6-11	4	80.0	1	20.0	5	100.0
12-14	1	100	0	0.0	1	100.0
Chi-Squared test=0.388, p = 0.824						

4.6.1.4 Association between months on ART and TC levels

The Chi-Squared test analysis supported the null hypothesis of no association between age and TC levels (χ^2=2.654, p=0.265) as indicated in table 53.

Table 53: Association between months on ART and TC levels

Months on ART	Borderline and below 5.2 mmol/L		Above 5.2 mmol/L		Total	
	n	%	n	%	n	%
0-12	5	100	0	0.0	5	100
>12	10	76.9	3	23.1	13	100
Chi-Squared test=2.654, p= 0.265						

4.6.1.5 Association between absolute CD4 count and TC levels

4.6.1.5.1 Association between CD4 count and TC levels for ages five and less than five years

The Chi-Squared test analysis supported the null hypothesis of no association between age and TC levels (χ^2=0.563, p=0.755) as indicated in table 54.

Table 54: Association between CD4 count for ages five and under

CD4 count (cells/mm^3)	Borderline and below 5.2mmol/L		Above 5.2mmol/L		Total	
	n	%	n	%	n	%
<750	3	100	0	0	3	100.0
750-1500	5	83.3	1	16.7	6	100.0
>1500	8	88.9	1	11.1	9	100.0
Chi-Squared test=0.563p- value = 0.755						

4.6.1.5.2 Association between CD4 count and TC levels for ages between six and 14years

The Chi-Squared test analysis rejected the null hypothesis of no association between absolute CD4 count and TC levels (χ^2=5.000, p=0.025) as indicated in table 55.

Table 55: Association between the CD4 count and TC levels for ages greater than five years

CD4 count (cells/mm^3)	Borderline and below 5.2mmol/L		Above 5.2mmol/L		Total	
	n	%	n	%	n	%
<200	0	0.0	0	0.0	0	0.0
200-500	0	0.0	1	100	1	100
>500	4	100	0	0.0	4	100
Chi-Squared test=5.000, p= 0.025						

4.6.1.6 Association between CD4 percentage and TC levels

The Chi-Squared test analysis supported the null hypothesis of no association between absolute CD4 percentage and TC (χ^2=0.3771, p=0.831) as indicated in table56.

Table 56: Association between the CD4 percentage and TC levels

CD4%	Borderline and below 5.2mmol/L		Above 5.2mmol/L		Total	
	n	%	n	%	n	%
<15	9	90	1	10	10	100
15-25	10	83.3	2	16.7	12	100
>25	1	100	0	0.0	1	100
Chi-Squared test=0.371p- value = 0.831						

4.6.1.7 Association between the viral load and TC levels

The Chi-Squared test analysis supported the null hypothesis of no association between absolute viral load and TC levels (χ^2=3.333, p=0.189) as indicated in table57.

Table 57: Association between viral load and TC levels

Viral load (Copies/ml)	Borderline and below 5.2mmol/L		Above 5.2mmol/L		Total	
	n	%	n	%	n	%
<40	6	75.0	2	25.0	8	100.0
40-1000	1	100.0	0	0.0	1	100.0
>1000	1	100.0	0	0.0	1	100.0
Chi-Squared test=3.333, p= 0.189						

4.6.1.8 Effect of ART regimen on the TC levels

The Chi-Squared test analysis supported the null hypothesis of no association between the effect of the regimen and TC levels (χ^2=0.003, p=0.955) as indicated - refer to table 58.

Table 58: Association between ART regimen and TC levels

ART regimen	Borderline and below 5.2mmol/L		Above 5.2mmol/L		Total	
	n	%	n	%	n	%
PI- containing	13	86.7	2	13.3	15	100.0
Without PI	7	87.5	1	12.5	8	100.0
Chi-Squared test=0.003p-value = 0.955						

4.6.2 Triglycerides

4.6.2.1 Frequency of hypertriglyceridemia

Of the two patients that had a maximum triglyceride test value subsequent to ART initiation, one had hypertriglyceridemia (50%). The other patient had the maximum triglyceride test value either at borderline or below 1.7 milimole per liter (refers to table 59).

Table 59: Frequency of hypertriglyceridemia (N=2)

Triglycerides	n	%
Borderline and below 1.7mmol/L	1	50
Above 1.7 mmol/L	1	50

4.6.2.2 Association between age and triglyceride levels

The Chi-Squared test analysis could not be applied to determine the association between age and triglyceride levels because of the small sample size as indicated in table 60.

Table 60: Association between age and triglyceride levels

Age (years)	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
Less than 5	0	0.0	1	100	1	100
6-11	1	100	0.0	0.0	1	100
12-14	0	0.0	0	0.0	0	0

4.6.2.3 Association between gender and triglyceride levels

The Chi-Squared test analysis could not be applied to determine the association between gender and triglyceride levels because of the small sample size as indicated in table 61.

Table 61: Association between gender and triglyceride levels

Gender	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
Male	1	100	0	0.0	1	100
Female	0	0.0	1	100	1	100

4.6.2.4 Association between the months on ART and triglyceride levels

The Chi-Squared test analysis was not applied to determine the association between months on ART and triglyceride levels because of the small sample size as indicated in table 62

Table 62: Association between the months on ART with triglyceride levels

Months on ART	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
0-12	0	0	1	100	1	100
>12	1	100	0	0.0	1	100

4.6.2.5 Effect of ART regimen on the triglyceride levels

The Chi-Squared test analysis was not applied to determine the association between ART regimen and triglyceride levels because of the small sample size as indicated in table 63.

Table 63: Effect of ART regimen on triglyceride levels

Regimen	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
PI- containing	0	0	1	100	1	100
Without PI	1	100	0	0	1	100

4.6.2.6 Association between the CD4 count and triglyceride levels

4.6.2.6.1 Association between the CD4 count and triglyceride levels for ages five and less than five years

The Chi-Squared test analysis could not be applied to assess the association between absolute CD4 count and triglyceride levels as the sample size was small (refer to table 64).

Table 64: Association between CD4 count and triglyceride levels for ages five and under

CD4 count (Copies/ml)	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
<750	0	0.0	0	0.0	0	0.0
750-1500	0	0.0	0	0.0	0	0.0
>1500	0	0	1	100	1	100

4.6.2.6.1 Association between the CD4 count and triglyceride levels for ages five and below

The Chi-Squared test analysis could not be applied to determine the association between CD4 count and triglyceride levels because of the small sample size as indicated in table 65.

Table 65: Association between CD4 count and triglyceride levels for ages greater than five years

CD4 count (Copies/ml)	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
<200	0	0.0	0	0.0	0	0.0
200-500	0	0.0	0	0.0	0	0.0
>500	1	100	0	0	1	100

4.6.2.7 Association between CD4 percentage and triglyceride levels

The Chi-Squared test analysis was not applied to determine the association between CD4% and triglyceride levels because of the small sample size as indicated in table 66.

Table 66: Association between CD4 percentage and triglyceride levels

CD4%	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
<15	1	100	0	0	1	100
15-25	0	0	0	0	0	0
>25	0	0	1	100	1	100

4.6.16 Association between the viral load and triglyceride levels

The Chi-Squared test analysis could not be applied to determine the association between viral load and triglyceride levels because of the small sample size as indicated in table 67.

Table 67: Association between the viral load and triglyceride levels

Viral load (Copies/ml)	Borderline and below 1.7 mmol/L		Above 1.7 mmol/L		Total	
	n	%	n	%	n	%
<40	0	100.0	2	0.0	2	100.0
40-1000	0	0.0	0	0.0	0	0.0
>1000	0	0.0	0	0.0	0	0.0

4.7 Laboratory testing for dyslipidemia and haematological abnormalities

4.7.1 Haemoglobin laboratory tests

Of the 168 patients in the study, 21.4% had an AZT containing regimen and 78.6% had a regimen without AZT. From the 36 patients that had AZT as part of their regimen, 25% patients

had at least one Hb laboratory test performed and 75% patients never had any Hb laboratory test performed (refer to figure 7).

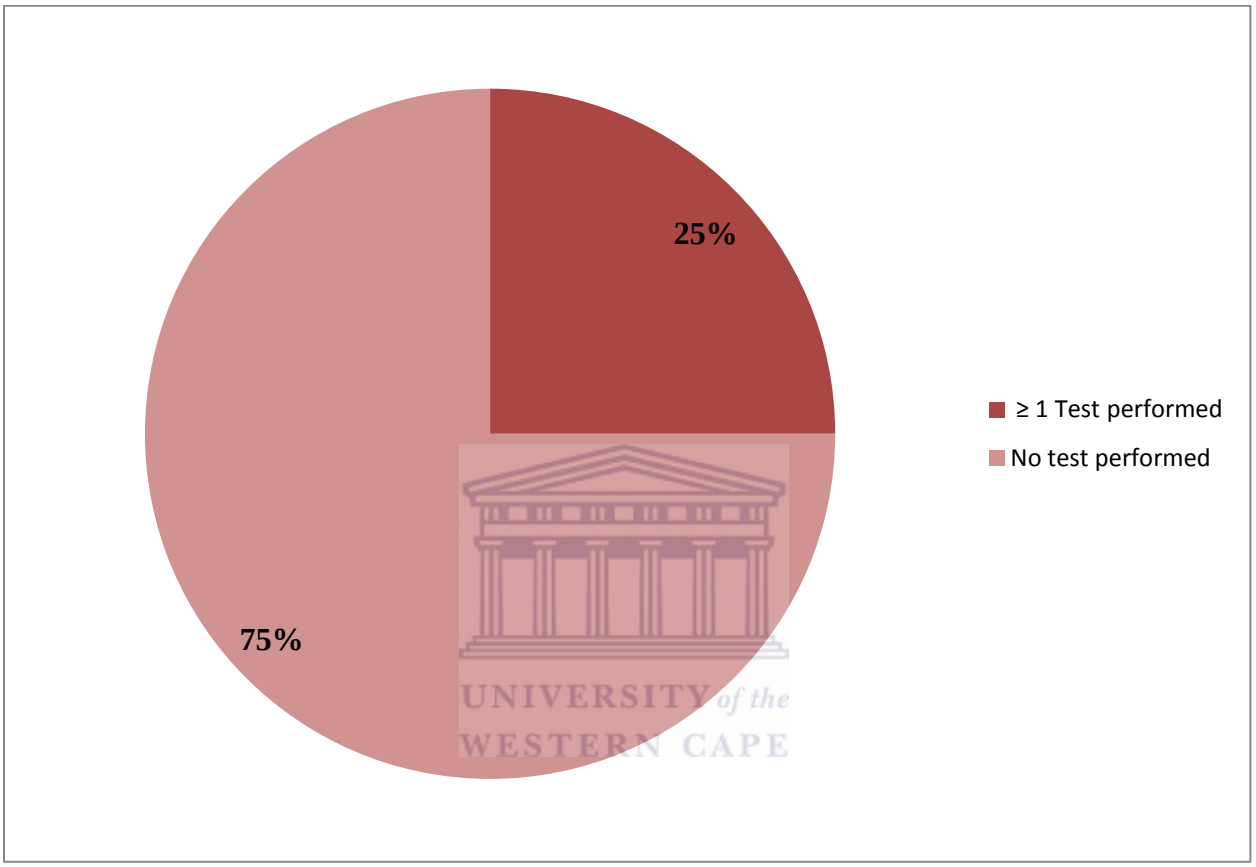

Figure 7: Patients with at least one Hb laboratory test performed (N=36)

4.7.2 Neutrophil laboratory tests

Of the 168 patients in the study, 94(55.9%) had at least one neutrophil laboratory test performed and 74(44.1%) patients never had a neutrophil laboratory test performed (refer to figure 8)

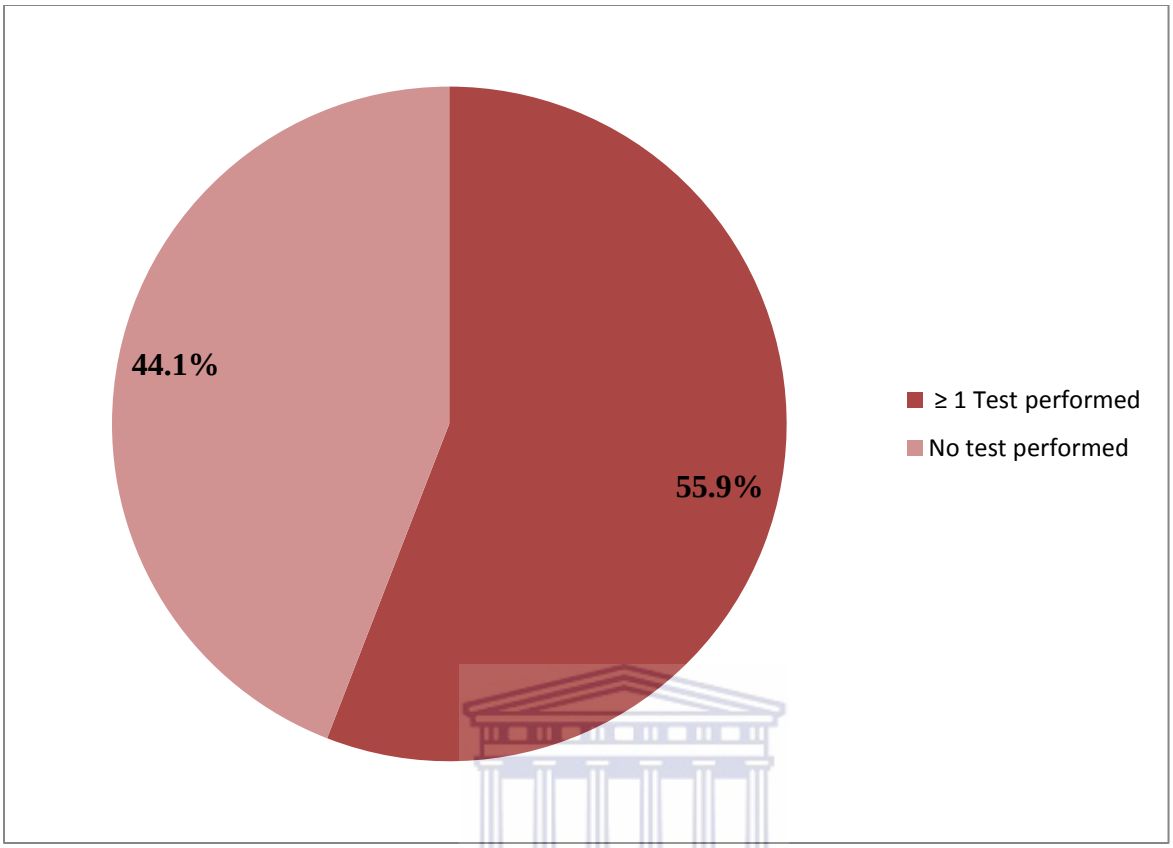

Figure 8: Patients with at least one neutrophil laboratory test performed (N=94)

4.7.3 TC laboratory tests

Ninety-seven (57.7%) patients had a regimen that contained a PI and 71(42.3%) had a regimen without a PI. From the 97 patients that had a PI as part of their regimen, 23(23.7%) patients had at least one TC laboratory test performed and 71(76.3%) patients never had a TC laboratory test performed(refer to figure 9).

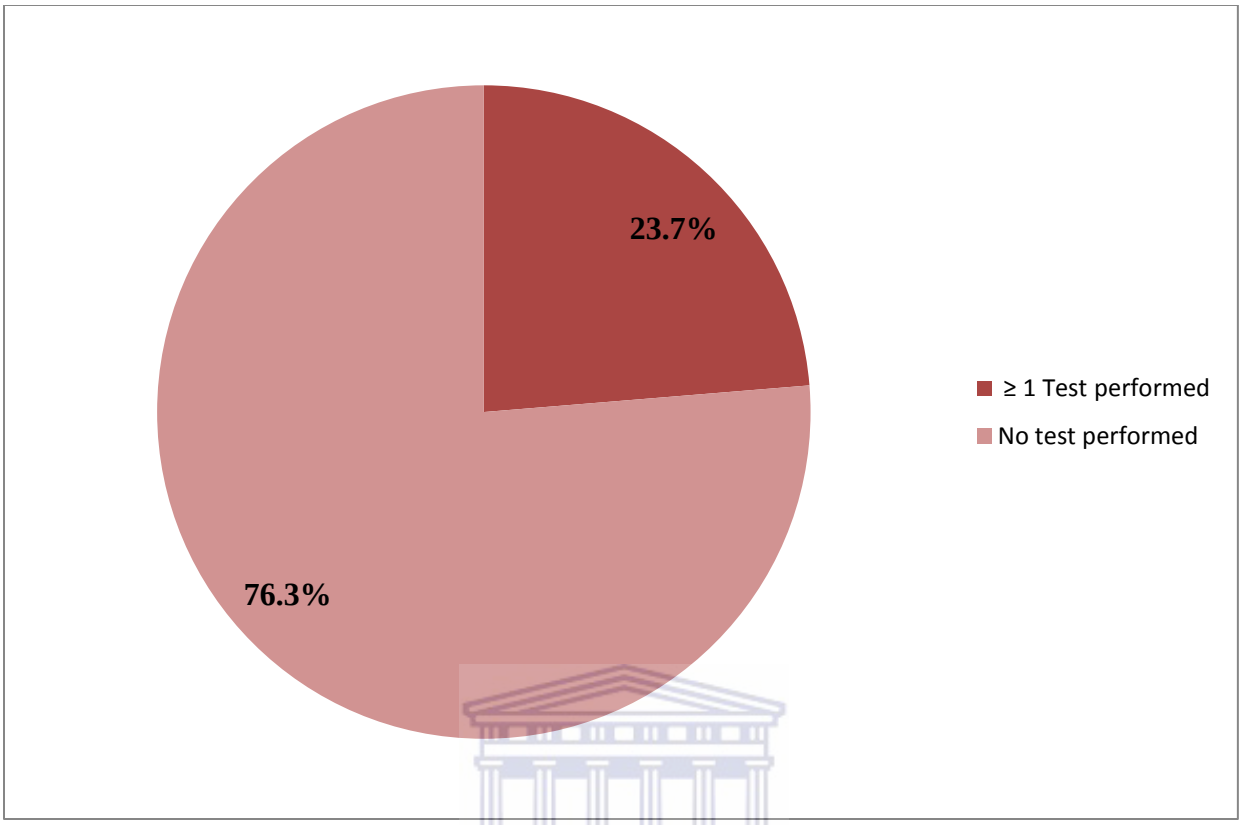

Figure 9: Patients with at least one TC laboratory test conducted (N=97)

4.7.4 Triglyceride laboratory tests

Out of the 97 study participants that were on a PI, two study participants had the triglyceride test performed (refer to figure 10).

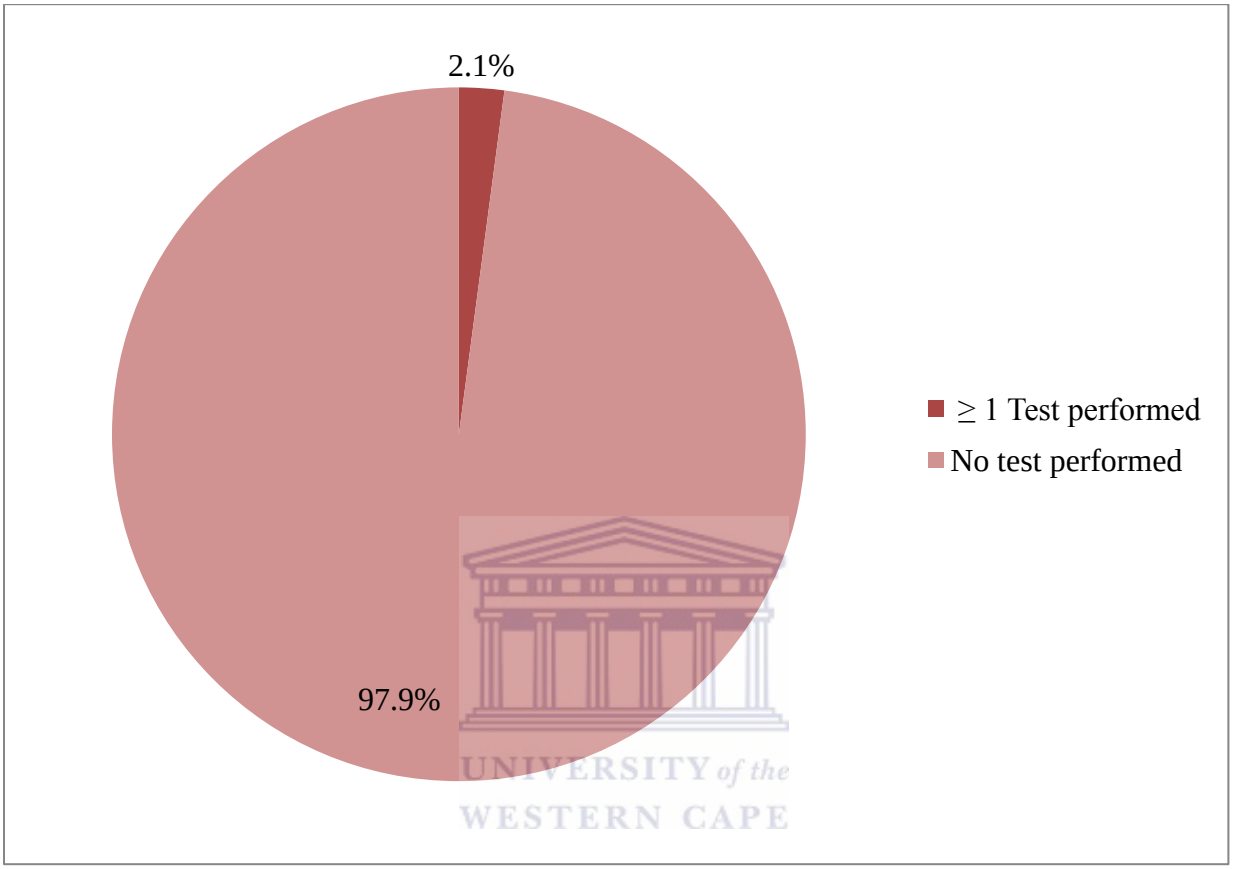

Figure 10: Patients with at least one triglyceride laboratory test performed (N=97)

Chapter 5 Discussion

This chapter presents an interpretation of the results presented in Chapter 4. It interprets the study findings within the South African context and against published literature.

5.1 Completeness of patient medical records

The facility is provided with patient medical record forms however, there is poor documentation of the patient clinical and laboratory data. In our study, baseline clinical records were complete in 53% of cases (ranging from 31.5% for viral load to 61.3% for WHO clinical staging). A similar study aimed at evaluating quality of patient data in health care clinics in the USA found that 40.5% of visits had complete baseline clinical information (Smith et al., 2005). This may be due to, *inter alia*, the prescribers' reluctance to order the tests because of limited knowledge – in the case of nurse-driven programmes – in ordering the necessary baseline laboratory tests (Horwood et al., 2010; Ruud et al., 2010), financial constraints at the facility (Sala, 2014; Yeap et al., 2010) and heavy workload (Phelps et al., 2013). Incomplete data hampers the provision of high quality care, the monitoring of patients over time, and their retention in treatment programmes. Communication and retention of patients is hampered when time is spent trying to find missing clinical information instead of talking to the patient about their care, resulting in a delay of providing quality care (Dovey et al., 2002; Elder & Hickner, 2005). Both data management and record-keeping are essential components when monitoring and evaluating HIV treatment. The clinical and laboratory data captured in facilities enables prescribers to make the right choices with regards to effectiveness and safety of ART and proper follow-up of the patient clinical progress. Failure to retain a high proportion of patients in care negates much of the potential benefit of ART treatment programmes, since most patients start ART with advanced

disease and are likely to die within weeks or months if therapy is discontinued. Treatment discontinuation raises similar concerns about drug resistance that incomplete adherence does (Forster et al., 2008).

Although South Africa has implemented the largest ART programme in the world, Cornel et al (2009) noted that the ability to monitor the programme closely has not kept pace with its expansion due to the lack of complete data on baseline clinical characteristics.

5.2 Lipid and haematological abnormalities

5.2.1 Haematological abnormalities

Haematological abnormalities such as reduction in Hb and neutrophil levels have been reported in children on ART (Enawgaw et al., 2014; Abebe & Alemseged, 2009; Resino et al., 2008; Tassiopoulos et al,2008; Feiterna-Sperling et al. 2007; European Collaborative Study, 2004; Jaquet et al., 2000). In Europe the prevalence of anaemia in children on ART ranges from 8% to 77% (European Collaborative study, 2004; Le-Chenadec; 2003). In Africa this prevalence ranges from 16.4% to 94% (Enawgaw et al., 2014; Renner et al., 2013; Njuguna et al., 2013; Abebe & Alemseged, 2009; Adeifa et al., 2006).

Anaemia was noted in 28.6% of children aged five or under in our study. These findings are consistent with the results of a meta-analysis conducted on a database which included published articles from Western and tropical countries representing data on 2,073 HIV-infected and 3,441 HIV-uninfected children on ART from 36 studies which yielded a prevalence range from 22% to 94% (Calis et al., 2008). Our findings are also supported by a retrospective study carried out by

Abebe & Alemseged (2009) in Ethiopia in children aged less than five years on ART which showed a 27.6% prevalence of anaemia. In our study, the prevalence of anaemia in children aged above five years was 50%. Similarly, a retrospective study in India by Shet et al (2009) showed a prevalence of 52.5% in the same age group.

Neutropenia was noted in 45.5% of children aged five years and below in our study. Similar to our findings, two sets of meta-analyses on studies in developed and developing countries involving children in the same age group on ART have reported the prevalence of neutropenia to be between 26 and 46% (Moyle et al., 2004; Kline et al., 1998). In contrast, a study conducted in Ethiopia by Abebe & Alemseged (2009) reported a 7.8% prevalence of neutropenia. The prevalence of neutropenia in children between six and 11 years and between 12 and 14 years was 21.7% and 65.6 %, respectively in our study. No studies on children in this age group were found in literature. A lower neutrophil count in individuals with African ancestry, known as 'ethnic neutropenia', is well-described in literature (Rezvani et al., 2001; Shaper & Lewis, 1971). This might accentuate the bone-marrow toxicity effects of neutropenia-inducing ARVs and of drug interactions between cotrimoxazole and AZT in the sub-Saharan African population (Moh et al., 2005).

5.2.2 Dyslipidemias

ART consisting of PIs or NNRTIs in combination with NRTIs has dramatically reduced morbidity and mortality among HIV infected children. However, with more widespread use of ART, there has been an increase in reports of dyslipidemias (Gortmaker et al., 2001).

In Europe, the prevalence range for dyslipidemia is between 10 and 70% (Kevin et al., 2011; Rhoads et al., 2011; Beraldo-Battistini et al., 2010; Resino et al., 2008; Solórzano-Santoset al., 2006; Farley et al., 2005; Jacquet et al., 2000). The prevalence of hypertriglyceridemia ranges from 13% to 71% (Lapphra et al., 2005; European Paediatric Lipodystrophy Group, 2004; Jaquet et al., 2000).

In our study, hypercholesterolemia was noted in 13.1% of children and hypertriglyceridemia in 50%.A similar retrospective study in the USA also found a hypercholesterolemia prevalence of 13% in children on ART (Farley et al., 2005). Other retrospective studies in Europe and the USA have found a higher prevalence of hypercholesterolemia in children ranging from 19% to 29% (Kevin et al., 2011; Resino et al., 2008; Jacquet et al., 2000).

A prospective paediatric AIDS clinical trials cohort in London found a 13% prevalence of hypercholesterolemia in children on ART (Tassiopoulos et al., 2008). After a duration of 50.4 months, an additional 13% developed hypercholesterolemia during follow-up for an incidence rate of 3.4 cases per 100 person-years (95% confidence interval [CI]: 3.0 to 3.9). The incidence rate of hypercholesterolemia during HAART with PI use was 4.8 cases per 100person-years (95% CI: 4.2 to 5.4) and the incidence rate seemed to be constant over the period of follow-up, suggesting that the proportion of children with hypercholesterolemia is likely to continue to increase over time. This in the long run may cause atherosclerosis and insulin resistance (Solórzano-Santos et al., 2006). The prevalence ranges of hypercholesterolemia maybe brought about by variations in dietary intake and also by genetic factors.

5.3 Factors associated with haematological and lipid profile abnormalities

5.3.1 Factors associated with haematological profile abnormalities

Haematological complications occur frequently in children with HIV infection and may be associated with a variety of factors including HIV infection itself, HIV-related conditions, ARVs or other drugs, age, gender, viral load, CD4 cell count and CD4 % (Rhoads et al., 2006; Moh et al., 2005). Anaemia and leucopoenia, especially neutropenia, have been documented in literature as the most common haematological abnormalities in HIV infected patients. The cause of the haematological abnormalities is multifactorial. Infiltration of the bone marrow by the HIV infection and the use of myelosuppressive medications, are some of the mentioned causes in literature (Gedefaw et al., 2013).

A significant association between neutropenia and viral load for children between 12 and 14 years was observed. The majority of children with neutropenia had an undetectable viral load. Similar to our findings, a study by Shet et al (2009) in India found a significant association between viral load and neutrophil levels in children. A significant association was observed between anaemia and viral load in children aged five years and below. In this age group, anaemia was noted in children with an undetectable viral load. This may be related to the increased risk of underlying anaemia attributed to co-infections and underlying malnutrition (Shet et al., 2009) and also positive responses to ART regimens may bring about a reduction in the viral load (Carter et al., 2005). Our findings are similar to those of a study conducted by Shet et al (2009) in which anaemia was associated with an undetectable viral load.

Despite no significant association with the other independent variables, haematological abnormalities were observed in the majority of the children included in our study. Anaemia and neutropenia were noted in children on regimens containing AZT and regimens without AZT, although this association was not significant. A study conducted by Pacheco et al (2003), using retrospective data from a cohort in the USA, reported haematological abnormalities in HIV infected children on ART regimens. This may have been due to the mitochondrial toxicity of ART regimens through the inhibition of the gamma polymerase enzyme. Contrary to our findings, results from other studies have associated regimens containing AZT with haematological abnormalities (Ejeliogu et al., 2014; Renner et al., 2013). AZT-based ART has been shown to be a risk factor for the development of anaemia and neutropenia in HIV positive children as it causes myelosuppression (Ejeliogu et al., 2014; Renner et al., 2013; Johannessen et al., 2011; Okechukwu et al., 2010).

Haematological abnormalities were noted in patients with high CD4 % and CD4 count but this association was not significant. Recovery of the immune system implies the effectiveness of the ART regimens. However, these regimens have been associated with abnormalities such as impaired haematopoiesis and immune cytopenias (Adeifa., 2006). Our findings are similar to a study conducted by Shet et al (2009) in India and Renner et al (2013) in the USA which found no association between haematological abnormalities and CD4 count and CD4 %. However, contrary to our findings, anaemia was associated with CD4 counts and CD4% in a study conducted in Uganda by Ruhinda et al (2012). In vitro studies of primary peripheral-blood lymphocyte cultures from individuals without HIV infection who are exposed to ART and who

have haematological abnormalities have shown dose and time dependent mitochondrial DNA depletion accompanied by decreased cell proliferation (Ruhinda et al., 2012).

In our study, haematological abnormalities were noted in the majority of children using cotrimoxazole although this association was not significant. Cotrimoxazole is prescribed in HIV infected children for the prevention of Pneumocystis carinii infection. It exerts its effects at two sequential points in the folate metabolic pathway i.e. sulphamethoxazole competitively inhibits the incorporation of p-amino-benzoic acid and thus blocks dihydrofolate synthesis and trimethoprim acts by inhibiting the reduction of dihydrofolate to tetrahydrofolate by dihydrofolate reductase, thereby exhibiting its effects on the haematopoitic system. Similar to our findings, a controlled clinical trial performed in Europe by Dryden-Peterson et al (2013), found no association between changes in haematological abnormalities and the use of cotrimoxazole.

5.3.2 Factors associated with dyslipidemia

We found a statistically significant association between absolute CD4 count and TC levels for children aged between 12 and 14; hypercholesterolemia was noted in children with a high CD4 count. A USA study showed that children taking PI-based ART, particularly those who adhere to their regimens and respond well to therapy, carry an increased risk of hypercholesterolemia as evidenced by an association between TC levels and undetectable viral loads, increased CD4 percentage and CD4 count (Carter et al., 2006). These findings reflect a possible confounding effect of ART, which in this case was PI-based. Our findings are also supported by studies

conducted in the USA (Farley et al., 2005) and Thailand (Lapphra, 2005), which reported a significant association between TC levels and absolute CD4 count.

Literature cites the use of PI-containing regimens as a factor contributing towards lipogenesis by a mechanism that is not well described in literature. Although not significant in our study, dyslipidemia was more common in children on a PI-based regimen than any other regimen. Some children on non-PI regimens also experienced dyslipidemias and this may be attributed to their use of a D4T-containing regimen. This is consistent with studies in the USA which reported dyslipidemias in children on D4T (Carter et al., 2006; Solórzano-Santos et al., 2006).

During the course of HIV infection, dyslipidemias may occur and these episodes increase with the progression of HIV infection mainly due to reduction of leptin levels in hepatocytes inducing lipogenesis (Calza et al., 2004). We found no significant relationship between viral load and TC levels to indicate that hypercholesterolemia is associated with HIV disease progression.

In our study, dyslipidemia was noted in older children (ages between 6 and 14 years) although this bore no statistical significance. Adolescents present with more dyslipidemias than pre-pubertal children in the general population and this could be due to the hormonal changes occurring during puberty (Taylor, 2004).

Some studies have reported an association between gender and TC levels such as the European Paediatric Lipodystrophy Group which showed a higher risk of girls developing dyslipidemias (Kim et al., 2009; Farley et al., 2005; European Paediatric Lipodystrophy Group, 2004).

However, ours and one other study conducted in Madrid showed no difference in hypercholesterolemia prevalence between males and females (Alves, Oliveira & Brites, 2008).

A similar study to ours investigating the same group of independent variables viz., age, gender, CD4 percentage and viral load found no association between these variables and TC levels (Strehlau et al., 2012; Lapphra et al.,2005).

5.4 Laboratory testing for dyspilidemia and haematological abnormalities

Guidelines are developed for various reasons, including: to bridge the gap between evidence and practice; to minimise variations in practice; to improve health outcomes and to improve quality of care and to reduce costs (Schünemann et al., 2006).

This study showed poor adherence to Hb, neutrophil, TC and triglyceride laboratory testing guidelines with the majority (>70%) of the patients on PIs and AZT never having been exposed to a single laboratory test. Our findings show a larger percentage of untested children than a West African cohort study by Renner et al (2013) in which laboratory testing during follow-up on ART was infrequent for 40.6% of children. In this cohort study the adherence to laboratory testing may have been more favourable due to the inclusion of only urban health facilities which were considered to deliver better health care (IeDEA Pediatric Working Group, 2013). The lack of testing in our study could be attributed to limited physical and financial resources, prescriber reluctance in requesting the laboratory test and the limited skills and numbers of staff relative to patient numbers (Yeap et al., 2010).

Decisions regarding initiation, changes in ART and progress in the patient's clinical condition should be guided by monitoring the laboratory parameters of viral load, CD4 count and CD4% in children. Results of these laboratory tests provide clinicians with key information regarding the virological and immunological status of the patient and the risk for disease progression to AIDS. In a large cohort study of paediatric patients conducted by Cornel et al., (2009), HIV infected children whose baseline laboratory tests were performed later than the recommended national and international guidelines subsequently started their ART at a later stage and this increased their risk of early mortality.

The presence of the NHLS laboratory services in South Africa and its support to the different regional laboratories has enhanced proper management of HIV and TB. This has, in turn, improved the quality of lives for people living with HIV/AIDS. In recent years, however, there have been reports of large debts owed to the NHLS by some provincial health departments and this could compromise future delivery of these services and ultimately further undermine the monitoring of patients on ART (Sapa, 2014).

5.5 Study limitations

1. Only one study site was used and the findings are, therefore, not generalisable to other Cape Metropole CHCs.
2. The patients' nutritional status, opportunistic infections and the body mass index are all factors associated with haematological and lipid profile abnormalities but these were not included in the study due to incompleteness of records.

3. The specific ART regimen taken at the time when the highest Hb, neutrophil, TC and triglyceride values were recorded was not used in the analysis. Instead, we identified whether a patient had ever been on a PI- or AZT- containing regimen during the course of therapy and used this data in assessing associations with lipid and haematological abnormalities. Similarly the duration on ART was not PI or AZT specific.
4. No control groups i.e. children not on ART were used in the study and as such, confounding variables such as age-related dyslipidemias unrelated to ART were not taken into consideration.
5. The sample size used for the study was small and may not have generated enough statistical power to find associations using the Chi-Squared Test.

Chapter 6 Conclusions and recommendations

This chapter presents the conclusions on the findings of the study and links them to the initial research questions. A list of recommendations for improved practices and further studies are also presented.

6.1 Conclusions

This study demonstrates poor record-keeping practices and poor adherence to laboratory testing paediatric guidelines at the CHC. However, in the absence of routine baseline and follow-up laboratory test data, the study provides an alternative parameter i.e. the highest haemoglobin, neutrophil, TC and triglyceride laboratory value for measuring the prevalence of haematological and lipid abnormalities. While this parameter may not be useful for routine monitoring of adverse effects, it provides a snapshot estimate of prevalence for a site providing ART.

When compared to the literature, our study demonstrated a higher prevalence of neutropenia and similar prevalence of anaemia and hypercholesterolemia in children on ART. Although there was no statistical significance between PI-containing and AZT-containing regimens and lipid and haematological abnormalities, respectively, the associations between selected immunological markers and these dependent variables reflect a possible confounding effect by ART although our sample size was too small to verify this.

6.2 Recommendations

6.2.1 Improving practices

With a view to improve practices in the management of HIV in children, the following recommendations are proposed:

- To optimize adherence to routine monitoring tests, health care providers must view patient outcomes as the goal of the patient's health care experience. An appreciation for the importance of testing as a tool for monitoring adverse drug reactions on ARVs and progress on therapy to achieve these outcomes must, therefore, be engendered in the training of health care providers.
- Certain entries on the patient medical folders, such as laboratory testing data received from the NHLS, could be completed by a data clerk to reduce the workload of prescribers.
- Standard Operating Procedures (SOPs) and algorithms for recommended laboratory tests for children on specific ART regimens should be accessible to health care workers at the CHC and adherence to these guidelines should be monitored by relevant clinical managers.
- Patients should be educated about adverse effects through patient friendly pamphlets which also outline the recommended laboratory testing schedule to empower them to take ownership of their health status and to remind the prescriber about laboratory monitoring tests.

6.2.1 Further research

To improve secondary data used in research and to build on the existing research presented in this thesis, the following are recommended:

- Including the ART regimen and the months on ART with every laboratory test requested from the NHLS will link important patient information with the NHLS data thereby creating a more useful database for research purposes.
- A follow-up qualitative study to understand the reasons for poor record-keeping and poor adherence to laboratory testing guidelines.
- Longitudinal studies on the same patient cohort to address research questions on, *inter alia*, the long-term use of ART in children.

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Appendix I Data collection tool

1.PATIENT DETAILS:

UNIQUE ID: ……

DATE OF COLLECTION:……

CAPTURED BY:……

Gender: M F

Date of birth:	
Folder number:	

2. PATIENT MEDICAL HISTORY:

Year diagnosed:

3. SOCIAL ASSISTANCE:

Lives in what sort of dwelling : informal dwelling / formal house / hostel / other (specify)		
Number of rooms:	Number of adults in household:	Refrigerator: Y N
Has patient disclosed HIV status: Y N To whom:		
Source of income? (circle) Employed / Grant / Pension / Friends or Family / Unemployed		

4. PRE-ARV COUNSELLING:

SESSION:	DATE/S	COUNSELLOR	GROUP	TX PARTNER ATTENDED?	COMMENTS:
General HIV education & healthy living					
ARV's					
Adherence planning					
Other					

Patient agreed to home visit Y N	Attends a support group? Y N
What is client's understanding (in their own words) for wanting ARVs?	

5. PSYCHO-SOCIAL READINESS:

	Y	N		Y	N
Have they attended all the required counselling sessions?			Do they have a treatment partner?		
Have they disclosed to anyone?			Have they been attending the clinic regularly?		

TREATMENT MONITORING PER VISIT

VISIT #..... **DATE OF VISIT:.....**

HISTORY AND EXAMINATION

WT (kg)	HT (cm)	MONTHS ON ART	MONTHS ON REGIMEN	MONTHS ON TB RX	NOTES

INVESTIGATIONS **ASSESSMENT**

TB M/C/S	CD4	CD4%	VL	ALT	OTHER	HIV COND/OI'S, TB	N/O/R	ADV EVENT	N/O/R	GRADE

PLAN AND TREATMENT

Adherence &counts		DRUG			CORRESPONDENCE	OUT	IN	NEXT VISIT
Drugs and dosages	DRUG	DRUG	DOSE	FREQ				
	ARV1							
	ARV2							**REFERRED**
	ARV3							
	ARV4/other							
	ARV5/ other							
	ARV6/other							
	Cotrimoxazole							
	INH							
	Fluconazole							

VISIT #..... **DATE OF VISIT:.....**

HISTORY AND EXAMINATION

WT (kg)	HT (cm)	MONTHS ON ART	MONTHS ON REGIMEN	MONTHS ON TB RX	NOTES

INVESTIGATIONS **ASSESSMENT**

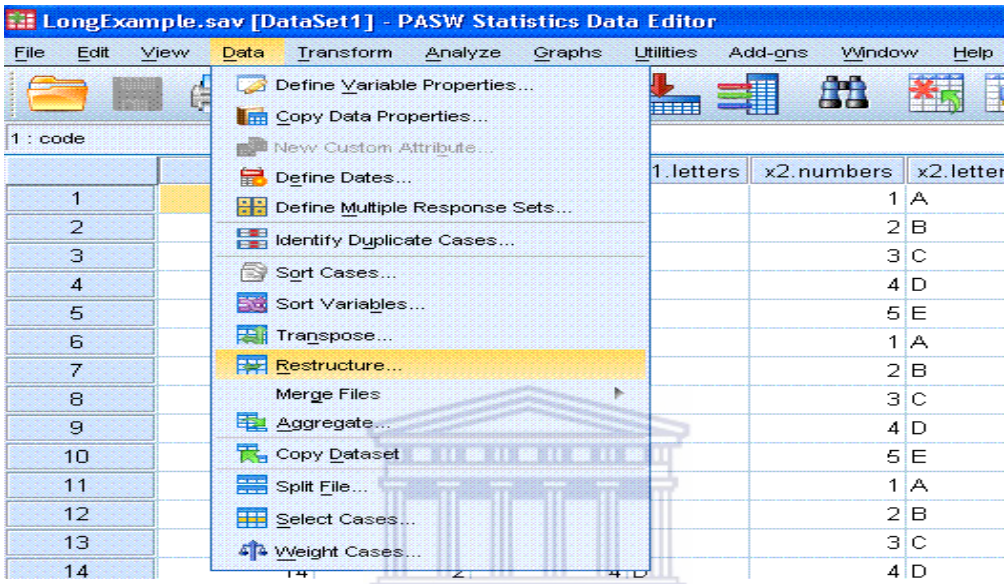
TB M/C/S	CD4	CD4%	VL	ALT	OTHER	HIV COND/OI'S, TB	N/O/R	ADV EVENT	N/O/R	GRADE

PLAN AND TREATMENT

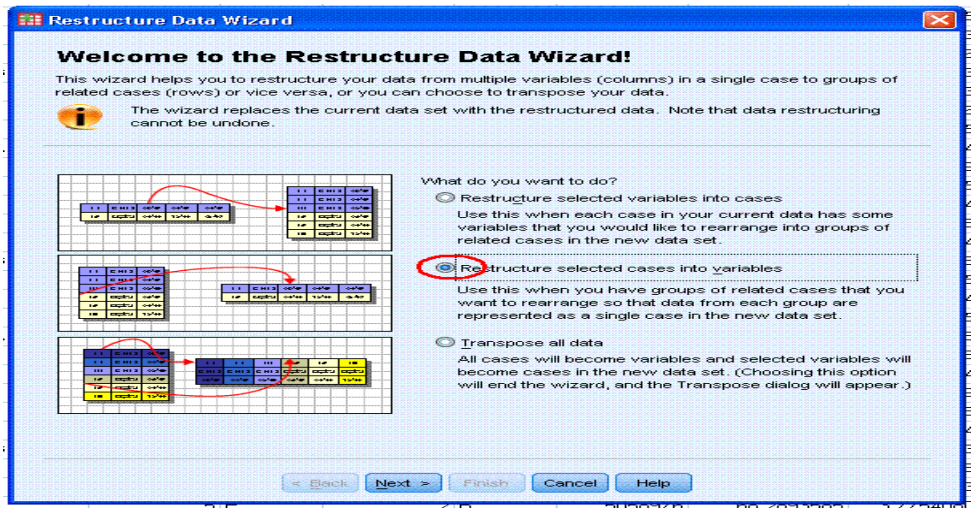
Adherence &counts		DRUG			CORRESPONDENCE	OUT	IN	NEXT VISIT
Drugs and dosages	DRUG	DRUG	DOSE	FREQ				
	ARV1							
	ARV2							**REFERRED**
	ARV3							
	ARV4/other							
	ARV5/ other							
	ARV6/other							
	Cotrimoxazole							
	INH							
	Fluconazole							
	ARV5/ other							
	ARV6/other							
	Cotrimoxazole							
	INH							
	Fluconazole							

Appendix II: Reshaping a long format data set to a wide format data set

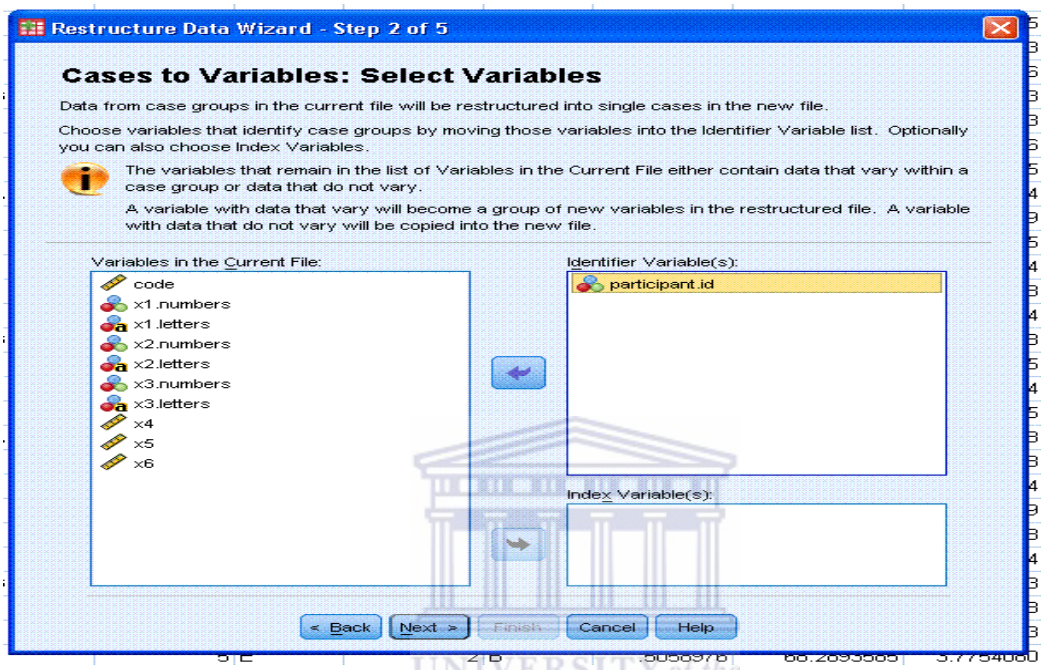
1. Open the excel spreadsheet in the SPSS software.
2. Click data on the tool bar.
3. Click restructure.
4. The screen should look like this.



6. Select “restructure selected cases into variables”, then click next.

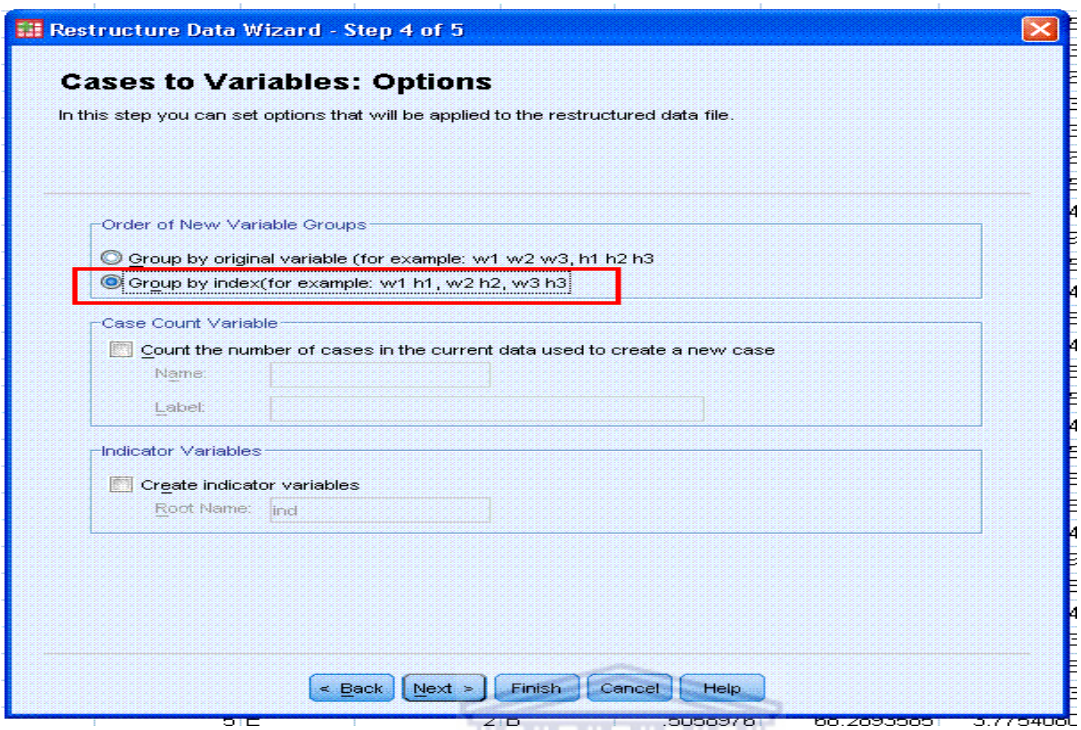


5. In the box that says variables in the current file, select the identifier variable which in this case is the folder number, then click the arrow outside the box to push it to the box labelled identifier variable. Click next.



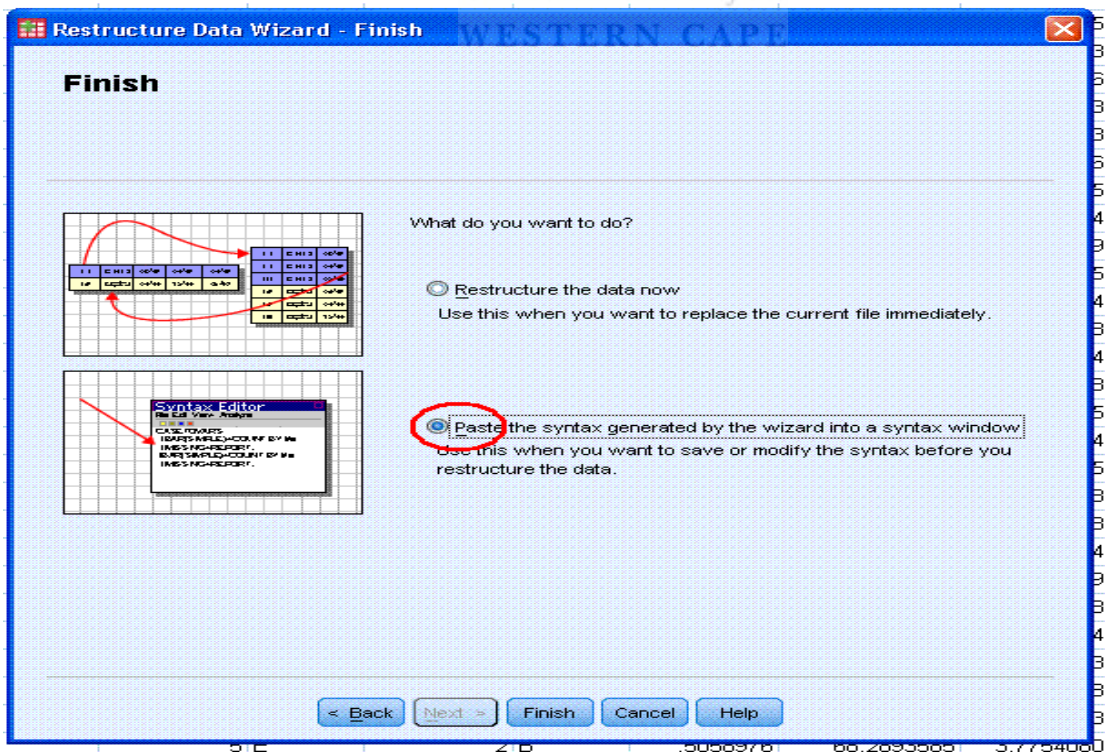
6. With the next table we do not need to select the sorting, just click next.

7. In the next table select "Group by index(for example: w1 h1, w2 h2, w3 h3)"



8. Then click next.

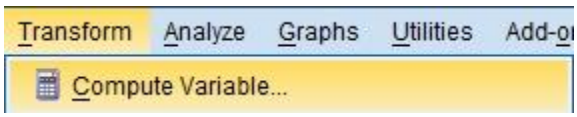
9. Click finish if "Restructure the data now" has been selected.



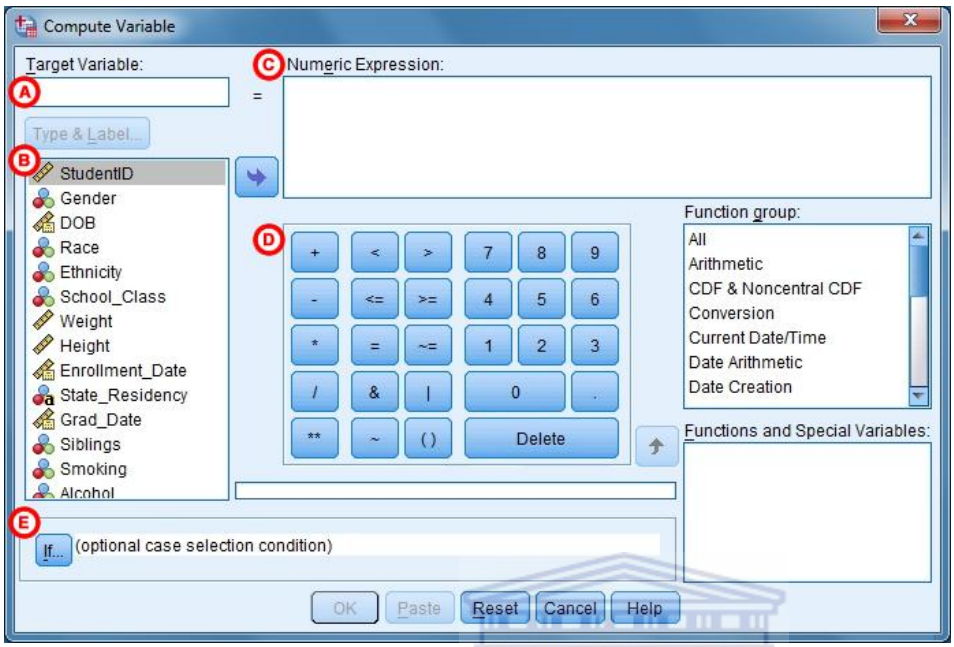
Appendix III: Manual to select (isolate) highest laboratory value

S/N	STEPS
1.	Change the variable in question from the string to numeric format.
2.	Select transform on the tool bar.
3.	Select compute variables.
4.	Type in the name of the new variable in the "target variable box".
5.	Next is the functional group box.
6.	Select "statistical" in the functional group box.
6.	Select MAX below the functional group box. Double click it so it appears in the numeric expression box.
7.	Within the brackets in the formula in the numeric expression box, transfer the cholesterol from cholesterol 1 to cholesterol 34 each separated by a comma and no spaces in between.
8.	NOTE: AT THE END OF RUNNING THE EXECUTION, CHECK IF THE NEW VARIABLE MATCHES THE OLD VARIABLES THAT WERE USED TO CREATE THIS NEW VARIABLE.

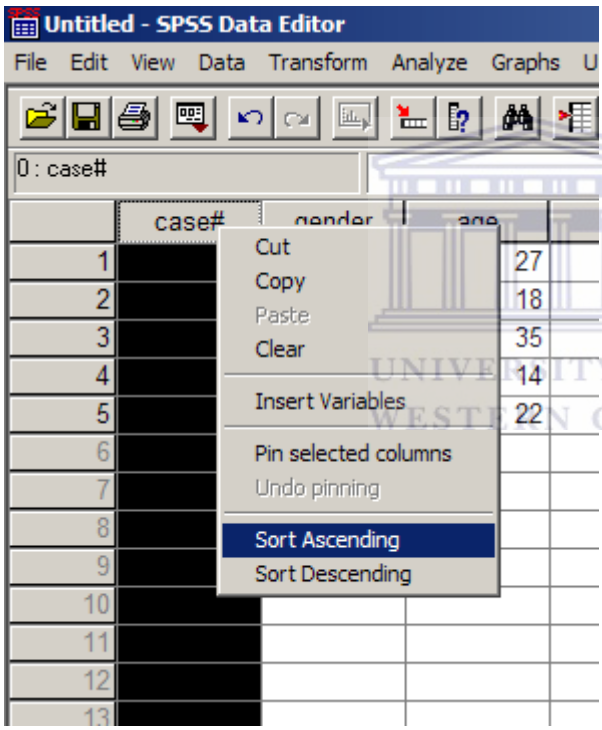
- **APPEARANCE OF THE TOOL BAR**

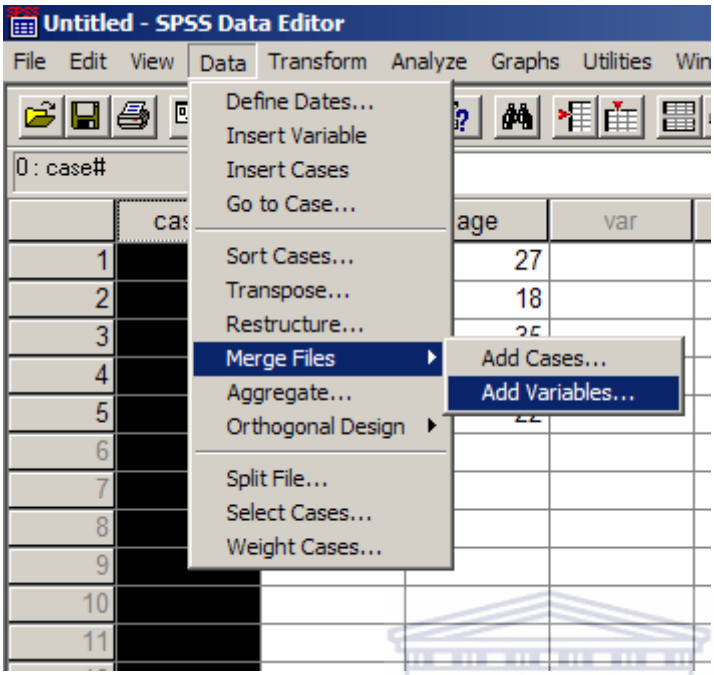


- **APPEARANCE OF THE COMPUTE VARIABLE DIALOG BOX ON THE SCREEN**



Appendix IV: Manual to merge the patient clinical data with NHLS data set

	Steps
1	Both files need to have a common indexing key(patient folder number). This would be a unique identifier for each case in your data set. Both data files should provide different data for the same set of cases.
2	The keys have to be sorted in ascending order for SPSS to be able to merge the files, so make sure both files are sorted in ascending order before trying to merge. 
3	Open the first file that you wish to merge. Next, under the 'Merge Files' item in the 'Data' menu, select 'Add Variables.'

	
4	Select the file you wish to merge
5	SPSS will give you a warning regarding sorted key variables. Make sure both files were sorted in ascending order before trying to do a file merge.