Virologic and Immunologic Responses in Patients on Highly Active Antiretroviral Therapy in Vhembe District, South Africa: A Retrospective Study

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A mini-dissertation submitted in partial fulfilment of the requirements for the degree of Master of Public Health, Department of Public Health, School of Health Sciences, University of Venda

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DECLARATION

I, Aniekan Edet, hereby declare that this mini-dissertation titled; "Virologic and Immunologic responses to Highly Active Antiretroviral therapy in Vhembe district, South Africa: A retrospective study", is my original work and has not been submitted for any degree at this or any other academic institution; and that all citations, materials and sources used have been duly acknowledged by a complete list of references.

Signature: …… Date: ……

Aniekan Edet

DEDICATION

I dedicate this work to God for his guidance and for good health throughout my study. I also dedicate this work to all health care personnel who work tirelessly providing health care services to all persons infected or affected by HIV, in the midst of all challenges they are faced with in South Africa.

ACKNOWLEDGEMENTS

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ABSTRACT

Background: South Africa presently has a very high HIV burden. It has adopted the UNAIDS "90-90-90 targets" to curb its HIV burden. This target aims to attain sustained viral suppression in 90% of all persons receiving antiretroviral therapy. This is supported by several studies. Studies to observe if patients are achieving and sustaining viral suppression in Limpopo, South Africa, are few.

Objective: To investigate the viral and immunologic responses of patients in Vhembe District to highly active antiretroviral therapy (HAART) between the 1st of January 2004 and 31st of July 2016.

Methodology: This was a retrospective medical record review conducted in Vhembe District in rural Limpopo. It included the medical records of 1247 individuals from Thohoyandou Community Health Centre. Analysis was done using SPSS 24.0. To model the factors associated with virologic and immunologic responses, each independent variable was tested for association with the dependent variable (viral suppression and CD4 count increase of ≥ 50 cells/µL from baseline to 6 months). The independent variables included age, year of initiation, gender, marital status, baseline BMI, haemoglobin, clinical stage and estimated creatinine clearance. The Pearson Chi square (X^2) was used for all categorical independent variables and the t-test, for all continuous independent variables, to test for association. The estimate used was a 95% confidence interval, and a p-value of < 0.05 was considered significant.

Results: The study showed that 52.6% of individuals were in clinical stage I at baseline. Viral suppression (viral load < 50 copies/ml) at 6 months was 64% (n = 648), 72% (n =193) at 60 months and 94% (n = 16) at 132 months. Fifty-nine percent had consistent viral suppression for a period of at least 6 months. Consistent viral suppression (viral load < 50 copies/ml on at least one consecutive occasion without any intervening viral load > 50 copies/ml) for at least 54 months was only 14%, while 2.3% had a delay in switching from a failing regimen. The mean CD4 count at baseline was 227 cells/µL, and 538 cells/µL at 60 months. The mean CD4 cell count increase from baseline to 6 months was 190 cells/µL. The immuno-virologic discordance was 27%. Patients with higher baseline CD4 count and females were significantly (p = 0.001 and 0.031 respectively) more likely to achieve viral suppression at 6 months. Those below 45 years and females were

significantly (p = 0.011 and 0.043 respectively) more likely to achieve adequate CD4 count increase at 6 months.

Conclusions: The proportion of individuals with viral suppression in the District increased from 6 months onwards, and is fairly adequate. However, sustainability of viral suppression, once attained, is low. Adequate immunologic response, however, seems high. Males and age group above 45 years appear to have poorer responses to HAART.

Key words: Virologic response, Immunologic response, Highly Active Antiretroviral Therapy

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LIST OF ABBREVIATIONS

- 3TC - Lamivudine
- ABC - Abacavir
- AIDS – Acquired Immune Deficiency Syndrome
- ART – Antiretroviral therapy
- ARV – Antiretroviral drugs
- ATV/r – ritonavir boosted Atazanavir
- AZT - Zidovudine
- BMI – Body Mass Index
- d4T - Stavudine
- EFV - Efavirenz
- FTC - Emtricitabine
- HAART – Highly Active Antiretrovial Therapy
- HIV – Human Immunodeficiency Virus
- LPV/r – ritonavir boosted Lopinavir
- NNRTI – Non-Nucleoside Reverse Transcriptase Inhibitor
- NRTI – Nucleoside Reverse Transcriptase Inhibitor
- PEP – Post Exposure Prophylaxis
- PEPFAR – US President's Emergency Plan For AIDS Relief
- PI – Protease Inhibitor
- SPSS – Statistical Package for the Social Sciences
- STI – Sexually Transmitted Infections
- TDF - Tenofovir
- UNAIDS – Joint United Nations programme on HIV/AIDS
- VL – Viral Load
- WHO – World Health Organisation

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CHAPTER ONE

INTRODUCTION

1.1 **Background of the study**:

Globally, an estimated 35 million people are living with Human Immunodeficiency Virus (HIV). Over twenty-four million of these are living in Sub-Saharan Africa (UNAIDS, 2014a). With 6.19 million people living with HIV and over 340,000 annual incident cases in 2013, South Africa's HIV burden was the highest in the world (Statistics South Africa, 2015a; Whiteside et al., 2015). As at the end of 2016, the HIV prevalence and incidence rate had further increased to 7.03 million individuals, and 380,000 individuals per annum respectively (UNAIDS, 2016). In order to control the global HIV burden, UNAIDS developed the "90-90-90 targets". South Africa has adopted these targets. It states that by the year 2020, 90% of all people living with HIV will know their status, 90% of those who know their status will be on lifelong ART and 90% of those on ART will achieve viral suppression. Modelling suggests that achieving these targets will enable the world to end the Acquired Immunodeficiency Syndrome (AIDS) as an epidemic by 2030 (UNAIDS, 2014a).

Several studies have shown that the transmission of HIV is markedly reduced among sero-discordant partners when the infected partner is virally suppressed. One study found a 'zero transmission' rate (Bruun et al., 2011). Viral suppression therefore forms the basis of many interventions that aim at achieving 'zero new HIV infections'. One of these intervention is the "test and treat" approach that aims to reduce HIV transmission. Furthermore, the World Health Organisation (WHO) has recommended different definitions of viral suppression and virologic failure for low-to-medium income and high income countries. For low-to-medium-income countries, it recommends that viral failure and viral suppression be defined as a VL > 5000 copies/ml and VL < 1000 copies/ml respectively. A VL of ≥ 200 copies/ml and a VL of ≤ 50 copies/ml defines viral failure and viral suppression respectively for high-income-countries. (McMahon et al., 2013). The South African National Department of Health (2012), however, recommends the use of VL ≤ 50 copies/ml as definition for viral suppression and 2 VL results >1000 copies/ml to define viral failure. Bartlett et al. (2012) state that maintaining the VL at ≤ 50 copies/ml is associated with the most durable clinical benefit.

The CD4 cell response to ART is more variable. It increases rapidly in the first month of ART by 75 – 100 cells/µL with a more gradual response thereafter (Meintjes et al., 2014). The CD4 count of some patients however fails to increase despite viral suppression. Several causes have been suggested for this discordant CD4 cell response. Insufficient thymic activity and ongoing viral replication despite good viral suppression, may account for this (Corbeau and Reynes, 2011). Some studies have investigated the implication of this immuno-virologic discordance. Zoufaly et al. (2011) found that compared with those whose CD4 count increases with viral suppression, patients with immuno-virologic discordance had increased risk for developing AIDS and other complications, especially in the first 6 months of therapy. Several factors may be associated with viral suppression, adequate CD4 cell response and immuno-virologic discordance.

The history of Antiretroviral (ARV) use in the public health sector in South Africa was marked by an initial denial of the existence of AIDS and opposition to the use of Highly Active Antiretroviral Therapy (HAART). This resulted in uncontrolled spread of HIV and law-suits against the then national government. This continued until October 2003, when the government announced that it would be rolling out HAART in public health facilities. This however only began in 2004 with United States President's Emergency Plan for AIDS Relief (PEPFAR) and Global Fund providing most of the initial funding (Nattrass, 2008). During the initial period, most patients were initiated on Antiretroviral Therapy (ART) in hospitals which were far away from their homes. This had a negative impact on retention on treatment. The government therefore changed this policy to provide care at health facilities closer to patients. As at the end of 2013, there were over 3000 public health facilities, including clinics that initiated and monitored patients on HAART. In Vhembe District, there were 131 public health facilities providing HAART as at 2013. Presently the South African government is the largest funder of its ART programme, contributing R19.9 billion in 2013/14. The rest comes from donor agencies such as PEPFAR (Bekker et al., 2014).

1.2 **Problem Statement**:

Every year, much funding is allocated by the South African government to control the spread of HIV infection. This has resulted in a steady increase in the number of people initiated on ART. One of the goals of providing ART is to achieve viral suppression and thereby reduce the possibility of HIV transmission. In 2013, South Africa had an annual HIV incidence rate of 340,000. This increased to 380,000 at the end of 2016 (Statistics South Africa, 2015; UNAIDS,

2016). Also, HIV was the leading cause of death among females aged 25 – 64 years in Vhembe District in 2013. It was also the second most common cause of death in males of the same age group in Vhembe District (Massyn et al., 2015). These figures remain high. There is therefore a need to evaluate the effectiveness of the massive roll-out of ART, especially with the "test and treat" guideline introduced in South Africa in September, 2016. This will be in terms of virologic and immunologic responses.

1.3 **Rationale for the study**:

HIV remains an important cause of morbidity and mortality in South Africa. In 2013, HIV was the third leading underlying natural cause of death in South Africa (Statistics South Africa, 2015$_b$). Available studies that focused on virologic and immunologic response to ART in South Africa were conducted mostly in KwaZulu Natal and Khayelitsha in the Western Cape. In Limpopo, the closest study on this subject was in Ndlovu Medical Centre, Sekhukhune District. This study found that 63% of patients achieved viral suppression at 3 years after initiation of therapy (Barth et al., 2011). There is no known published study conducted on this subject in Vhembe District.

1.4 **Significance of the Study**:

The expected outcomes of this study may be beneficial to different sectors of society. The study will determine the proportion of patients virally suppressed on HAART, the proportion of patients with low CD4 counts that require prophylactic drugs, those with adequate immunologic response, the proportion of patients on second or third-line HAART as well as the factors contributing to viral suppression. In addition, the findings will contribute to knowledge required by the Department of Health to review the implementation of the ARV policy/guidelines.

The above may also give health care workers in the district a better understanding of patient response to ARVs and how to improve the implementation of the guidelines. It may also provide further questions to which researchers will seek an answer. These may all lead to improved patient care.

1.5 **Purpose and Objectives of the Study**

1.5.1 **Purpose of the Study**:

The purpose of this study is to investigate the virologic and immunologic responses in patients on Highly Active Antiretroviral Therapy in Vhembe District, South Africa.

1.5.2 **Objectives of the Study**:

- To determine the response of the Human Immuno-deficiency Virus, as depicted by viral load, in patients on HAART for at least 6 months
- To determine the response of the immune system, as depicted by CD4 counts, in patients on HAART for at least 6 months.
- To identify the factors associated with viral and immunological response to antiretroviral therapy.

1.6 **Definition of terms**:

- **Virologic response** – This will be defined in terms of viral suppression and viral failure. HIV is said to be suppressed when the viral load is less than 50 copies/ml. Viral failure is defined as VL >1000 copies/ml on 2 occasions after detailed adherence counselling (South Africa National Department of Health, 2012). This study will adopt both definitions.
- **Immunological response** – Immunological response is defined in terms of CD4 count response. There is no consensus regarding the definition for immunologic response (Bartlett et al., 2012). For the purpose of this study, immunological response will be considered to be adequate when there is an increase in CD4 count from baseline of at least 50 cells/µL at 6 months after initiation of therapy.
- **Highly Active Antiretroviral Therapy** (HAART) – The World Health Organisation (2015) states that "the standard antiretroviral therapy consists of a combination of at least three drugs (often called highly active antiretroviral therapy or HAART) that suppress HIV replication". The drugs are usually from at least two different classes. In this study, HAART will be used interchangeably with ART.

CHAPTER TWO

LITERATURE REVIEW

2.1 **Overview of Literature**

This chapter reviews literature of studies carried out in different parts of the world, of how persons infected with HIV respond to antiretroviral therapy at different times after initiation therapy. The focus will be on immunologic and virologic responses as depicted by CD4 count and Viral load (VL) respectively. It will critically examine findings and differences based on gender, age, type of antiretroviral drugs used, immunological and clinical status at the time of initiation. Immuno-virologic discordance is also reviewed. This chapter will be broadly divided into two parts: data-based literature review and conceptual literature review.

2.2 **Data-based Literature Review**

2.2.1 **Highly Active Antiretroviral Therapy (HAART)**

2.2.1.1 **Antiretroviral regimen**

From 2004, the recommended first-line therapy for adults in the public sector in South Africa was Stavudine + Lamivudine + Efavirenz or Nevirapine. Second-line agents consisted of Zidovudine + Didanosine + Lopinavir/ritonavir (National Department of Health, 2004). From 2010, the recommended first-line therapy in South Africa became Tenofovir or Stavudine or Zidovudine + Lamivudine or Emtricitabine + Efavirenz or Nevirapine while the recommended second-line therapy in the public health sector became Tenofovir or Zidovudine + Lamivudine or Emtrictabine + Lopinavir/ritonavir (South African National Department of Health, 2010). Third-line regimen was determined by an expert committee after detailed review and resistance testing. Post-exposure prophylaxis regimen was made up of 3TC + AZT with lopinavir/ritonavir added in high risk cases (South African National Department of Health, 2012). These drugs all have their different mechanisms of action, toxicities and resistance profile.

2.2.1.2 **Triple therapy versus monotherapy and dual therapy**

The cost of antiretrovirals have fallen over the years. Prior to the year 2000, the cost for one person on a first-line therapy per year was about $2,000. This was reduced to $1,200 per person per year, after the launch of the Accelerated Access Initiative (Vella et al., 2012). The cost has continued to

decrease since then. However, with the number of people infected with HIV and eligible for ART, the cost of managing the disease remains very high. This has led to studies comparing the effectiveness of monotherapy or dual therapy; either from the time of initiation or after initial viral suppression; to a HAART regimen. The aim of these are to reduce the cost as well as the side-effects. In most cases protease inhibitors (PI) were the drug of choice due to the fact that PIs have a high genetic barrier to resistance (Paton et al., 2015). The PI most commonly used was ritonavir boosted darunavir (DRV/r) because it is taken once a day. In a study to compare the effectiveness of PI-monotherapy with PI-triple regimen in patients that had achieved viral suppression, Paton et al. (2015) found that "PI-monotherapy is an acceptable alternative for long-term clinical management of HIV infection". PI-monotherapy maintained viral suppression in 58% of patients in 3 – 5 years of the study which was done in the United Kingdom. Valantin et al. (2012) and Aribas et al. (2012) came to similar conclusions at 96 weeks and 144 weeks respectively. Valantin and colleagues (2012) compared DRV/r monotherapy with DRV/r triple therapy for 96 weeks and came to the conclusion that DRV/r monotherapy is "durable and efficacious for maintaining virologic suppression in HIV-1 patients". Dual therapy has also been attempted with the combination of dolutegravir with lamivudine. These researchers however recognise that one drawback of the antiretroviral monotherapy is that it require more frequent monitoring for viral failure. This negatively impacts on the possibility of the strategy to reduce cost.

However, other studies comparing triple therapy to monotherapy and dual therapy came to the conclusion that triple therapy was efficient for a longer period and showed much better virological response (Mathis et al., 2011; Romanelli et al., 2006).

2.2.2 **Virologic response**

2.2.2.1 **HIV response to HAART – Global trends:**

The World Health Organisation recommends that at least 70% of patients on ART be virally suppressed at twelve months after initiation. It further recommends different definitions for viral suppression and viral failure based on resources available to each particular country. For low-to-medium-income countries, it recommends that viral failure and viral suppression be defined as a VL > 5000 copies/ml and VL < 1000 copies/ml respectively. A VL of ≥200 copies/ml and a VL of ≤ 50 copies/ml defines viral failure and viral suppression respectively for high-income-countries (McMahon, et al., 2013). Presently, the South African National Department of Health (2012)

however, recommends the use of VL ≤ 50 copies/ml as definition for viral suppression and 2 VL results >1000 copies/ml to be viral failure.

In the United Kingdom, 82% of patients attained viral suppression at twelve months after initiation of therapy. (McMahon et al., 2013). In sub-Saharan Africa, 76% of people on ART have achieved viral suppression (UNAIDS, 2014b). In South Africa 77% and 74% of patients achieved viral suppression at 12 months and 60 months, respectively, after initiation of therapy (Shisana et al., 2013), while in Kakai, Uganda, 92% attained < 400 copies/ml at 24 weeks after initiation. This figure decreased to 84% at 48 weeks (Billioux et al., 2015). In Rio de Janeiro, Brazil, Cardoso et al. (2014) found that 77%, 76%, and 68% of patients attained a VL of < 400 copies/ml at 6, 12 and 24 months respectively. In another study to determine the incidence of virologic failure in Latin America and the Caribbean, Caeser et al. (2015) found that 7.8%, 12.7% and 18.2% of ART-naïve patients had viral failure at 12, 36 and 60 months after initiation on ART respectively. The researches cited above reveal a trend in which the percentage of patients virally suppressed decreases over time. Questions raised are: Will this trend continue if these patients were to be monitored over a longer period of time? What factors were contributing to this trend?

However, in one study conducted in the private sector in India on patients on ART, Gaikwad et al. (2015) found that 79%, 81% and 87% of patients attained viral suppression at 6, 12 and 60 months, respectively, after initiation of ART. This was the only study found in which the proportion of patients that attained viral suppression consistently rose for the duration of the study.

2.2.2.2 **Viral load and HIV transmission**

Frequent mutations in the HIV genome has made it extremely difficult for scientists to develop a vaccine or cure. This has led to different approaches that aim to end the HIV epidemic. One of these approaches aims at eliminating transmission. This includes a multifaceted approach that involves education, use of condoms and reducing viral load to an undetectable level. Interim results from the ongoing PARTNER study found that overall HIV transmission rate among sero-discordant partners, who had sex without condoms, was zero when they had a viral load of < 200 copies/ml on ART at most, 12 months previously. They stated that there is however uncertainty over the upper limit of risk, especially for receptive anal intercourse (Bruun et al., 2014). The implication of this is that when a patient on ART experiences viral blips, which is defined as an

increase in VL > 50 copies/ml but < 1000 copies/ml and a subsequently returns to < 50 copies/ml; there is a probability of transmission.

Also, some studies conducted to examine the relationship between community viral load and HIV incidence found that reduced community viral load resulted in reduced HIV incidence in that community, "independent of unsafe sexual behaviours and sharing used syringes" (Wood et al., 2009; Montaner et al., 2010).

Furthermore, several factors may influence HIV transmission, irrespective of viral load. Ondoa et al. (2015), found that genital HIV shedding occurred only when the plasma viral load was detectable. They stated that ART only alters VL but not genital cytokines. The secretion of these genital cytokines are promoted by sexually-transmitted infections (STIs), and these cytokines promote HIV replication. Their findings were supported by Champredon et al. (2015), who investigated the effects of STI co-infection on genital viral load among individuals on suppressive ART. They found no significant effect of STI co-infection on genital viral load. These two studies further support the idea that suppressing viral load is an effective way to achieve zero new HIV infections.

However, other studies have found that genital viral shedding occurred even in women and men with sustained suppressed plasma VL, irrespective of STIs (Cu-Uvin et al., 2010; Lambert-Niclot et al., 2012). Therefore, the possibility of HIV transmission still exists even when there is an undetectable plasma viral load.

2.2.2.3 **Factors associated with HIV response to HAART**

It is known that poor adherence to antiretrovirals is associated with failure to suppress HIV. The best indicator of adherence and response to treatment is virologic response. More than 90 – 95% adherence is required to achieve viral suppression (Billioux et al., 2015). Other factors have also been shown to be associated with delay in or failure to attain viral suppression. Cardoso et al. (2014) found that fewer years of formal education, being a woman and younger age group, were associated with greater incidence of viral failure. Their finding, that a younger age was associated with greater incidence of viral failure was supported by Caeser et al. (2015), who also found that infection via injection drug use (versus heterosexual contact) was associated with viral failure. This finding may however be related to the lifestyles of injection drug users, who are usually addicts and homeless and are less likely to be adherent to antiretroviral therapy.

Another factor associated with higher incidence of viral failure is a high viral load at initiation. A high CD4 count and better clinical stage at initiation is however associated with earlier viral suppression (Billioux et al., 2015). This study supports the World Health Organisation's HIV treatment guideline released in September 2015, which advocates for a "diagnose-and-immediate treatment" approach.

2.2.3 **CD4 cell count**

2.2.3.1 **CD4 cell count – general principles**

This is the standard test to assess the prognosis for progression to AIDS or death, to formulate the differential diagnosis in a symptomatic patient and to make therapeutic decisions regarding antiviral treatment and prophylaxis for opportunistic infections. It is the most reliable predictor of prognosis. Normal values range from 500 to 1400 cells/mm^3 (Bartlett et al., 2012). With HIV infection, there is progressive depletion of CD4 cells.

There is however variability in CD4 test results when measured. Bartlett et al. (2012) state that the 95% confidence interval range for true count of 200 cells/mm^3, for example, is 118 – 337 cells/mm^3. It suggests that results that are inconsistent with prior trends should be repeated. It then lists the factors that may contribute to this variation. These include analytical variation, seasonal variations, intercurrent illness and corticosteroids. Diurnal changes also occur with the lowest values at 12h30 and peak values at 20h30.

2.2.3.2 **CD4 cell response to HAART**

The CD4 response to ART is also highly variable. It increases for most patients, more for some than for others. It may never attain normal values in some, while others attain normal values after some years on ART. Meintjes et al. (2014) state that CD4 count increases rapidly in the first month of ART by 75 – 100 cells/µL, with a more gradual increase, thereafter, of 50 – 100 cells/µL per year. Studies have been conducted to monitor the CD4 response to ART. Cardoso et al. (2014) observed a median increase of 107 cells/µL, 151 cells/µL and 242 cells/µL at 6, 12 and 24 months, respectively, after initiation of ART in a study to review the effectiveness of first-line ART in Brazil. Engsig et al. (2014) found that 15% of the patients did not achieve a CD4 count of > 200 cells/µL after three years of viral suppression. Luz et al. (2015) found a sustained increase in median CD4 count from baseline throughout the 5 years of the study. The median CD4 cell count

increased from 154 cells/µL to 259 cells/µL at 6 months to 413 cells/µL at 5 years. The increase was steeper in the first 2 years. This has been supported by other similar research and therefore demonstrates that there will be sustained CD4 count increase among patients remaining on therapy, even among patients who do not have a steep CD4 response. No studies were found that showed zero CD4 increase or even a decrease. Some showed a poor response. However, all the studies reviewed demonstrated a sustained CD4 count increase in patients on ART.

2.2.4 **Immuno-virologic discordance**

The therapeutic goal of antiretrovirals is sustained immune recovery, in the form of CD4 count increase, and viral suppression. Most patients achieve both but some fail to attain immune recovery despite sustained viral suppression. Immuno-virologic discordance has been used to describe this failure to have adequate CD4 count increase in the presence of viral suppression. As stated earlier, CD4 count best predicts clinical progression. It is also used to determine when to initiate prophylaxis for some opportunistic infections (Bartlett et al., 2012). The question therefore arises if there is any clinical implication for this discordant response. Some researchers have even gone a step further, attempting to determine which factors will predict the occurrence of a discordant immuno-virological response.

Different studies have estimated the prevalence of immune-virologic discordance (ID) to be between 8 – 42% (Cassotti et al., 2011). The measured prevalence depends on the value of CD4 count increase from baseline considered to be adequate response. There is however no generally acceptable definition for adequate immune response. Most studies reviewed use a CD4 increase of > 50cells/µL from baseline at 6 months post-ART initiation. Cassotti et al. (2011) had a measured prevalence of 9% using an adequate CD4 count response, defined as ≥ 350 cells/µL after 12 months of ART. Anude et al. (2013) and Muzah et al. (2012) both defined adequate CD4 response as an increase of ≥ 50 cells/µL at 6 months of ART and had measured prevalence of 33% and 24% respectively.

Zoufaly et al. (2011) showed that patients with ID had an increased risk of developing AIDS. This risk was greatest in the first 6 months but decreased by 65% afterwards. The amount of this decrease increased with the duration of time virally suppressed. It may be critically important to maintain viral suppression in these patients. Therefore, conditions such as tuberculosis, as well as immunisations such as influenza or pneumococcal vaccine; HIV superinfection, which have been

shown to increase viral load, should be avoided (Bartlett et al., 2012). In 2014, Zoufaly et al. further investigated the relationship between non-AIDS and AIDS event patients with ID. They found that compared with CD4 responders, patients with ID may be at increased risk of developing non-AIDS conditions. Takuva et al. (2014), concluded that "patients on ART with poor recovery early in treatment are at greater risk of progression to new AIDS diagnosis or death despite viral suppression". Engsig et al. (2014) found that patients with CD4 count ≤ 200 cells/μL after three years of viral suppression had substantially increased mortality, compared to those who achieved CD4 count > 200cells/μL. These researches suggest that discordance between immune and viral responses may result in morbidity and mortality.

Considering the above, being able to predict the patients that are at risk of developing ID, is important. Muzah et al. (2012) found that CD4 count > 200 cells/μL at baseline, moderate anemia, older patients and patients on zidovudine, had an increased risk of ID. Anude et al. (2013) concurred with Muzah et al. (2012), but also showed that poor adherence and male gender were contributory factors. In a study in southern Ethiopia, investigating the trends of immune and virological responses to HAART, Hirigo et al. (2015) found that weight < 50kg and living in rural areas were important predictors of developing ID.

2.3 **Conceptual-based literature review**

This aspect of the literature review will attempt to describe the framework or theory that underlies this study. "A conceptual framework represents the researcher's synthesis of literature on how to explain a phenomenon. It maps out the actions required in the course of the study given his previous knowledge of other researchers' point of view and his observations on the subject of research" (Regoniel, 2015).

2.3.1 **Cellular kinetic theory**

This theory will be used to explain the immune system responses to HIV infection. The theory itself describes how cells multiply and differentiate in response to internal and external stimuli. After HIV transmission, the virus enters cells by binding to CD4 receptors and co-receptors. After entry, it multiplies with the help of its enzymes, destroying the host CD4 cell. This process continues, leading to a reduction in CD4 cell count and increasing viral load. This depletes the effectiveness of cell-mediated immunity, exposing the human body to opportunistic infections. The enzymes and co-receptors are targets for HAART, and initiation on HAART leads to reduction

in viral load and immune recovery in the form of CD4 cells rebound (Bartlett et al., 2012). Several independent variables may influence the responses to HIV infection and HAART. Some of these include adherence to HAART, which may also be influenced by socio-demographical factors such as age, gender, marital status and educational status. Clinical variables such as baseline CD4 count, viral load, haemoglobin and clinical stage, may also influence the viral and immunological responses to HAART.

CHAPTER THREE

METHODOLOGY

3.1 **Study design**

The study adopted a retrospective design. It reviewed the secondary medical records of individuals who had been receiving HAART between 1st January 2004 and 31st July 2016. No direct contact with any participant occurred.

3.2 **Area of study**

Vhembe District is one of the five districts of Limpopo Province. It is the northern-most district of the province and covers an area of 25,597km^2. It is predominantly rural and its district municipal office is located in Thohoyandou. It is bordered to the northwest by Zimbabwe and Botswana, to the southeast by Mozambique, Mopani to the south, Capricorn to the southwest and Waterberg District to the west. It has four local municipalities including Musina, Mutale, Makhado and Thulamela (Main, O. 2015). According to the 2011 Census, Vhembe District has a population of 1,294,722 people, 65.2% of whom are 15 years and above. It has an annual population growth of 0.78%. 54.4% of the population is female and 45.6% are males. Over ninety-eight percent of the population is black, with 1.1% and 0.4% white and Indian groups, respectively (Main, 2015). As at 2011, it had a medical scheme coverage of 7.2%. Most of its population therefore depends on the public health sector for health care (Massyn et al., 2014). By 2013, the district had 131 public health care facilities which provided both diagnostic and therapeutic antiretroviral care (Vhembe District Department of Health unpublished data, 2015).

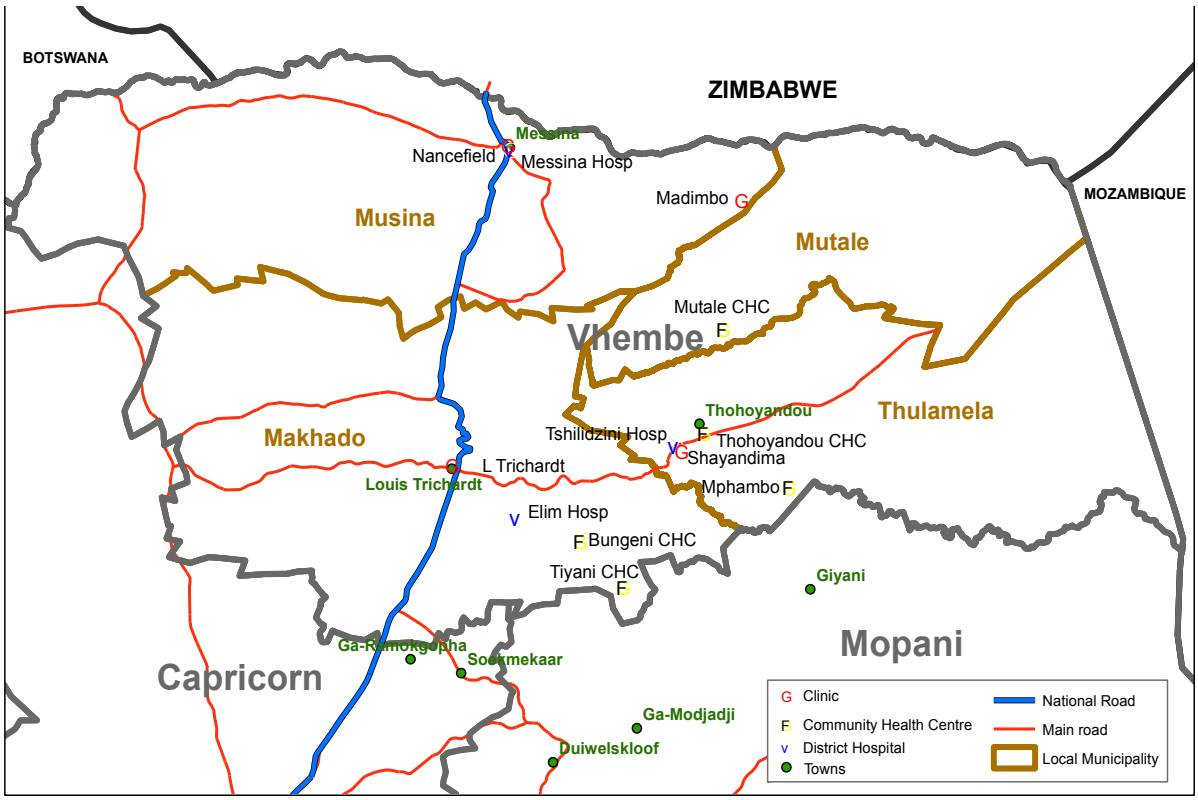

Figure 1: Map of Vhembe District showing its local municipalities and some health-care facilities. (Courtesy: Audrey Mbatha, Statistics South Africa, 2015).

3.3 **Study population and sampling**

3.3.1 **Study population**

The study population included all patients in Vhembe District initiated on HAART before 31st July 2016. By the end of December 2014, there were 44,450 persons remaining on ART in Vhembe District. (Unpublished data Vhembe District Department of Health, 2015).

3.3.2 **Target population**

Among the study population, only those that met the eligibility criteria were included in the sample. The eligibility criteria included the following;

- Must have been receiving HAART for at least 6 months.

- Must have at least one result of CD4 count or viral load test results taken at the South African National Department of Health recommended intervals post initiation, with a range of ± 3 month before 12 months and ± 5 months after 12 months post-initiation for each of the test.

3.3.3 **Sample size**

The records of 1247 individuals were reviewed from stored electronic records and paper folders in Thohoyandou Community Health Centre in Vhembe District. Due to the limited time, only this health facility was chosen randomly and all the patients that met the eligibility criteria in the facility was included in the study.

3.4 **The data collection instrument**

A data collection form was designed to collate the data. The data collection form was divided into four parts describing the demographics, and to achieve each specific objective. These include:

Section A - Demography

Section B – Virologic response to HAART

Section C – Immunologic response to HAART

Section D – Factors associated with viologic and immunologic responses to HAART.

These factors were chosen because they can assist in forming target groups for which interventions can be made, and they could be easily retrieved from tier.net.

3.5 **The validity and reliability of the instrument**

3.5.1 **Validity of instrument**

3.5.1.1 **Face validity**

The researcher ensured that the instrument is able to achieve its objectives precisely by conducting a review of a sample of the data collection form by non-experts in the field of HIV. Corrections were made based on their subjective review.

3.5.1.2 **Content Validity**

This was ensured by carrying out a detailed literature review and also by reviewing the instrument with an expert in the field of HIV research. Corrections were made based on the literature review and expert advice.

3.5.2 **Reliability of the instrument**

This ensures that the same result will be obtained if the instrument is tested on the same sample again. The reliability of the data collection form was measured using interrater reliability method of reliability testing. The medical records of forty patients were collected on day 1 from available electronic medical records by the researcher, and the medical records of the same sample of patients were retrieved again by a health record worker after a 7 day interval. The Cohen ƙ coefficient was calculated and found to be 0.79, and a range of 0.61 to 1.0 was within the acceptable limit (Liddy et al., 2011).

3.6 **Method of data collection**

The data used for this study was obtained from secondary data in electronic registers (tier.net) and paper folders at Thohoyandou Community Health Centre. At the health care facility, the medical records of patients on HAART that met the eligibility criteria were collated by filling the data collection form from information abstracted from the electronic registers and paper folders. The CD4 count, VL results and socio-demographic and clinical characteristics of patients were retrieved.

3.7 Data analysis

The data collected was analysed using Microsoft Excel 2010 and SPSS software version 24.0. The data was first coded and fed in Microsoft Excel and exported to the SPSS programme to generate descriptive and inferential statistics. During data cleaning, duplicate entries were identified and removed. The records of 1912 individuals were reviewed and 1247 met the eligibility criteria. The dataset for socio-demographic variables and clinical variables were summarised. Frequencies and proportions were calculated excluding individuals with missing variables at fixed points when calculating proportions. The proportions calculated include the proportion of virally suppressed, the proportion with sustained viral suppression, those with adequate immunologic response and those with immuno-virologic discordance.

To model for factors associated with virologic and immunologic responses, each independent variable was tested for association with the dependent variable (viral suppression and CD4 count increase of ≥ 50cells/μL from baseline at 6 months) taking one at a time. The independent variables included age, year of initiation, gender, marital status, baseline BMI, haemoglobin, clinical stage and estimated creatinine clearance. The Pearson Chi square (X^2) was used for all categorical independent variables and the T-test, for all continuous independent variables, to test for association. The estimates used was a 95% confidence interval, and a p-value of < 0.05 was be considered significant. Categorical variables, such as gender, are presented using frequency tables and bar charts. Continuous data such as CD4 count, are described numerically with the mean and standard deviation and presented graphically and using tables.

3.8 **Ethical considerations**

3.8.1 **Permissions to conduct study and ethical clearance**

The proposal was presented to the Department of Public Health and the Higher Degree Committee of the School of Health Sciences. Corrections were made after the presentations. An application was then be made to the University of Venda Research and Ethics Committee for Ethical Clearance and this was granted. Approval was also obtained from the Department of Health in Polokwane and Vhembe District to conduct the study. These were then submitted to the head of the Thohoyandou Community Health Centre for final approval to have access to the required data.

3.8.2 **Anonymity**

In order to protect patient anonymity, only de-identified data was used. The data collection form had no provision for names of individuals whose data was used for the study. Clinic folder numbers were used to enable the researcher match their electronic data with their paper folders.

3.8.3 **Confidentiality**

All identifiable data the researcher came across was kept confidential. He will under no circumstance provide such data to a third party. The researcher will also ensure that the internet security of laptops used will be up-to-date to prevent data theft.

3.8.4 **Justice**

This principle was observed by ensuring no group of individuals were excluded based on their sexual orientation, religious, racial or social background.

3.8.5 **Beneficence**

The best interest of all individuals whose data was used for the study was always assured as all decisions involving the data always considered this. The data was not exploited for any other reason apart from this study.

3.9 **Scope and Limitations of study**

The study reviewed the viral load and CD4 count response of all patients on HAART before the 31st July 2016 in Vhembe District. It was conducted using only one health care facility. Using more facilities would have required obtaining permission at each facility in order to have access to electronic data and data on paper folders. Obtaining these could have taken a few weeks to several months, and financial and time resources for the study was limited.

Data stored both in electronic and paper folders were incomplete. These missing data could have the potential of altering the outcomes of the study. Also, although multivariate logistic regression analysis was planned, multiple missing data precluded its use, as it would have affected the reliability of the study.

Some of these patients recorded as lost to follow-up may have actually died. This could also influence some outcomes and there was no data on adherence, which is an important confounding variable that could also influence the outcomes of the study.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter deals with the presentation of the findings of the study. It will be divided in four sections. These will include; demography, viral response, immunologic response and factors associated with these responses.

The medical records of 1912 patients were reviewed, of which 1247 met the inclusion criteria. Both electronic (tier.net) and paper records were reviewed. Data such as marital status, baseline body mass index, haemoglobin and creatinine clearance, was not available on tier.net and as a result was retrieved from paper records. All individuals did not have all required results as prescribed by national guidelines. For instance, some had viral loads at 24 months after initiation but did not have results at 36 months or even 48 months, with their next results being at 60 months. This created gaps. The proportions were calculated based on those that had results at the fixed point in time, excluding those that did not have results for that particular time.

4.2 Demography

One hundred and ninety-one individuals in the cohort were initiated in 2013. This corresponded to 15.4%. A further 184 (14.8%) were initiated in 2014 and 183 (14.7%) in 2015. Only 0.3% of the cohort were initiated in 2004. This is depicted in the Figure 2.

Of the 1247 individuals that met the inclusion criteria, 1237 had their age recorded. Of these, 940 (75.3%) were female. The marital status of 528 patients was not recorded. Of the 719 recorded, 362 (50%) were single, 326 (45%) were married, 27 (4%) were widowed and 4 (1%) were divorced. Previous exposure to antiretroviral therapy could be due to post-exposure prophylaxis, PMTCT or HAART. Cumulatively, only 1% had any form of previous exposure to antiretroviral therapy before commencing lifelong HAART. At baseline, 53% were on stage I, 14% were on stage II, 8% were on stage III and 2% were on stage IV. The baseline clinical stage of 283 (23%) patients was not known. Ninety-nine percent (1232) of the patients did not have their BMIs recorded. Most individuals were initiated at a haemoglobin level between 10 to 13g/dl, while 25% (89) were initiated at a haemoglobin of greater than 13g/dl.

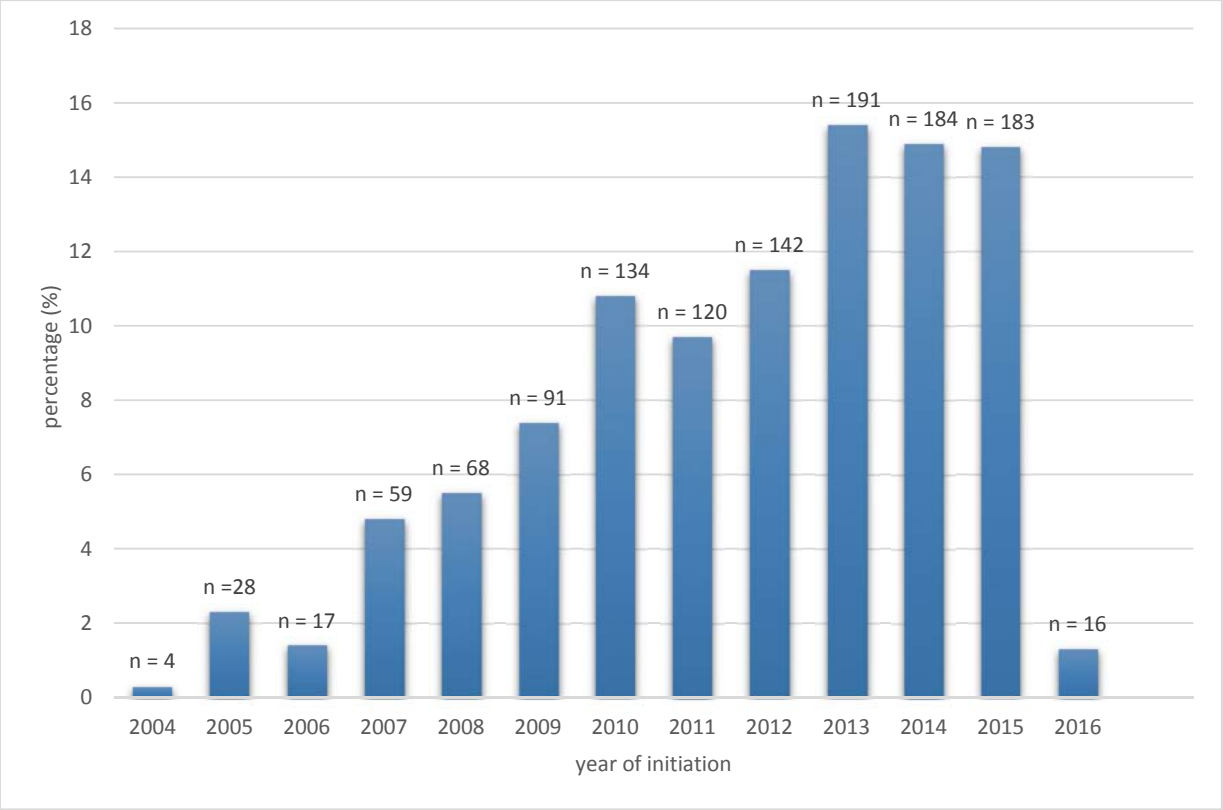

Figure 2: Year initiated on HAART

The renal function in this case is as depicted by the estimated glomerular filtration rate (GFR). This is calculated from the creatinine level using Cockcroft-Gault equation. These findings show that 59% (257) had a normal or stage 1 chronic kidney disease GFR of ≥ 90ml/min/1.73m^2. This also showed that 137 patients (31%) had a GFR that placed them in stage 2 chronic kidney disease (60 - 89 ml/min/1.73m^2). Eight hundred and eleven patients did not have their GFRs recorded. At the end of the study, 137 (11%) patients had been transferred to other health care facilities, 22 (2%) had died while on treatment and 128 (10%) had been lost to follow-up. This left 948 (77%) retained on treatment at the facility. Further review showed that 2.5%, 10.4%, 13.6% and 73.5% demised, were lost to follow-up, transferred out and retained in care in 2010 and before, respectively, while 1.4%, 10.4%, 10.8% and 77.6% demised, were transferred out, lost to follow-up and retained in care after 2010 respectively. There was no significant difference ($p < 0.05$) in the percentage retained in care or demised when the values were compared after 2010, and 2010 and before.

Table 1: Socio-demographic and clinical characteristics of cohort

Characteristics	Total	%	N
Gender:			1247
• **Male**	307	24.6	
• **Female**	940	75.4	
Age (years):			1237
• **< 15**	67	5.4	
• **16 – 29**	308	24.9	
• **30 – 44**	582	47	
• **45 – 60**	237	19.2	
• **> 60**	43	3.5	
Marital status:			719
• **Married**	326	45.3	
• **Single**	362	50.3	
• **Divorced**	4	0.6	
• **Widowed**	27	3.8	
ART exposure:			1236
• **PMTCT**	8	0.6	
• **PEP**	2	0.2	
• **HAART**	1	0.1	
• **None**	1225	99.1	
Baseline clinical stage:			1236
• **I**	650	52.6	
• **II**	176	14.2	
• **III**	104	8.4	
• **IV**	23	1.9	
• **Unknown**	283	22.9	
Baseline BMI:			15
• **< 18.5**	2	13.3	
• **18.5 – 24.9**	5	33.3	
• **25 – 29.9**	4	26.7	
• **≥ 30**	4	26.7	
Baseline haemoglobin (g/dl):			356
• **< 6**	4	1.1	
• **6 – 9.9**	59	16.6	
• **10 – 13**	204	57.3	
• **> 13**	89	25	
Baseline eGFR (ml/min/1.73m^2):			436
• **16 – 29**	1	0.2	
• **30 – 59**	41	9.4	
• **60 – 89**	137	31.4	
• **≥ 90**	257	58.9	

Patient outcomes:			1235
• **Transfer out**	137	11.1	
• **Still on treatment**	948	76.8	
• **Demised**	22	1.8	
• **Lost to follow-up**	128	10.4	

When the HAART regimen was reviewed, no patient was on third-line regimen throughout the period under study. Three (0.2%) out of 1288 patients were on second-line at baseline. This was still three (1.2%) out of 259 at 72 months. The maximum number of individuals on second-line ART at any point throughout the study was four. There was one incidence of change from first-line to second-line and back to first-line.

Table 2: Proportion on HAART regimen

Duration on HAART	First-line (%)	Number of individuals	Second-line	Number of individuals	Third-line
Baseline	99.8	1225	0.2	3	0
6 months	99.8	1211	0.2	3	0
12 months	99.6	1056	0.4	4	0
24 months	99.6	793	0.4	3	0
36 months	99.5	613	0.5	3	0
48 months	99.6	473	0.4	2	0
60 months	99.5	366	0.5	2	0
72 months	98.8	256	1.2	3	0
84 months	98.9	177	1.1	2	0
96 months	98.0	98	2	2	0
108 months	96.4	54	3.6	2	0
120 months	100	25	0	0	0
132 months	100	18	0	0	0

4.3 Virologic responses to Highly Active Antiretroviral Therapy

4.3.1 Viral suppression:

As stated earlier, not all patients had their results taken at the specific intervals required for the study. Six months after starting HAART, 64% were virally suppressed (n = 648). At 12 months, 70% (n = 577) were suppressed. 70% were also suppressed at 24 months (n = 453) and 69% at 36 months (n = 392). The proportion virally suppressed almost consistently increased with duration on HAART. The total number of patients remaining on HAART however decreased with time. This may have contributed to the almost consistent increase in the proportion virally suppressed. At 132 months, 94% (n = 16) were suppressed.

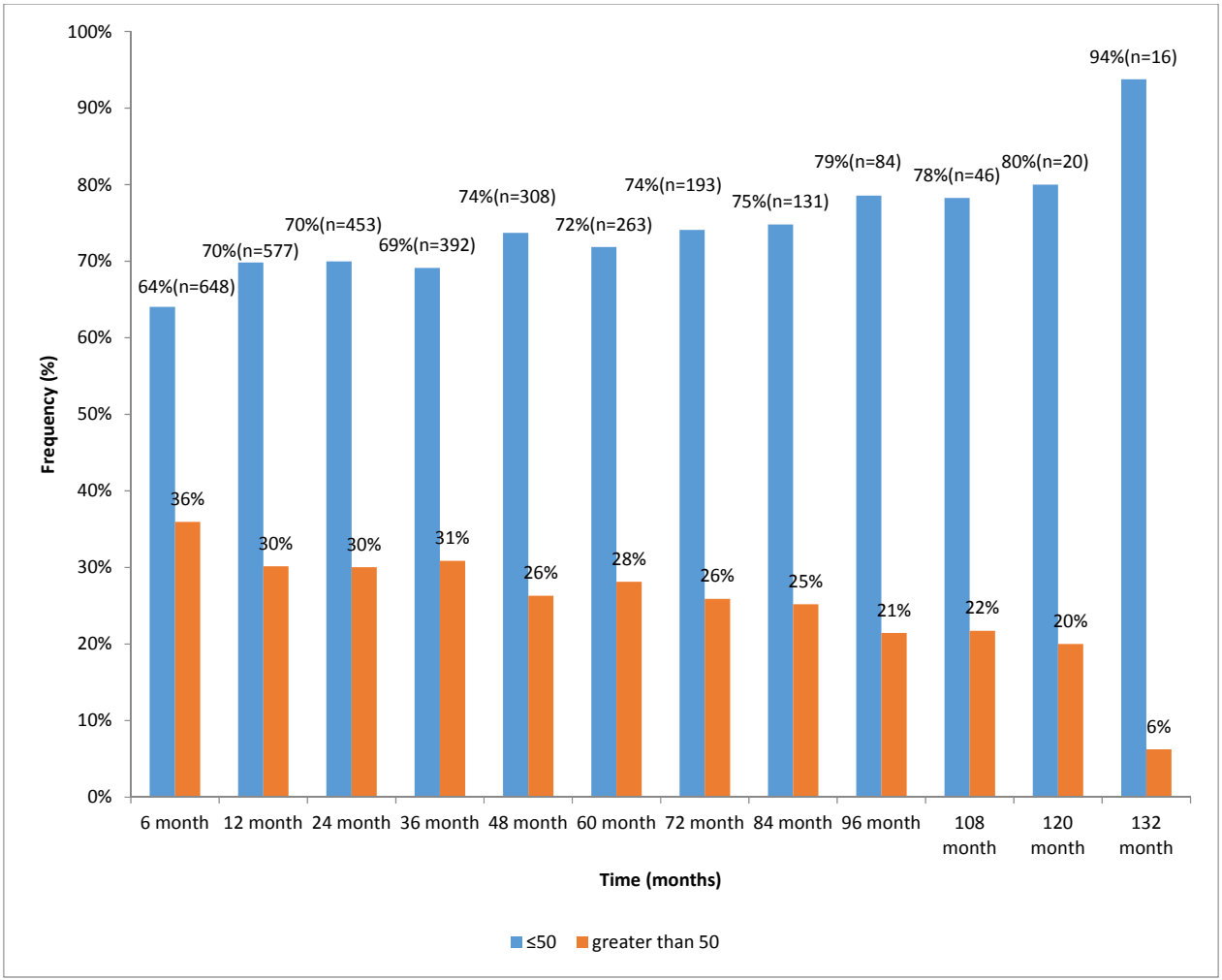

Figure 3: Proportion virally suppressed < 50 copies/ml at fixed intervals

Figure 4 depicts the values when the definition of viral suppression becomes less than 400 copies/ml instead of less than 50 copies/ml.

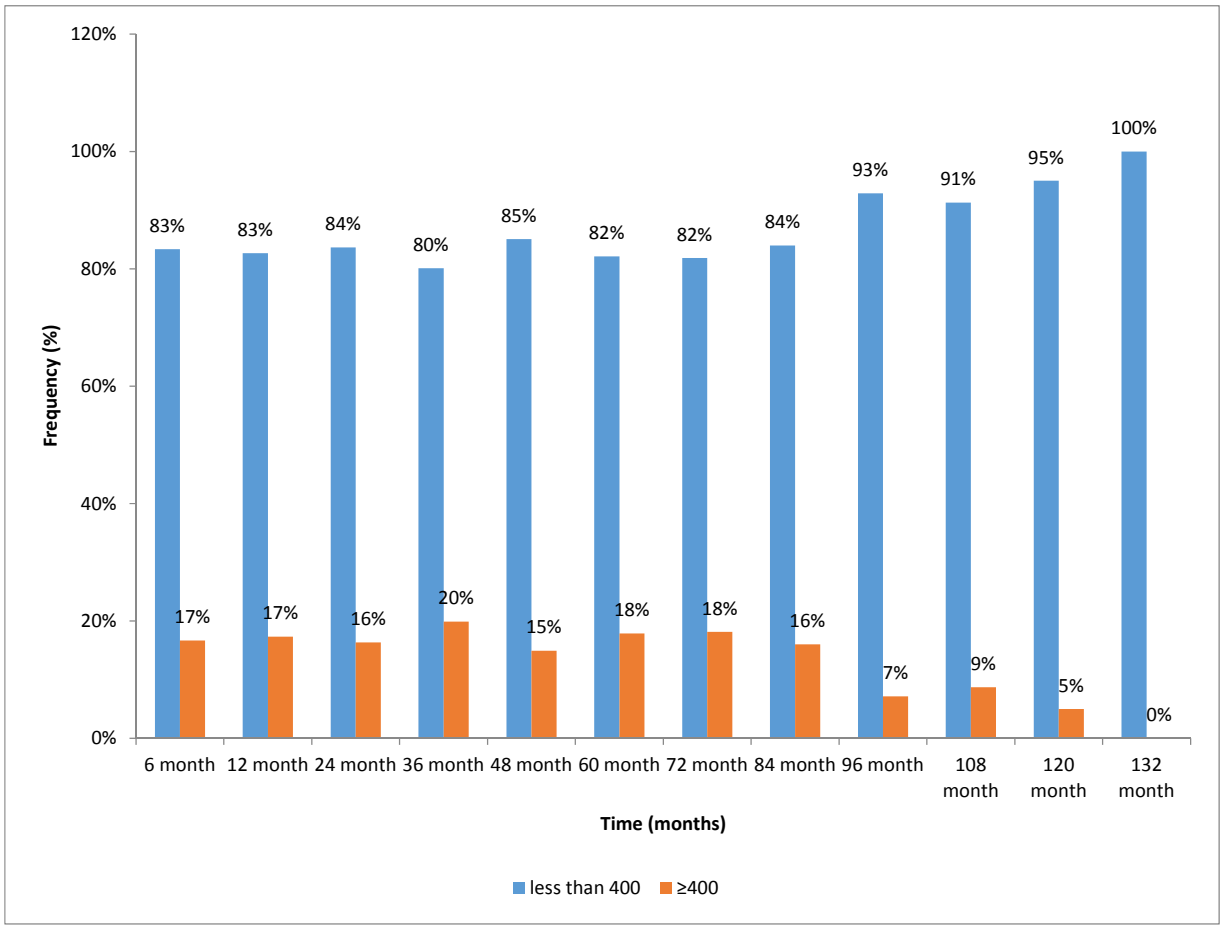

Figure 4: Proportion virally suppressed (<400 copies/ml) at fixed intervals

4.3.2 Sustainability of viral suppression:

This section reviews how consistently patients were able to maintain viral suppression. Those with consecutive virally suppressed results are reviewed. The proportion with at least one consecutive virally suppressed result was 59% (N = 882). This is shown in the table below.

Table 3: Proportion with sustained viral suppression

Number of consecutive virally suppressed result	Percentage	N	Minimum duration consistently suppressed
One	59%	882	6 months
Two	38%	547	18 months
Three	32%	334	30 months
Four	26%	148	42 months
Five	14%	22	54 months

4.3.3 Incidence of viral load blips:

As stated earlier, viral blip is defined as an increase in viral load from less than 50 copies/ml to a maximum of 1000 and a subsequent return to less than 50 copies/ml (Bartlett et al., 2012). However for this study, the proportion of those with results between 50 and 1000 copies/ml at specific intervals are calculated. At 12 months, this percentage was 18.5%. The range for this proportion varied between 6.3%, at 132 months to 18.5% at 12 months. At most times, the proportion was between 17% and 19%. This will be depicted in Figure 4.5.

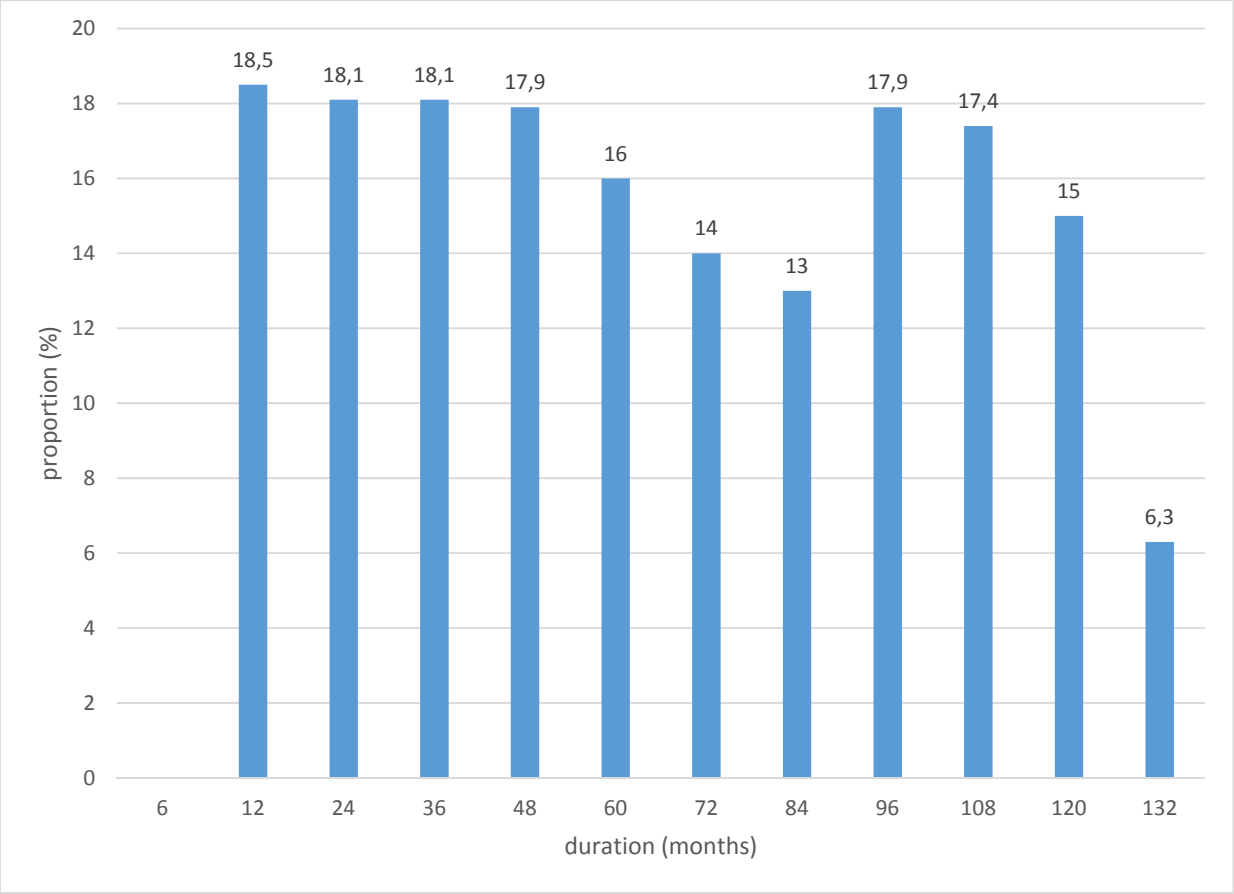

Figure 5: Prevalence of viral blips

4.3.4 Delay in regimen change:

The South African national guidelines recommend a regimen change when there are two viral load results greater than 1000 copies/ml at least 12 weeks apart. This will define virologic failure. Delay in regimen change could lead to accumulation of resistance to NRTIs, in particular. There were however some patients that met this criteria but were kept on the same failing regimen for much longer. Twenty-eight (2.3%) out of 1230 had 2 or more viral loads > 1000copies/ml at least 6 months apart and still remained on the failing regimen.

Table 4: The proportion with delayed regimen change

Have ≥ 2 Viral loads recorded	**Number of individuals**	**Percentage (%)**
Have ≥ 2 consecutive viral loads >1000 copies/ml	28	2.3
Do not have ≥ 2 consecutive viral loads >1000 copies/ml	1202	97.7
Total	1230	100.0

4.4 Immunologic responses to Highly Active Antiretroviral therapy

The immunological responses is reviewed from three aspects. These include the mean CD4 counts at specific times, the mean CD4 count increases from baseline and the immunologic discordance at 6 months from initiation.

4.4.1 Mean CD4 cell counts:

This aspect reviews actual CD4 counts at specific times from baseline to after initiation on HAART. The mean CD4 count at baseline was 227 cells/µL. This increased to 372 cells/µL at 6 months. The increases was consistent until at 60 months after initiation, at which point it was 538 cells/µL. At 120 months, the mean CD4 count was 487 cells/µL. No individual had CD4 cell count result recorded at 132 months. This is depicted in Figure 6 below.

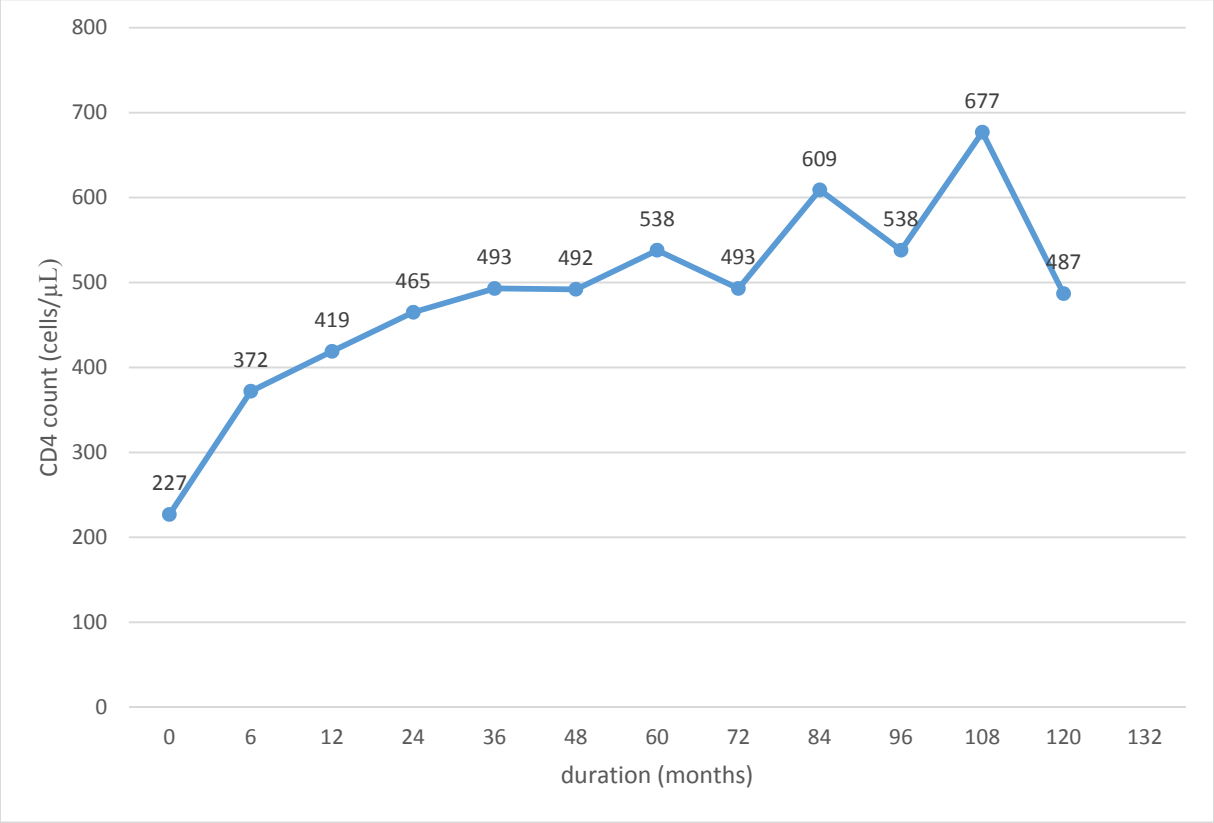

Figure 6: The mean CD4 count at fixed intervals

4.4.2 Mean CD4 cell count increases from baseline:

For a patient to be able to have an increase from baseline calculated, the patient has to have the baseline CD4 count recorded. The mean increase at 6 months was 190 cells/µL, 372 cells/µL at 60 months and 520 cells/µL at 120 months. There was no significant difference (p = 0.952) in achieving CD4 count of ≥ 500 cells/µL at 60 months when individuals with a baseline CD4 count of ≤ 200 cells/µL were compared with those with a baseline CD4 count > 200 cells/µL.

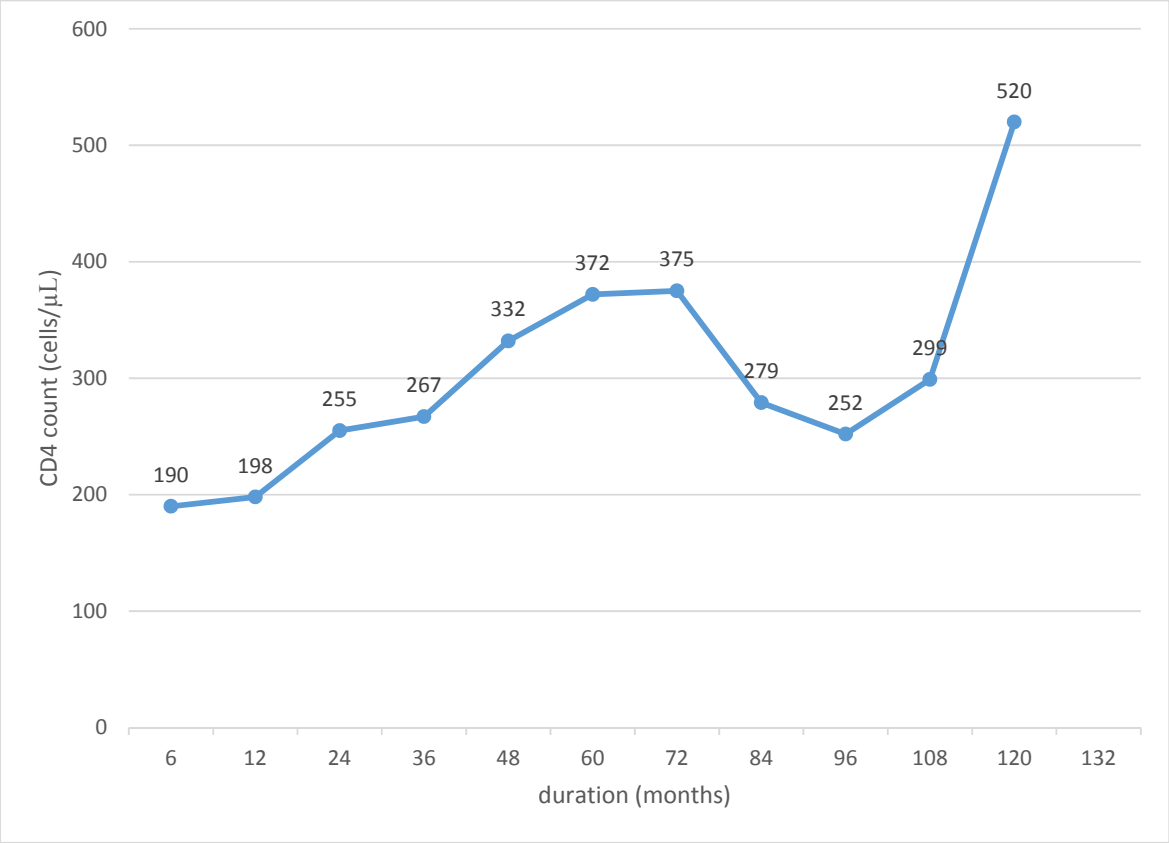

Figure 7: The mean CD4 count increases from baseline

4.4.3 Immuno-virologic discordance at 6 months from baseline:

For this study, immune-virologic discordance is defined as inadequate CD4 count increase (an increase of less than 50 cells/µL) despite adequate viral suppression (viral load of less than 50 copies/ml) at 6 months after initiation on HAART. The calculated immune-virologic discordance for this cohort was 27% (n = 415). Therefore, out of 415 patients with viral suppression at 6 months, 111 of them had inadequate CD4 count response at 6 months.

Table 5: **Immuno-virologic discordance at 6 months after initiation**

CD4 count increase at 6 months	**Number of individuals**	**Percentage**
> 50 cells/µL	304	73.25%
< 50 cells/µL	111	26.75%
Total	415	100.00%

4.5 Factors associated with viral suppression

Each of the independent variable was tested for association with the dependent variable (viral suppression at 6 months). The independent variables tested include age, marital status, gender, previous antiretroviral exposure, baseline clinical stage, haemoglobin, body mass index and creatinine clearance. The chi-square (X^2) test (for all categorical independent variables) and t-test (for all continuous variable), tests of association, were used. The $p < 0.05$ was considered significant. This is shown in Table 6 Analysis could not be done using previous ART exposure and BMI due to the small size of individuals having these data recorded.

Table 6: Factors associated with viral suppression at 6 months

Variable	Value	df	p-value
Age	2.334[a]	4	0.675
Marital Status	2.182[a]	3	0.535
Gender	8.420[a]	1	0.004*
Baseline clinical stage	5.643[a]	4	0.227
Baseline Haemoglobin	2.460[a]	3	0.483
Baseline creatinine clearance	1.430[a]	3	0.698
Baseline CD4 count	10.571[b]	1	0.021*
Duration on HAART (months)	1.041[b]	1	0.783

[a]Chi-square value; [b]T-test;*statistically significant

From the table above, only baseline CD4 count and gender were significantly associated with viral suppression at 6 months. Further analysis revealed that for each additional unit increase in CD4 count, the likelihood for viral suppression increased by 0.2%. Also, women were significantly (p

= 0.031) more likely to be virally suppressed at 6 months. Age and baseline clinical factors were not significantly associated.

However, at 60 months, age and baseline clinical stage were significantly associated with viral suppression. Further analysis revealed that the extreme age groups (0 – 15 years and > 60 years) were less likely to achieve viral suppression. Also clinical stage IV were less likely to achieve viral suppression when compared with the other clinical stages. Table 7 depicts this below.

Table 7: Factors associated with viral suppression at 60 months

Variable	Value	Df	p-value
Age	12.955[a]	4	0.011*
Marital Status	1.065[a]	3	0.786
Gender	0.954[a]	1	0.329
Baseline clinical stage	20.070[a]	4	0.000*
Baseline Haemoglobin	0.695[a]	3	0.874
Baseline creatinine clearance	0.722[a]	3	0.868
Baseline CD4 count	1.438[b]	1	0.110
Duration on HAART (months)	0.961[b]	1	0.992

[a]Chi-square value; [b]T-test; *statistically significant

To assess for factors associated with consistent viral suppression, the independent variables were further tested for association with individuals with at least one consecutive virally suppressed result. Age, gender and baseline clinical stage were significantly associated with this. This is depicted in Table 8. Further analysis revealed extreme age groups (0 -15 years and > 60 years), male and baseline clinical stage IV were less likely to have at least one consecutive viral suppression. Other independent variables showed no significant association.

Table 8: Factors associated with having at least one consecutive virally suppressed result

Variable	Value	Df	p-value
Age	10.606[a]	4	0.031*
Marital Status	0.468[a]	3	0.926
Gender	4.410[a]	1	0.036*
Baseline clinical stage	11.742[a]	4	0.019*
Baseline Haemoglobin	0.968[a]	3	0.809
Baseline creatinine clearance	2.573[a]	3	0.462
Baseline CD4 count	0.631[b]	1	0.117
Duration on HAART (months)	0.868[b]	1	0.662

[a]Chi-square value; [b]T-test; *statistically significant

4.6 Factors associated with Immunological responses

Each of the independent variables was tested for association with the dependent variable (CD4 increase > 50 at 6 months). The Pearson Chi-square (X^2) test (for all categorical independent variables) and the t-test (for all continuous independent variables) was used. The $p < 0.05$ was considered significant. Factors associated with adequate CD4 response (>50cells/µL at 6 months) were age, gender and baseline creatinine clearance. Further analysis revealed that age groups < 45 years was significantly ($p = 0.011$) more likely to achieve adequate CD4 count increase, compared to those aged 45 years and older. Also females had a significantly ($p = 0.043$) higher chance of attaining adequate CD4 count response at 6 months.

Table 9: Factors associated with adequate immunologic response

Variable	Value	df	p-value
Age	22.330[a]	4	0.000*
Marital Status	3.181[a]	2	0.204
Gender	12.253[a]	1	0.000*
Baseline clinical stage	3.525[a]	4	0.474
Baseline Haemoglobin	3.241[a]	2	0.198
Baseline creatinine clearance	9.418[a]	3	0.024*
Baseline CD4 count	2.160[b]	1	0.092
Duration on HAART (months)	3.075[b]	1	0.101

[a]Chi-square value; [b]T-test; *statistically significant

CHAPTER FIVE

DISCUSSION OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter discusses findings of the study. This is structured based on the specific objectives of the study. The conclusions and recommendations are also presented in this chapter.

5.2 Discussions

5.2.1 Demography

The study found that most (78.4%) patients were initiated on HAART after 2010. This is probably because of changes in the South African National Department of Health (NDoH) guidelines that have occurred since 2010 which increased uptake into the antiretroviral therapy program. The 2010 NDoH guidelines recommended starting patients on HAART when they had a CD4 count of ≤ 350 cells/μL if they were pregnant or had HIV/TB co-infection (NDoH, 2010). With a HIV/TB co-infection incidence of 270,000 per annum in South Africa, many persons living with HIV qualified to be initiated on lifelong HAART (WHO, 2014). The previous NDoH guideline only allowed for those with a CD4 count of < 200 cells/μL and those with clinical stage IV HIV to be initiated on HAART (NDoH, 2004). Other possible contributors to this increase post-2010, were further changes in eligibility criteria for HAART through new NDoH guidelines, introduction of Nurse Initiated and Maintenance of Antiretroviral Therapy (NIMART) in 2010 and the fact that patients were initiated in any clinic closest to their homes, compared to the previous practice of initiating patients on HAART in hospitals only (Cameron et al, 2012).

Also, more females were initiated on HAART than males and most people in the cohort were between the ages of 16 and 44 years. Johnson (2012) found that women appeared to have more access to HAART compared to men and children. This is because pregnancy and breastfeeding are used as entry points for women by testing for HIV and initiation on lifelong antiretroviral therapy during this period. No such entry point exists for men and most children at present. However, some entry points have been suggested for males. One of these is any visit by males to health care facilities annually and during antenatal visits of their female partners (Ham et al., 2016). Some have suggested testing of males during circumcision. De Allegri et al. (2015), however, state that "findings suggested that using antenatal care and curative services as the exclusive entry points

into HIV testing may not be sufficient to reach large portions of the male population". Other strategies will therefore need to be developed to reach males and children. The fact that there are more women on HAART than men may be due to the fact that more women are infected with HIV. WHO (2016) however recognises that men account for only 30% of those that have tested for HIV. The WHO further recommends and supports the free distribution of HIV test kits. This is an innovative way of achieving the UNAIDS 90-90-90 targets of 90% for all persons infected with HIV knowing their status. Other studies in Kenya have found that self-testing had doubled the frequency of HIV testing among men (Gichangi et al., 2016). The present guidelines recommend voluntary counselling and testing when individuals present for post-exposure prophylaxis.

This study however showed that only 0.9% of the cohort had any form of previous antiretroviral exposure. This proportion is quite low considering that guidelines recommending post-exposure prophylaxis has been available for more than six years. The researcher did not find other studies determining the prevalence of previous PEP use among HIV-infected individuals. These antiretroviral drugs are generally given to reduce the risk of HIV transmission after exposure to any fluid that may have a risk of HIV transmission. This may contribute to the high incidence of HIV in South Africa as, although post-exposure prophylaxis (PEP) is not 100% effective, it has been shown to reduce HIV transmission in non-occupational exposures (Gay & Cohen, 2008).

A review of the clinical stages at which patients were initiated revealed that most patients were initiated at stage I, while few were initiated in stage IV. This is in line with improvements in guidelines that have occurred along the years, starting with a CD4 count of < 200cells/µL to the present "diagnose and treat", irrespective of CD4 count, guideline. At a CD4 count of < 200 cells/µL, most patients will be in the later clinical stages. A further analysis revealed that most individuals in the 0-15 year age groups had their clinical stage unrecorded. This is compared to less than a quarter of those in the other age groups. Therefore for some reason, most children are not being staged clinically.

Recording the body mass index in paper files was very poor. Of the 1247 patient records reviewed, few individuals had their BMIs recorded. Of those patients had their haemoglobin recorded at baseline, 17.7% of these were anaemic. Anaemia was defined as having a haemoglobin of < 10g/dl. Another study, comparing the prevalence of anemia before and after antiretroviral therapy initiation in Ethiopia, found that anaemia prevalence was 21.2% before initiation on lifelong

antiretroviral therapy. This study also found significant ($p < 0.001$) improvement in anaemia prevalence after starting HAART. The prevalence reduced to 11.5%. This was associated with better immunologic response (Tesfaye & Enawgaw, 2014). Most individuals in the cohort had an (estimated glomerular filtration rate) eGFR placing them as stage I chronic kidney disease or normal renal function (Levey et al, 2011). Later WHO HIV clinical stages (III and IV) appeared not to be associated with advanced chronic kidney disease or anaemia according to this study. This was not an expected finding, as the later stages indicated a more advanced disease, and anaemia of chronic disease will be expected or in some cases HIV-associated nephropathy.

Finally, a patient could have one of four outcomes. These outcomes were as defined by the health care facility. The outcomes included transferred out, still on treatment, demised or lost to follow-up. A patient is said to have been lost to follow-up after two missed clinic visits along with failure to contact the patient or convince the patient to continue treatment. An important finding of this study is that the transfer processes were not adhered to completely. Details of laboratory results and specific antiretroviral drugs patients were initially started, were not recorded when they were transferred to another health facility. This is a serious omission, as these details are required for resistance-testing especially when choosing third-line ART. The study also found that 76.8% were still on treatment or retained in care, 1.8% had demised and 10.4% met the criteria for being lost to follow-up. A study in Cameroun investigating the determinants of retention in care in an antiretroviral therapy program, found that at 18 months of follow-up, 75% were retained in care, 2% were dead and 22% had been lost to follow-up (Tsague et al., 2008). Another similar study in Zambia, found a 73.9% retention rate at 1 year of follow-up (Lembela, 2013).

5.2.2 Virological responses

Results of this study showed a pattern in which the proportion virally suppressed increased with the passage of time. However, the data recorded for individuals in this study also reduced consistently with time. The proportion suppressed was therefore calculated based on the number of individuals who had data recorded at a particular point in time. There was no difference when comparing viral suppression of those that started HAART in 2010 and before to those that started HAART after 2010. The proportion of patients virally suppressed in this study are quite different from other studies. In Rio de Janeiro, Brazil, Cardoso et al. (2014) found that 77%, 76%, and 68% of patients attained viral suppression at 6, 12 and 24 months respectively. This may be due to the

methodology used. In this research, the records of different patients was reviewed over a period of time while in the Brazilian study, exactly the same patients were reviewed over similar intervals. However, in a study in India, investigating the use of once daily antiretroviral therapy, Gaikwad et al. (2015) found that 79%, 81% and 87% of patients attained viral suppression at 6, 12 and 60 months, respectively, after initiation of ART. This was the only other study in which the proportion virally suppressed increased with time. Reviewing viral suppression at a group level is important but it is very important to know how sustainable viral suppression is on an individual basis once achieved.

The present guidelines recommend the initiation on HAART once diagnosed with HIV. One major aim of this is to prevent HIV transmission through achieving viral suppression. One large study has even shown a zero transmission among sero-discordant couples when the infected partner had a viral load below 200copies/ml one year previously (Bruun et al., 2014). This study therefore attempted to review how consistently patients were able to maintain viral suppression or how sustainable viral suppression was after attainment. Only 59% of the sample were able to maintain one consecutive viral suppression. These patients maintained viral suppression over a period of at least 6 months. When reviewed for those able to maintain viral suppression consecutively for at least 54 months, only 14% of the sample achieved this. This means that the probability of being consistently virally suppressed when reviewed over a 54-month period was only 14%. These figures may have a negative impact on the use of viral suppression as a prevention strategy.

The WHO (2013) Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection state that the risk of HIV transmission when the viral load is less than 1000copies/ml is very low. Further analysis was done to review those that had viral blips at specific intervals between 6 months and 132 months. The mean of this proportion was similar to Ibrahim et al. (2012), who found that 12.6% of individuals experienced viral blips in a study in Brazil. This study reviewed viral blips and virologic failure in Rio de Janeiro, Brazil.

Another important finding of this study is the delay in change of regimen when a patient fulfils the criteria for viral failure. The South African National Department of Health (SA NDoH) defines virologic failure as two consecutive viral loads > 1000copies/ml at least 3 months apart after adequate adherence support. This, however, was not the case for some of the patients who had more than two consecutive viral load results > 1000copies/ml but were left on the same failing

regimen. Studies have shown that delaying a change from a failing regimen leads to accumulation of resistance, which could make future regimen less effective (Agwu et al., 2008). This can also increase the cost and affect provision of these life-saving drugs to individuals who need them.

5.2.3 Immunological responses

With the initiation of HAART, immune recovery, in the form of CD4 count increase, is expected. Meintjes et al. (2014) state that CD4 count increases rapidly in the first month of ART by 75 – 100 cells/µL with a more gradual increase thereafter of 50 – 100 cells/µL per year. Cardoso et al. (2014) observed a median increase of 107 cells/µL, 151 cells/µL and 242 cells/µL at 6, 12 and 24 months, respectively, after initiation of ART. The mean increases at these times were higher for this study. This difference may be due to the fact that the period under review for this study spanned over several guidelines which recommended different baseline CD4 counts for initiation on HAART. The CD4 count increased almost consistently throughout the period under review. This increase in CD4 count reduced the risk of developing several opportunistic infections (Bartlett et al., 2012). Also, very few patients met the WHO criteria for immunologic failure which requires a CD4 count remaining consistently below 100 cells/µL or falling below 100 cells/µL after an initial immune recovery (WHO, 2013). This means that most patients had adequate immune recovery among the sample.

The proportion with immuno-virologic discordance was similar to the findings by Anude et al. (2013) and Muzah et al. (2012), who investigated immuno-virologic discordance in Nigeria and South Africa respectively, both defined adequate CD4 response as an increase of ≥ 50 cells/µL at 6 months of ART and had measured prevalence of 33% and 24% respectively. Zoufaly et al. (2011) showed that the risk for developing AIDS was greatest in the first 6 months in those with immuno-virologic discordance. That was beyond the scope of this study.

5.2.4 Factors associated with viral suppression and adequate immunologic response

Viral suppression forms one of the basic elements for many current strategies that aim at reduced HIV transmission. The PARTNER study found a zero HIV transmission rate among discordant couples who practise ‘condomless’ sexual intercourse when the infected partner had a viral load of < 200 copies/ml (Bruun et al., 2015). This forms the basis for the review of viral suppression and factors that influence it. The only factors found to be significantly associated with early viral suppression were baseline CD4 count and gender, with individuals with higher CD4 counts and

females more likely to achieve viral suppression at 6 months. This was contrary to a study reviewing factors associated with viral suppression in Vietnam which found that gender had no influence on viral suppression (Rangarajan et al., 2016). This study, however found that stigma and social isolation had negative association with viral early suppression. The difference observed may have been due to cultural differences. Another study also found that higher baseline CD4 count was associated with viral suppression (Billioux et al., 2015). When the factors that are associated with viral suppression in the long-term was reviewed, baseline clinical stage and age were significantly associated with viral suppression. Gender and baseline laboratory results had no significant association. Bello et al. (2016) however found that absence of prior exposure to antiretrovirals before initiation on lifelong HAART was associated with viral suppression at 10 years post-initiation. They also found that age, race and baseline CD4 count had no influence on viral suppression in the longer term. Mujugira et al. (2016) however agreed with this study that younger age is associated with viral suppression.

Similar factors were found to be significantly associated with at least one consecutive virally suppressed result. These were age, gender and baseline clinical stage. Independent variables such as previous ART exposure, baseline BMI, creatinine clearance, haemoglobin and CD4 count had no significantly association. This may have been because the number of patients with previous ART exposure and those that had their BMI recorded were very few. Missing data of some other individuals may have also had influence on the outcomes.

Immune recovery following initiation of HAART results in reduced incidence of opportunistic infections, and improved clinical well-being (Bartlett et al., 2012). However, not all individuals initiated on HAART experienced adequate immune response. There has been no universally acceptable definition for adequate immune response. For this study, a CD4 count increase greater than 50 cells/µL at 6 months is regarded as adequate. Several factors have been shown to be associated with adequate immune response following commencement of lifelong antiretroviral therapy. Machado et al. (2007) found that younger age, baseline CD4 count and number of antiretrovirals in HAART regimen were associated with adequate immunological response. In this study, younger age (< 45 years) was also found to be associated with good immunological response. Gender was not. This was contrary to the study by Nash et al. (2008) who found that gender was associated with adequate immune response. Nash and colleagues (2008) however agreed with Machado et al (2007) and Tirfe et al (2013) that baseline CD4 count was associated

with good immune response. There was however no significant association with baseline CD4 count in this study, as was the case in the study by Teshome and colleagues (2013). Baseline clinical stage was also found not to be associated with adequate immune response in this study.

Almost all individuals throughout the period under review were on a first-line regimen with efavirenz or nevirapine as the non-nucleoside reverse transcriptase inhibitor. Few individuals were on a second-line regimen that had mostly ritonavir boosted lopinavir and rarely atazanavir/ritonavir as the protease inhibitor. As stated earlier, one patient was switched from first-line to second-line and then back to first-line ART. This is not what is recommended by the South African NDoH, as a switch back to a failed regimen will result in the accumulation of more resistance mutation (Bartlett et al., 2012). No patient in the cohort was on a third-line regimen.

5.3 Conclusions

Several conclusions can be derived from this study, and they include:

- Among those retained in care, the proportion virally suppressed increased almost consistently throughout the study.
- Sustainability of viral suppression appeared quite low when individuals were reviewed over a longer period of time.
- Some of the individuals in this study experienced a delay in switching from a failing regimen or a complete failure to switch during the period under review.
- The mean baseline CD4 count at initiation had increased over the years and most individuals had adequate immunologic response.
- Females have a higher chance of achieving (early) viral suppression at 6 months. There is no gender differences at (the longer term) 60 months.
- For every unit increase in CD4 count, there is a 0.2% increase in the likelihood of achieving viral suppression at 6 months.
- People in the extreme age groups (< 15 years and > 60 years) are less likely to achieve viral suppression at 60 months. So were those in WHO clinical stage IV at baseline.
- Also extreme age groups, males and individuals in clinical stage IV were less likely to have sustained viral suppression.
- Younger age (< 45 years) was associated with good immunologic response and baseline CD4 count had no influence on immunologic response.

5.4 Recommendations

Based on the above, the following recommendations are made:

- Data should be completely recorded in both electronic and paper folders at all health care facilities.
- Details of patients should be completely transferred with patients who are being transferred to other health facilities. Health care workers will need to be trained to do this.
- There is a need to investigate possible strategies to incorporate more men living with HIV into the antiretroviral programme.
- There is need to investigate the prevalence of post-exposure prophylaxis use in the district. It is also important to determine what proportion of the population knows about PEP.
- There may be a need to retrain health care workers on how to conduct a WHO clinical staging for HIV.
- It will be important to determine how sustainable viral suppression is in patients once they achieve it on a wider scale. This is important because it will determine how effective the "test-and-treat" approach will be in the control of HIV transmission.
- Health care workers, especially in clinics, will require more training to avoid delays in switching from a failing regimen or a switch back to a failing regimen.
- Target groups who are less likely to achieve viral suppression or adequate immunologic response should be properly investigated and strategies to improve their outcomes should be instituted.

Despite the limitations earlier stated, a larger sample size increases the likelihood of finding associations and differences where there is one. Therefore, the study could serve as a pilot for similar research in Vhembe District.

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APPENDIX I

DATA COLLECTION FORM ON IMMUNOLOGICAL AND VIROLOGICAL RESPONSES OF PATIENTS IN VHEMBE DISTRICT ON HAART.

INSTRUCTION: Kindly fill in the appropriate responses as appropriate. Confidentiality is guaranteed.

SECTION A: Socio-demographics

1. Clinic folder number: …..……
2. Year of initiation: ……..……..
3. Age:

0 – 15	1	
16 – 29	2	
30 – 44	3	
45 – 60	4	
> 60	5	

4. Gender:

Male	1	
Female	2	

5. Marital status:

Never married	1	
Married	2	
Divorced	3	
Widowed	4	
Co-habitation/Life-partner	5	

SECTION B: HIV response to HAART

6. Viral load (copies/ml) at:

	Code	< 50	51 – 399	400 – 1000	> 1000
		A	B	C	D
Baseline	1				
6 month	2				
12 month	3				
24 month	4				
36 month	5				
48 month	6				
60 month	7				
72 month	8				
84 month	9				
96 month	10				
108 month	11				
120 month	12				
132 month	13				

7. Number of consecutive viral load < 50 copies/ml:

SECTION C: Immunologic response to HAART

8 CD4 count (cells/µL) at:

	Code	CD4 count	CD4 count increase from baseline
Baseline	1		
6 month	2		
12 month	3		

24 month	4		
36 month	5		
48 month	6		
60 month	7		
72 month	8		
84 month	9		
96 month	10		
108 month	11		
120 month	12		
132 month	13		

9. Meets criteria for immunologic failure:

SECTION E: Factors associated with HIV response and Immunologic response to HAART

10. Previous antiretroviral exposure:

PMTCT	1	
PEP	2	
HAART	3	
NONE	4	

11. Clinical stage at baaseline:

I	**A**	
II	B	
III	C	
IV	D	
Unknown	E	

12. Body mass index at initiation (kg/m^2):

< 18.5	1	
18.5 – 24.9	2	
25 – 29.9	3	
≥30	4	

13. Haemoglobin level at initiation (g/dl):

< 6	1	
6 – 9.9	2	
10 – 13	3	
>13	4	

14. Creatinine clearance (ml/min):

≤ 15	1	
16 – 29	2	
30 – 59	3	
60 – 89	4	
≥ 90	5	

15. Antiretroviral regimen at:

	Code	First-line	Second-line	Third-line
		A	B	C
Baseline	1			
6 month	2			
12 month	3			
24 month	4			
36 month	5			
48 month	6			

60 month	7			
72 month	8			
84 month	9			
96 month	10			
108 month	11			
120 month	12			
132 month	13			

16. Patient outcome:

			Duration on HAART at last event
Default	**1**		
Transfer out	2		
Still on treatment	3		
Demised	4		
Lost to follow-up	5		

APPENDIX II

LETTER OF INTRODUCTION

University of Venda
DEPARTMENT OF PUBLIC HEALTH

TO: WHOM IT MAY CONCERN (Department of Health)

FROM: MPH PROGRAMME COORDINATOR (University of Venda)

DATE: 16 – 07 – 2015

SUBJECT: HEALTH FACILITY VISIT BY DR. EDET A.

I hereby introduce and confirm that Dr. Edet A is currently a registered Master of Public Health (MPH) student at the University of Venda. He will be working on his research project entitled: ***Virologic and Immunologic Response to Antiretroviral Therapy in Adults up to 2 Years after Initiation in the Vhembe District*** and as such, he needs preliminary information pertaining to this topic.

It will be highly appreciated if you can give him the assistance he may need.

Regards,

Dr. Augustine K Tugli (MPH programme co-ordinator)
Department of Public Health
School of Health Sciences
Tel: +27 15 962 8828
Cel: +27 837940174
E-mail: Tugli.augustine@univen.ac.za

UNIVERSITY OF VENDA
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UNIVERSITY OF VENDA
Signature:.....
2015 -07- 17
OFFICE OF THE DEAN
SCHOOL OF HEALTH SCIENCES

APPENDIX III

PERMISSION TO CONDUCT STUDY

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

Enquiries: Latif Shamila (015 293 6650) Ref:4/2/2

Edet A
University of Venda
Private Bag X505
Thohoyandou
0950

Greetings,

RE: Virologic and immunologic responses in patients on highly active antiretroviral therapy in Vhembe District South Africa

The above matter refers.

1. Permission to conduct the above mentioned study is hereby granted.
2. Kindly be informed that:-
   - Research must be loaded on the NHRD site (http://nhrd.hst.org.za) by the researcher.
   - Further arrangement should be made with the targeted institutions, after consultation with the District Executive Manager.
   - In the course of your study there should be no action that disrupts the services.
   - After completion of the study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   - The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   - The above approval is valid for a 3 year period.
   - If the proposal has been amended, a new approval should be sought from the Department of Health.
   - Kindly note, that the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated.

Head of Department

06/10/2016
Date

18 College Street, Polokwane, 0700, Private Bag x9302, POLOKWANE, 0700
Tel: (015) 293 6000, Fax: (015) 293 6211/20 Website: http/www.limpopo.gov.za

APPENDIX IV

ETHICAL CLEARANCE

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Dr A Edet

Student No:
15004539

PROJECT TITLE: Virologic and immunologic responses in patients on highly active antiretroviral therapy in Vhembe District South Africa.

PROJECT NO: SHS/16/PH/16/1808

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof PO Bessong	University of Venda	Supervisor
Prof HA Akinsola	University of Venda	Co-Supervisor
Dr A Edet	University of Venda	Investigator - Student

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE

Date Considered: August 2016
Decision by Ethical Clearance Committee Granted
Signature of Chairperson of the Committee: .....
Name of the Chairperson of the Committee: Prof. G.E. Ekosse

University of Venda
PRIVATE BAG X5050, THOHOYANDOU, 0950λ LIMPOPO PROVINCEλ SOUTH AFRICA
TELEPHONE (015) 962 8504/8313 FAX (015) 962 9060
"A quality driven financially sustainable, rural-based Comprehensive University"

APPENDIX V

LETTER OF PROOF OF EDITTING

SCHOOL OF HUMAN AND SOCIAL SCIENCES

21 January 2017

School of Health Sciences
University of Venda
Private Bag X5050
Thohoyandou
0950

Dear sir/madam

This letter serves to certify that I have proof-read Dr.A.Edet's mini-dissertation, titled, "Virologic and Immunologic Responses in Patients on Highly Active Antiretroviral Therapy in Vhembe District, South Africa: A Retrospective Study".

The proof-reading entailed editing some parts of it, where I felt it would make the document more understandable; for example, to avoid wordiness, redundancy; sub-dividing long sentences into shorter ones, for clarity; rephrasing sentences, etc. However, I have not tempered with the content of the mini-dissertation, except where I found that this constituted repetition or made the content confusing.

The mini-dissertation is presently ready for examination/presentation.

Thank you for your time.

Sincerely

V.T. Bvuma

Mobile: 083 423 9227

University of Venda

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