PREVALENCE, PREDICTORS AND HIV DISEASE PROGRESSION IN IMMUNOVIROLOGICAL DISCORDANT HIV PATIENTS AT 12 MONTHS OF FIRST LINE ANTIRETROVIRAL THERAPY IN ZAMBIA

By

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DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or at any other University, and does not represent any interest group(s).

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I, Professor Lloyd Mulenga, having supervised and read this dissertation, I am satisfied that it is the original work of the author under whose mane it is presented. I confirm that the work has been completed and approve it for final submission.

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CERTIFICATE APPROVAL

This dissertation of Dr Suilanji Sivile has been approved as fulfilling part of the requirements for the award of the degree of Master of Medicine (internal Medicine and Infectious Diseases) by the University of Zambia.

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DEDICATION

This work is dedicated to my parents: Edgar Bernard Sivile and Margaret Kancheya. It is no easy to raise a child, and their constant encouragement, correction and guidance have not been in vain.

ABSTRACT

Combined antiretroviral therapy (cART) has improved mortality and morbidity among HIV-infected patients. However, a third of HIV-infected patients still present to care with advanced disease despite the rollout of cART. Some of these patients fail to appropriately reconstitute their immune system despite being on effective cART signified by a suppressed viral load. This phenomenon is termed immunovirological discordance. These patients remain immunocompromised and could still be at risk of opportunistic infections and subsequent mortality. As the HIV population is getting older, immune senescence and its impact on discordance has become topical. Understanding the prevalence and predictors of this phenomenon is crucial for the HIV response.

A cross-sectional study was conducted in 20 health facilities throughout Zambia selected based on probability proportion to size method. Adult HIV patients with a suppressed viral load at 12 months of first line cART were enrolled. Relevant blood samples were drawn and a questionnaire was completed with the aid of the hospital chart . Adequate immune response was defined as an increase of baselines CD4cell count to >200cells/μL at 12 months of ART and/or an absolute CD4cell count change of >150cells/μL. We used multivariate logistic models to identify predictors for immunovirological discordance.

360 patients were enrolled. 57% were females. 68% were 25-44 years old. 17% had a CD4cell count below 200cells/μL at 12 months of ART and 54% had an absolute CD4cell count change of less than 150cells/μL. Females were 2 times more likely to have a CD4cell count above 200cells/μL (OR 2: 95%CI 1.00-3.62;P=0.028) and patients with a body mass index >25kg/m^2 were 4 times more likely to have a high CD4 count compared to those underweight (OR 4:95% CI 1.29-13.73; P=0.017) . A baseline CD4cell count below 200cells/μL was a predictor for an absolute CD4cell count change of less than 150cells/μL (OR12:95% CI 4.04-33.41; P= <0.0001). Hepatitis B virus positive status (OR 0.03:95% CI 0.003-0.25; P= 0.001) and baseline WHO stageIV/III disease (OR 0.01:95% CI 0.01-0.59; P=0.0001) were predictors for suboptimal CD4cell response. Patient's age, Positive RPR, TNF levels and CRP levels were not associated with suboptimal CD4cell recovery. There was no association between WHO Clinical Stage at 12 months of cART with immunovirological discordance.

In patients with viral load suppression at 12 months of cART, immunovirological discordance is common. Baseline CD4cell count, male sex, baseline low BMI, HBV infection and baseline WHO clinical stage III/IV could predict immunovirological discordance. Markers of morbidity such as high CRP levels and advanced WHO clinical staging at 12 months of cART are not necessarily associated with suboptimal immune response. Early commencement of cART may prevent immunovirological discordance, a finding which supports the 'test and start' strategy. Further investigation in understanding the immunology of discordance and its clinical outcomes are proposed.

***Key Words**: HIV, combined antiretroviral therapy, CD4 cell count, Zambia, Immunovirological discordance, viral Load*

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ABBREVIATIONS AND ACRONYMS

3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
cART	combined anti-retroviral therapy
AZT	Zidovudine
CD_4	Cluster of differentiation
DHHS	Department of Human and Health Services
FBC	Full blood count
GPPF	Graduate Proposal Presentation Forum
HAART	Highly Active Anti-retroviral Therapy
HCV	Hepatitis C Virus
HBV	Hepatitis B virus
Hb	Hemoglobin
HIV	Human immunodeficiency virus
IL-1	InterLeukin-1
INSTI	Integrase strand transfer inhibitor
IVDU	Intravenous drugs users
LFT	Liver Function Tests
LPV-r	Lopinavir-ritonavir
NNRTI	Non-Nucleoside Reverse Transcriptase
NVP	Nevirapine
PI	Protease Inhibitor
POC	Point Of Care
RNA	Ribonucleic Acid
TNF	Tumor Necrosis Factor
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION AND BACKGROUND

1.1 Introduction

Combined antiretroviral therapy (cART) has improved mortality and morbidity among HIV-infected patients. One of the goals of cART is to restore and preserve immunological function in HIV-infected patients. Other benefits of cART include reduction in transmission, and improvement in the duration and quality of life among people living with HIV (PLHIV). However, mortality in the early stages of cART still remains at 10- 15 % and a third of patients still present with advanced HIV disease signified by a CD4 cell count below 200cell/μL and WHO clinical stage III/IV opportunistic infections [1, 2].

HIV infection damages the immune system thereby allowing a myriad of opportunistic infections to cause both morbidity and mortality. The immune recovery in persons on combined antiretroviral therapy (cART) is one the best predetermining factor of both morbidity and mortality. In fact, high levels of mortality and morbidity are observed in the first few months after cART commencement because the immune system is yet to recover. In the absence of immune recovery, PLHIV remain vulnerable to infections.

The CD4 cell count is the primary marker of immune status in people with PLHIV. It is used to assess both disease progression and immune recovery in patients on cART. It also acts as a surrogate marker for successful cART. Currently, CD4 cell count is still used to assess for treatment failure where viral load is not available. The CD4 cell count typically increases by 20-30 cell/μL/months in the first 3 months after commencing cART, and then 5-10 cells/μL/months till the second year of cART. Thereafter, it will increase by 2-5cell/μL/month. With good virological response, the increase at 12 months averages 100-150cell/μL. A CD4 cell count of < 200 cell/μL at 12 months is considered suboptimal. This is mainly because AIDS-defining illnesses have been shown to significantly occur below this threshold.

A proportion of patients on cART are known to have suboptimal immune recovery despite virological suppression. This phenomenon is termed immunovirological discordance. These patients have low CD4 cell count despite virological suppression, which is usually defined as a viral load below 50 copies/mL. This phenomenon could have profound implications on the monitoring, clinical state and management of the HIV populations on cART.

It is not uncommon for clinicians to use the clinical status or indeed the CD4 cell count as a yardstick for the diagnosis of cART treatment failure. This practice arises due to the scarcity of the viral load in low and middle income countries like Zambia. However, the use of the immunological and clinical criteria for treatment failure may be incorrect in the presence of immunovirological discordance and result in unnecessary change in treatment regimen.

A state of severe and perpetual immune compromise could lead to increased mortality and morbidity despite adequate and potent cART. Therefore, a quick velocity of immune recovery in the shortest time is crucial in dropping the high mortality and morbidity characteristic of early cART. The management of immunovirologically discordant patients is challenging and would require extensive prophylactic care and/or immune bolstering approaches. Anecdotal data suggests that some ARVs regimens could have a separate immune bolstering capacity.

The cause of immunovirological discordance is not clearly known but is thought to be due to patient's biological characteristics. Factors associated with the management of HIV disease state are also thought to play a role.

The proportion of patients in Zambia who have this discordance at twelve months of first line cART is not known. The factors that determine immunovirological discordance, and the impact it has on the quality of life and treatment programs in the Zambian HIV population have not been investigated.

We investigated the proportion of virologically suppressed patients at 12 months of first line cART who have immunovirological discordance in Zambia. We also investigated the factors associated with suboptimal CD4 cell recovery at 12 months on first line cART and the clinical progression of HIV disease in these patients.

1.2 Statement of the Problem

Despite the potency of cART, a proportion of patients with a suppressed HIV viral load fail to achieve a CD4 cell recovery above a critical threshold associated with opportunistic infections. There is need to know the prevalence of immunovirological discordance (non-responders) and better understand factors associated with immune non-response in Zambia.

1.3 Study Justification

Currently, CD4 cell monitoring is still the most widely used method for monitoring response to cART. This study could assist in further justifying the need to expedite the rollout of the use of viral load levels for monitoring.

This study will also help in consolidating the definition of immunological failure. The definition of immunological failure has had many changes and has not been domesticated to the Zambian context. This study gives an opportunity to assess the relevance of the prevailing definition and perhaps show a threshold of immune recovery that would better predict immune response.

Currently, the predictors of immune response are still poorly understood especially in the Zambian context. This study will clarify the predictors of immune discordance. The knowledge of predictors of immunovirological discordance would help clinicians to identify potential discordants before commencement of cART and also to give an explanation to those patients who have poor immune response.

Management of immune non-response is always a clinical dilemma. This study could help to influence clinician in the selection of first line regimen. High rates of immune non-response and high risk of the occurrence of AIDS defining illnesses in immune non-responders will support the need to use and study immune boosters, vaccines and prophylaxis in this population.

Findings of this study could further support the need of early initiation of cART. High rates of immunovirological discordance would support the idea that patients must be started on cART before the immune system collapses because it may never recover.

The exact rate of immune discordance in Zambia is still not known and this finding will add to the body of knowledge.

1.4 Research Questions

Among patients on cART for 12 months, what is the proportion of patients with immunovirological discordance and what are the factors that are associated with immunovirological discordance? Do immunovirologically discordant patients have a worse HIV disease clinical state compared to the appropriate immune responders?

1.5 Hypothesis

1.5.1 Null Hypothesis Among patients starting cART, less than 30% will be immunovirologically discordant defined as a CD4 cell count <200cells/μL or an absolute CD4 cell count increase of <150 cells/μL, with a viral load <50 copies/mL after 12 months of first line cART.

1.5.2 Alternative hypothesis

Greater than 30% of patients on cART on 12 (±3) months will have immunovirological discordance.

1.6 Objectives

1.6.1 General Objectives

To investigate the proportion and predictors of immunovirological discordance at 12 months of first line cART in Zambia.

1.6.2 Specific Objectives

I. To determine the proportion of patients on first line ART for 12 months with a CD4 cell count <200 cells/μL and VL <50 copies/mL.
II. To determine the proportion of patients on first line ART for 12 months with an absolute CD4 cell count raise of < 150 cells/μL and VL < 50cp/mL.
III. To identify risk factors associated with immunovirological discordance at 12 months.
IV. To determine the association between immunovirological discordance and WHO clinical staging at 12 months of ART.

CHAPTER TWO
LITERATURE REVIEW

2.1 Low CD4 Cell Counts at Presentation to Care

In sub-Saharan Africa and Zambia in particular, late presentation for ART care is still common despite the rollout of ART. A meta-analysis done by Siedner et al. on the trends of CD4 cell count at presentation to care and treatment initiation in sub-Saharan Africa from 2002 to 2013 showed that the trend has not changed and that the median CD4 cell count is 152 cells/μL on initiation [3]. Other studies have shown that about 60% of patients have a CD4 cell count of less than 100cell/μL at presentation to care [4].

2.2 CD4 Cell Count Monitoring and Recovery

CD4 cell monitoring is inaccurate in monitoring response to cART and therefore, major guidelines like DHHS 2015 guidelines on the treatment of HIV patients recommends that viral load should be used for monitoring response to cART and the CD4 cell count could be used to assess the need for prophylaxis. However, CD4 cell monitoring is still the most widely used method for monitoring cART in Zambia and other resource limited settings due to the inherent high cost and complexity of the viral load testing [4].

There is no consensus on the expected rise of CD4 cell count and the threshold of immune recovery considered appropriate whilst on cART. However, Autran B. et al. showed that CD4 cell recovery after commencement of cART normally comprise of three phases. The first phase is a rapid phase with an increase of 20-30 cell/μL/month during the first 2 to 3 month. This is followed by a second phase with an increase of 5-10 cell/μL/month, which lasts for about 2 year. The third phase an increase of 2-5 cell/μL/month lasting for the rest of the patient's treatment time. It expected that a patient would have an increase of 150 cell/μL after one year of treatment.

It is also known that a CD4 cell count below 200 cells/μL before commencement of cART is a marker of advanced HIV disease and defines AIDS. Several studies have shown that a CD4 cell count below 200 cell/μL at 12 months after commencement of cART is a risk factor for both AIDS defining illnesses and non AIDS defining illnesses [5]. In a prospective study by Benjamin et al. done in Zambia, it was found that a CD4

cell count below 100cell/μL at 6 months after commencement on cART had an increased likelihood of mortality with an adjusted hazard ratio of 2.25 and a CD4 cell count of below 200cells/μL at 12months of cART had an adjusted Hazard ratio of 3.41 [6]. Thus, studies done elsewhere that have assessed immune recovery on cART have used 200cell/μL as a threshold for immune response [11, 12].

Even though the first 12 months is insufficient time to assess full immune recovery, it is widely believed that suboptimal immune recovery in the short term is predictive of poor immune recovery in the long term. This particular fact was demonstrated by Kye-Hyung et al who showed that a CD4 cell count slope of less than 20 cell/μL/month in the first 6 month of commencing cART is indeed suboptimal and predictive of poor long term CD4 cell recovery [7].

The previous Zambia consolidated guidelines for the treatment and prevention of HIV defined immunological treatment failure as a persistent CD4 cell count below 100 cell/μL and patients who met this criteria were switched to second line treatment because of the scarcity of viral load testing in Zambia [8]. It is now well established that clinical and immunological monitoring does not accurately identify patients that are failing treatment [9]. A study done in paediatric patients by Ginwalla et al. at UTH in Lusaka Zambia showed that 28% of paediatric patients with immunological failure had suppressed viral loads while 32% of patients with virological failure had good immunological response [10].

2.3 The Rate of Immunovirological Discordance

Various studies have been done to determine the rate of immune non-response and to establish factors associated with this phenomenon. Elsewhere, the rate has been shown to be between 9% and 37% [11, 12]. Boris et al showed that the rate of this discordance in black South African is 37% at 12 months of cART [12]. In this study, the nadir CD4 cell count on presentation was 97 cells/μL. Prabakar found the rate to be 12% in a cross sectional study done in India. From these findings, it is shown that about 30% of patients remain immunosuppressed despite potent cART. These findings have also raised the question of the clinical outcome of the immunovirological discordant patients.

2.4 Immunovirological Discordance and Opportunistic Infections

Different studies have found that there is an intermediate occurrence of opportunistic infections in immune non-responders [13]. Kaufmann et al showed that insufficient immune recovery in the short term leads to elevated risks of Centre for Disease Control and Prevention category C and D events [14]. Benjamin et al in a prospective study done in Zambia showed that the risk for mortality is much higher in PLHIV who still remain with CD4 cell count <200cells/μL while on cART [4]. Another prospective cohort study in South Africa by Takuva et al found an adjusted hazard ratio of 2.8 for CD4 cell count recovery below 50 cells/μL against CD4 cell counts above 200cells/μL at 12months [15].In a large multicenter study by Zoufrey et al, immunovirological discordance was an independent predictor for new AIDS defining illnesses. Immunovirological discordance hard an adjusted hazard ratio of 3.1 and the risk was greatest in the first 6 months of being suppressed [16].

In addition Gutiérrez et al showed that despite having the same risk for AIDS defining illness, non- immune responders have increased risks of mortality from non–AIDS illness compared to immune responders. This has led to additional fears concerning subclinical non-immune responders. This could in part be attributed to the chronic state of immune activation and inflammation as one of the underlying causes of immunovirological discordance. Chronic state of inflammation is known to be associated with various diseases including non-communicable diseases such as cardiovascular diseases [17].

2.5 Factors Associated With Immunovirological Discordance

Several factors have been individually associated with immune-virological discordance; some of these factors are discussed here.

Age has been reported to have a significant impact on immune recovery: the older the patient is, the more likely that he or she will experience delayed immune reconstitution [18]. Boris et al showed that individuals >40 years old were less likely to have a CD4 cell count of >200cell/μL at 12months. In addition, some cohort studies suggest that immune recovery in older patients may hide a more profound functional impairment,

as evidenced by a persistent increased risk of AIDS-related events in these patients even after adjusting for CD4 cell counts [7,19].

Another factor presumed to affect immune recovery during HAART is concurrent viral hepatitis. In a recent meta-analysis, Miller et al showed that increases in the CD4+ T cell count during HAART are significantly lower in the course of hepatitis C virus (HCV) co infection [20].

Nadir CD4+ T cell count is the most common and most reliable determinant of suboptimal immune recovery during HAART [14, 21]. From a mechanistic standpoint, a simplistic view is that the nadir CD4+ cell count predicts the immune response due to simple depletion in immune reserves. However, it has been shown that differences in CD4+ T cell nadir are indicators of differences in immunological regulatory functions over T cell homeostasis that might affect immune recovery capacity [22]. Therefore, the sole quantification of nadir CD4+ T cell count may fail to qualitatively estimate the immunological mechanism(s) that hinder CD4+ T cell count rescue, indicating a need for a more detailed assessment of immunological gaps associated with nadir-driven T cell homeostasis [23].

The effect of Tuberculosis on immune response on cART has been studied with conflicting results. Abate et al in a study done in Ethiopia to assess the effect of incident tuberculosis on immunological response of HIV patients on HAART concluded that the proportion of patients with impaired immune restoration was significantly higher among patients who developed incident TB during HAART [24]. Several other investigators have suggested similar findings [25-27]. Boris et al in assessing factors predicting discordant viral and immunological response to ART therapy in HIV clade C infected Zulus/Xhosas in South Africa found that TB infection at time of HAART initiation did not undermine CD4 cell recovery. In contrast, individuals with TB at baseline tended to be at decreased risk for failure to recover to a CD4 cell counts below 500cells/ μL at 30 months (OR 0.58, 95% CI). He also found that other patient characteristic like gender baseline BMI or haemoglobin were not associated with CD4 count restoration at 12 months or 30 months in virologically suppressed patients [7].

2.6 The Mechanism of Immunovirological Discordance

The mechanism of suboptimal immune recovery has been investigated in terms of factors that influence reduction in CD4 cell production and those factors that enhance CD4 cell destruction.

Pierre-Marie et al found that increased apoptosis of CD4 cell supersedes their reduced production in terms of contribution to suboptimal immune recovery [28].

The pathogenesis of immunological response may be secondary to specific failure of the T cell armamentarium machinery i.e. failure of the bone marrow to produce the hematopoietic stem cell or to a deficiency in thymic output. Decreased bone marrow progenitor cell growth and abnormal stromal microenvironment have been described in patients with HIV/AIDS. The failure to restore circulating CD4 cells during ART may partly be caused by the deficiency in thymopoietin and several studies have demonstrated a trend towards smaller thymuses and lower thymopoietin levels in immune non-responders [29, 30]. Other studies have demonstrated that there is a relative deficiency in IL-7 (which is a vital cytokine for thymocyte development) in immune non-responders.[31]

Immune non-responders are characterised by augmented levels of CD4 cell loss in the periphery. It has been found that the degree of immune activation and the increased levels of CD4 memory cells at commencement of ART are predictive of poor immune recovery [23]. It is further postulated that this finding can be used to predict patients at risk of immune non-response. Ongoing viral replication even in the presence of undetectable viral load is thought to be one of the persistent drivers of immune activation together with other factors like gut mucosal microbial translocation. Indeed, Brenchley et al demonstrated that high levels of plasma lipopolysaccharides in HIV infected patient's correlates with immune hyperactivity [32, 33]. Byakwaga et al showed that the kynurenine pathway of tryptophan catabolism independently predicts poor CD4+ T cell count recovery and increased mortality among HIV-infected Ugandans initiating ART and may be an important target for interventions. This pathway is induced by immune activation in dendritic cells and monocytes and its products are neurotoxic and act as immune activators, inhibitors of T cell proliferation and contribute to microbial translocation.[34]

Haas et al showed that there might be a genetic component to immune-virological discordance. He found a significant association between CD4+ T cell recovery and polymorphism in genes encoding for TNF related apoptosis inducing ligands, TNF-α, Bcl-2 interactive molecules, and IL-15/IL-15 receptor α chains.[35]

2.7 Treatment of Immunovirological Discordance

Work has been done in determining the strategies in improving immune recovery in patients that have sub-optimal immune response. Interleukin-2, a molecule that sustains CD4+ cell count in the periphery has been investigated with promising results [36, 37]. However, the DHHS guidelines currently recommend the use of interleukin immunotherapy only in the context of clinical trials. Interleukin-7 has also demonstrated a sustained dose dependent increase in naïve and memory CD4 + cells but its use is still controversial and DHHS guidelines recommend it only in the context of clinical trials [38].

Studies to optimise antiretroviral therapy regimens in the context of suboptimal immune recovery have also been done. Protease inhibitors have been shown to have a better effect on CD4+ cell recovery compared to NNRTI's [39]. Thymidine analogues like AZT have been shown to hamper the recovery of the CD4+ cell count possibly due to the associated mitochondrial toxicity and oxidative stress accumulation [40, 41]. Wilkins et al showed that the CCR5 antagonist (maraviroc) enhances CD4+ cell response [42]. Other newer classes of drugs such as fusion inhibitors and INSTIs which provide the strongest suppression of HIV have shown greater immunological efficacy.

There has been an interest in dampening the excessive immune activation with immunosuppressive agents which could theoretically increase the CD4+ cell response. Unfortunately, conclusive trials in assessing the benefits of administration of immunosuppressive drugs to immune non-responders are still lacking [43].

Chisanga et al showed that a nutritional intervention did not improved T cell subsets recovery in the HIV positive and malnourished Zambian population [44].

CHAPTER THREE
METHODOLOGY

3.1 Study Design

We conducted a cross sectional study of patients on first line cART for 12 months. This was a sub-study of **the Viral Load Suppression and Acquired HIV Drug Resistance among HIV-1 Infected Zambians at 12 Months of Combination Antiretroviral Therapy surveillance**, which had ethical approval by Biomedical Research Ethics committee of the University of Zambia reference No. 007-08-16. This study had ethical approval from ERES reference number: 2016 JULY 009.

3.2 Study Sites

This study took place at 20 different site distributed throughout Zambia. These sites were selected according to probability proportion to size criteria. See Table 3.1.

Table 3.1: Study Site Facilities

Central province	**Copperbelt**	**Lusaka**	**Southern/ western**	**Luapula /Eastern Northern**
Kayosha Clinic	Kalulushi General Hospital	Chaisa (Chifundo) Clinic	Macha Hospital	Kasenengwa Clinic
Kapiri Mposhi District Hospital	Wusakile Mine Hospital	Chipata Clinic	Livingstone Hospital	Petauke district Hospital
Nalubanda RHC	Malcom Watson Hospital	Kabwata Clinic	Namwala Hospital	Samfya Stage II Clinic
Serenje District Hospital	Masala New Clinic	University Teaching Hospital	Limulunga Clinic	Luwingu district hospital

3.3 Survey Populations

The study was performed on HIV infected populations receiving cART at 12 months. Since patients do not visit clinics necessarily at 12 months, a margin of ±3 months was added to the time point to improve its feasibility.

3.4 Study Process

The study took place at the 20 facilities between 12 September 2016 and 7 October 2016. A study nurse picked from the respective health facility and a research assistant oriented in the protocol enrolled the patients at the respective facilities. Both the study nurse and the research assistant received orientation in the protocol for this study and in good clinical practice.

Patient who had been on cART for 12 months ± 3 months were identified from the facility registers. A simple random selection of every third patient was made in the register till a list of 23 candidates was made. These were contacted and informed to come for enrolment at the health facility on the date of their convenience within the period of enrolment at that facility. A consent form was given to each patient. An HIV-1 and HIV-2 test was administered to each patient who had given consent. Only the patients that were NOT infected with HIV-2 were enrolled in the study. While HIV-2 infection could suppress the CD4 cell count, the viral test used in this study could not test for HIV-2 viraemia.

The study questionnaire was administered to each enrolled patient and supplementary information was obtained from the chart review of each patient's file. Thereafter, each patient had 12 mls of bloods drawn and anthropometric measurements taken. The patients were thereafter allowed to go home and given an appointment for the results. The results were explained to the patients by the study nurse and the investigators. Appropriate clinical interventions were made according to the Zambian guidelines for managing HIV patients on those patients who required them.

3.5 Laboratory Methods

12 mls of blood was drawn from each patient. 2 EDTA bottles were filled with 4 mls each for viral load and HIVDR genotype while one EDTA bottle was filled with 2 mls of blood for CD4 cell count and Hb. Another 2 mls was filled into a heparin bottle for

biochemistry. Hepatitis B surface antigen test and RPR were done by point of care strip test on site.

3.5.1 Transportation and Storage

The viral load and HIVDR genotype samples were kept at room temperature for 2 hours before being stored at -20 degrees Celsius. The CD4 cell count samples were kept at room temperature and were processed within 24 hours at the respective centres. The sample for biochemistry was stored at -20 degrees Celsius.

The viral load and biochemistry samples were transported to the central lab at UTH from respective centres in liquid nitrogen containers at -80 degrees Celsius. The samples were considered infectious during transportation and the triple packaging system was used in tight container and absorbent material to avoid leakage. These samples were stored at -20 at UTH lab while waiting processing.

3.5.2 Sample Processing

CD4 cell count samples were processed using BA FACSCount System. They were all processed within 48hrs of collection. The viral load samples were processed using COBAS TaqMan 96 analyser.

3.6 Eligibility Criteria

3.6.1 Inclusion criteria

i. Patient who met the following were included in the study:
ii. HIV-infected adult above 18 years of age patients able to provide informed consent
iii. Patients on cART for 12 (±3) months and are still on cART at the time of enrolment.
iv. Patients with self-reported good compliance to cART.
v. Only patients with viral load < 50 copies/ml were analyzed.

3.6.2 Exclusion criteria

The following patients were excluded from this study:

i. HIV infected patients with HIV-2 or HIV1/HIV-2 co-infection.

ii. Acutely ill patients based on vitals and self-report.

iii. Long term use of steroids or other immune suppressing therapy

3.7 Sample Size

The following assumptions were made to calculate the sample size:

I. The rate of immune-virological discordant rate of 30% (obtained from previous studies).

II. The virological suppression rate of 85%.

Using OpenEPI website for calculating cross-sectional studies with a confidence interval of ±5% and power of 80 %. The needed sample size for the assumed rate of 30 % is 323 and the total patients required for recruitment in view of the virological suppression rate of 85% was 380.

3.8 Sampling

A simple random sampling method was used in which every third patient in the ART register who met the inclusion criteria was selected.

8.9 Study Variables

The CD4 cell count was the dependent variable and we included included demographic and laboratory variables for independent variables as shown in Table 3.2.

Table 3.2: List of variables

Independent variables	Dependent variables
Age	CD4 cell count at 12 months
Sex	
BMI	
Facility	
IVDU/ Alcohol use/Smoking	
ARV regimen	
History of PTB	
Nadir CD4 cell count	
WHO staging at 12 months	
Baseline WHO clinical staging	
Current Hemoglobin	
HBsAg/RPR/RBS	
CRP	

3.10 Outcomes

3.10.1 Primary outcomes

The primary outcome was the proportion of patients with immunovirological discordance which was be defined as a CD4 cell count of less than 200 cells/μL or an absolute increase in CD4 cell count of less the 150 cells/μL at 12 months of cART in the presence of virological suppression. Virological suppression was be defined as VL of <50 copies/mL.

3.10.2 Secondary Outcomes

We assessed the relation between the following variables and immunovirological discordance:

- Age
- Sex
- Baseline CD4 cell count

- Baseline WHO stage
- Adherence
- Type of facility
- HBV infection
- BMI
- Baseline Hb
- CRP

We also assess the correlation between WHO HIV/AIDS clinical stage and immunovirological discordance.

3.11 Data Analysis

Descriptive statistics were used to calculate the prevalence. For continuous variables, we looked at the mean, standard deviation and range. Bivariate analysis was done to assess associations (Pearson Chi- Square; or Fisher's exact tests if Chi-Square assumptions were not satisfied) with $P< 0.05$ at 95% Confidence level. Univariate and Multivariate logistic regression were employed to control for confounders.

We had a check list and Microsoft excel 2010™ was used for data entry, cleaning and validation, then data was transported to STATA version 14.0 for analysis.

3.12 Ethical Considerations

Phlebotomy was done by trained nurses using antiseptic technique and care was taken to cause as little discomfort as possible. No names were used in this study either on the questionnaires or on the lab forms. Enrolled patients were given special numbers and the data was kept in a secure place to ensure privacy.

The patients who were found to have immunovirological discordance were directed to their ART centers for counseling and prophylaxis as per standard Zambian guidelines for managing HIV/AIDS. Those found to have high viral loads were also directed to their ART centers as cases of ART treatment failure and managed as per standard guidelines.

The study assistants and study nurses who participated in this study were trained in the principles of Good Clinical Practice as part of the orientation process. The

investigators conducted the study in accordance with the proposed protocol, International Conference on Harmonization (ICH) regulations and guidelines, FDA regulations and guidelines, ethical principles that have their origin in the Declaration of Helsinki and all applicable Zambian regulations. Ethical approval was granted by ERES converge with certificate number 09-JULY-2016. Permission to carry out the study was also granted by UTH and the selected clinics through the MOH.

3.13 Study Limitations and Strengths

This is a cross sectional study and by its nature it excluded patients who could have died in the first 12 months of ART giving a survival bias. Therefore, the findings of this study can only be looked at as a snapshot of the problem of immunovirological discordance.

This was a nested study in a countrywide surveillance of suppression rates and HIVDR. This also meant that the data collecting tools had to be merged with the data collecting tools for the parent study. In this process, some of the details had to be changed. For instance, the eligibility criterion in this study was initially supposed to include only those patients with baseline CD4 cell count below 200 cells/μL but we compromised to include all patients on ART regardless of baseline CD4 cell count.

This study also failed to bring out data on the relationship between different ART regimen and immune response. This is because almost all patients recruited were on Tenofovir, Lamivudine and Efavirens regimen.

We had planned to include flow cytometry for CD4 cell subtype to give insight in to the mechanisms of immune non-response but this was not feasible in view of the countrywide nature of the study.

To our knowledge, this is the first study done in Zambia in country wide nature with a good samples size looking at the CD4 cell count changes in patients who are known to be virologically suppressed assessing the probable relationship with various demographic and laboratory parameters. While the threshold of good immune recovery is uncertain, we demonstrated in this study that sufficient immune recovery can be assessed.

CHAPTER FOUR
RESULTS

4.1 introduction

The study was conducted between 10th September, 2016 and the 7th October, 2016. We screened 427 patients in 20 different facilities. Of those screened, 360 patients had a viral load test of less than 50 copies/ml and these were enrolled for analysis in this study. Among those enrolled, 332 patients had baseline CD4 cell count and 306 patients had CD4 cell count at 12 months. Of those screened, 8 patients had HIV-2 infection and these were excluded from the study. Figure 4.1 shows the enrollment process.

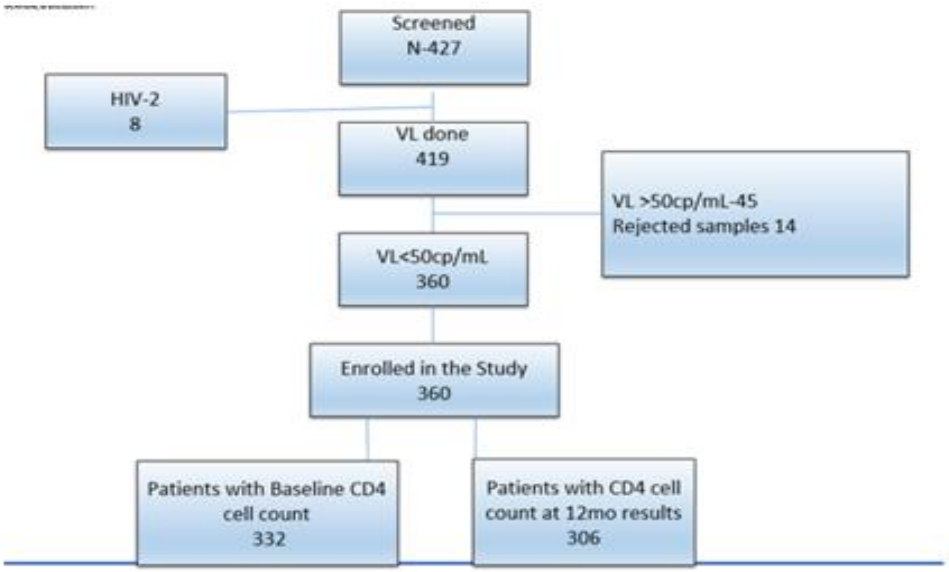

Figure 4.1: The Study Process Outcome

4.2 Baseline Characteristics

The baseline characteristics of the patients enrolled are shown in Table 4.1. Most of the patients enrolled were between the age of 25 to 44 years old (68%), and 57% of the patients enrolled were females.

Table 4.1: Demographic Characteristics of HIV-1 patients in the Immunovirological discordant study after 12 months of combined ART in Zambia, 2016.

N= 360		
Characteristic	Frequency	(Percent)
Age Group		
19-24	28	[7.8]
25-44	245	[68.1]
45-74	87	[24.1]
Total	360	[100.0]
Sex		
Male	156	[43.5]
Female	203	[56.5]
Total	359	[100.0]
Marital Status		
Single	48	[13.7]
Cohabiting	8	[2.3]
Married	214	[61.1]
Divorced	34	[9.7]
Widowed	46	[13.2]
Total	350	[100.0]
Employment Status		
Unemployed	209	[58.1]
Employed	151	[41.9]
Total	360	[100.0]

Note: Sample size was 360. Some variables have less than sample size because of missing data.

Table 4.2 shows baseline laboratory characteristics. The mean baseline CD4 cell count was 283 cell/µL (SD194.2) and a range of 1 cell/µL to 1100 cell/µL. In this study, we enrolled patients on Option B-plus who started ART at higher CD4 cell count than the guideline indication of a CD4 cell count below 350 cell/µL at the time of the study. The mean CD4 cell count at 12 months was 426 cell/µL with a range of 2 cell/µL to 1149 cell/µL. The mean Haemoglobin at baseline and at 12 months was comparable with 12.7g/dL and 12.8g/dL respectively. All the patients analysed had a viral load <50 copies /mL (Range: < 20 to 49).

Table 4.2: Laboratory Parameters of HIV-1 patients in the immune-virological discordant study after 12 months of combined ART in Zambia, 2016

N=360					
Characteristic	Observations (n)	Mean	[Std. Dev.]	Min	Max
Baseline CD4	332	282.6	[194.2]	1	1100
CD4 Count at 12 months	306	426.3	[237]	2	1149
Baseline Haemoglobin	198	12.7	[6.1]	8.8	19
Current Haemoglobin	198	12.8	[2]	9.6	19.9
Random Blood Sugar	355	4.5	[1.1]	3	5.9
CRP	423	6.13	[18.08]	0.009	193.9
TNF	369	81.15	212	0.593	1403
Viral Load	360	-	-	<20	49*

*Note: All participants had their viral load suppressed <50 copies/ml

4.3 Comparison of CD4 cell count bands at baseline with at 12 months

The proportion of patients with baseline CD4 cell count < 200 cells/μL, <100 cells/μL and <50 cells/μL was 40 %, 17% and 9% respectively (table 4.3). Among those patients who had a baseline CD4 cell count of <200 cells/μL, 40.1% failed to increase the CD4 cell count beyond 200 cells/μL. The proportion of patients with CD4 cell count at 12 months of cART of < 200 cells/μL in the entire sample was 16.7% (Table 4.3).

Table 4.3: CD4 Cell Count Bands at Baseline and at 12 Months of cART

CD4 Cell count Bands (cell/μL)	Baseline CD4 cell count			CD4 cell count at 12 months of cART		
	Frequency	Percent	Accu. %	Frequency	Percent	Accu. %
1-49	30	9.1	9.1	3	1.0	1.0
50-99	27	8.1	17.2	7	2.3	3.3
100-199	76	22.9	40.1	41	13.4	16.7
200-1189	199	59.9	100	255	83.3	100
Total	332	100		306	100.0	

Figure 4.2 illustrates the transition of the CD4 cell counts between the baseline and that at 12 months of cART. While 83.3 percent of the patients had increased their CD4 cell counts to cross the threshold of 200 cells, some patients still had low CD4 cell counts. Of note, 3.3 percent still had CD4 cell counts below a 100 cell/μL.

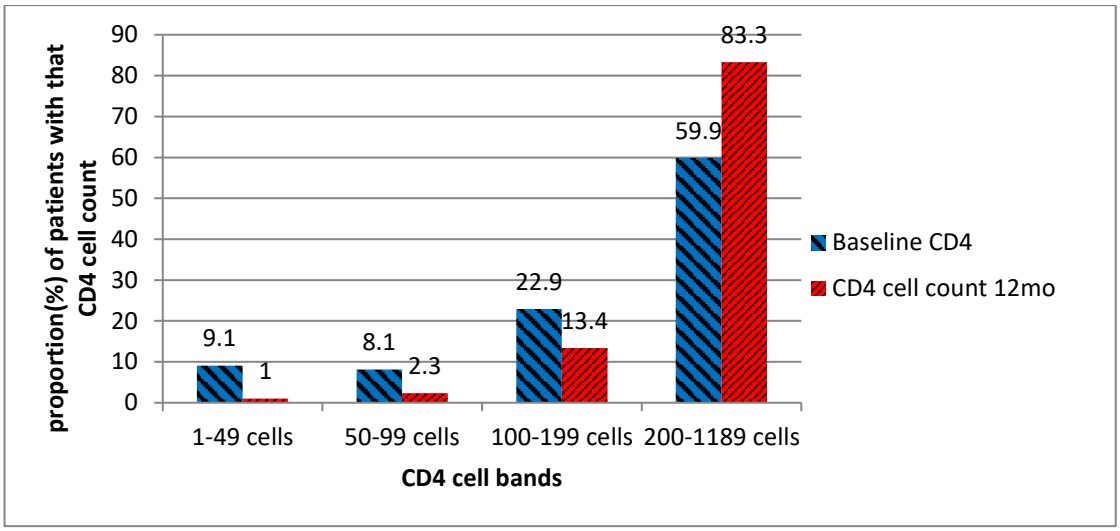

Figure 4.2: Baseline CD4 cell count verses CD4 cell count at 12 months

4.4 Absolute CD4 Changes after 12 months of cART

We also found that 58% of patients failed to have a CD4 cell count increase above the 150 cells/μL threshold, and 27% of patients had a CD4 cell count increase of only <20 cell/ μL (Table 4.4).

Table 4.4: Shows the absolute increase in CD4 cell count between baseline CD4 cell count and CD4 cell count at 12 months in each band

CD4 cell count difference in bands (cells/μL)	frequency	percentage	Cumulative (%)
<20	117	27.40	27.40
20-50	22	5.15	32.55
51-100	56	13.11	45.67
101-150	55	12.88	**58.55***
151-200	47	11.01	69.56
200-950	130	30.44	100.00
total	427	100	

Table 4.5 shows that the mean change in the CD4 cell count after 12 months of ART increased with increasing baseline CD4 cell count bands. This signifies the relation of low baseline CD4 cell count and failure to have an appropriate increase in CD4 cell count.

Table 4.5: CD4 cell count mean change in each baseline CD4 cell count band.

Baseline CD4 cell count band (cells/μL)	Mean CD4 cell count change within this band (cells/μL)
<50	33
50-100	39
101-200	85
201-1100	396

4.5 Predictors of Immunovirological discordance

Table 4.6 shows the bivariate analysis of baseline patients and laboratory characteristics with the CD4 cell counts above 200 cells/μL. Table 4.7 shows the multivariate analysis. During the logistic regression analysis, age was not a significant predictor of immunovirological discordance in both bivariate and multivariate analysis. Males were 2 times more likely to have poor CD4 cell recovery compared to females [OR 2: CI 1.00- 3.62; P= 0.028].

Patients with baseline WHO stage IV disease were more likely to have a poor CD4 cell recovery compared to those with baseline WHO stage I disease [aOR: 1.99 CI : 1.4-1.99 p= <0.0001]. However, there was no relationship between a CD4 cell count at 12 months <200 cells/μL and the WHO clinical stage at 12 months of ART in this virologically suppressed sample.

Overweight patients were more likely to have a good CD4 cell count response compared to the underweight patients [OR: 4.2 CI: 1.29-13.7 P=0.017] and normal weight patients were more likely to have a good CD4 cell count response compared to underweights [OR: 2.3 CI: 1.14-4.8 P=0.021].

Patients who were infected with the Hepatitis B Virus (HBV) were more likely to have insufficient CD4 cell count response compared to those who were not infected [aOR 1.97 CI: 1.97-1.997 p= 0.001].

The level of health care facility, reported adherence, RPR and haemoglobin levels did not predict immunovirological discordance in this study. Predictors were the same when we used the absolute CD4 cell count change of <150 cells/μL as the dependent variable.

We also looked at the relationship between absolute CD4 cell count change and a CD4 cell count at 12 months of cART (Table 4.6 and 4.7). Among patients who have a CD4 cell count at 12 months of <200 cells/μL, 91% had an absolute CD4 increase of <150 cells/μL. Further stated, patients who had an absolute CD4 cell count increase of > 150 cells/ μL were more likely to have a CD4 cell count at 12 months of >200cells [aOR: 27 CI: 6.08-120.4 p=<0.0001].

Table 4.6: Bivariate Analysis of Background Characteristics of HIV-1 patients in the immunovirological discordant study after 12 months of combined ART in Zambia, 2016

N= 360					
	Immuno-Virological Discordance				
Characteristic	**<200 cells**		**≥200 cells**		**P-Value**
CD4 at 12 months	**17%**		**83%**		
	n	**(%)**	**n**	**(%)**	
Age Group					0.202
19-24	1	[2.0]	24	[9.4]	
25-44	36	[70.6]	170	[66.7]	
45-74	14	[27.4]	61	[23.9]	
Total	51	[100.0]	306	[100.0]	
Sex					**0.027***
Male	28	[54.9]	97	[38.2]	
Female	23	[45.1]	157	[61.8]	
Total	51	[100.0]	254	[100.0]	
Facility					**0.051***
health centre	38	[74.5]	153	[60.0]	
Hospital	13	[25.5]	102	[40.0]	
Total	51	[100.0]	255	[100.0]	
Baseline WHO					**<0.0001*****
stage 1	24	[52.1]	159	[67.4]	
stage 2	5	[10.4]	47	[19.9]	
stage 3	11	[22.9]	28	[11.9]	
stage 4	7	[14.6]	2	[0.8]	
Total	48	[100.0]	236	[100.0]	
Adherence					0.581
non-adhered	14	[27.5]	60	[23.8]	
Adhered	37	[72.5]	192	[76.2]	
Total	51	[100.0]	252	[100.0]	
Baseline Body Mass Index					**0.020***
Underweight	16	(34.8)	38	[16.4]	
Normal	26	(56.5)	144	[62.1]	
Overweight	4	(8.7)	40	[17.2]	
Obese	0	(0.0)	10	[4.3]	
Total	46	(100.0)	232	[100.0]	
Haemoglobin (Hb)					0.273
<8.5g/dL	4	[13.8]	6	[5.0]	
8.5 - 10.0 g/dL	2	[6.9]	10	[8.3]	
>10.0 g/dL	23	[79.3]	104	[86.7]	
Total	29	[100.0]	120	[100.0]	
CD4 Difference					**p<0.0001*****
Below 150	42	[91.3]	113	[47.5]	
150 and above	4	[8.7]	125	[52.5]	
Total	46	[100.0]	238	[100.0]	
Hepatitis B					**0.066***
Negative	47	[92.2]	249	[97.7]	
Positive	4	[7.8]	6	[2.3]	
Total	51	[100.0]	255	[100.0]	
RPR					**0.059***
0-1	49	[96.1]	221	[86.7]	
2-30	2	[3.9]	34	[13.3]	
Total	51	[100.0]	255	[100.0]	

Note: Pearson's chi-square test was used to obtain p values. For frequencies less than 5, fisher's exact was used. * $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$

Table 4.7: Predictors for immunovirological discordance in HIV-1 patients after 12 months of combined ART in Zambia, 2016

N= 360

Characteristic	Odds Ratio	95% CI	P-Value	Adjusted Odds Ratio	95% CI	P-Value
Age Group						
19-24	Ref.					
25-44	0.2	[0.03-1.5]	0.117			
45-74	0.2	[0.02-1.5]	0.108			
Sex						
Male	Ref.					
Female	2	[1.10-3.6]	**0.028****			
WHO at 12 mo.						
stage 1	Ref.					
stage 2	0.5	[0.17-1.8]	0.317			
Stage 3	0.3	[0.04-1.7]	0.163			
Baseline WHO						
stage 1	Ref.					
stage 2	1.5	[0.54-4.1]	0.450	1.3	[0.45-3.9]	0.606
stage 3	0.4	[0.18-0.9]	**0.028****	0.4	[0.13-0.9]	**0.048****
stage 4	0.04	[0.01-0.2]	**<0.0001*****	0.01	[0.01-0.6]	**<0.0001*****
Baseline Body Mass Index						
underweight	Ref.					
Normal	2.3	[1.14-4.8]	**0.021****			
overweight	4.2	[1.29-13.7]	**0.017****			
CD4 Difference						
Below 150	Ref.					
150 and above	11.6	[4.04-33.4]	**<0.0001*****	27.1	[6.0-120.4]	**<0.0001*****
Hepatitis B						
negative	Ref.					
Positive	0.3	[0.08-1.0]	**0.058***	0.03	[0.003-0.3	**0.001****
Health Facility						
health centre	Ref.					
hospital	1.9	[0.99-3.8]	**0.054***			

Note: Logistic Regression Analysis was used to obtain Odds ratios and p values.
Exponentiated coefficients; 95% confidence intervals in brackets
* $p < 0.1$, ** $p < 0.05$, *** $p < 0.001$

Table 4.8 shows a bivariate analysis of CRP and a CD4 cell count of below 200 cells/μL. We did not find a correlation between a CRP, using a laboratory threshold of 10mg/L, with the CD4 cell counts below 200 cells/μL.

Table 4.8: Results for associations of CRPs and CD4 cell count at 12 months (<200/200+). CRPs (categorized as <10/ 10+) using Pearson Chi-Square

CRP	CD4 at 12mo <200	CD4 at 12mo >200	Total
<10mg/L	45 83.33%	239 85.97%	284 85.54%
>10mg/L	9 16.67%	39 14.03%	48 14.46%
Total	54 100%	278 100%	332 100%

Pearson Chi-Square (l)=0.2544 p= 0.614

CHAPTER FIVE
DISCUSSION

This study found that 40 % of patients had a baseline CD4 cell count below 200 cells/μL with a mean baseline CD4 cell count of 282 cells/μL (SD ±192). After 12 months of cART, 17% had a CD4 cell count still below 200 cells/μL, 38 % of patients those who had a baseline CD4 cell count <200 cells/μL failed to increase beyond 200 cells/μL and 58% had an absolute CD4 cell count change of less than 150 cells/μL. The CD4 cell count mean at 12 months was 436 cells/μL (SD ±237). While HAART is effective in reducing viral load, this study found that HAART does not sufficiently restore the immunological status at early time point of treatment in between 38 % to 58% of cases . Even though a precise measurement or threshold for sufficient immune response has not been agreed upon, the findings of this study do show that the proportion of immunovirological discordance is significant. The threshold of 150 cells/μL increase in 12 months is in keeping with the expected normal kinetics of the CD4 cell increase shown by Autran et al [3][45]. The fact that this threshold correlated well with the threshold of 200 cells gives more credit to these findings and suggests that the use of 150 cells/μL increment threshold is reasonable. The 38%-58% immunovirological discordant rate found in this study is consistent with other studies [12]. The prevalence found could become even larger when survival bias in this cross-section study is accounted for, especially that mortality before this 12th month time point is expected to be higher in individuals with a lower baseline CD4 cell count.

This study was conducted when the commencement criteria for ART was a CD4 cell count of <350 cells/μL and a number of participants were started with CD4 cell count above 500 cells/μL in the option B plus category or indeed based on other reasons other than the CD4 cell count. Subsequently, the mean baseline CD4 cell count was much higher than the one found in earlier studies when the commencement criterion was at 250cells/μL. However, CD4 bands above shows that about 40% of patients still present to care in a severe immunocompromised state and that about 10% of patients still come to care with a CD4 cell count <50 cells/μL. This is an unfortunate results to have in this era of option B plus and test and treat.

The proportion of CD4 cell count increase of < 150 cells/μL of 58% found in a sample with 40 % of participants with CD4 cell count above 200 cells/μL and a maximum baseline of 1100 cells/μL suggests that while the nadir CD4 cell count is the strongest predictor of discordance, insufficient immune response does occur even when the initiation CD4 cell count is much higher than the classical 200 cells/μL. In this regard, Okulicz JF et al in study published in JAMA in 2015 showed that a delay of ART commencement of 12 months from the estimated day of seroconversion in the early periods of HIV infection does affect immune normalization among patients with CD4 cell count of above 500cells/μL [46]. Events in the acute and early stage of HIV infection seem to play a significant role in immune recovery. It is thought that the initial CD4 response in early HIV infection is in a triphasic manner comprising of an initial rapid and profound loss of peripheral-blood CD4 cells followed by a spontaneous but transient recovery in CD4 cell counts after which there is a progressive decline in CD4 counts. Le T et al found that the initial 4 months from the day of HIV infection during which a transient and spontaneous CD4 recovery occurs is the best time to start ART. Initiation of ART during this 4 months window after the infection of HIV was found to be associated with an enhanced likelihood of recovery of CD4+ counts [47]. Therefore, the above findings and literature suggests that the proposed 'test and treat' recommendation in the 2016 Zambian guideline for the treatment and prevention of HIV may not be sufficient to prevent insufficient immune recovery. While we acknowledge that 'test and treat' is crucial in controlling the HIV pandemic, we should be thinking of prompt diagnosis and treatment immediately after HIV infection, and if possible during the acute HIV phase. The time of testing and treatment after HIV infection seem to be very important in order to preserve a normal immune system in HIV disease.

The high rates of discordance found in this study suggest that a perfect solution to HIV infection is a cure or treatment that should have the capacity to rapidly restore the immune system. This feature is unfortunately missing in the current therapy (HAART). Such a therapy should probably employ immunological methods tackling the postulated mechanisms of discordance stated in the literature review.

The relation between Body Mass Index (BMI) or nutrition and immune restoration is of special interest. While nutritional supplementation is not effective in improving immune restoration on ART [42].Baseline BMI was found to be a predictor of immune

restoration in this study and other studies [48, 49]. We found that overweight (BMI> 24 kg/m^2) was significantly predictive of good immune recovery compared to both normal BMI and underweight. A large multinational study done in North America showed similar findings. It further showed that the optimal BMI for immune recovery is around 30kg/m^2 [50]. The exact mechanism for this process is not known but the adipose tissue is known to have both immunological and endocrine functions which could influence CD4 replacement. Knowing exact mechanism for this process could give opportunities for possible therapy for suboptimal immune recovery. The overall benefit of this positive relationship between overweight and CD4 recovery in HIV patient is still unclear, in view of the known deleterious effect of overweight and obesity in terms of non-communicable diseases.

Hepatitis B viral infection has profound effect on the immune system and its progression in the body is really dependent on the immune system. Idoko J et al in a study done in Nigeria found similar findings like ours while investigation the impact of hepatitis B virus infection on human immunodeficiency virus response to antiretroviral therapy [51]. This study showed that there is an inverse relationship between the HBV DNA viral load and the CD4 cell count. It further found that HBeAg+ status is a predictor of poor CD4 cell count even in the absence of HIV infection. General, patients with chronic HBV infection are known to have a lower CD4 cell count compared to normal subject and those with HCV. It is known that there is an insufficient cellular immune response which is critical for the ineffective virus clearance and liver damage in chronic hepatitis B [52]. You J et al found that there is a substantial linear dose-response relationship and strong independent predictive effect of HBV viral load on CD3+ and CD4+subpopulations which could suggests the possibility of a causal relationship between them, and indicates the importance of HBV viral load in the pathogenesis of T cell hyporesponsiveness in these patients [53]. CD4 cell count level also has a prognostic value in predicting the clinical course of HBV infection, and the determination of the therapeutic response [54]. The relation between HIV, HBV and the immunity is a subject that requires further studies but current research shows that HBV does worsen HIV and HIV worsens HBV infection, and both infections negatively affects the CD4 cell count.

The inability of this study to show a correlation between immunovirological discordance with inflammatory markers CRP and TNF is peculiar with regards to the

probable pathogenesis of immunovirological discordance and is not in keeping with previous studies.

This study did not show that the immunovirological discordant patients had a worse WHO clinical stage at 12 months compared to those with appropriate response. This is a finding that has been inconsistent in previous studies. For instance, in a multicentre study done in Germany by Zoufaly et al comprising of 14433 patients [14], Immunovirological discordance and prior AIDS diagnosis were independently associated with new AIDS events (hazard ratio, 3.10; 95% CI, 1.09–8.82; P 5 .03). Takuva et al in a cohort study done in South Africa comprising 4129 patients on ART for 6 months with suppressed viral load; compared to those with an absolute CD4 cell count increase of >200 cells/μL, patients with a CD4 cell count increase of 0-49 cells/μL were more likely to develop AIDS [aHR:2.0 CI: 1.2-2.4] and those with a CD4 cell count increase of 50-199 cells/μL were also more likely to develop AIDS [aHR: 1.5 CI: 0.9-2.2]. Again, the survival bias in this cross sectional study would explain this finding since immune non-responders are at a higher risk of AIDS or death.

The predilection of immunovirological discordance to the male sex is a consistent finding in previous studies [13]. The cause for this finding is difficulty to explain. It is possible that this is due to a constitutive nature of the different sexes. While molecular and genetic reasons could play a major role here, social and clinical confounders could be at play as well. Generally, females are thought to have better health seeking behavior and are healthier than males.

In view of the known high mortality in the early times of ART and this huge finding of immunovirological discordance, it is necessary that more work continues to be done in the area of prophylaxis against opportunistic infections while these patients remain immunosuppressed.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In patients with viral load suppression, 17% remained severely immunosuppressed and between 38% and 58 % had immunovirological discordance despite effective ART for 12 months. This study found that the baseline CD4 cell count, male sex, low BMI, HBV infection and baseline WHO clinical stage III/IV were predictors of immunovirological discordance. Immunovirological discordance was not associated with a worse WHO stage at 12 months of ART in this study nor with inflammatory markers.

6.2 Recommendations

i. The findings of this study support the test and treat recommendation since patients who are started on ART with low CD4 cell count may never restore their immune system.
ii. To strengthen viral load monitoring in places where it is not available and to adopt the use of POC technologies for viral load test.
iii. Adopt the use novel HIV testing technologies such as self-testing so that patients test closer to the time of infection when the immune system is still intact.
iv. Further studies on the influence of HBV infection the immune system in HIV patients.
v. Further studies in the immunology of immunovirological discordance and possible remedies.

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APPENDIX

APPENDIX I: Immunovirological Discordance Data Collection Tool

1	**FACILITY CODE PATIENT #..... ART #.....** **DATE……. RESIDENCE……..**	
2	**Sex: □ Male □ Female □ DOB: / /**	
3	**Education level: □ Primary** **□ Grade 8-9** **□ Grade 10-12** **□ Tertiary**	**Employment status: □ Yes □formal □informal** **□ No** **Salary/month: □ <K1,000 □ K1,000-5,000** **□5,000-10000 □ K10,000-15,000** **□ >K15,000**
4	**Other comorbidities:** **□ HTN □ DM □ CKD □ SCD □ Asthma** **□ CLD □ Hip B □ Other: ……** **Other drugs besides ARVs** **Septrin □ Yes □ No** **Corticosteroids □ Yes □ No** **Chloramphenicol □ Yes □ No** **Chemotherapy □ Yes □ No** **Others ……**	**Smoking □ Yes □ No** **Substance abuse □ Yes □ No** **Substance name:** • **Ever took alcohol? □ Yes □ No** • **Currently taking alcohol? □ Yes □ No** • **Quantity: _____** • **Type: _____**
5	**Date diagnosed HIV: ____ /_____ /____** **Baseline Hb……..WCC…….. RPR……..** **Baseline CD4 …….HBsAg……BMI……** **CD4 profile Date** **1) _____ ____ /_____ /____** **2) _____ ____ /_____ /____** **3) _____ ____ /_____ /____**	**Date started ART: ____ /_____ /____** **ART Regimen: □ TDF/ XTC/ EFV** **□TDF/ XTC/ NVP** **□ABC/ 3TC/ EFV** **□ABC/3TC/ NVP** **□AZT/ 3TC / NVP** **□AZT/3TC/ EFV** **□TDF/3TC/ LPV/r** **□others ……**
6	**ART history:** ***Reported adherence □ Good □ Poor** **WHO Staging at initiation …… Incident of TB in last 12 months** **Others OIs** **……** **…….** **…….**	
7	**Viral Load at 12 months** ☐ **BMI at 12 months** ☐ **HCV antibodies** ☐	**CD 4 cell count at 12 month** ☐ **WHO staging at 12 months** ☐ **OIs……** **……** **……..**

APPENDIX II: WHO Clinical Staging

WHO Clinical Staging of HIV/AIDS for Adults and Adolescents	
Clinical Stage	Clinical Conditions or Symptoms
Primary HIV Infection	Asymptomatic Acute retroviral syndrome
Clinical Stage 1	Asymptomatic Persistent generalized lymphadenopathy
Clinical Stage 2	Moderate unexplained weight loss (<10% of presumed or measured body weight) Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis) Herpes zoster Angular cheilitis Recurrent oral ulceration Papular pruritic eruptions Seborrheic dermatitis Fungal nail infections
Clinical Stage 3	Unexplained severe weight loss (>10% of presumed or measured body weight) Unexplained chronic diarrhea for >1 month Unexplained persistent fever for >1 month (>37.6°C, intermittent or constant) Persistent oral candidiasis (thrush) Oral hairy leukoplakia Pulmonary tuberculosis (current) Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia) Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis Unexplained anemia (hemoglobin <8 g/dL) Neutropenia (neutrophils <500 cells/µL) Chronic thrombocytopenia (platelets <50,000 cells/µL)
Clinical Stage 4	HIV wasting syndrome, *Pneumocystis* pneumonia Recurrent severe bacterial pneumonia Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site) Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs) Extrapulmonary tuberculosis Kaposi sarcoma Cytomegalovirus infection (retinitis or infection of other organs) Central nervous system toxoplasmosis HIV encephalopathy Cryptococcosis, extrapulmonary (including meningitis) Disseminated nontuberculosis mycobacteria infection Progressive multifocal leukoencephalopathy Candida of the trachea, bronchi, or lungs Chronic cryptosporidiosis (with diarrhea)

	Chronic isosporiasis Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis) Recurrent nontyphoidal *Salmonella* bacteremia Lymphoma (cerebral or B-cell non-Hodgkin) Invasive cervical carcinoma Atypical disseminated leishmaniasis Symptomatic HIV-associated nephropathy Symptomatic HIV-associated cardiomyopathy Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

APPENDIX III

Participant Information Sheet for the Prevalence, Predictors and HIV Disease Progression in Immune-Virological Discordant HIV Patients at 12 Months of First Line cART in Zambia Study

Introduction

My name is…….. I am acting on behalf of Dr Suilanji Sivile, a master of medicine student school of medicine at the University Of Zambia at UTH, kindly request your participation in the above mentioned study. This study is in partial fulfilment for the award of Master of Medicine in Internal Medicine.

I would like to explain to you the purpose of the study and then you can decide whether you would like to take part. If you agree to take part in this study, you will be asked to sign this consent form in the presence of a witness.

Nature and purpose of the study

This is the study being conducted to determine the burden of immune-virological discordance, its predictors and outcome in Zambia.

Procedure of the study

If you agree to participate in this study, we will obtain information using a data entry sheet. Your contact details will be required. Blood samples will be collected for laboratory test for; CD4 cell count and Viral Load.

Possible risks and discomforts

You may experience needle stick pain from the collection of blood.

Possible benefits

You will not accrue any direct benefit from this study. However, the results of your CD4 cell count and viral load test will assist in your HIV care.

Confidentiality

All information obtained is strictly confidential. Data that will be collected, analysed and reported on will not include your name and therefore cannot be traced to you.

Consent

Your participation is strictly voluntary. You will not suffer any consequences if you decide not to participate in this study. You may also withdraw from the study at any time for any reason without consequences to you.

Thank you for considering participation in this study .If you have any questions, concerns and clarification, please contact Dr Suilanji Sivile or The university of Zambia Research ethics committee on the following addresses;

Dr Suilanji Sivile

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APPENDIX IV:

CONSENT FORM

I, _____ hereby confirm that the nature of this clinical study has been sufficiently explained to me. I am aware that my personal details will be kept confidential and I understand that I may voluntarily, at any point, withdraw my participation without suffering any consequences. I have been given sufficient time to ask questions and seek clarifications, and of my own free will declare my participation in this research.

I have received a signed copy of this agreement.

_____ _____ __

_____

Name of Participant (Print) Participant (Signature or thumbprint)

Date

_____ _____

_____

Witness (Print Name) Witness (Signature)

Date