PREVALENCE AND PREDICTORS OF IMMUNOLOGIC FAILURE AMONG HIV PATIENTS ON HAART IN SOUTHERN ETHIOPIA

by

KESETEBIRHAN DELELE YIRDAW

submitted in accordance with the requirements

for the degree of

MASTER OF PUBLIC HEALTH

at the

## UNIVERSITY OF SOUTH AFRICA

SUPERVISOR: PROFESSOR SP HATTINGH

November 2014

Student number: 4836-405-3

DECLARATION

I declare that **PREVALENCE AND PREDICTORS OF IMMUNOLOGIC FAILURE AMONG HIV PATIENTS ON HAART IN SOUTHERN ETHIOPIA** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.
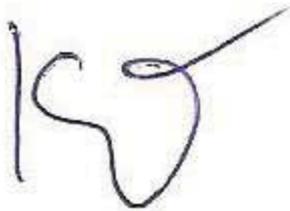
Kesetebirhan Delele   November, 2014

Full name and signature   **Date**

ACKNOWLEDGEMENTS

My heartfelt gratitude goes to my parents who laid down the foundations for my life that have led to its being the way it is now, and my lovely wife, Fitih Zelalem, who has been considerate, patient and supportive throughout my education even at times when it was necessary for me to be absent from the family so that I could succeed, and our baby girl, Gelila, whom I am grateful to have and dedicate this dissertation to.

In addition, I would like to thank the following people who contributed to the completion of this current research:

- Special thanks to my supervisor, Professor SP Hattingh, for her insightful guidance and unrelenting support throughout.
- My gratitude also goes to the dedicated team of health care workers in Butajira and Hosanna Hospitals who provide care to HIV patients who made this study possible.
- Heartfelt thanks to Kabtamu Tolossie who guided me with statistical aspects of the study, Mrs. Rina Coetzer for professionally formatting the dissertation, Emebet Haile who did the editing.

Last but not least, I would like to thank Dr. Zewdu Gashu, Ato Kemal Woliyu, and Ato Tekalign Mekuria, all UNISA students, who provided me with technical information that made life at UNISA easy for me.

PREVALENCE AND PREDICTORS OF IMMUNOLOGIC FAILURE AMONG HIV PATIENTS ON HAART IN SOUTHERN ETHIOPIA

STUDENT NUMBER: 4836-405-3
STUDENT: DR KESETEBIRHAN DELELE YIRDAW
DEGREE: MASTER OF PUBLIC HEALTH
DEPARTMENT: HEALTH STUDIES, UNIVERSITY OF SOUTH AFRICA
SUPERVISOR: PROFFESOR SP HATTINGH

ABSTRACT

Immunologic monitoring is part of the standard care for patients on antiretroviral treatment. Yet, little is known about the routine implementation of immunologic monitoring in Ethiopia. This study assessed the pattern of immunologic monitoring, immunologic response, level of immunologic treatment failure and factors related to it among patients on antiretroviral therapy in selected hospitals in southern Ethiopia. A retrospective longitudinal analytic study was conducted using documents of patients started on antiretroviral therapy.

A total of 1,321 documents of patients reviewed revealed timely immunologic monitoring were inadequate. Despite overall adequate immunologic response, the prevalence of immunologic failure was 11.5% (n=147). Having WHO Stage III/IV of the disease and a higher CD4 (cluster differentiation 4) cell count at baseline were identified as risks for immunologic failure.

These findings highlight the magnitude of the problem of immunologic failure. Prioritizing monitoring for high risk patients may help in effective utilisation of meager resources.

Key concepts: Human Immunodeficiency Virus; CD4 cell; highly active antiretroviral therapy (HAART); adherence; immunologic treatment failure; antiretroviral treatment failure; prevalence; patient; predict.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXURES

ABBREVATIONS

ABC	Abacavir
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
AZT	Zidovudine
CD4	Cluster of differentiation 4
D4T	Stavudine
DNA	Deoxyribonucleic acid
EFV	Efavirenz
FACS	Fluorescent Activated Cell Sorter
FHAPCO	Federal HIV/AIDS Prevention and Control Office
FMOH	Federal Ministry of Health
FTC	Emitricitabine
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
INRUD-IAA	International Network for the Rational Use of Drugs Initiative on Adherence to Antiretrovirals
KHB	Shanghai Kehua Bio-Engineering Co. Ltd
MDG	Millennium Development Goal
MSF	Médecins Sans Frontières
NGO	Non-Governmental Organisation
NVP	Nevirapine
LPV/r	Ritonavir boosted lopinavir
PEPFAR	President's Emergency Plan for AIDS Relief
RNA	Ribonucleic acid
SNNPR	Southern Nations Nationalities and Peoples' Region
TB	Tuberculosis
TDF	Tenofovir
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNISA	University of South Africa
USA	United States of America
WHO	World Health Organization
3TC	Lamivudine

CHAPTER 1

ORIENTATION TO THE STUDY

1.1   INTRODUCTION

The Human Immunodeficiency Virus (HIV) is a retrovirus that infects people across all ages and from all walks of life (Zuckerman, Banatvala, Griffiths, Schoub & Mortimer 2009:899). The most common means of transmission among adults is unprotected sexual intercourse, while among children mother to child transmission accounts for much of it (Zuckerman et al 2009:899).

The Joint United Nations Programme on HIV/AIDS (Acquired Immunodeficiency Deficiency Syndrome) (UNAIDS) (2014:A35) further states that, in 2013, 35 million people had already been infected with the HIV virus worldwide. The impact of the virus is being mitigated by the use of highly active antiretroviral therapy also referred to as HAART. Access to this therapy has been increasing and this is reflected in the number of people taking antiretroviral medication. By 2013, the number of people on HAART had already reached 12.9 million globally while it increased to 8.4 million in sub-Saharan Africa (UNAIDS 2014:A67, A69).

In Ethiopia, access to life saving antiretroviral medication, also referred to by its acronym, ARVs started on a massive scale in 2005, and by 2012 it had reached some 270,000 people according to Federal Ministry of Health (FMOH) and Federal HIV AIDS Prevention and Control Office (FHAPCO & FMOH) (2012:33) and UNAIDS (2013b:A85, A91). Treatment has been successful, as demonstrated by the improvement in the survival rate in many settings in Ethiopia (Alemu & San Sebastian 2010:online; Tsegaye &Worku2012:105). This has changed the course of the epidemic and made HIV a chronic manageable disease.

As important as it is to get people who need the treatment to be on HAART, it is even more important to ensure that patients adhere to the treatment so that they benefit from it in the long run as well. This is because poor adherence to treatment is associated with increased risk of drug resistance and thus increased risk of treatment failure (El-Khatib,

Katzenstein, Marrone, Laher & Mohapi 2011:online; Jaka, Mshana, Liwa, Peck & Kalluvya 2009:7). This statement is supported by Beyene, Gedif, Gebre-Mariam and Engidawork (2009:1010) who further state that achieving adherence to treatment of ≥95% is not always possible for all people on HAART because of, for example, lack of family support, unemployment, alcohol intake, poverty and the presence of other conditions and diseases that could limit insight and knowledge. In this regard, Keiser, Tweya, Braitstein, Dabis, MacPhail and Boulle (2010:254) report that the longer a person is on a failing HAART regimen, the higher the mortality risk. For this reason, monitoring HAART treatment response, especially in the immunological and virological, aspects which are challenging issues in many resource limited chronic HIV care settings, becomes very important. In addition, detection and management of treatment failure could be compromised if the monitoring is not up to standard (Azzoni, Foulkes, Liu, Li, Johnson, Smith, Kamarulzaman, Montaner, Mounzer, Saag, Cahn, Cesar, Krolewiecki, Sanne & Montaner 2012:online; Bélec & Bonn 2011:1255)

This study, therefore, intends to investigate the level of immunologic monitoring, the level of treatment response to antiretroviral medication as well as the prevalence of treatment failure and its determinants in selected areas in Ethiopia with a focus on immunologic criteria.

1.2   BACKGROUND TO THE RESEARCH PROBLEM

In this section, a brief background of HIV and HAART in the world with specific reference to Ethiopia is given.

1.2.1   HIV infection, disease causation, and HAART

The HIV is a viral infection from the family *Retroviridae* and subfamily of *Lentivirinae*. Among adults, it is predominantly spread by unprotected sexual intercourse among heterosexuals. Other important means of transmission include unprotected sexual intercourse between men, the sharing of sharp materials such as needles, and exposure of mucous membranes or broken skin to bodily fluids for example blood and pus. Among children, mother to child transmission (during pregnancy, delivery or breast feeding) accounts for most of the transmission (Zuckerman et al 2009:899).

The path to HIV disease progression is viral replication in great numbers. This is inevitably accompanied by destruction of immune cells (especially cluster of differentiation 4 cells, also known as CD4 cells) that weakens the body's defence mechanisms and gradually leads to an escalation of susceptibility to various life threatening opportunistic infections (Longo, Fauci, Kasper, Hauser, Jameson & Loscalzo 2012:1510). The stage of the disease whereby an HIV infected person experiences serious opportunistic infections is called Acquired Immunodeficiency Syndrome (AIDS). It takes an average of 10-15 years for the disease to reach this stage after infection. Once in this stage, even the short term mortality is extremely high (Longo et al 2012:1500; Zuckerman et al 2009:910).

To mitigate the impact of this disease, the use of HAART is warranted (Longo et al 2012:1580). Antiretroviral therapy works by suppressing viral replication which leads to halting and reversing of the destruction of immune blood cells. With this therapy, the immune system starts to regain its ground and the frequency of opportunistic infections declines (Longo et al 2012:1568). With further restoration of the immune system, it is possible for affected individuals to regain their health status depending on other factors like the timing of treatment initiation, presence of co-morbid medical conditions, nutrition, psychosocial support, life style, and the quality of care. Achieving meaningful control of the pandemic requires access to life saving HAART for all infected people who need the treatment (Department of Health and Human Services [USA] 2013:K-1; Longo et al 2012:1568).

1.2.2 HIV and AIDS globally

The number of people affected globally had reached 35.3 million in 2012. All nations are affected but the most affected are the least developed and developing nations from sub-Saharan Africa and South Eastern Asia. By 2012, 9.7 million people had begun receiving antiretroviral therapy in sub-Saharan Africa alone (UNAIDS 2012:19, 24). The global AIDS related mortality reached a peak in 2005 at 2.3 million yearly deaths. With widespread access to HAART, this number had decreased to 1.5 million by 2013 (UNAIDS 2014:A95).

1.2.3 HIV and AIDS in sub-Saharan Africa

Sub-Saharan Africa is the worst affected by the HIV/AIDS pandemic, accounting for two thirds of all HIV-infected individuals in the world. From the latest information, it is indicated that 70% of new HIV infection in the world for 2012 also came from the same region (UNAIDS 2013b:A24, A27). Access to HAART has gradually increased, and by 2012, 68% of all HIV infected people in the region who needed the treatment urgently had access to it. For this reason, mortality had also been decreasing, having declined to 1.1 million by 2013 from a peak of 1.8 million recorded in 2005 (UNAIDS 2013b:A85; UNAIDS 2014:A56).

1.2.4 HIV and AIDS in Ethiopia

The prevalence of HIV in Ethiopia among adults age 15-49 is 1.2% (1.1-1.4%) according to the latest estimates (UNAIDS 2014:A9). The estimated number of all people with HIV was around 790,000 (720,000-890,000) for 2013. In the same year, new HIV infections were estimated to be 21,000 (15,000-30,000). This number declined from 51,000 in 2005 (UNAIDS 2014:A38). Access to HAART had then been ensured through the President's Emergency Plan for AIDS Relief (PEPFAR) and Global Fund programmes utilized throughout the country that started in 2005. This resulted in the fact that 68% of the 400,000 people who need the treatment commenced with HAART (UNAIDS 2013b:A85). Various studies in Ethiopia showed this roll out of treatment improved survival of people with HIV/AIDS (Alemu & San Sebastian 2010:online; Mulissa, Jerene & Lindtjørn 2010:online; Tsegaye & Worku2012:105; UNAIDS 2013b:A80).

1.2.5 The goal of HAART and adherence

Achieving the goal of HAART, that is sustained viral suppression to an undetectable level, requires a high degree of adherence to antiretroviral medications (≥95%). Unless this level of adherence is maintained throughout the therapy, treatment will not be effective. In order to make sure that patients are adherent to treatment, it should be monitored by responsible professionals and caregivers (FHAPCO & FMOH 2008a:82).

There are various types of adherence to HAART medication that need monitoring under ideal conditions. These include adherence to medication refill, adherence to timing in taking the medication, adherence to the prescribed dosing of medication, and so on

(WHO 2003:118). There are various tools that can be used to monitor adherence: which may include self-report, pill count, review of treatment or drug dispensary records, electronic devices, and/or biochemical assays that measure the blood levels of ARV drugs. In Ethiopia though, only the first two are available in routine clinical care settings (FHAPCO & FMOH 2008a:130; Turner 2002:S143).

A number of studies conducted in Ethiopia have demonstrated variable levels of adherence to HAART in various clinical settings. Azmeraw and Wasie (2012:120) report that 80% of patients showed an optimal adherence (at ≥95%) among 340 children in two referral hospitals in Northwest Ethiopia. Among adult patients, Giday and Shiferaw (2010:191) report on the level of adherence of ≥95% as being 88% for patients investigated for one month. Kebede and Wabe (2012:67) report a 95% adherence for HAART among 24 tuberculosis (TB)/HIV co-infected patients in a hospital in Southwest Ethiopia. In addition, in a study conducted in a university hospital in Southwest Ethiopia which evaluated the timing of ARV intake in addition to adherence to doses, 303 of the patients (95%) were adherent based on dose adherence during the week before the actual interview. The same study also indicated the adherence including timing and food intake was at 72.4% (Tiyou, Belachew, Alemseged & Biadgilign 2010:online). All the above studies used self-report as the measure of adherence. A different set of studies used other means to evaluate adherence besides self-report. One such study is a multi-country survey of adherence in East Africa, in which Ethiopia was one of the countries. This study revealed that the percentages of patients with optimal adherence to antiretroviral medications based on self-reporting adherence, adherence to appointment date, and pill count were 94%, 86%, and 76% respectively (Chalker, Andualem, Gitau, Ntaganira, Obua, Tadeg, Waako, Ross-Degnan & International Network for the Rational Use of Drugs Initiative on Adherence to Antiretrovirals (INRUD-IAA) 2010:online).

The above reported discrepancy between different measures of adherence is noted in other studies as well. Beyene et al (2009:1010) report optimal adherence among 93% of the study participants using a 15-day self-report, while it was 88% when using an unannounced pill count. This shows that self-report as a measure of adherence can be unreliable for some patients as they tend to overestimate adherence due to social desirability or recall bias. A similar problem exists when using pill count as a measure of adherence: conscious of being monitored, patients may dispose of pills before showing

up for the clinic visit in order to make it look like they took it (Department of Health and Human Services 2013:K-2; Turner 2002:S144; WHO 2003:118).

Authors in nearly all the referred to research studies mentioned above (Beyene et al 2009:1010; Department of Health and Human Services 2013:K-2; Turner 2002:S144; WHO 2003:118) uniformly agree that the means or tools used in routine practice to assess adherence in Ethiopia (self-report and pill count) are not sensitive indicators for all types of adherence to ARV treatment. In practical terms, this means that a lot more patients on HAART have poor or suboptimal adherence than reported in published literature and that identifying all these patients is going to be a persistent challenge.

1.2.6 Monitoring HAART treatment response

If left unattended, the consequence of poor adherence to HAART is drug resistance and treatment failure (El-Khatib et al 2011:online). Therefore, the best thing to do besides assessing and promoting adherence to therapy is to monitor response to HAART and identify patients who have treatment failure as soon as possible. This may also involve the changing of the current antiretroviral treatment regimen to second line regimen. Early intervention will preserve the effectiveness of second line ARVs and prevents transmission of drug resistant strains to others (Jaka et al 2009:6).

The WHO (2010a:48) refers to three means used to monitor response to HAART, namely clinically, immunologically, and virologically. The WHO (2010a:49) is of the opinion that, successful treatment means suppressed viral replication, which is measured by viral load monitoring. Suppressed viral replication eventually leads to increase in CD4 cell count; and this is the basis for immunological monitoring. Tsegaye and Worku (2012:105) state that after a period of time on HAART, the amount of CD4 cells gained is somewhat predictable: an increase by roughly 50 to 150 cells is expected after six months on treatment, after which the rate of increase decreases slightly. The increase in CD4 cell count eventually leads to improvement of the immune status and this is reflected by decrease in the occurrence of opportunistic infections. This forms the basis for clinical monitoring: the better the immunity, the lower the probability of a person experiencing opportunistic infections for example tuberculosis, oral candidiasis, toxoplasmosis, etc. The gold standard to monitor HAART treatment response is viral load measurement (FHAPCO & FMOH 2008a:159). But since this is not available in

routine care in Ethiopia, clinical and immunologic criteria are mostly used. Clinical monitoring is recommended at every visit, while immunologic monitoring is recommended at baseline and every six months for the first year and every year after that, plus whenever the clinicians deem it necessary (FHAPCO & FMOH 2008a:159). The same tools are used to identify treatment failure and effect the decision to change the current regimen the patient is on.

In resource limited settings where viral load is not available for routine clinical practice, the WHO (2010a:48) recommends using clinical and immunologic criteria. However, immunologic criteria have poor sensitivity and positive predictive value in picking virological failure. However, they have good negative predictive value and thus can be used to rule out treatment failure (Keiser, MacPhail, Boulle, Wood, Schechter, Dabis, Sprinz &Egger 2009:1221; Rawizza, Chaplin, Meloni, Eisen, Rao, Sankale´, Dieng-Sarr, Agbaji, Onwujekwe, Gashau, Nkado, Ekong, Okonkwo, Murphy & Kanki 2011:1285; Reynolds, Nakigozi, Newell, Ndyanabo, Galiwongo, Boaz, Quinn, Gray, Wawer & Serwadda 2009:697). Clinical criteria are the least effective in terms of accuracy as well as in terms of detecting treatment failure in time to remedy it. For this reason, the WHO (2010a:48) recommends the utilisation of immunologic criteria supplemented by targeted viral load testing when available in these settings to lessen any unnecessary changing of the regimen of treatment.

1.3   STATEMENT OF THE RESEARCH PROBLEM

A number of studies in sub-Saharan Africa have assessed the prevalence of immunologic failure and have come up with variable results. El-Khatib et al (2011:online) report the prevalence of immunologic failure to be 19% among 456 patients followed for a median period of 15 months in a South African clinic. In Uganda, after a median follow-up time of 22 months, immunologic failure was 11% among 1,133 patients (Reynolds et al 2009:697). In Nigeria, Rawizza et al (2011:1286) reported that the prevalence of immunologic treatment failure after a median follow-up of 33.2 months was 32.2% among 9,690 patients. In another study in Nigeria, though, the prevalence of first line HAART failure was reported to be very low, 1.19% among 9,449 patients during the study period (Eze & Uyagu 2009:46). Jaka et al (2009:6) report that, in one clinic in Tanzania, the prevalence of immunologic treatment failure was 17.1% among 362

patients followed for a mean duration of 29 months. In short, these studies show that different settings have different levels of immunologic failures.

For Ethiopia, data on immunologic failure among patients on HAART is available only for paediatric patients. Bacha, Tilahun and Worku (2012:online) report immunologic failure of 8.2% in selected hospitals in Addis Ababa among 1,186 children with a mean age of six years and mean follow-up period of 37 months. In another study in Jimma university in Ethiopia, among a smaller cohort of paediatric patients (mean age six years, mean follow-up duration 13.7 months), the percentage of immunologic failure was 11.5% among 96 patients (Workneh, Girma & Woldie 2009:78). Not much similar data could be found for adult patients despite an extensive search. However, one study was reported that the prevalence of patients on second line HAART in selected health facilities across Ethiopia, among 7,451 clients actively on follow-up at 24 months, was 2.13% (Assefa, Kiflie, Tesfaye, Haile-Mariam, Kloos, Edwin, Laga & Van Damme 2011:online). This is comparable to what is reported by WHO (2009:53), but much higher than that in studies reported from 48 Médecins Sans Frontières (MSF) supported programmes in resource limited countries (Pujades-Rodriguez, O'Brien, Humblet & Calmy 2008:1307).That study showed change to second-line regimens at a rate of 4.1 per 1,000 person-years after 20 months of follow-up among 48,000 patients.

Because of difficulties in monitoring treatment response in many settings and difficulty in the utilisation of available resources by health care workers, the actual prevalence of treatment failure is believed to be higher (Azzoni et al 2012:online; Bélec & Bonn 2011:1251). Only one publication could be found that has assessed adherence to immunologic monitoring of children with HIV in Ethiopia (Berhan 2011b:205) despite a wide literature search for adult patients in Ethiopia indicating the level of adherence to immunologic monitoring guidelines. Furthermore, the researcher could not find any study indicating the prevalence and determinants of patients with immunological failure that may benefit targeted viral load monitoring in the Ethiopian care setting. Information obtained from this study is vital for clinicians that manage patients at facility level and for decision makers at health system level for the scaling up and the proper utilisation of immunological and viral load tests.

1.4   DEFINITIONS OF KEY CONCEPTS

In this section the researcher outlines the definitions, descriptions and application of the terms related to this study.

1.4.1   Human Immunodeficiency virus

According to the *Macmillan Dictionary Online* (2013, Sv "HIV"), the HIV is defined as a virus that causes AIDS. Longo et al (2012:1506) define HIV more technically as a retrovirus which has two major types, HIV-1 and HIV-2. It infects cells of the immune system which leads to progressive damage to the immune system, which leads to diminished defence mechanisms and increased susceptibility to various infections that lead to ill health and death if left unattended (Zuckerman et al 2009:897, 904). For the purpose of this study, HIV infection refers to that by HIV-1 because, in the Ethiopian context, HIV-1 is the predominant type of the virus known to infect people (Longo et al 2012:1506).

1.4.2   Describing CD4 cell

The CD4 is a special type of immune cell referred to as a *lymphocyte* with a marker on its surface called cluster of differentiation or CD4, that which is responsible for coordinating the defence mechanisms of the body (FHAPCO & FMOH 2008a:36). The term 'CD4 cell count' refers to the number of CD4 cells within a micro litre of blood. A machine called FACS (Fluorescent Activated Cell Sorter) Count is used to determine CD4 cell count. In the Ethiopian context, the normal value in HIV uninfected people ranges between 600-1,300 per micro litre, with a mean value closer to the lower end (FHAPCO & FMOH 2008a:38). For HIV infected patients, this number decreases significantly as the disease progresses to a more advanced stage (Longo et al 2012:1519).

1.4.3   Highly active antiretroviral therapy (HAART)

The *Oxford Dictionary Online* (2013, Sv "HAART") defines the term HAART as a drug therapy (or regimen) for HIV infection containing at least three antiretroviral drugs. The WHO (2013:15) refers to HAART as a combination of three or more antiretroviral drugs given for life in order to effect the sustained suppression of the HIV virus. The combination of antiretroviral medications used in Ethiopia as first line therapy includes

one of tenofovir, zidovudine, or stavudine, plus lamivudine, plus either nevirapine or efavirenz (FHAPCO & FMOH 2008a:122).

1.4.4 Adherence

According to the *Macmillan Dictionary Online* (2013, Sv "adherence"), the term 'adherence' means "accepting and following a prescribed programme". A patient or client is said to have good adherence for antiretroviral therapy if one follows the treatment regimen as prescribed in terms of dosage, timing of medication intake, and timing of medication pick up from the treatment facility (Department of Health and Human Services 2013:122). In this study, adherence to antiretroviral therapy was assessed by the timing of medication pick up from the treating facility (FHAPCO & FMOH 2008a:100; FHAPCO & FMOH 2008b:66).

1.4.5 Treatment failure

The *Macmillan Dictionary Online* (2013, Sv "treatment") defines the term "treatment as medical care provided to improve a situation". In this study, antiretroviral therapy is the treatment or medical care provided to improve the health of those with the disease as a result of HIV infection. 'Failure' is defined as a lack of success in accomplishing an intended objective (*Oxford Dictionaries Online 2013*, Sv "failure"). The WHO (2010a:50) defines antiretroviral treatment failure as "the state of failure for HAART to suppress HIV viral replication while it is being taken". The same definition was used in this study to refer to treatment failure. This is further elaborated in the next section.

1.4.6 Antiretroviral treatment failure

The WHO (2010a:50, 51) recommends three criteria to define antiretroviral treatment failure namely clinical, immunologic and virologic. Clinical failure for adults and adolescents is when a patient develops a new or recurrent WHO HIV disease stage four condition or WHO HIV stage three conditions for example pulmonary tuberculosis and a severe bacterial infection. For children, this includes WHO HIV disease stage three or four conditions as per the WHO classification, except TB. Immunologic failure is diagnosed when there is a decline in the CD4 cell count by 50% from the peak level achieved during treatment or when it falls to or below the baseline CD4 cell count level,

or when it is persistently below 100 cells/micro litres. Virologic failure is when the plasma viral level is more than 5,000 copies. All three definitions are used in the Ethiopian context, but for the purpose of this study, only immunologic criteria were used (FHAPCO & FMOH 2008a:155).

1.4.7 Prevalence

The term 'prevalence' refers to the proportion of individuals having a characteristic within a given population (*Oxford Dictionary Online 2013,* Sv "prevalence"). Webb and Bain (2011:34) define prevalence as the "proportion of behaviour or disease among a particular population at a given time". In this study, the prevalence of immunologic treatment failure among HIV patients on HAART was determined as a proportion expressed as a per cent.

1.4.8 Patient or client

A patient is someone who is receiving medical care because of illness while a client is a person who seeks professional help from for instance a lawyer or a doctor (*Macmillan Dictionary Online 2013,* Sv "patient"). Glynn and Drake (2012:3) describe a 'patient' as a term derived from the Latin term meaning 'sufferance'. The term 'patient' in this study is regarded as being synonymous with the term 'client', both designating a person who has a confirmed diagnosis of HIV through laboratory tests and who has been prescribed antiretroviral medication at the selected setting in Ethiopia used as a source of data in this research.

1.4.9 Predict

The *Macmillan Dictionary Online (*2013 *Sv "predict"*) defines the term 'prediction' as a "statement about what will happen in the future or the process of making such a statement". Polit and Beck (2012:738) provide a more technical definition of prediction, stating that "prediction is the use of objective data in forecasting a relationship between variables of interest". Therefore, a predictor variable is a variable that has a measurable influence on a different variable of interest. In this study, independent variables including age, gender, baseline CD4 cell count and others were used to predict or

forecast the occurrence of immunologic treatment failure in order to develop a care plan for patients at risk of immunologic treatment failure.

1.5   OPERATIONAL DEFINITIONS

Polit and Beck (2012:736) define an operational definition as a selected meaning when there are various meanings for a term.

In this research, clarification for the following terms relative to their specific use in the study must be given. The terms are adherence to medication refill and immunological treatment failure.

1.5.1   Adherence to medication refill

Antiretroviral therapy involves a lifelong treatment. This means that medications must be picked up at regular time intervals from treatment facilities in order for treatment to continue. A patient who has run out of medication but fails to collect more from a treatment centre for one month is labelled as being 'lost to follow-up'. A patient who has not collected medication for more than three consecutive months is labelled as a 'drop out' (FHAPCO & FMOH 2008a:130).

1.5.2   Immunological failure

Although the WHO (2010a:50, 51) recommends the use of three types of criteria with which to define antiretroviral treatment failure, namely clinical, immunologic and virologic, the focus of this study is on immunologic criteria which the WHO defines as follows:

- A falling of follow-up CD4 cell count to baseline (or below), or
- A 50% fall from on-treatment peak value, or
- CD4 cell count levels persisting below 100 cells/mm^3.

These same criteria were used in this study to identify patients with immunologic failure.

1.6   RESEARCH AIM/PURPOSE

The aim of the study is to describe the prevalence of immunologic failure and identify its determinants among people living with HIV receiving treatment at selected hospitals.

1.7   RESEARCH OBJECTIVES

The objectives of this study are to:

- Assess the pattern of immunologic (CD4 cell count) monitoring in selected hospitals in southern Ethiopia
- Assess the pattern of immunologic (CD4 cell count) response for patients on HAART in selected hospitals in southern Ethiopia
- Describe immunologic treatment failure and identify its predictors among patients on HAART in selected hospitals in southern Ethiopia

1.8   RESEARCH QUESTIONS/HYPOTHESES

The research questions posed in this study are as follows:

- What is the pattern of immunological monitoring in the selected hospitals in southern Ethiopia?
- What is the level of CD4 cell count response of patients on HAART in the selected hospitals in southern Ethiopia?
- What do the results reveal with respect to the extent of and factors characterising immunologic treatment failure among patients on HAART treatment in selected hospitals in southern Ethiopia?

1.9   PARADIGM

In the quantitative research design, reality exists independent of the observer. A number of measures are taken to make sure the observation of phenomena is objective and free of bias, unaffected by the beliefs of the observer. Values are assigned to variables by counting or measuring with an instrument (as for example, a checklist, questionnaire, or structured interview schedule) in an objective manner (Polit & Beck 2012:13). This

design is appropriate for this study because the aspect of the dependant variable being studied; that is, immunologic treatment failure is determined by examining and interpreting the patterns of quantitative measures of CD4 lymphocyte counts (WHO 2010a:50, 51).

1.10   RESEARCH DESIGN

This study has a quantitative, correlational and longitudinal design. Since this is a quantitative, non-experimental study, the researcher simply observes what is going on without interference or manipulation. In some cases, manipulation would not be possible, as in the case of gender. In other cases, manipulation is possible but wouldn't be ethical, as in the case of providing a beneficial supplement for one group and withholding it from another just to study the additional effects it may have (Polit & Beck 2012:227). Exposure and outcome status is determined when the condition of interest is experienced by the study subjects. Nor is there randomization of study subjects into exposure and control group. That is exactly what is happening in this study. Patients' information gathered in their natural environment during routine clinic visits is used to evaluate prevalence and determinants of immunologic failure. It would be unethical to manipulate who gets what sort of treatment by allocating different intervention categories to various patients just to satisfy one's curiosity about who is likely to experience treatment failure. So this design is appropriate for this study (LoBiondo-Wood & Haber 2010:310). The next two items (see 1.10.1 and 1.10.2) focus on the elements of this design.

1.10.1   Correlational elements

Polit and Beck (2012:226) state that in the correlational study design, the association between variables which the researcher cannot manipulate is determined. In addition to assessing the mere association between variables, this type of design addresses the issue of whether one variable (a dependant variable) is predicted by another (an independent variable). In this study, the major aim was to assess the prevalence of immunologic treatment failure and factors that predict the development of immunologic treatment failure. With respect to the second aim, independent variables (for example age, gender, and poor adherence history) that may be correlated to immunologic treatment failure are studied, while in the first, features of patients with immunologic

failure are described and hence, this study has a descriptive component as well. In a descriptive research design, a health event of interest is simply described (LoBiondo-Wood & Haber 2010:200,201).

1.10.2   Longitudinal elements

In a longitudinal study design, a group of subjects with common characteristic of interest are followed for some length of time. The outcome of interest is measured repeatedly as the follow-up continues (Webb & Bain 2011:100). A longitudinal study design can have two forms: prospective and retrospective. In the prospective longitudinal design, subjects are followed for a time, and observations are made for the occurrence of outcomes of interest, while in the retrospective longitudinal design, both the exposure and outcome of interest have already occurred in the past, and the exposure precedes outcome (Kestenbaum 2009:37).

In this study, a group of patients on HAART were followed and the outcome of interest, i.e. immunologic treatment failure, was evaluated at various times. Clearly, patients take the treatment for a while before they experience treatment failure. So, there is a timed sequence of events. All these features make a longitudinal design appropriate for this study. Both exposure and outcome status were determined at the start of the study and hence that makes it a retrospective study (Chernick 2011:12). This design is particularly advantageous for this study. This is because, since the specific objective includes assessment of the pattern of immunological monitoring and whether immunologic failure was dealt with appropriately and in a timely fashion, health care workers may modify their behaviour and exercise greater vigilance than they normally do in response to their awareness of a prospective study being done addressing these issues (Polit & Beck 2012:225).

1.11   SAMPLING

Sampling is the process of selecting part of the accessible population with the intention of studying the selected cases and making inferences about the population from which it is drawn. Taking a sample and studying it has a number of advantages. This includes saving time and minimizing the cost associated with collecting data from a large population (LoBiondo-Wood & Haber 2010:224).

1.11.1   Site sampling

In this study, a total of 22 hospitals made up the site population. Twenty were under government control and two administered by non-governmental organizations (NGO).There are also 110 antiretroviral treatment providing health centres in the Southern Nations Nationalities and Peoples' Region (SNNPR).All 22 of the hospitals formed the site target population and hence the site sampling frame. Two of them have been accessible and convenient for the investigator to draw data from. In this study, these hospitals will be referred to as Hospital A and Hospital B. Convenience sampling is chosen as these facilities have been accessible to the investigator. These facilities are accessible to the investigator since they are found in the place where the investigator lives (Hospital A) and works (Hospital B).

1.11.2   Data source sampling

The population of clients' on HAART in the two hospitals at the time of sampling was as follows: 881 in hospital A and 1,266 in hospital B. Documents of all patients enrolled for HAART in the selected facilities formed the site population.

1.11.2.1 Eligibility criteria

The eligibility criteria are the ones that specify which subgroup of the population is to be studied (Polit & Beck 2012:274). For this study, the eligibility criteria were as follows listed as inclusion and exclusion criteria.

Inclusion criteria

- The documents of people living with HIV on antiretroviral therapy enrolled and started on ART between September 1, 2005 until December 31, 2012
- Age ≥5 years old (because for patients of an age lower than this immunologic monitoring is rare as a result of the difficulty in taking blood samples)
- Patients who had two or more CD4 cell count tests

Exclusion criteria

- Having taken HAART for less than six months
- Being a case transferred from a facility other than the study facilities for continuation of treatment
- Being a case that does not fall within the time frame of the study

All patients' documents fulfilling the eligibility criteria in the study in the two hospitals were accessible; and hence, formed the accessible population. The sample was selected through a sampling process. It was important for the researcher to select a representative sample from the population of each hospital's ART clinic, a sample which accurately and directly represented the characteristics as reflected by the population (Polit & Beck 2012:283).For the purposes of this study, the sample was selected from the total population of clients' from hospitals A and B whose documents complied with the eligibility criteria for this study.

1.11.2.2 Respondent/participant sampling technique

Simple random sampling of all accessible patients fulfilling the eligibility criteria was used to determine the required study sample.

1.11.2.3 Respondent/participant sample size

The total sample size required was estimated for the combined group of accessible patients on HAART treatment in the two hospitals. Sample size calculations were conducted assuming random sampling for a finite population based on the estimation of a 50% proportion, at a 5% significance level and 2.5% precision as described by Daniel (2009:192). The formula used to estimate the sample size for the study was:

$$n= [z^2p(1-p)N]/[(N-1)d^2+z^2p(1-p)]$$

Where

n = calculated sample size

N = population size

z = critical value at the chosen significance level

p = proportion to be estimated

d = precision

Thus, for this study taking N=2,147, z=1.96, p=0.50 (50%) and d=0.02 (2 %), sample size was thus 1,133.703 which is rounded off to 1,134. Assuming the proportion of missing records to be 15%, the sample size was inflated to 1,303. This means after applying the eligibility criteria, of the remaining patients' files, 1,303 patients' files were randomly selected. In other words, proportionate sampling was used (Daniel 2009:13).

Permission was obtained from the management of hospitals under study, and the ethics committee of the Regional Health Bureau of The Southern Nations Nationalities and Peoples' Region and the National Research Ethics Review Committee of Ethiopia (see Annexure B to F) to access the list of all clients that were started on. A list was compiled indicating the patients' file number. The statistician then applied the eligibility criteria to assess which patient records on the list fulfilled the eligibility criteria and deleted those who did not fulfil it.

1.12   DATA COLLECTION

This study utilised a self-designed document analysis checklist. Personal and social information was not collected from human data sources.

1.12.1   The data collection instrument

The data collection instrument used in this study is provided in Annexure A.

1.12.2   Administering the data collection instrument

The existing medical records were used as a data source. The advantage of using existing records was to save time and reduce cost than if data was collected prospectively. In addition, since the researcher was familiar with the records and the data collection procedures, appropriate precautions were taken where the records and the data entered had limitations (LoBiondo-Wood & Haber 2010:270, 279).

1.12.3   Pretesting the data collection tool

The data collection document checklist and data cleaning program codes were pretested prior to data collection and cleaning. This was done by a statistician among transferred-in patients found in one of the study facilities. The purpose of the pre-test was to assess the adequacy of the program codes, the length of time required to do the data collection, and cleaning, and to make sure the clarity of the procedures was adequate (LoBiondo-Wood & Haber 2010:280). A full description of this process is given in chapter 3.

1.12.4   Data collection procedure

Trained data clerks at each facility provided the statistician with electronic version of patients' records onsite, where he applied eligibility criteria and identified charts that were eligible for inclusion in the study. This list was passed to data collectors who abstracted patients' records then after.

The data collection instrument utilised to collect the information was a self-designed document checklist which consisted of the following four sections:

Section A included the age, and gender. Such demographic variables were used to characterise the study population.

Section B included clinical characteristics in the form of baseline CD4 cell count, baseline WHO stage, and other variables that might have affected treatment failure, such as, occurrence of tuberculosis, a history of treatment interruption, and weight status. All these variables were used to characterise immunologic monitoring, immunologic response, and immunologic treatment failure.

Section C included immunologic information like timing of immunologic (CD4 cell count) monitoring, magnitude of CD4 cell count, and occurrence of immunologic failure.

1.13   DATA MANAGEMENT AND ANALYSIS

In this study, Epi Info^{TM} 3.5.1 statistical software (United States Centers for Disease Control and Prevention 2007) and Stata/IC 12.0 (StataCorp LP) were used to undertake both descriptive and inferential statistics.

The purpose of descriptive statistics is to summarize and organise raw data so as to understand the information contained in it in a simple way (Daniel 2009:20). A number of descriptive statistical measures were used in this study to summarise data. These included frequency distribution depicted in the form of tables or graphs used for categorical variables. Data from section C was used to address the following objectives:

- Describe the expected pattern of immunological monitoring as well as the observed immunological (CD4 cell count) monitoring (section C.1). This was shown graphically.

- Describe the pattern of CD4 cell count response after HAART initiation (section C.2).

- The prevalence of immunologic treatment failure was calculated as proportion with 95% confidence interval (section C.3). Mean and standard deviation were used as summary statistics whenever the variable was normally distributed or median and inter quartile range when it was not. These are the so called univariable statistics that are used to describe a single variable.

Inferential statistics must go one step further from just describing and summarising variables and compares two or more variables in order to assess similarity or difference amongst them in order to reach a conclusion (LoBiondo-Wood & Haber 2010:310,318). In this study, variables from sections A and B of the checklist were used to characterise pattern of immunologic monitoring, immunologic response, and to characterise patients with immunologic treatment failure as compared to those without immunologic treatment failure. A Chi- square test was used to compare variables in the nominal or ordinal scale, while Student's t-test was used to compare the mean of continuous variables if they were normally distributed; otherwise non-parametric tests were used.

Time to event, that is time from HAART initiation to immunologic treatment failure, was determined using survival analysis as described in Chernick (2011:164). The use of this technique was justified for two reasons. Firstly, immunologic treatment failure is a time dependent variable. This means the risk of immunologic treatment failure increased with time as the exposure to antiretroviral medications increased. Secondly, it was expected that patients would have different length of follow-up duration. In addition, the variation in follow-up duration was accounted for in this technique. Factors thought to affect the

probability of developing immunologic treatment failure were studied by applying a multivariable regression model using the Cox proportional hazards model as explained in Daniel (2009:648,659).

1.14 DATA AND DESIGN QUALITY

This section deals with data and design quality as it applies to this study.

1.14.1 Quality of research design

In this section the quality of the research design is discussed, in terms of internal and external validity.

1.14.1.1 Internal validity

Internal validity refers to the extent to which it is possible to draw a conclusion from the study for the fact that there is a true causal relationship between an independent and dependant variables, independent of the influence of other variables (Webb & Bain 2011:230).

Threats to the internal validity include the absence of temporality, the presence of historical events that might have influenced one group only, maturation or fatigue, attrition, and selection bias. In this study, since it was a longitudinal study, the investigator evaluated whether predictor variables occurred prior to the occurrence of the outcome of interest (Polit & Beck 2012:495).

The survival analysis carried out involved the analysis of choice and therefore, by its being conducted, the researcher took into consideration statistically the effects of variable duration of follow-up. Selection bias is a common threat to internal validity but in this study, since cases were randomly selected from the selected facilities and the Cox regression analysis was used, the investigator was able to provide a control for the difference in baseline characteristics (Polit & Beck 2012:228,450).

1.14.1.2 External validity

External validity refers to the generalizability of the findings of the study concerning the studied population to similar groups of people outside the study. Generalisation to the accessible population in this case would be straightforward as there was simple random sampling of all eligible cases such that the selection took place without any bias. But, generalization to the target population should be made carefully as convenience sampling was used to select study sites (Polit & Beck 2012:250).

1.14.2 Quality of the data gathering instrument

In this section, the quality of the data gathering instrument is described in terms of its reliability and validity.

1.14.2.1 Reliability and validity

Polit and Beck (2012:332) state that "reliability refers to the accuracy and consistency of information obtained in a study." Since this study was retrospective and based on existing medical records, a number of potential measurement biases were essentially avoided. These include interviewer bias, recall bias, and the Hawthorn effect. One particular variable that needed to be measured and might have had some influence on the outcome variable, that is immunologic treatment failure, was adherence. Self-reported adherence could have been unreliable because of a social desirability bias. For this reason, the investigator measured patients' adherence to their appointment for a drug refill. This measure of adherence also prevented misclassification errors with respect to adherence that could have occurred if one relied on self-report to assess adherence. (LoBiondo-Wood & Haber 2010:279)

1.15 ETHICAL CONSIDERATIONS

This section addresses ethical considerations as applied to this study.

1.15.1 The institution/site

After getting approval for the proposal from the Department of Higher Degrees of the Department of Health Studies at The University of South Africa (UNISA), ethical approval was sought from the National Research Ethics Review Committee, and the

Southern Nations Nationalities and People's Regional Health Bureau of Ethiopia. Permission was also obtained from the directors of each of the hospitals from whose HIV clinics data was going to be used for the purpose of this study. (See Annexures D, E, F and G)

1.15.2   Scientific integrity of the research

This is a quantitative study and such studies are planned from beginning to end. This helped in ensuring that the standards used at various levels were visible and accounted for in the research process. Preparing a study is only half of the job, though, and making sure that everything is implemented as planned is equally important. Guidelines at every level were followed from start to finish and the investigator faithfully upheld the desired standards at various levels. Whenever that was not possible for operational reasons, the researcher provided alternatives to compensate for the deficit, with the intention of openly discussing such during the reporting of results about the limitations of the study.

1.15.3   Ethical principles

In this section, the three basic ethical principles and how they relate to this study is discussed.

1.15.3.1   Beneficence

According to LoBiondo-Wood and Haber (2010:250), research and the research process must maximise benefit (beneficence) and minimise harm (non-malfeasance) at all times. In this study, there was no interaction of the researcher with study subjects. Therefore, the possibility of harm being inflicted during the study was remote.

1.15.3.2   Respect for human dignity

LoBiondo-Wood and Haber (2010:250) state that this principle entails ensuring that the researcher will respect the confidence of research subjects, and recognize that they have the right to decide for themselves. This is ensured through the implementation of informed consent whenever it applied. In this study, in fact, since there was no

interaction between study subjects and investigator or data collector, there was no need for informed consent.

1.15.3.3   Justice

LoBiondo-Wood and Haber (2010:251) state that this principle deals with the right to fair treatment and the right to privacy. In this study the privacy of patients was respected in that information about them was kept in confidence. Access to electronic records and computer sat study sites was limited to the trained data clerks already working in the study facilities. The computer used to update the electronic records was password locked and medical records were kept in a medical records room which is secured under normal circumstances and this security was maintained throughout the study. Since random selection of all eligible patients was to be used, it was ensured that all eligible would have an equal chance to be involved in the study, and thus, equality was taken into consideration as well.

1.16   SIGNIFICANCE OF THE STUDY

This study may help decision makers in Ethiopia to identify gaps in the quality of care pertaining especially to immunologic monitoring. In addition it may provide valuable information in providing an estimate of patients who experience immunologic failure and need targeted viral load testing. This will facilitate the estimating of the potential number of people who may need second-line therapy in years to come.

For health care providers at the point of care, the study will prove a help in addressing operational issues relevant to monitoring immunologic response. The analysis of specific risk factors for immunologic failure will also help them to identify and pay attention to those patients who are in greater risk of immunologic failure and addressing the barriers to their adherence.

For people living with HIV in the study area, and beyond, the information obtained from this study could be beneficial to improve the quality of care and ultimately contribute to the betterment of their health status.

1.17 SCOPE AND LIMITATIONS

The scope of this study is limited in many ways. Firstly, the study focused on patients in antiretroviral care in the Southern Nations Nationalities and People's Region and no other regions in Ethiopia. Secondly, the study was conducted among patients in selected hospitals and not health centres. Thirdly, existing client records were used as the data source with all the limitations such may have, including human error in reporting, the omitting of patient information, and the lack of other pertinent information.

1.18 STRUCTURE OF THE DISSERTATION

This dissertation is divided into the following chapters:

Chapter 1: Orientation to the study

Chapter 2: Literature review

Chapter 3: Research methodology

Chapter 4: Presentation and discussion of the results of the research

Chapter 5: Conclusions, limitations and recommandations.

1.19 CONCLUSION

This chapter is an orientation to the study. Separate sections have described the background to the study, the research problem, key concepts, and objectives of the study. This was followed by the research methodology which specified the research design, sampling, data collection, and data analysis plan to be used in order to achieve these objectives. The final section identified possible ethical issues and limitations that might arise in the course of the study and outlined the means employed to handle them.

All in all, it is the researcher's strong belief that the findings of this study might fill in a knowledge gap which now exists with respect to the management of HIV patients on antiretroviral therapy. The health care system might benefit specifically from the estimate provided of the prevalence of immunologic treatment failure among adults and children and factors associated with it.

The next chapter will deal with the review of the relevant literature which formed the basis for this study.

CHAPTER 2

LITERATURE REVIEW

2.1   INTRODUCTION

The previous chapter briefly addressed several issues related to this study. Initially it addressed the background to the study, the origin of the problem, and the problem statement. Next it addressed the specific objectives of the study. This was later followed by the methodology in brief to be used so as to answer the study question in ethically and scientifically sound ways. The major components of this section included the sampling plan, the data collection tool, the data cleaning plan, the data analysis plan, and ethical issues relevant to the study.

This chapter commences by outlining the purpose of literature review. This will be followed by the actual review.

2.2   PURPOSE OF THE LITERATURE REVIEW

According to Polit and Beck (2012:57), the purpose of a literature review is to familiarize oneself with existing knowledge. That will serve two main purposes: the identification of existing knowledge gaps or problems and the identification of existing solutions to the problem. A literature review can further be used to outline research directions in terms of: refining research questions, selecting an appropriate study design to answer the research question, developing or using an existing data collection and analysis plan, creating an outline to serve as the basis for interpretation of the research findings, and the exploration of improved means to finding a solution to a problem.

For those who will ultimately use the research, a literature review can also be used to enhance evidence based practice in designing a policy or choosing an appropriate policy (Polit & Beck 2012:58). In this study, the researcher conducted an extensive review of the literature and this will delineate the existing knowledge in relation to HIV/AIDS and ART globally with special reference to Ethiopia with respect to immunologic treatment failure, and factors associated with it. Also the pattern of

immunologic response among patients on treatment and the practices used in immunologic monitoring in different settings were reviewed. In order to achieve a comprehensive review of the most recent and relevant literature, various research engines were used to obtain research articles; at the same time, international and local HIV treatment guidelines were reviewed in order to get an idea of existing standards and practices.

2.3   CLASSIFICATION AND HISTORY OF THE HIV VIRUS

Zuckerman et al (2009:869) state that HIV is a virus from the family Retroviridae and subfamily Lentivirinae. They further elaborate that this family of viruses is unique because, in the various type of it, genetic material is encoded with ribonucleic acid (RNA) instead of deoxyribonucleic acid (DNA). The HIV is further classified into two subtypes, HIV-1 and HIV-2. HIV-1 has well defined subgroups designated M, N, O, and P while HIV-2 has subgroups designated A to H (Longo et al 2012:1506).

Historically, HIV/AIDS was first reported in the United States of America among homosexual men who exhibited Kaposi's Sarcoma and Pneumocystis Carinii Pneumonia (Weiss 2008:201). Later, it was observed among injection drug users, sex workers, and blood recipients, which led to the thinking that this disease was probably transmitted by an infectious agent. This was followed by the isolation of HIV-1 and HIV-2 in 1983 and 1986 (Barré-Sinoussi, Chermann, Rey, Nugeyre, Chamaret, Gruest, Dauguet, Axler-Blin, Vézinet-Brun, Rouzioux, Rozenbaum & Montagnier 1983:869; Clavel, Mansinho, Chamaret, Guetard, Favier, Nina, Santos-Ferreira, Champalimaud & Montagnier 1987:1180). Even though HIV was first isolated from human samples, Heeney, Dalgleish, and Weiss (2006:463) state that HIV has a zoonotic origin with HIV-1 coming from chimpanzees while HIV-2 developed in Sooty Mangabey monkeys in West Africa.

2.4   EPIDEMIOLOGY OF HIV/AIDS

This section addresses the epidemiology of HIV with particular attention to the risk factors and its distribution.

2.4.1 Transmission and risk factors

Zuckeman et al (2009:899) state that unprotected sexual intercourse, vertical transmission, and injection drug use continue to constitute the major risk factors for the transmission of HIV. Even though almost everyone is at risk, the WHO (2013:14) identifies men who have sex with men, sex workers, and injection drug users as those most at risk for HIV infection. Next in the tier of vulnerability to infection are adolescents, especially girls, orphans, street children, prisoners, people with disabilities and migrant and mobile workers. In general, the higher the level of the virus in the blood or body fluids of a source case, the higher the risk of transmission (FHAPCO & FMOH 2008a:39).

2.4.2 Prevalence and geographic distribution
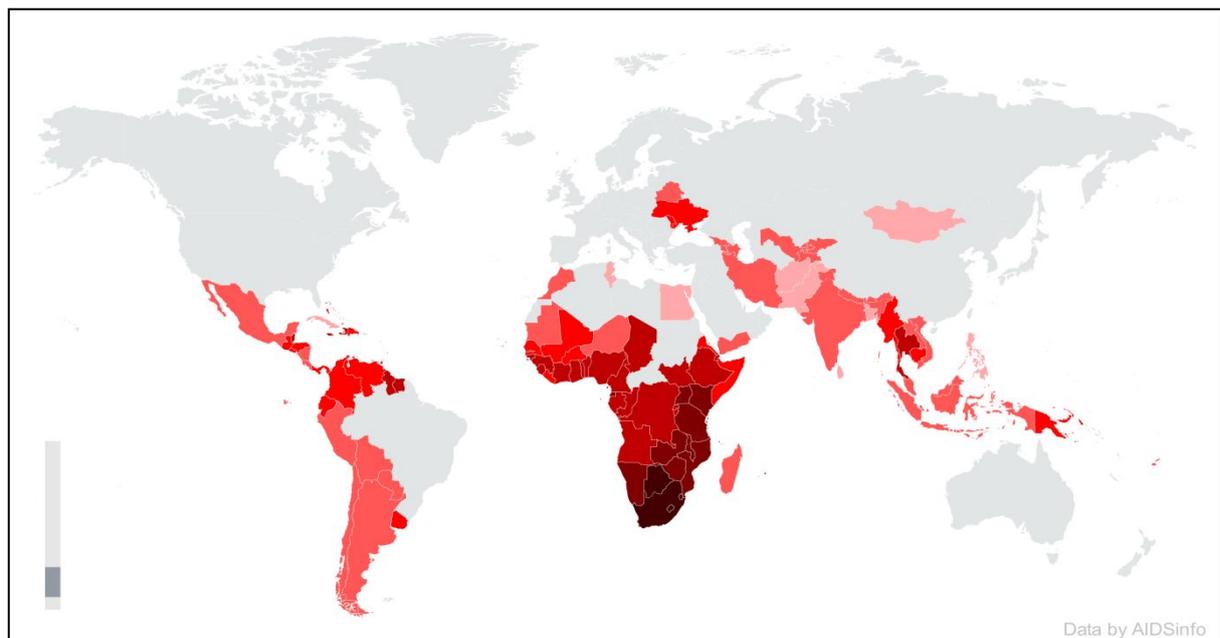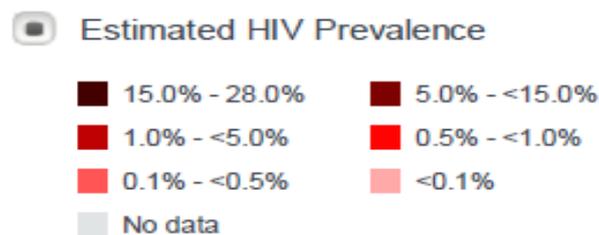
(UNAIDS 2013a:online)

Figure 2.1   Geographic distribution of HIV: estimated HIV prevalence by country
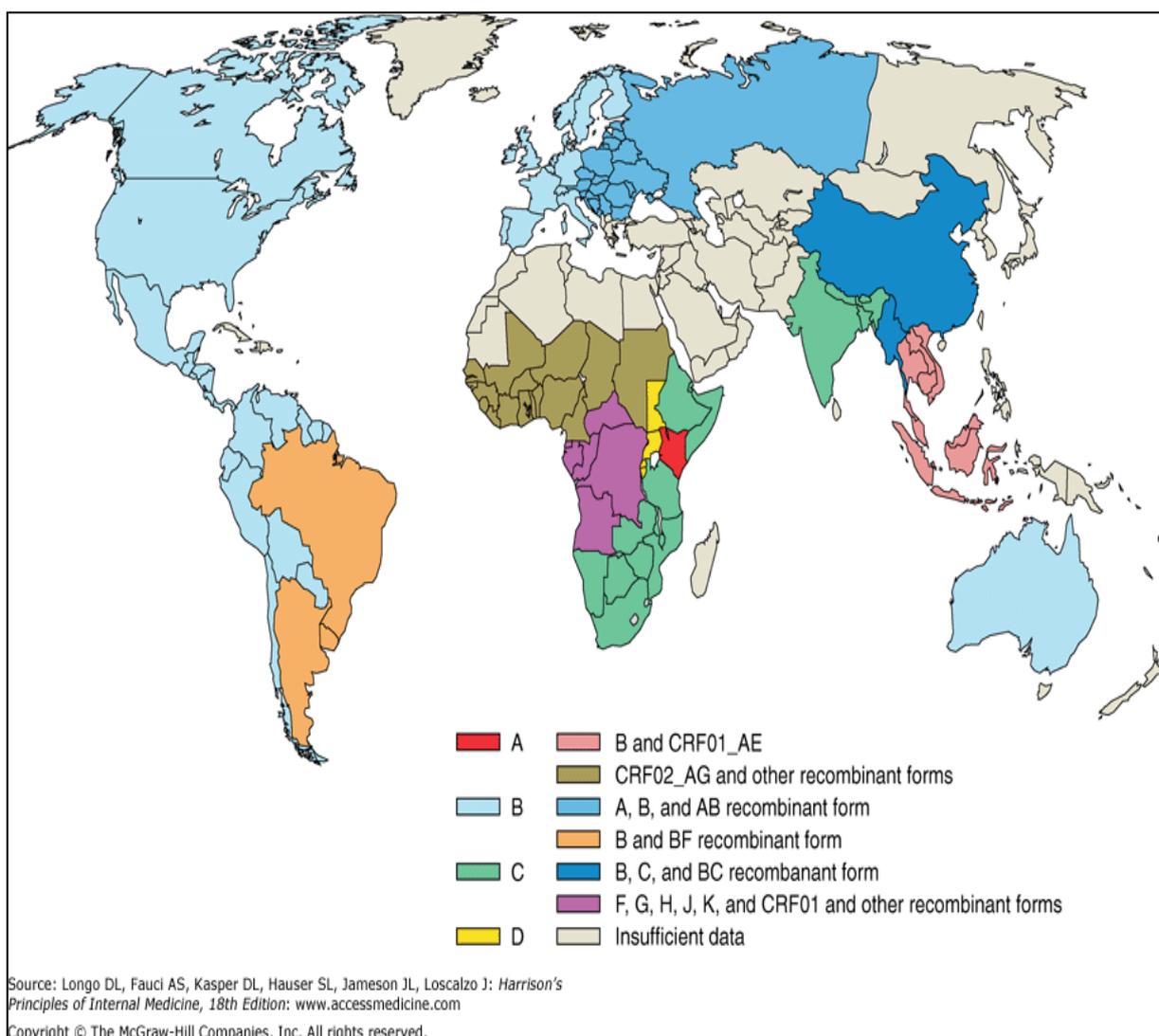
(Longo et al 2012:1513)

Figure 2.2   Geographic distribution of HIV-1 subtypes and recombinant forms

As can be seen from figures 1 and 2, the HIV pandemic affects sub-Saharan Africa the most even though no region of the world is spared. The geographic distribution of the various subtypes of HIV-1 also indicate different predilections in various regions, as for example, subtype B for western countries, while it is subtype C for eastern Africa (Longo et al 2012:1513). The UNAIDS (2013a:online) estimated the global adult HIV prevalence in 2012 to be 0.8% (range: 0.7 to 0.9%) from which it can be projected there were then 35.3 million people with HIV. It further estimated the number of new infections and deaths in 2012 among adults and children to be 2.3 and 1.6 million respectively. The global trend for both new infections and mortality is a decreasing one.

2.4.3 The state of the epidemic in Ethiopia

The prevalence of HIV in Ethiopia among adults age 15-49 is 1.2% (1.1-1.4%) according to the latest estimates (UNAIDS 2014:A9). The estimated number of all people with HIV was around 790,000 (720,000-890,000) for 2013. In the same year, new HIV infections were estimated to be 21,000 (15,000-30,000). This number declined from 51,000 in 2005 (UNAIDS 2014:A38). The annual HIV mortality rate is declining as evidenced by the fact that there were 45,000 deaths in 2013 as opposed to 120,000 in 2005 (UNAIDS 2014:A57). As can be seen from figure 2.2, the subtype C of HIV-1 is responsible for the epidemic in Ethiopia.

2.4.3.1 *The state of the epidemic in Southern Nations Nationalities and People's Region of Ethiopia*

The Ethiopian Health and Nutrition Research Institute (EHNRI) and FMOH (2012:36) estimate that in the Southern Nations Nationalities and People's Region, the prevalence of HIV from the latest estimates for adults was 0.7%. This puts the estimated number of people with HIV at 97,236. Thus, prevalence in the study area is well below the national prevalence of 1.2% (EHNRI & FMOH 2012:6).

2.5 PATHOGENESIS OF HIV/AIDS

This section discusses how HIV causes AIDS, and what makes it difficult to treat and cure.

2.5.1 HIV infection and replication

To gain entry into cells the virus attaches its surface structure, gp120 (see figure 2.3), to CD4 receptors found mainly on CD4 T lymphocytes. Once attachment is established, it fuses its membrane with that of the cell and releases its RNA and enzymes to the inside of the cell (see figure 2.4). This is followed by the enzyme reverse transcriptase making a DNA copy of the viral RNA. The new viral DNA is integrated within the host DNA of the infected CD4 cell. Then, as the cell carries out its routine chores, new viral components are produced. Once produced, the different components are assembled together and new viruses are released (FHAPCO & FMOH 2008a:36).

gp41

Matrix
Capsid

Lipid
membrane

RNA

gp120

Reverse
transcriptase

B
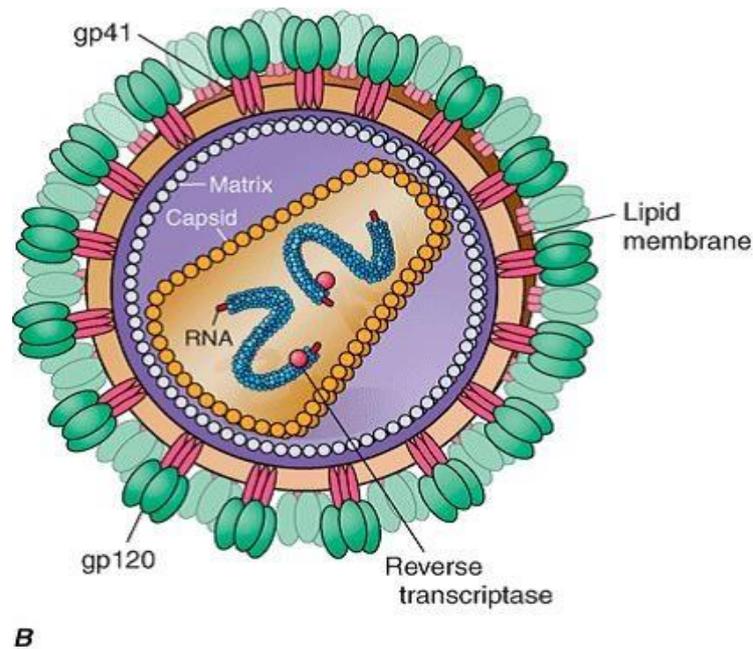
Figure 2.3   Structure of HIV-1, including envelope structures (gp120 envelope, gp41), genetic material (RNA), enzyme, inner membrane (matrix), and core protein (capsid)

Life Cycle of HIV

1. Viral RNA and reverse transcriptase enter the cell.

Human chromosome

Human cell nucleus

2. DNA copy synthesized by reverse transcriptase.

3. RNA degraded: second DNA strand synthesized.

Viral DNA

4. DNA circularizes or integrates into human chromosome.

5. With host cell activation viral DNA is transcribed, yielding viral messenger RNAs and viral genome RNA.

6. Viral RNAs are translated, yielding viral enzymes and structural proteins.

7. Viral membrane proteins are transported to host cell membrane.

8. Final viral assembly and budding take place.
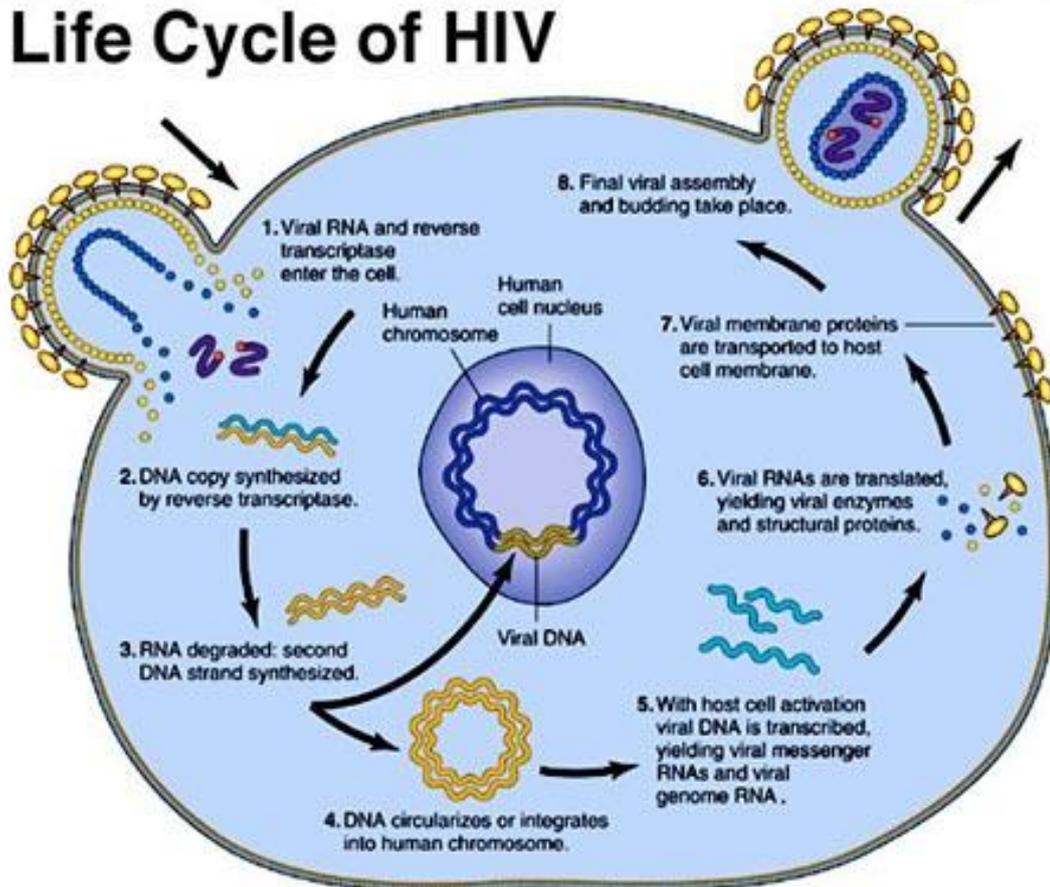
Figure 2.4   Life cycle of HIV

This process repeats itself time and time again, producing hundreds of millions of viruses per day, each cycle producing a genetically different copy as a result of mutations and the recombination of viral genetic material. This continuous high replenishment of viral particles creates the opportunity for adaptation to selective pressure from the immune system or antiretroviral drugs and drug resistance (Zuckerman et al 2009:904).

2.5.2 Immune activation and CD4 T-cell depletion

The availability of the viral antigen in the body activates the immune system, which leads to the creation of activated CD4 cells (Grossman, Meier-Schellersheim, Paul & Picker 2006:290; Sodora & Silvestri 2008:440). Activated CD4 cells are further infected and lead to more viral particles being released in the body. This positive feedback process leads to chronic activation of the immune system, which in turn results in the depletion of CD4 cells and the dysfunction of the immune system. The CD4 cells are depleted because of the destructive effect of the virus directly or indirectly through the cell death of infected cells programmed by the immune system (Levy 2009:151). Since CD4 T cells play a central role in the defence mechanism of the body, their depletion coupled with the dysfunction of the immune system leads to enhanced susceptibility of affected individuals to opportunistic infections and malignancies which ultimately lead to ill health and to death in those who are not treated (FHAPCO & FMOH 2008a:40).

2.5.3 Reservoirs of HIV infection

A small portion of infected CD4 cells survive and those cells are converted into resting or memory cells that lie dormant for a long time, up to ten years (Zuckerman et al 2009:904). Even in patients on antiretroviral medication, HIV in such cells is not affected as long as the cells remain dormant (Chun & Fauci 2012:1262). However, when such cells get activated and start to function, full viral replication capability resumes. It is this type of immune cells that serves as a reservoir of HIV infection. This forms the Achilles heel for any attempt to cure HIV (Chun & Fauci 2012:1262; Levy 2009:150; Katlama, Deeks, Autran, Martinez-Picado, Van Lunzen, Rouzioux, Miller, Vella, Schmitz, Ahlers, Richman & Sekaly 2013:2110). What is even more important is the fact that the genetic material of any dominant virus is continuously archived in these cells including drug resistant strains, which updates and maintains HIV latency.

2.6   IMMUNE RESPONSES TO HIV INFECTION

As with any infection, the immune system reacts when it detects a foreign antigen in the body (see figure 2.5). In the case of HIV, this results in a cell-mediated and humoral (antibody) mediated response (Levy 2009:153). The antibody response is rather effective, especially with the neutralizing antibodies because they target the more stable surface proteins, but it comes months after the infection occurs, by which time, usually new variants of the virus are present, as a result of which, the virus manages to continue to reproduce, circumventing the body's defence mechanism (Longo et al 2012:1534).
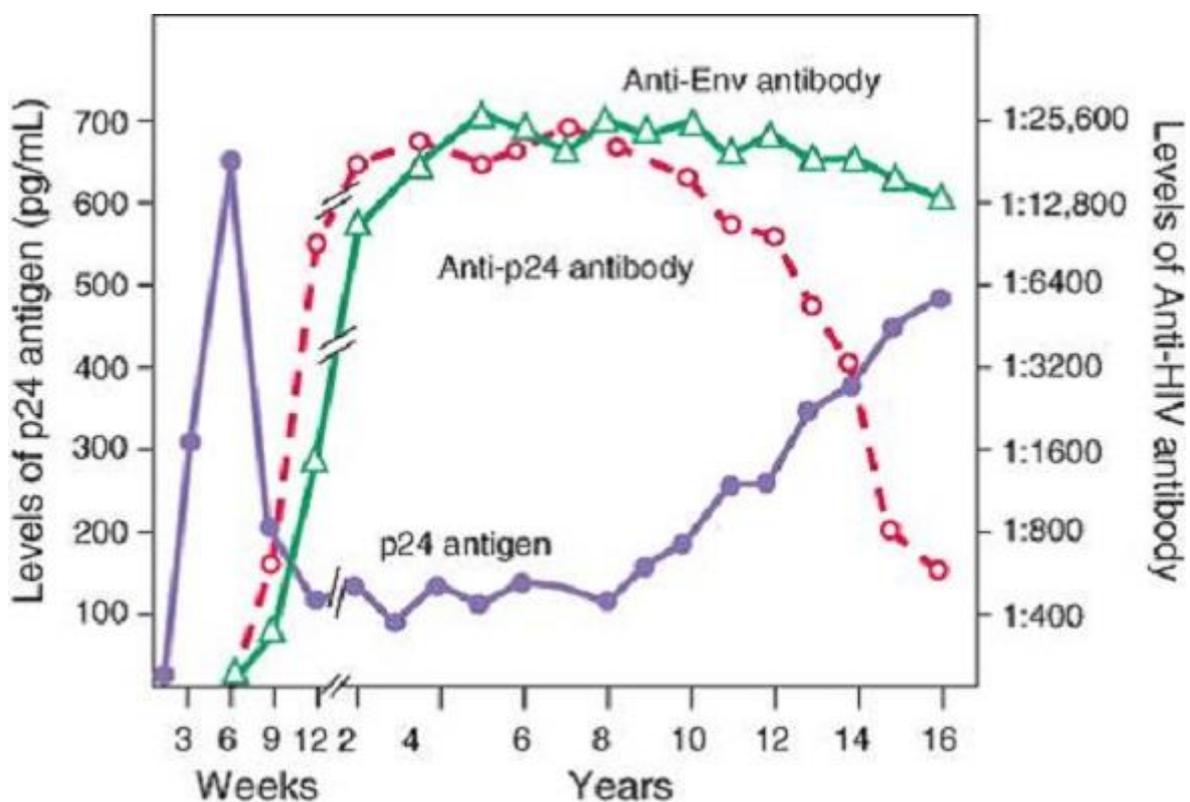
(Longo et al 2012:1534)

Figure 2.5   Antibody responses to HIV infection

2.7   LABORATORY DIAGNOSIS OF HIV INFECTION

The FMOH (2010b:95) states that the diagnosis of HIV is made through detection of HIV specific antibodies in the body for anyone older than 18 months. Rapid antibody tests are employed to serve this purpose. According to the WHO (2012b:35), the use of

rapid tests instead of laboratory based tests (enzyme immune assays) makes it possible for HIV counselling and testing services to be accessible at point of care in health facilities. A serial testing algorithm is used in order to make sure that a person's HIV status is determined accurately (WHO 2012a:36). There are various combinations of tests in the world, but the testing algorithm used in Ethiopia at present is displayed in figure 2.6 (FMOH 2010b:98).

The HIV (1/2) Antibody Colloidal Gold test from Shanghai Kehua Bio-Engineering (also known as KHB test), which has high sensitivity, is used initially to screen patients. If the result is negative, then the person is HIV negative. If positive, a confirmatory test is carried out using HIV 1/2 Stat-Pak™ Dipstick. If the result is positive, then the individual is HIV positive, while if negative, a tie breaker test is done using the Uni-Gold™ HIV test (FMOH 2010b:100). The limitation of this test algorithm is that it cannot detect patients in the window period (in the first three months after infection); that is, patients with recent HIV infection are at a stage where anti-HIV antibodies used for the detection purpose are not produced by the immune system yet. For this reason, a negative result of HIV with a such testing algorithm only means the person in question was not infected with HIV three months prior to the test (FHAPCO & FMOH 2008a:39). For this reason, repeat testing is recommended if there was exposure within three months prior to testing.

Earlier detection is possible these days with the development of test technology that detects the viral genetic material directly. This is used in diagnosing infants exposed to HIV (FMOH 2010b:96; WHO 2010b:4).

The WHO (2012a:35) states that HIV testing is not a simple series of lab tests. It must be accompanied by pre-test counselling and post-test counselling sessions in order to make sure that the person being tested understands what the test is for and why the testing is necessary and what should be done to reduce future risks. For HIV positive patients, the counselling provides an opportunity to educate them about the care they need and where they may find it in addition to giving them advice on positive living (FMOH 2010b:44).

HIV Testing Algorithm
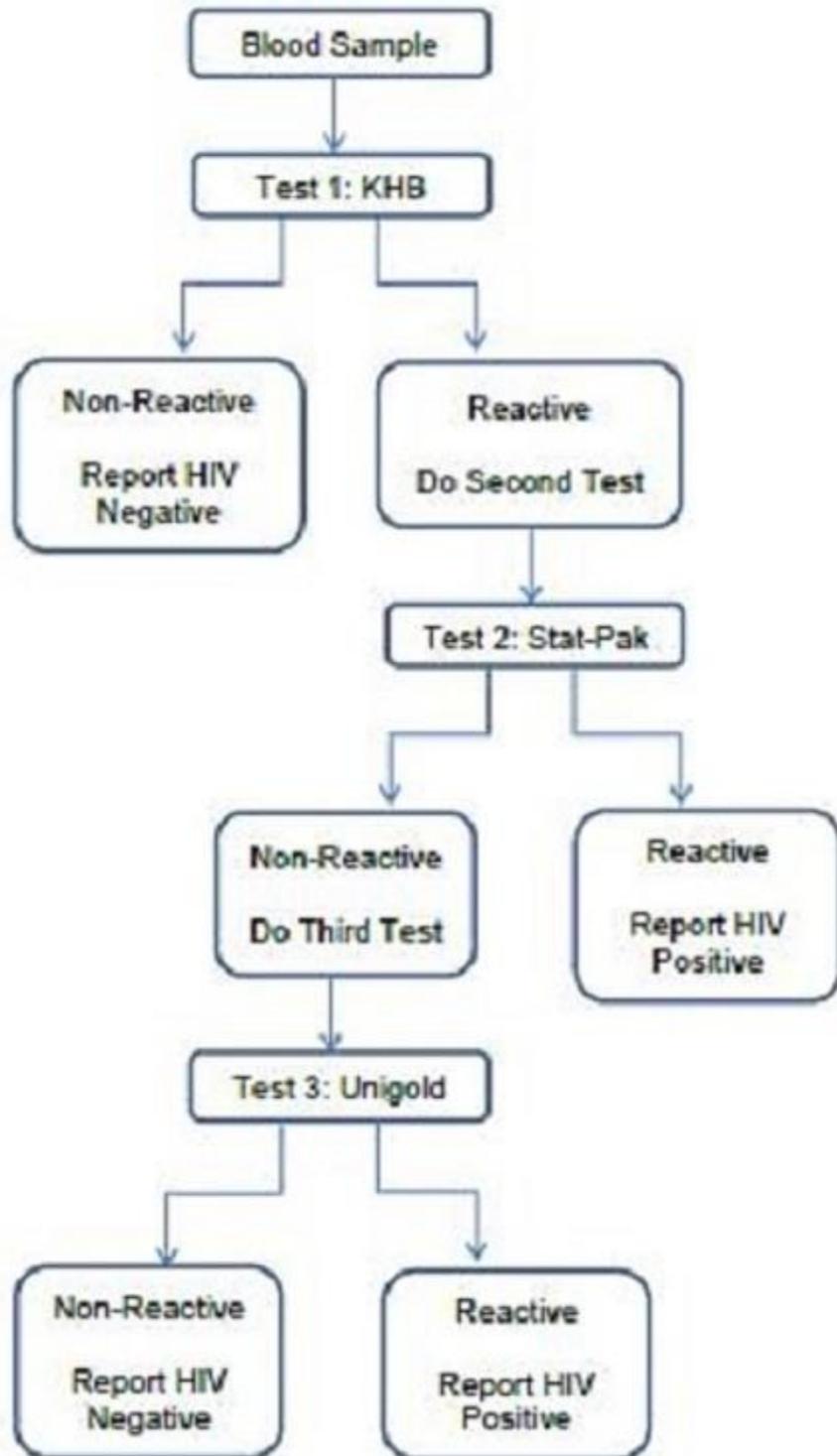
(FMOH 2010b:100)

Figure 2.6   HIV testing algorithm in Ethiopia

2.8   THE NATURAL HISTORY OF HIV INFECTION

The natural history of HIV follows three general patterns: rapid progressors, typical progressors, and long term non-progressors. Typical progressors account for 90% of patients and it takes eight to ten years before they develop AIDS symptoms. Rapid progressors account for five per cent of individuals infected, and it takes them a much shorter time to develop AIDS, between three to five years. Finally, long term non-progressors are patients who remain clinically asymptomatic for more than 15-20 years (FHAPCO & FMOH 2008a:39; Levy 2009:153; Sabin & Lundgren 2013:314). In any of these types of cases, untreated people go through three phases which are discussed next.

(Longo et al 2012:1520)
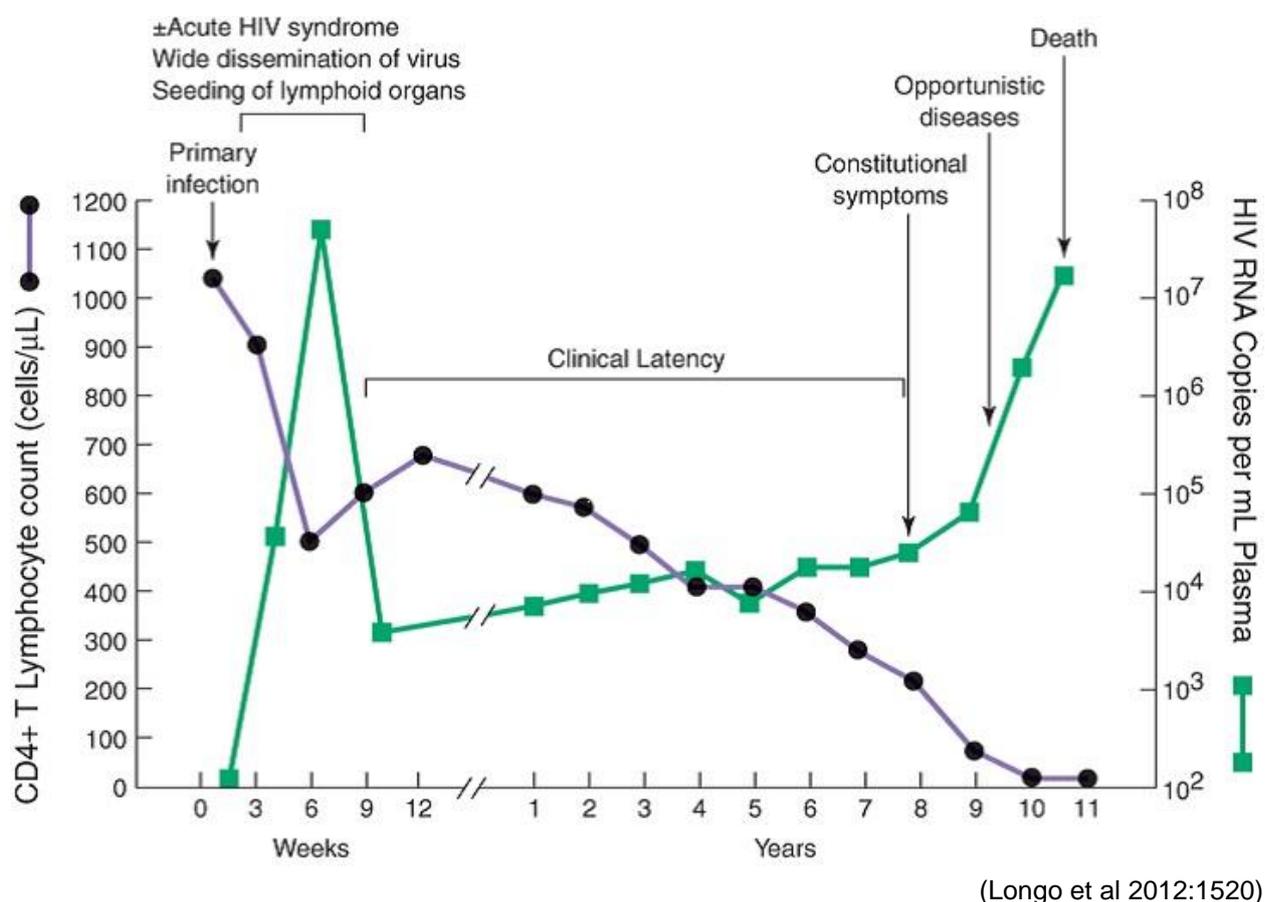
Figure 2.7   Natural history of HIV infection in untreated individual: virological, immunological, and clinical progression of typical progressors

2.8.1   Acute phase

This phase follows HIV infection and lasts around six to nine months (Zuckerman et al 2009:509). Initially there is uncontrolled viral replication and rapid destruction of CD4 T lymphocytes (see figure 2.7). As the body mounts its defence, CD4 cells show some recovery and viral replication drops rapidly but fails to clear and is maintained around a certain level called the viral set point. This marks the end of this phase. The level of viral copies at this stage could range between 1,000 and 100,000. Zuckerman et al (2009:511) explain that in most individuals, this phase is accompanied by the acute HIV syndrome, which is characterised by the acute onset of a constellation of flu like symptoms including fever, rashes, inflammation of lymph nodes, and pharyngitis. This condition is self-limiting and resolves by itself.

2.8.2 Asymptomatic phase

This phase begins with the establishment of a viral set point (Longo et al 2012:1520). There are no clinical symptoms during this stage, but viral replication increases gradually over the years, accompanied by a gradual decrease in CD4 T lymphocytes (see figure 2.7). This phase could last three to 20 years depending on the viral set point. The lower the viral set point, the longer the duration of this phase and vice versa (Longo et al 2012:1520).

2.8.3 Symptomatic phase

Longo et al (2012:1540) state that this phase is marked by the onset of clinical symptoms. In untreated patients, nonspecific, mild infections appear in the form of skin fungal infections, herpes zoster, ear, and sinus infections. As the number of CD4 cells declines to below 200 per micro litre of blood, the more serious AIDS defining infections follow. The FHAPCO and FMOH (2007:2) identify the common AIDS defining conditions as oral candidiasis, severe bacterial infections, chronic diarrheal diseases, Pneumocystis Carinii Pneumonia, tuberculosis, and Kaposi's sarcoma. The authors further state that, as time goes on, these opportunistic infections become more frequent and eventually lead to death.

2.9   ANTIRETROVIRAL THERAPY

By definition this is therapy directed at the HIV virus (Longo et al 2012:1563). The coming sections further discuss this therapy from different perspectives.

2.9.1 Historical perspective

Zidovudine (AZT) was the first antiretroviral drug ever used for treating HIV. It was used as mono therapy and marked the beginning of a revolution towards managing this disease (Fischl, Richman, Grieco, Gottlieb, Volberding, Laskin, Leedom, Groopman, Mildvan, Schooley, Jackson, Durack, King and the AZT Collaborative Working Group 1987:187). The short term benefit was marked but a different study demonstrated its limitation in regard to the long term benefit, since there was no difference in survival, disease progression, or death comparing the effects of AZT and a placebo (Seligmann, Warrell, Aboulker, Carbon, Darbyshire, Dormont, Eschwege, Girling, James, Levy, Peto, Schwarz, Stone, Weller, Withnall, Gelmon, Lafon, Swart, Aber, Babiker, Lhoro, Nunn & Vray 1994:873). This was the result of drug resistance occurring with mono therapy. This finding resulted in the launching of combination therapies starting with dual drug use in the form of zidovudine with didanosine or zalcitabine (Aber, Aboulker, Babiker, Bragman, Breckenridge, Carbon, Charreau, Chene, Collis, Cooper, Darbyshire, Dormont, Fiddian, Flepp, Gazzard, Goebel, Hooker, Lange, Luthy, Peto, Reiss, Seligmann, Stone, Thomis, Vella, Walckenaer, Warrell, Weller, Wilber, Yeni, Yeo & Withnall 1996:286). Such studies showed that dual therapy is superior to mono therapy and paved the way for subsequent studies using three drugs. Three drugs combinations are the mainstay of therapy at present. Zuckerman et al (2009:917) state that studies done with early initiation of HAART identified challenges with adherence, and toxicity, for which reason the need for eligibility criteria became evident in order to balance those factors with respect to long term benefits.

2.9.2 Eligibility criteria

Eligibility criteria for the initiation of antiretroviral therapy are based on CD4 cell count and the staging of the disease. Currently, the WHO (2013:92) recommends initiation for all adult and adolescent (>14 years) patients with a CD4 cell count below 500, with those below 350 or those whose disease stage is advanced being given top priority. The same criteria apply for children above five years of age. For children below five years of age, all are eligible for initiation but priority is being given to those whose

disease is at advanced stage. The CD4 cell count cut-off for adults and adolescents was 350 during the 2010 revision of eligibility criteria according to the WHO (2010a:25).

2.9.3 Access to antiretroviral therapy

Access to antiretroviral therapy has been progressively increasing all over the world, and especially in low and middle income countries, where it reached 9.7 million people in 2012 (UNAIDS 2013b:6). This represents great progress in considering all eligible for the treatment. For sub Saharan Africa, the most affected region by the HIV/AIDS pandemic, nearly 56% of eligible people has been put on HAART (see figure 2.8) (UNAIDS 2012:23). For Ethiopia, 68% of the 400,000 people who need the treatment have started HAART (UNAIDS 2013b:A85). Treatment eligibility cut-off for these assessments was at CD4 cell count of ≤350/mm^3 according to the WHO (2010a:25) treatment guideline.
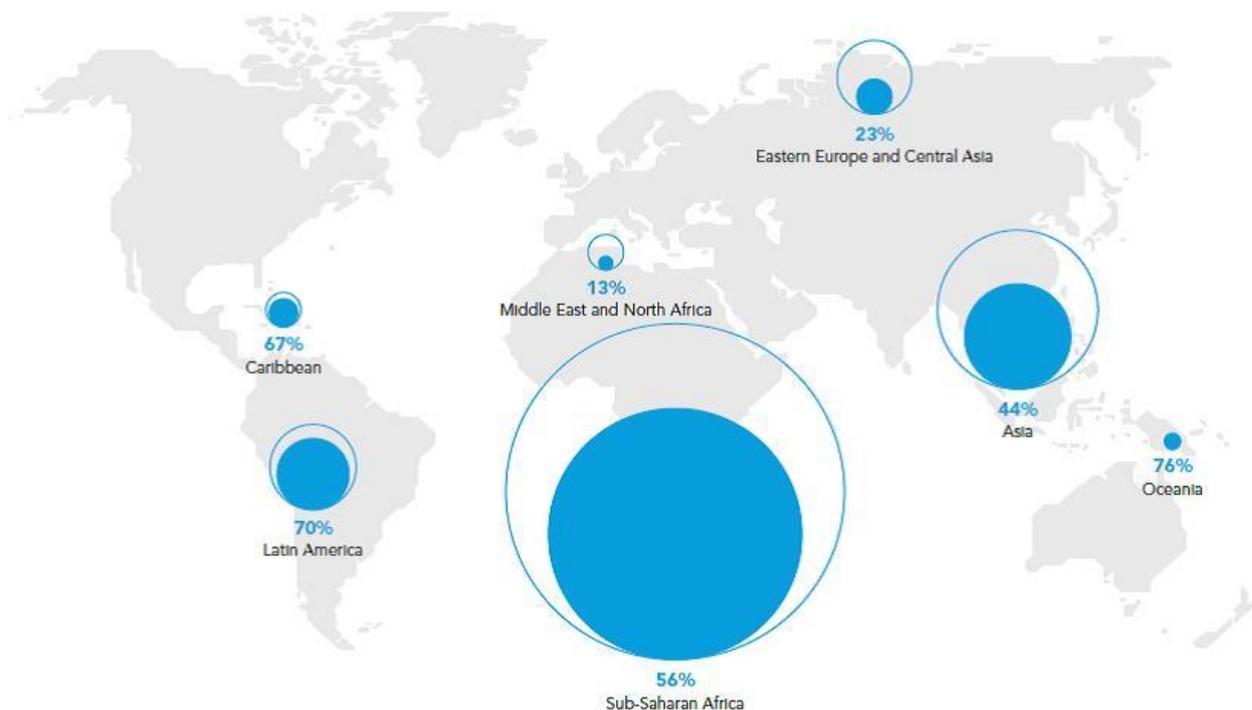
(UNAIDS 2012:23)

Figure 2.8   Eligibility for antiretroviral therapy versus coverage, low- and middle-income countries, by region, 2011

UNAIDS (2013b:A85) plans to put 15 million people on antiretroviral therapy by 2015 as part of fulfilment of HIV component of Millennium Development Goal (MDG) 6 (United Nations 2012:38).

2.9.4 Antiretroviral drug classes

Various classes of drugs have been invented over the years. There are five classes of drugs in clinical practice globally. These include nucleoside reverse transcriptase inhibitors (as for example, zidovudine, tenofovir, lamivudine, didanosine, abacavir), non-nucleoside reverse transcriptase inhibitors (example nevirapine, efavirenz), protease inhibitors (as for example ritonavir boosted lopinavir, ritonavir and boosted atazanavir), fusion inhibitors (example maraviroc), and integrase inhibitors (like raltegavir) (Department of Health and Human Services 2013:P-1; Longo et al 2012:1563). Three combinations of drugs from at least two different classes must be used in order to ensure effective treatment. All drugs listed in the examples are available in Ethiopia except for the fusion inhibitors and integrase inhibitors (FHAPCO & FMOH 2008a:122).

2.9.5 Goal of therapy

The WHO (2013:176) states that the goal of antiretroviral therapy is to achieve viral suppression to an undetectable level. It further clarifies that achieving this goal means very limited viral replication, meaning improved immunologic and clinical outcomes. In order to achieve this, adherence to treatment must be more than 95% (FHAPCO & FMOH 2008a:82).

2.10 ADHERENCE PREPARATION BEFORE INITIATING ANTIRETROVIRAL THERAPY

Adherence preparation for ART is an important step before initiating treatment. This is because poor adherence to treatment can lead to drug resistance and ineffectiveness of the medication being taken (WHO 2012b:10). It is said that the first regimen taken is the most effective. Even if the treatment regimen is changed once the first regimen fails, drug resistance may continue to pose a problem as there is cross resistance among drugs within the same class (FHAPCO & FMOH 2008a:103).

The WHO (2013:89) states that once eligibility is determined, adherence preparation should start as soon as possible by assessing patient's willingness, readiness for, and knowledge about HIV/AIDS and ART. This should be followed by addressing the

knowledge gap of the client in a coordinated way so as not to overwhelm the client with too much information. So, such counselling sessions must be planned ahead of time, when possible, to address specific issues in multiple visits. A systemic review done by Bärnighausen, Chaiyachati, Chimbindi, Peoples, Haberer and Newell (2011:946) show the added value in having a structured education program rather than informal sessions in providing adherence education. Two to three well-planned visits are usually adequate to address the most important aspects of treatment, namely the effectiveness of the therapy, the medication dosage, the drug side effects, opportunistic infections, recommended nutritional intake, personal hygiene, and the risk of transmission to others. Once a common understanding is established, all potential barriers to adherence in the individual (for example, the lack of a care giver, mental illness, substance abuse, forgetfulness) or the health system (for example distance to facility) must be discussed including the means to overcome them (WHO 2013:176). The WHO (2013:178) states that client specific arrangements need to be made to address any major barrier. Such assessment should be made continuously even after treatment is commenced in order to identify any potential problem and manage it. Treatment should be initiated once the client and provider agree on all important aspects of the treatment.

2.11 ADHERENCE ASSESSMENT DURING ANTIRETROVIRAL THERAPYINTAKE

The WHO (2013:181) states that in routine care settings, adherence can be assessed by viral load measurement, pill count, pharmacy refill records, or self-report. Additional complex adherence monitoring tools are also available according to Turner (2002:S143) which include electronic devices, and biochemical assays that measure blood level of antiretroviral drugs. The level of adherence in different care settings is reported below.

2.11.1 Level of adherence to HAART in developed countries

Cohen, Meyers and Davis (2013:online) calculate that the mean medication possession ratio, which is a proxy measure of adherence to picking up a prescription refill, among 8,721 patients on ART in the United States of America (USA) insured with Medicaid was 81% (with a standard deviation of 15%). The level of adherence was higher for patients who took single tablet regimens once a day. This study was representative as it was conducted in 11 different states. In another study with a wider geographic coverage in North America that spanned Canada, the USA and Puerto Rico, the mean level of self-

reported adherence over the 30 days prior to the data collection day was 85.7% (with a standard deviation of 21.8) among 1,875 patients receiving ART (Phillips, Webel, Rose, Corless, Sullivan, Voss, Wantland, Nokes, Brion, Chen, Lipinge, Eller, Tyer-Viola, Rivero-Méndez, Nicholas, Johnson, Maryland, Kemppainen, Portillo, Chaiphibalsarisdi, Kirksey, Sefcik, Reid, Cuca, Huang & Holzemer 2013:online). The level of adherence in North America was, in general, comparable to that found in a study in Italy where in it was reported that adherence among 2,763 patients who received ART in March and May 2010 was 100% and 79% respectively in the week prior to the data collection. No association was found between self-reported adherence and the frequency and number of pills taken (Gianotti, Galli, Bocchiola, Cahua, Panzini, Zandonà, Salpietro, Maillard, Danise, Pazzi, Lazzarin & Castagna 2010:156). In a meta-analysis that included 9,931 HIV patients in 23 observational studies done in Spain, Ortegoa, Huedo-Medinab, Vejoa and Llorcac (2011:286), it was reported that the level of adherence more than 90% was 54% (95%CI: 49-59%). The findings of this study showed levels of adherence much lower than those reported from other studies. The authors explained that this was because 52% of studies used more than one tool to assess adherence, like for example, pharmacy dispensary records, plasma drug concentration, left over pill count, viral load and electronic devices in addition to self-report, and emphasised the need to use multiple strategies in order to unmask overestimation of adherence as obtained from self-reports.

2.11.2 Level of adherence to HAART in sub-Saharan Africa

Studies from Kenya, Uganda, and Cameroon show that the level of adherence to ART ranges between 61% and 98%. The actual level varies depending on the type of adherence measure (in the form of self-report, record monitoring, or plasma drug levels) employed and the time patients have been on treatment. (Boruett, Kagai, Njogo, Nguhiu, Awuor, Gitau, Chalker, Ross-Degnan, Wahlström and Tomson 2013:online; Mghamba, Minzi, Massawe & Sasi 2013:online; Roux, Kouanfack, Cohen, Marcellin, Boyer, Delaporte, Carrieri, Laurent & Spire 2011:S42).

2.11.3   Level of adherence to HAART in Ethiopia

Most of the studies done on the level of adherence in Ethiopia are based on the self-report of adherence. Azmeraw and Wasie (2012:120) reported that in their study, 80%

of patients had optimal adherence (at 95%) among 340 children in two referral hospitals in Northwest Ethiopia. Among adult patients, Giday and Shiferaw (2010:191) have reported adherence of ≥95% among 88% of patients followed for one month. Kebede and Wabe (2012:67) report a 95% adherence for HAART among 24 TB/HIV co-infected patients in a hospital in Southwest Ethiopia.

A study that assessed adherence for the month prior to the study found adherence to be 80.2% among 349 patients in central Ethiopia (Dagnew 2009:89). Another study in a university hospital in Southwest Ethiopia evaluated the timing of pill intake in addition to adherence to doses. The finding of this study was that 303 (95%) of the study subjects were adherent based on dose adherence during the week before the actual interviews while adherence including timing and food intake was found to be 72.4% (Tiyou et al 2010:39).

Chalker et al (2010: online) report that adherence to appointment date was 86% while the adherence by to the regimen measured by self-reporting and by pill count was 94%, and 76% respectively. A tendency of overestimate adherence was observed in another study by Beyene et al (2009:1010); in that study, optimal adherence among 93% study participants was reported when15-days of self-report were used, while it was 88% when the measure was on unannounced pill count. While adherence has been suboptimal in most studies reviewed, a degree of overestimation found in data based on self-report leads one to assume the true level of adherence to be lower in most study-settings than reported.

2.12   MONITORING OF ANTIRETROVIRAL THERAPY

The WHO (2013:131) identifies three options for monitoring treatment response. These are virological, immunological and clinical options. With virological monitoring, the level of plasma RNA is measured as an indicator of the viral load. It is recommended that viral load measurements be taken at baseline followed by another measurement taken at six months after antiretroviral treatment is initiated. Hereafter, it should be measured yearly. Viral load measurement is the gold standard for assessing treatment response because a decrease in the level of viral load is highly associated with improved clinical outcome (Murray, Elashoff, Iacono-Connors, Cvetkovich & Struble 1999:799).

With immunological monitoring, the number of CD4 T lymphocytes in the blood is measured. The WHO (2013:131) recommends that the CD4 cell count be determined at baseline and every six months during treatment. The CD4 cell count is a measure of the strength of the immune system and is a good predictor of patient survival (Egger, May, Chêne, Phillips, Ledergerber, Dabis, Costagliola, D'Arminio, De Wolf, Reiss, Lundgren, Justice, Staszewski, Leport, Hogg, Sabin, Gill, Salzberger & Sterne 2002:125). Clinical monitoring is a documentation of the clinical stage of the patient and must be done at every visit. In resource poor settings, viral load monitoring, which is the gold standard, is too expensive to use, for which reason the WHO (2013:133) recommends immunological and clinical monitoring.

2.12.1   The pattern of immunologic monitoring in developed countries

One multi-national study by Podlekareva, Reekie, Mocroft, Losso, Rakhmanova, Bakowska, Karpov, Lazarus, Gatell, Lundgren and Kirk (2012:online) evaluated the frequency of immunologic monitoring among patients started on ART as part of an evaluation of the provision of care for patients in chronic HIV care. It included 5,859 patients from 34 countries in Europe and Argentina. The study found that the median number of CD4 cell count tests done on a patient varied considerably among the different regions of Europe. West-Central European countries had the highest number of tests per patient per year at 3.5, followed by northern and southern European countries having three annually and Eastern-Central European countries having an average of 2.5 per year. This indicates that, countries from the aforementioned regions have more tests than the WHO recommends for CD4 cell count testing, and implies that a more stringent monitoring strategy exists in those countries (WHO 2013:132). In eastern European countries, the level of monitoring is two tests per patient per year, as per the recommendation, while in Argentina, it is slightly lower, 1.5. In general, viral load testing followed the same pattern and from this one can conclude that the system of monitoring treatment response among those in the study population Europe met the set targets.

2.12.2   The pattern of immunologic monitoring in sub-Saharan Africa

In general, there is limited published literature addressing the issue of laboratory monitoring for patients on ART in sub-Saharan Africa including immunologic monitoring.

Fawibe, Olafimihan, Salami, Desalu and Odeigah (2010:290) report that in a teaching hospital in Nigeria providing chronic HIV care, evaluation of the baseline and follow-up CD4 cell count was documented for 56% and 28% of patients respectively among those who had had follow-up for at least one year (N=440) over a period of 12 months. A prospective study in Kenya that evaluated the effect of electronic reminders for ordering CD4 cell count tests reveals that overdue baseline CD4 cell count tests were 29% (N=1482) at baseline. The percentage of requests for these overdue tests was 42% and 36% respectively for patients in intervention and control clinics (Were, Shen, Tierney, Mamlin, Biondich, Li, Kimaiyo & Mamlin 2011:152). These studies point to a much lower frequency of lab monitoring for ART patients in the study settings.

In resource limited settings such as existing in most sub-Saharan African countries, there are a number of challenges that hamper the effective implementation of immunologic monitoring. Zachariah, Reid, Chaillet, Massaquoi, Schouten and Harries (2011:39, 40) state that maintaining the traditional laboratory based or non-point of care CD4 cell count tests is problematic because of technology, logistic, and patient factors. The authors further explain that the problem with the technology is that the machines are expensive and prone to breakdown and require regular maintenance. Even when they are present and functional, they require skilled workers to run them, which there is a scarcity of. Logistics issues include problem with sustained availability of reagents, test tubes, and other supplies. In Malawi, such factors contributed to the progressive decline of functional CD4 cell count machines from 52 in 2009 to 41 in 2010. Taking this into consideration, it would not be surprising to get a much lesser frequency of monitoring in resource poor settings.

2.12.3   The pattern of immunologic monitoring in Ethiopia

Literature is scanty on this issue in Ethiopia, but the available data reveals a similar picture of suboptimal CD4 cell count monitoring in chronic HIV care settings in Ethiopia similar to that in settings in other sub-Saharan African countries. Berhan (2011b:205) reports that in a multicentre study that included seven public hospitals in Ethiopia, 6th and 24th month follow-up CD4 cell count tests were done for 68% and 37% of children respectively (N=1,163). Another study in northwest Ethiopia showed that only 21% of patients had had follow-up CD4 test in the previous six months (Alemayehu, Bushen & Muluneh 2009:359).

2.13 IMMUNOLOGIC RESPONSE AFTER TAKING ANTIRETROVIRAL THERAPY

According to the Department of Health and Human Services (2013:C-7) a minimum of a threefold decrease in viral load measurement in the first three to six months of therapy is an indication of adequate treatment response, while sustained suppression is said to occur when there is undetectable viral replication during follow-up. The same guideline further clarifies that it takes a year or two to achieve complete suppression in patients with proper follow-up.

In patients with treatment judged to be successful, the CD4 cell count increases by 50-150 in the first year of treatment. A further yearly increase of 50 to 100 cells per micro litre of blood is expected per year in patients with suppressed viral replication (Department of Health and Human Services 2013:C-5). The rate of the CD4 cell increment is lower for patients with a low starting CD4 cell count and for the elderly in spite of viral suppression (Althoff, Justice, Gange, Deeks, Saag, Silverberg, Gill, Lau, Napravnik, Tedaldi, Klein & Gebo 2010:2473).

2.13.1 The pattern of immunologic response in developed countries

In a study conducted in San Diego primary infection cohort, the rate of CD4 cell recovery and the peak CD4 cell count achieved after 48 months of HAART was lower for patients who initiated treatment later as well as for patients with a lower CD4 cell counts at baseline (Le, Wright, Smith, He, Catano, Okulicz, Young, Clark, Richman, Little & Ahuja 2013:222). In a different study done among members of the military, the United States Military HIV Natural History Study, the first six months showed the greatest increase of CD4 recorded, at 93-151 CD4 cells. Then after, the yearly CD4 cell increment was 22-36 CD4 cells per year. Furthermore, it was shown that patients who started treatment with a lower CD4 cell count (≤200) were seen to follow a trajectory of CD4 cell recovery lower than those that started when there was higher CD4 cell count, for which reason their mean CD4 cell count after 12 years on HAART failed to reach 400, while those with a higher baseline CD4 cell count managed to have a CD4 cell count above 500 (Lifson, Krantz, Eberly, Dolan, Marconi, Weintrob, Crum-Cianflone, Ganesan, Grambsch & Agan 2011:online).

Touloumi, Pantazis, Chaix, Bucher, Zangerle, Kran, Thiebaut, Masquelier, Kucherer, Monforte and Meyer (2013:online) report that immunologic response was similar among B (predominant subtype in most developed nations) and non-B subtypes except for subtype A of the HIV virus, which showed a lower increase of CD4 cells in the first three months followed by a higher rate of long term CD4 cell recovery after controlling for gender, sexual orientation, injecting drug use, age at initiation of HAART, prior antiretroviral drug exposure, time from sero-conversion, initial HAART regimen, and viral load at baseline. Apart from that, they reported a similar pattern of CD4 cell recovery, which was an initial rapid CD4 cell count increase in the first three months and a slower one thereafter.

2.13.2   The pattern of immunologic response in sub-Saharan Africa

Kanters, Nachega, Funk, Mukasa, Montaner, Ford, Bucher and Mills (2013:online) explain that in their prospective cohort study involving 5,271 patients on HAART in Uganda, the largest increase in CD4 cell count was recorded in the first year of therapy. After five years of therapy, they reported that CD4 cell recovery had reached a plateau. In their study, the baseline CD4 cell count was the strongest predictor of CD4 cell recovery, and adolescents experienced the greatest CD4 increase. A different study comparing clinical outcomes between patients with and without Kaposi's sarcoma in South Africa found that the pattern of CD4 cell recovery still held a similar pattern of a greater initial step increment followed by a protracted and gradual increase later in the course of treatment. Patients with Kaposis Sarcoma were also found to be less likely to show an increase of 50 in their CD4 cell count in the first six months as compared to those without Kaposis Sarcoma (Maskew, Fox, van Cutsem, Chu, MacPhail, Boulle & Egger 2013:online).

Nakanjako, Ssewanyana, Nabatanzi, Kiragga, Kamya, Cao and Mayanja-Kizza (2013:online) demonstrate that patients in Uganda with lowest CD4 cell count increase having been on HAART for four years despite suppressed viral replication (also called suboptimal immune responders) tend to have lower baseline CD4 cell count as compared to optimal immune responders. They further substantiate this by demonstrating that cells from these patients have impaired T-cell proliferation (to which CD4 cells are part of) in vitro studies in reaction to stimulation with antigens from

common pathogens infecting HIV patients hinting at depletion of reserves. This same phenomenon was observed in vitro study among patients in the USA (Lederman, Calabrese, Funderburg, Clagett, Medvik, Bonilla, Gripshover, Salata, Taege, Lisgaris, McComsey, Kirchner, Baum, Shive, Asaad, Kalayjian, Sieg & Rodriguez 2011:1222).

2.13.3   The pattern of immunologic response in Ethiopia

In general, in Ethiopian patients on HAART, CD4 cell increment was found to be related with duration of HAART, the greatest increase observed in the first six to twelve months. A pattern of slight decrease in mean CD4 cell count in later years of follow-up was also noted (see figure 2.1). Age, functional status, and baseline WHO stage were all not found to be associated with CD4 cell increment (Berhan 2011a:294; Tsegaye & Worku 2012:107; Kassa, Gebremichael, Alemayehu, Wolday, Messele & Van Baarle 2013:online; Reda, Biadgilign, Deribew, Gebre & Deribe 2013:online).

(Reda et al 2013:online)
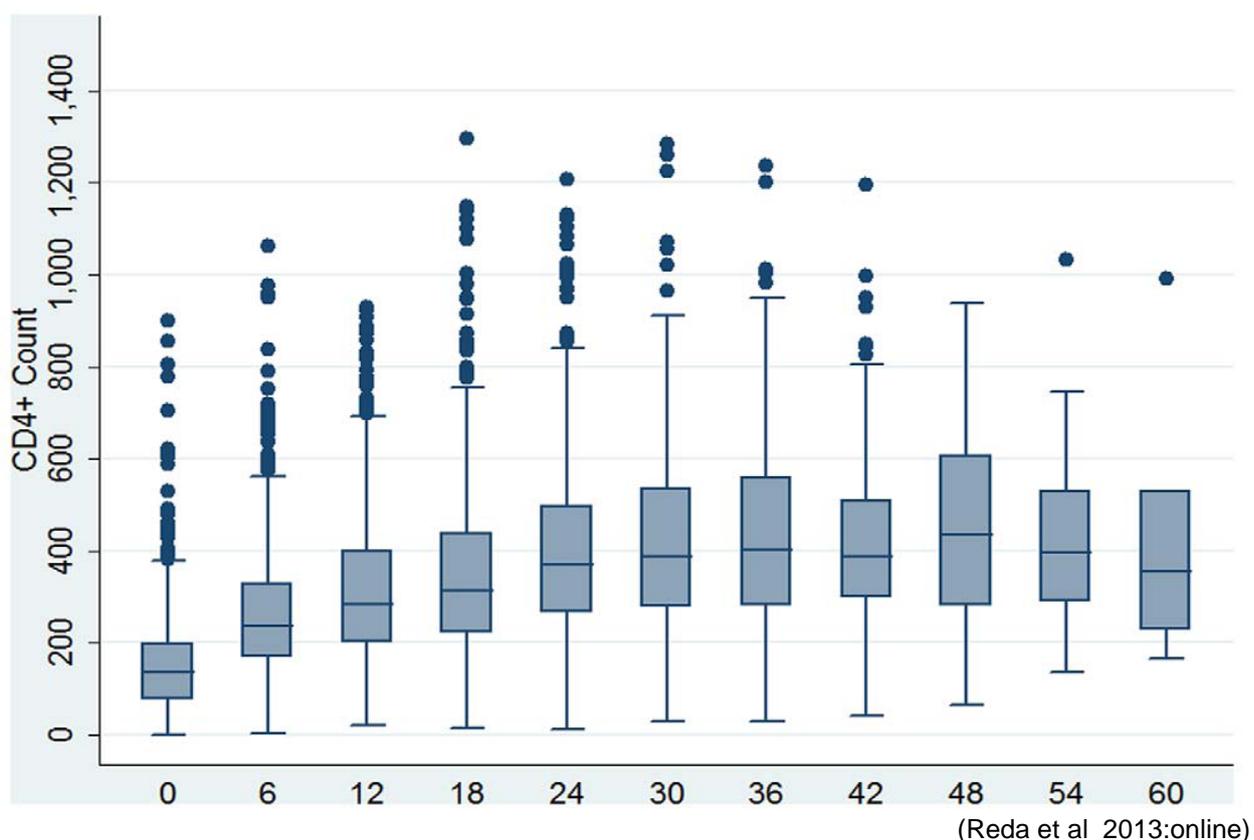
Figure 2.9   Box plot for changes in magnitude of CD4 cell count among the cohort of patients on anti-retroviral treatment across months of retrospective follow-up over five years

2.14 IMMUNOLOGICAL TREATMENT FAILURE

The consequence of poor treatment adherence is treatment failure, which is then managed by switching treatment to another effective regimen than the patient was taking when the treatment failed (Department of Health and Human Services 2013:K-2; WHO 2013:176). The criteria used to measure adequate treatment response cannot be used to decide which ones require treatment switching especially for those with a poor immunological response, as they may actually have adequate viral suppression. Even for viral load measurement, a temporary rise of plasma RNA could be wrongly assessed for treatment failure (Department of Health and Human Services 2013:C-5, C-7). For this reason, in order to identify those with immunological failure, the WHO criteria must be used to declare a patient as having immunologic treatment failure. In keeping with the objective of this study, only immunological criteria are further discussed.

2.14.1 Immunological treatment failure criteria

Immunologic failure is when there is a decline of CD4 cells by 50% from the peak level achieved during treatment or when it goes to or below the baseline CD4 cell count level, or when it is persistently below 100 (WHO 2010b:50). These criteria should be applied only after a minimum of six months of therapy with HAART. Interpretation of immunologic criteria should be made carefully since patients with suppressed viral replication may have immunological failure. This is because these criteria have low sensitivity and positive predictive value for virological failure (WHO 2010b:48). But, since they have very high negative predictive value, they can be used to identify those who may need a further work up with viral load measurement. This strategy will prevent unnecessary switching to second line therapy (WHO 2013:135).

2.14.1.1 The prevalence of immunologic treatment failure

Since developed nations use virological parameters, the so-called gold standard, to define treatment failure (WHO 2013:133), data is in general lacking on prevalence and determinants of immunologic treatment failure among patients on HAART for those nations. For this reason, discussion for this and the next section is limited to sub-Saharan Africa.

2.14.1.1.1   The prevalence of immunologic treatment failure in sub-Saharan Africa

A number of studies in sub-Saharan Africa have assessed the prevalence of immunologic failure and have come up with variable results, but in general, most studies point to a conclusion that, in most settings, the prevalence of immunologic treatment failure has been more than 10% (range 10-32%) (El-Khatib et al 2011:online; Jaka et al 2009:6; Rawizza et al 2011:1286; Reynolds et al 2009:697). In these studies, the follow-up duration was variable, ranging from 15 to 33 months. From the reviewed literature, one study in Nigeria has a contradictory finding. In this study the prevalence of first line HAART failure was reported to be very low, at 1.19% among 9,449 patients during the study period, but the follow-up duration was not reported (Eze & Uyagu 2009:46).

2.14.1.1.2   The prevalence of immunologic treatment failure in Ethiopia

For Ethiopia, data is available only for paediatric patients. Bacha et al (2012:online) report immunologic failure to exist for 9.2% of 1,186 children with a mean age of six years and a mean follow-up of 37 months. In another study among a smaller cohort of 96 paediatric patients (with a mean age six years, mean follow-up duration of 13.7 months) immunologic failure was at 11.5% (Workneh et al 2009:78). Despite an extensive search, similar data could not be found for adult patients. Assefa et al (2011:online) report that the prevalence of patients on second line HAART among 7,451 clients actively on follow-up at 24 months was 2.13%. But because of difficulties in monitoring treatment response in many settings and difficulties concerning the utilisation of available resources by health care workers, the actual prevalence of treatment failure is expected to have been higher (Azzoni et al 2012:online; Bélec & Bonn 2011:1255). The level of patients on second line HAART was comparable to what the WHO (2009:79) reports but much higher than that reported by 48 MSF supported programs in resource limited countries (Pujades-Rodriguez et al 2008:1307), which was a switch to a second-line regimen of 4.1 per 1,000 person-years after 20 months of follow-up among 48,000 patients.

2.15   DETERMINANTS OF IMMUNOLOGIC TREATMENT FAILURE

Few studies have been conducted to assess for predictors of immunologic treatment failure among patients on HAART. Kassa et al (2013:online) demonstrated that at six

months post HAART initiation, among patients in Addis Ababa, Ethiopia, the odds of immunologic failure were 5.6 times higher for patients with a baseline CD4 cell counts of less than 100 as compared to those with a higher CD4 cell counts (95% CI 1.6-20.1; p value=0.008). The same study found that patients in an advanced disease stage at treatment initiation were also found to be more likely to experience immunologic failure than those who were asymptomatic (an odds ratio of 4.3, 95% CI 1.4-13.4; p value=0.01). The problem with this assessment is that the authors used a different set of criteria to define immunologic treatment failure than what the WHO (2010a:50) has recommended. They used the criteria 'an increase of CD4 cell count by fewer than 50 cells/µl at month six,' and the criteria 'fewer than 100 cells/µl at months 18 and 24 of HAART'. These criteria are rather similar to what other authors have defined as a sub-optimal immunologic response, which is completely different from immunologic treatment failure (Lederman et al 2011:1222; Maskew et al 2013:online; Nakanjako et al 2013:online). For this reason, any comparison of their findings with those in other studies is difficult.

A similar problem exists with the interpretation of a study conducted in Nigeria that assessed immunologic failure. The criteria the authors used were: a fall of the CD4 cell count to baseline or below; or an increase of CD4 cell count by less than 50cells/mm^3 at one year after HAART initiation, which is still different than the WHO recommendation (2010b:50). The findings of the study were that patients with immunologic failure were more likely to be of the male sex and of an age less than 30 years (OR 1.46; 95% CI 1.04-2.45; p value=0.04) (Anude, Eze, Onyegbutulem, Charurat, Etiebet, Ajayi, Dakum, Akinwande, Beyrer, Abimiku & Blattner 2013:online). Overall, there is limited literature on predictors of immunologic treatment failure as defined by the WHO (2010b:50) for use in resource limited settings.

2.16 INTERVENTION FOR THOSE WITH TREATMENT FAILURE

Since one of the most common causes of treatment failure is poor treatment adherence, any attempt to manage treatment failure by simply switching the HAART drug regimens are futile, as the same problems that caused the treatment to fail in the first place may result in treatment failure for the new regimen as well (Department of Health and Human Services 2013:H-2, H-3).

2.16.1   Managing poor treatment adherence

This should be done by addressing barriers to adherence at the various levels which are discussed below.

2.16.1.1   Factors related to the health system

The WHO (2013:183) states that such factors include the high cost of travel or treatment, use of regimens with a high pill burden or a high level of severe side effects, the frequency of drugs being "out of stock", and the lack of adequate and trained health care workers to provide high quality care. The WHO further states that these factors could be addressed if the health care system were designed based on the recommended continuum of care and followed the public health approach. Such system would provide care free of charge, and provide fixed dose combination drugs with minimal side effects and pill burden. In addition, there would need be some task shifting used to address the overburdened health care providers through deployment of adherence supporters to assist in providing appropriate counselling, adherence preparation, and patient tracing (WHO 2008:14). Decentralization and integration of services would help in addressing accessibility issues.

2.16.1.2   Factors related to the individual

The WHO (2013:183) states that such personal factors include forgetfulness, not appearing for clinic visits or not taking medications at home for fear of being seen taking HAART medication, the lack of a care providers for guidance and co-morbid conditions such as poor mental health and substance abuse. The most important intervention for removing such barriers would be adequate adherence preparation and on-going personalised follow-up during therapy in the form of education and counselling concerning the issues that prove troublesome, both for patients and their care givers. This could be done by adherence supporters or community health workers and could help those providing care identify needs and get the proper care for patients, with consultation to health care providers when required. A review by Bärnighausen et al (2011:944) has demonstrated the effectiveness of such interventions in multiple countries. In addition to this, the WHO (2013:183) recommends the use of text messaging to manage forgetfulness when it is applicable.

2.16.2   Managing treatment failure

Patients with treatment failure should be managed by switching treatment to an appropriate regimen. In developed countries, doing this can be facilitated by the determination of resistance patterns, which is very useful in selecting patient specific regimens that will be able to effectively suppress viral replication in the face of drug resistance (Department of Health and Human Services 2013: H-1). In resource poor settings, though, this cannot be done because these tests are too expensive. For this reason, the WHO (2013:147,150) has come up with standardised second line regimens to use when failure is diagnosed with known first line regimens based on the most likely resistance pattern expected to occur (see tables 2.1 A and B). These regimens are used in Ethiopia except emitricitabine (FTC).

TABLE 2.1a   SUMMARY OF PREFERRED SECOND-LINE ART REGIMENS FOR ADULTS AND ADOLESCENTS

Target population	Preferred second-line regimen[a]	
Adults and adolescents (≥10 years)	If d4T or AZT was used in first-line ART	TDF + 3TC (or FTC) + ATV/r or LPV/r
	If TDF was used in first-line ART	AZT + 3TC + ATV/r or LPV/r

(WHO 2013:147)

TABLE 2.1b   SUMMARY OF RECOMMENDED FIRST- AND SECOND-LINE ART REGIMENS FOR CHILDREN AND ADOLESCENTS

	Children	First-line ART regimen	Second-line ART regimen
LPV/r-based first-line regimen	Younger than 3 years	ABC + 3TC + LPV/r	No change[a]
		AZT + 3TC + LPV/r	
	3 years and older	ABC + 3TC + LPV/r	AZT + 3TC + EFV
		AZT + 3TC + LPV/r	ABC or TDF[b] + 3TC + EFV
NNRTI-based first-line regimen	All ages	ABC + 3TC + EFV (or NVP)	AZT + 3TC + LPV/r[c]
		TDF[b] + 3TC (or FTC) + EFV (or NVP)	
		AZT + 3TC + EFV (or NVP)	ABC or TDF + 3TC[c] (or FTC) + LPV/r[c]

(WHO 2013:150)

AZT = zidovudine; d4T = stavudine; TDF = tenofovir; 3TC = lamivudine; FTC = emitricitabine; LPV/r = ritonavir boosted lopinavir; ABC = abacavir; NVP = nevirapine; EFV = efavirenze

2.17   CONCLUSION

This chapter dealt with a number of issues related to HIV and pertinent to this study. Initially, it dealt with the pathogenesis of the disease. This was followed by its epidemiology at various levels: global, African, and Ethiopian. The review also addressed treatment objectives of antiretroviral therapy and how treatment can be monitored with particular attention to immunologic criteria. Finally, determinants of treatment failure were briefly discussed. Chapter 3 addresses issues related to research methodology as used in this study.

CHAPTER 3

RESEARCH METHODOLOGY

3.1   INTRODUCTION

This chapter deals with the research methodology. It has four components: the research design, the research method, validity and reliability, and ethical considerations.

The research design pertains to the general approach the researcher chooses to answer the research questions. Research designs depend on the paradigm chosen. For quantitative studies, the design is further specified by aspects relating to the presence of interventions, the presence of comparison, and the timing of measurement (Polit & Beck 2012:58,59). The research methods outline the specifications with respect to the study population, aspects of sampling, data collection, and data analysis. Validity and reliability will be discussed further with respect to both study design and data collection instrument.

The purpose of the research methodology is to systematically address the research problem. The aim of the study was to generate knowledge by describing the practice of immunological monitoring at the facility level, and identifying determinants of immunologic failure among people living with HIV in selected hospitals. The specific objectives of this study were to:

- assess the pattern of immunologic monitoring in selected hospitals in southern Ethiopia
- assess the pattern of CD4 cell count response of patients on HAART in selected hospitals in southern Ethiopia
- describe immunologic treatment failure and identify its predictors among patients on HAART in selected hospitals in southern Ethiopia

The following sections of this chapter will address the details of research design, research methodology, validity and reliability, and ethics.

3.2   RESEARCH DESIGN

3.2.1   Paradigm

Polit and Beck (2012:736) defines paradigm as "a way of looking at natural phenomena that encompasses a set of philosophical assumptions and that guide one's approach to inquiry". It is like a model that is followed to answer research questions. There are two paradigms, positivist and naturalistic, whose distinction is made by answers to the following philosophical questions: what is the nature of the reality being studied (ontologic assumption); how is the researcher related to that which is being studied (epistemologic assumption); what is the role of researcher's values and views (axiologic assumption); and how is data acquired (methodologic assumption). (Polit & Beck 2012:13)

Quantitative research design is related to the positivist research tradition which dictates that, reality exists independent of the observer. A number of measures are taken to make sure the observation of phenomenon is objective and free of bias, unaffected by the beliefs of the observer including measurement of observations by an objective tool. A fixed and predetermined study design generates numerical data which is collected by counting or measuring a phenomenon of interest. (Polit & Beck 2012:13). In contrast to this, qualitative research design follows the positivist tradition where subjectivity and values of the investigator are deemed inevitable and demanded. The investigator's interaction with what is studied generates the information. A flexible study design, that which evolves as the study progresses, is followed. Narrative type of data is collected and analysed. (Burns & Grove 2009:22; Polit & Beck 2012:13)

3.2.2   Research design

Research design is defined as the overall plan to answer a research question (Polit and Beck 2012:741). In this study, a quantitative, correlational (or non-experimental) study, the researcher simply observes what is going on without interference or manipulation. Manipulation was not required nor was it possible in this study since all events occurred prior to data collection (Polit & Beck 2012:227). Since there was no intervention randomization of study subjects into exposure and control groups was not required. Patients' information gathered in their natural environment during routine clinic visits

was used to evaluate prevalence and determinants of immunologic failure. So this design was appropriate for this study. (LoBiondo-Wood & Haber 2010:310).

3.2.2.1 Quantitative elements

This design is appropriate for this study because the aspect of the dependant variable being studied; that is, pattern of immunologic monitoring, response and treatment failure were determined by examining and interpreting the pattern of quantitative measure of CD4 lymphocyte counts (WHO 2010a:50,51). The number of CD4 tests and magnitude of CD4 cell count and related data was measured by a pre-designed document checklist by data collectors making the data measurement independent of the observer. Also controlling mechanisms were used to analyse the data.

3.2.2.2 Correlational elements

This study is a correlational or non-experimental study as there was no intervention. In such correlational or non-experimental quantitative study design, the association between variables as they occur naturally is measured (Bruce, Pope & Stanistreet 2008:423). In addition to assessing the mere association between variables, this type of design can address the issue of whether one variable (a dependant variable) is predicted by another (an independent variable). In this study, the major aim was to assess the prevalence of immunologic treatment failure and factors that predict the development of immunologic treatment failure. With respect to the second aim, independent variables that correlated to immunologic treatment failure as well the degree of correlation were studied, while in the first, it intended to describe the point prevalence of immunologic failure, and describe other features of patients with immunologic failure and hence, this study had a descriptive component as well as a predictive analytic component (Burns & Grove 2009:13). In a descriptive research design, a health event of interest is simply described. (LoBiondo-Wood & Haber 2010:200,201; Polit & Beck 2012:226).

3.2.2.3 Longitudinal elements

In a longitudinal study design, subjects are followed for some length of time. Exposure and the outcome of interest are measured repeatedly as the follow-up continues. This is

in contrast to cross-sectional study where observation is made at one point in time (Polit & Beck 2012:181; Shaughnessy, Zechmeister & Zechmeister 2012:154,158).In this study, there were no distinctive cohorts or exposure groups that are followed. Two types of study designs exist based on the timing of data collection: prospective and retrospective. In the prospective design, a group of subjects is followed forward in time, and observations are made on the occurrence of outcomes of interest. In the retrospective design, both the exposure and outcome of interest has already occurred in the past and exposure precedes outcome (Polit & Beck 2012:187).

In this study, records of patients that were on HAART were followed and CD4 cell count monitoring, magnitude of CD4 cell count as well as the main outcome of interest, i.e. immunologic treatment failure, were evaluated at various times. Patients took treatment for some time before experiencing treatment failure. So there needed to be time sequence of events. All these features made the longitudinal design appropriate for this study. Both exposure and outcome status were already determined at the start of the study and hence that makes it a retrospective longitudinal study (Chernick 2011:12). This design was particularly advantageous for this study. This is because since the specific objective include assessment of the patterns of immunological monitoring and whether immunologic failure was being dealt with appropriately and in a timely fashion, health care workers might have modify their behaviour and been unnaturally more vigilant in reaction to their awareness of a prospective study being done addressing these issues (Polit & Beck 2012:225).

3.3   RESEARCH METHODS

'Research methods' refers to the specific techniques followed to structure a study including the specific means to collect and analyse data. It is specified by choices made in identifying study population, sampling technique, data collection approach, and analysis. (Polit & Beck 2012:12)

3.3.1 Population

'Study population' refers to the collection of all subjects being considered for a study that have some common characteristics (Polit & Beck 2012:273; Shaughnessy et al 2012:141). The study population in this study was medical records of HIV patients aged

five years and older who took HAART for at least six months in selected hospitals (Butajira and Nigist Eleni Mohamed Memorial, also called Hosanna) in SNNP regional state. Of these records, all were accessible for the investigator hence forming the 'accessible population'. At the time of sample size determination, there were a total of 881 patients in hospital A (Butajira hospital) and 1,266 in hospital B (Nigist Eleni Mohamed Memorial hospital).

3.3.2 Sampling

LoBiondo-Wood and Haber (2010:224) state that sampling is the process of selecting part of the accessible population with the intension to study the selected cases and make inferences about the target population. They further highlight advantages of sampling which includes saving time and cost associated with collecting data from large population. The 'target population' is the collection of all patients about which the researcher would like to make inferences about including patients beyond the accessible population (Polit & Beck 2012:273).

3.3.2.1 *Eligibility criteria*

These criteria are the ones that specify which subgroup of the study population is to be studied (Polit &Beck 2012:274). For this study, the eligibility criteria were as follows listed as inclusion and exclusion criteria:

Inclusion criteria

- People living with HIV on antiretroviral therapy enrolled and started on HAART ever since the program was started in the facility until 31 December 2012.
- Age ≥5 years

Exclusion criteria

- Patients who had been initiated on HAART and took it for less than six months,
- Patients who had fewer than two CD4 cell count tests, and

- Transferred in cases. These were cases that started ART in other facility than the study facility and transferred to the study facilities for continuation of treatment.

3.3.2.2 Sample size

The total sample size required was estimated for the combined accessible patients who were on HAART treatment in the two hospitals. Sample size calculations were done assuming random sampling for a finite population based on the estimation of a 50% proportion, at a 5% significance level and 2.5% precision (Daniel 2009:192). The formula used to estimate the sample size for the study was:

$n= [z^2p(1-p)N]/[(N-1)d^2+z^2p(1-p)]$

Where

n = calculated sample size

N = population size

z = critical value at the chosen significance level

p = proportion to be estimated

d = precision

Thus, for this study taking N=2,147, z=1.96, p=0.50 (50%) and d=0.02 (2%), the sample size was thus 1,133.703 which was rounded up to 1,134. Assuming the number of missing records to be 15%, the sample size was inflated to 1,303. This meant that after applying the eligibility criteria, of the remaining patients, 1,303 patients would be randomly selected. In other words, proportionate sampling was used (Daniel 2009:13).

3.3.2.3 Sampling process

The sample was selected through a sampling process. Any sampling plan should deliver a sample that accurately represents characteristics of the study population (Polit & Beck 2012:283). For the purposes of this study, sample was selected from the total population of clients that fulfilled the eligibility criteria. Of the two types of sampling designs, that is probability and non-probability sampling, probability sampling was chosen for this study (Shaughnessy et al 2012:144). In probability sampling, subjects are chosen randomly and have equal chance of being selected (Polit & Beck 2012:280;

Shaughnessy et al 2012:144). For this study, simple random sampling of all accessible patients fulfilling the eligibility criteria was planned.

Permission was obtained from the management of the hospitals under study and ethics committee of Regional Health Bureau of Southern Nations Nationalities and Peoples' region and National Ethics Review Committee of Ethiopia to access the list of all clients started on HAART. A list was compiled indicating the patient file number. The statistician then applied the eligibility criteria by going through the list and deleting those who didn't fulfil the eligibility criteria.

3.3.3 Data collection

This study utilized a self-designed document checklist. Personal and social details were not collected from human data sources.

3.3.3.1 Data collection instrument

Data collection was carried out by a self-designed document checklist (see annexure A) which consisted of the following three sections:

Section A included patients' age, and gender. These demographic variables were used to characterise the study population.

Section B This section included clinical characteristics in the form of baseline CD4 cell count, baseline WHO stage, and other variables that might predict treatment failure such as occurrence of tuberculosis, history of treatment interruption, and weight status. All these variables were used to characterise immunologic monitoring, immunologic response, and immunologic treatment failure.

Section C In this section immunologic information like timing of immunologic (CD4 cell count) monitoring (**section C.1**), timing and magnitude of CD4 cell count (**section C.2**), and timing and occurrence of immunologic failure (**section C.3**) were recorded.

3.3.3.2 Administering the data collection instrument

Data source were existing medical records. The advantage of using existing records was to save time and reduce cost than if data was collected prospectively. In addition, since the researcher was familiar with the records and the data collection procedures, appropriate precautions were taken where the records and the data entered had limitations (LoBiondo-Wood & Haber 2010:270, 279).

3.3.3.3   Pre-testing the data collection tool

The data collection tool and cleaning program codes were pre-tested prior to data collection and cleaning. The statistician did this among transfer-in patients. These were patients who started HAART treatment in other facilities than study hospitals and sent for follow-up to the study sites. Such patients were excluded from this study but had the same set of data as required for this study and hence provided ideal ground for pre-testing the data collection instrument. Twenty five such charts were randomly selected from one of the study facilities and the pre-test was carried out. The purpose of the pre-test was to assess the adequacy of the tool, the length of time required to do the data collection, and to make sure of the clarity of the procedures. (LoBiondo-Wood & Haber 2010:280; Shaughnessy et al 2012:168)

3.3.3.4   Data collection procedure

Data clerks at each facility provided electronic records to the statistician in the study hospitals. The database had a built in program to de-identify or remove patient identifiers like name and address. That program was run before the data was provided to the statistician. So total anonymity was ensured and even the researcher had no access to the identity or any personal information of the clients under study. After the statistician gave the data collectors the selected sample of cases, data collectors abstracted the data from medical records.

3.3.4   Data management and analysis

In this study, Epi Info[TM] 3.5.1 statistical software (United States Centers for Disease Control and Prevention 2007) and Stata/IC 12.0 (StataCorp LP) were used to undertake both descriptive and inferential statistics. P value was set at 0.05.

3.3.4.1   Descriptive statistics

The purpose of doing descriptive statistics was to summarize and organize raw data so as to understand the information contained in it in a simple way (Daniel 2009:20; Shaughnessy et al 2012:348). Data from section C of the checklist was used to address the following objectives:

- Assess the pattern of immunologic monitoring in selected hospitals in southern Ethiopia.
- Assess the pattern of CD4 cell count response for patients on HAART in selected hospitals in southern Ethiopia.
- The prevalence of immunologic treatment failure was calculated as a proportion with a 95% confidence interval.

A number of descriptive statistical measures were used in this study to summarise data. These include frequency distribution depicted in the form of tables for categorical and ordinal variables(Polit & Beck 2012:379; Shaughnessy et al 2012:122). The distribution of continuous variables (age and CD4 cell count) was displayed graphically in addition to summary of their mean, standard deviation, median, minimum and maximum values(Polit & Beck 2012:385; Shaughnessy et al 2012:355). Pattern of immunologic monitoring and response were examined graphically.

Pattern of immunologic monitoring was assessed using the national monitoring and evaluation standard for determination of follow-up CD4 cell count for patients on antiretroviral therapy. The timing for evaluation of patients was every six months the first year and yearly then after (FMOH 2010a:107). A CD4 cell count test was said to have been done timely if it was done within one month of that particular follow-up month the test was supposed to be done. The expected number of follow-up CD4 cell count tests was calculated for those patients who were still on follow-up in that facility. A bar chart was used to show and contrast expected and actual CD4 cell count tests that were determined timely. The use of bar chart is justified as it is used for categorical data with natural ordering (Chernick 2011:41). In this study, data are ordered by month of follow-up. Pattern of immunologic response was evaluated at cohort level every six months. All CD4 cell counts within two months of follow-up month of interest were used to

determine CD4 cell count response at that month. A box-plot was used to view this graphically. A box-plot is a graph that shows median, inter-quartile range, as well as outliers (Chernick 2011:41,42).

Descriptive survival analysis was used to assess the cumulative probability of immunologic failure. Survival analysis is used when analysing data with time dependent outcome that may or may not have complete follow-up information. Follow-up may be incomplete either because subjects don't experience event of interest or they are lost to follow-up before end of study (Daniel 2009:648). Result of analysis is shown in a life table as well as graphically by Kaplan-Meier curves for this study. Life table shows survival probability estimates for specific time intervals whereas Kaplan-Meier survival curves show continuous probability over time (Chernick 2011:159,160). In addition, Kaplan-Meier curves were drawn to graphically evaluate similarities and differences with respect to values of covariates on event free survival.

3.3.4.2   *Inferential statistics*

The Chi Square test, the log-rank test and multivariable analysis using Cox proportional hazards regression were used to analyse data. The P value was set at 0.05.

3.3.4.2.1   *The Chi square test*

The Chi square test is a non-parametric test that is used to compare two categorical or ordinal variables (Daniel 2009:598; Jacobsen 2011:207). It was used to identify variables that have effect on pattern of immunological monitoring and immunological response after HAART initiation.

3.3.4.2.2   *The log-rank test*

Daniel (2009:656) describes the log-rank test as a non-parametric test that is used to compare the survival experience of two or more groups. This test was used to supplement the graphical description and comparison of survival experiences between groups.

3.3.4.2.3   The Cox proportional hazards regression

Regression is a technique used to determine the effect of covariates on an outcome variable. In more practical terms, it is used to predict the value of a dependent variable given the value of independent variables (Daniel 2009:410). Cox proportional hazards regression techniques are those techniques used to model dependent variables that measures time to event data with or without the censoring of observations (Daniel 2009:659). For this study, the event was the first record of immunologic treatment failure. The term 'censoring' refers to the fact that observations may be incomplete in certain individuals because they discontinued follow-up in the selected facilities as a result of death, transfer to another facility or loss to follow-up. The hazard rate ratio is calculated during the Cox proportional hazards regression as compared to the odds ratio during the logistic regression or rate ratio during the Poisson regression (Daniel 2009:659). The term 'hazard rate' refers to the instantaneous risk of an event happening after a time 't' given that the subject survived up to time 't' (Hosmer, Lemeshow & May 2008:62).

3.3.4.2.3.1   Extensions of the proportional hazards regression model

In longitudinal studies, the values of some factors may vary with time. Two such variables in this study were adherence and tuberculosis status. The variable 'adherence' was assigned a value of '0' as long as patients were adherent to treatment with respect to their appointment to HAART medication prescription refill. If they failed to show up, this variable was assigned a value of '1' for the time of follow-up after the time of interruption. This enabled the investigator to compare if the risk of immunologic treatment failure varied between times after treatment interruption as compared to that before treatment interruption. In practical terms, this required splitting the observation time of patients into that up to the point at which treatment was discontinued, where the value to adherence was '0', and the time period after that, where the value given to adherence was '1'. So, the time an individual spent being adherent to appointments was taken into account. This is justified for this study since patients who failed to show up for their medication refill in effect discontinued treatment for at least more than two weeks and thus fell beneath the 95% adherence limit, thus putting themselves at risk of experiencing treatment failure (WHO 2012b:10). Tuberculosis was treated in the same way to assess if occurrence of tuberculosis was a predictor of immunologic failure. Such

modelling of time-varying covariates requires the use of extensions to the Cox proportional hazards regression modelling which was employed in this study (Collett 2003:251; Hosmer et al 2008:213;Machin, Cheung& Parmar 2006:176). In addition, tuberculosis cases after six months of therapy were considered during regression analysis. This was important in order to use it as proxy measure of follow-up WHO staging during treatment (treatment or T-staging).Finally, since samples were taken from two different sites, accounting for within site correlation was made by stratification of hazard ratios by site. The same was done for calculating the log-rank test (Hosmer et al 2008:208; Machin, Cheung & Parmar 2006:181).

3.3.4.2.3.2   Variable selection for Cox proportional hazards regression modelling

There are a number of techniques that can be used to develop a regression model. These include purposeful, stepwise and best subsets selection of covariates. In the first method variable selection is under the control of the data analyst, while in the latter two methods, covariates are selected by statistical procedures (Hosmer et al 2008:132). The former method of variable selection procedure was used in this study as recommended by Hosmer et al (2008:132).

3.3.4.2.3.3   Purposeful selection of covariates

In this section, the details of purposeful selection of covariates as used in this study are discussed as described by Hosmer et al (2008:132).

3.3.4.2.3.3.1 Step 1

In this step, a multivariable model containing all variables significant in the individual regression analysis at the 20-25 per cent level was generated.

3.3.4.2.3.3.2 Step 2

In this step, p-values from the Wald tests of the individual coefficients were used to identify covariates that might be deleted from the model. The deletion was made one variable at a time. P-value of the partial likelihood ratio test was used to determine whether the deleted covariate was significant or not.

3.3.4.2.3.3.3 Step 3

In this step, the effect of the deletion of covariates on coefficients of those variables still remaining in the model during Step 2 was evaluated. A change in coefficient was considered significant if its coefficient changed by 20 per cent or more. If a variable that had this much effect was deleted in Step 2, then it was added back to the model.

3.3.4.2.3.3.4 Step 4

In this step, variables left out in the initial multivariable model were added one at a time and checked to see if that made any significant change in the model or in the coefficients in the model. Then, partial likelihood ratio test was used to check whether there were significant changes to the model. Any variable with such an effect was kept in the model, thus giving a preliminary main effects model.

3.3.4.2.3.3.5 Step 5

In this step, the scale of continuous covariates found in the model was checked. This was graphically checked by plotting Martingale residuals from a model excluding the variable of interest and plotting that against the variable. A Lowess curve was then fitted to aid in estimating the functional form of the covariate in the log hazard. If this line was non-linear, then fractional polynomials analysis was used to decide if transformation was needed. If it was linear, the variable was unchanged in the final model, but if the transformation fit the data better, then the new term(s) was kept in the model to serve as the main effects model.

3.3.4.2.3.3.6 Step 6

In this step, there was testing done to assess whether or not there was any interaction of variables to be used in the final model. The interaction terms significant at the p-value of 0.05 were used to select interaction terms. A partial likelihood ratio test was used to decide if inclusion of such terms had statistically significant addition to the model. The model at the end of this step was called the preliminary final model.

3.3.4.2.3.3.7 Step 7

In this step, the preliminary final model was checked for key assumptions in regression analysis in order to come up with the final model. These included checking for collinearity between covariates, the fulfilment of proportional hazards assumptions, the existence of influential records, and the goodness-of-fit test.

- Collinearity between covariates

Extreme collinearity between covariates may produce clinically implausible results (Hosmer et al 2008:167). For this reason, whether there was any collinearity between covariates in the preliminary model was checked.

- Proportional hazards assumption

This assumption entails that the hazard function for values of a covariate remain equidistant over time (Machin et al 2006:121). Since the Cox proportional hazards regression modelling is based on this assumption, it was necessary to test for the fulfilment of this assumption after modelling. Hosmer et al (2008:167) suggest a two-step procedure to test for this assumption: evaluate score test based on the scaled Schoenfeld residuals and examine plots of the scaled and smoothed scaled Schoenfeld residuals obtained from the model. P values for score tests of covariates and the global test are>0.05 in cases in which this assumption is fulfilled. This evidence should be supported by a smoothed scaled Schoenfeld residuals plotting that shows no pattern of distribution, or must be randomly scattered about zero. In addition, the smoothed curve should have a slope close to zero.

- Checking for influential records

In this step, regression diagnostic statistics were examined to assess the presence of outliers or extreme values of a covariate, their influence on estimates of parameters, and their influence on the fit of the overall model. In order to assess this, values of score tests were plotted against the covariate of interest in a scatter plot for continuous variables and examined for outliers. Records of outliers were further examined to see if

those values were meaningful. The effect of the deletion of those values was checked to see if it significantly changed parameter estimates. The effect on parameter estimates was measured by calculating delta-betas. A record was said to have an influence if the change in parameter estimate, as measured by delta-beta ($\Delta\beta$), following its deletion form the data set was more than one standard error of the parameter in the preliminary model. In cases in which the deletion of a record had a big effect, the change in magnitude of the hazard ratio was examined as well. Those with a very high effect needed to be deleted before the final model was generated. (Collett 2003:242; Hosmer et al 2008:191)

- The goodness-of-fit test

The fit of the model was evaluated by using Cox-Snell residuals. If the model fit the data well, then the true cumulative hazard function conditional on the covariate vector was considered to have an exponential distribution with a hazard rate of one (Collett 2003:238).

3.4 RESEARCH DESIGN AND DATA INSTRUMENT QUALITY

Research design and data instrument quality must be kept to the highest possible standard in order to capture the true essence of reality. For this to happen, one must address these issues properly at design or analysis stage.

3.4.1 Quality of research design

Quality of research design is reflected in internal and external validity, which are discussed further below.

3.4.1.1 *Internal validity*

This refers to the extent to which it is possible to draw a conclusion from the study because there is a true causal relationship between independent and dependant variables (Polit & Beck 2012:244). But prior to checking internal validity, it is important to first establish that there is a relationship between the two variables to begin with. This is

termed statistical conclusion validity. The power of a study must be high to detect this which in turn is reflected in the sample size (Polit & Beck 2012:241).

Threats to internal validity include absence of temporality, attrition, and selection bias (Polit & Beck 2012:295; Shaughnessy et al 2012:187). In this study, temporality of independent variables was maintained during analysis by implementing statistical control of time-varying covariates. This means value of time-varying covariate occurring prior to diagnosis of immunologic failure was included in analysis while that occurring after the outcome was ignored (Collett 2003:251; Hosmer et al 2008:213). As for attrition bias, the researcher had little control over the prevalence of attrition but assessment was made to see if there was such bias by comparing if patients lost to follow-up differed by baseline characteristics than those remaining in care.

Selection bias is a common threat to internal validity but in this study since cases were randomly selected from the selected facilities and Cox regression analysis was used, the investigator was able to control for difference in baseline characteristics (Polit & Beck 2012:228,450; Shaughnessy et al 2012:453).

3.4.1.2   External validity

This refers to the generalizability of the findings of the study to different settings, and populations (Shaughnessy et al 2012:94). Generalization to the accessible population in this case would be straight forward as simple random sampling of all eligible cases was taken (Polit & Beck 2012:250). But generalization beyond that to the target population of HIV patients on HAART in SNNPR might not be made straight forward especially for prevalence estimate of immunologic failure since patients from only two sites were studied.

3.4.2   Quality of the data gathering instrument

Quality of data collection instrument refers to its validity and reliability of measuring concepts of interest.

3.4.2.1   Validity and reliability

Validity of a research instrument refers to whether an instrument is measuring what it is supposed to be measuring (Polit & Beck 2012:336; Shaughnessy et al 2012:164). For this study, the concepts being measured have been clearly defined (see section 1.4), hence there was a match between concepts intended to be measured and what was actually measured. Though data was collected retrospectively, the operational definitions used were identical to that used in clinical practice as outlined by the working guideline of HIV treatment. Polit and Beck (2012:332) state "Reliability refers to the accuracy and consistency of information obtained in a study." Since this study was retrospective and based on existing patient medical records a number of potential measurement biases were mostly avoided. These include interviewer bias, recall bias, and Hawthorn effect (Jacobsen 2011:58,70,83). One particular variable that needed to be measured and has influence on the outcome variable, that is immunologic treatment failure, was adherence. Self-reported adherence could be unreliable because of social desirability bias. For this reason, the investigator measured adherence to appointment to drug refill. This measure of adherence prevented misclassification error with respect to adherence than if one relied on self-report to assess adherence. All monitoring and evaluation tools have their own standard operating procedure which helped to uphold consistency of data (LoBiondo-Wood & Haber 2010:279). Apart from this, data was cross checked between medical records and registers as well as pharmacy records to ensure consistency and accuracy of data.

3.5   ETHICAL CONSIDERATIONS

Ethics can be defined as standards that guide how a person or a professional should behave or act (Polit & Beck 2012:154). According to the Belmont Report (Polit & Beck 2012:151) there are three main domains of ethics that must be addressed while conducting research. These include principles of beneficence, respect for human dignity and justice, which are discussed below in line with what was done to uphold each while this study was being carried out (Polit & Beck 2012:152; Zaidi 2014:75).

The principle of beneficence is about maximizing benefit and minimizing harm at all times while conducting research (LoBiondo-Wood & Haber 2010:250; Zaidi 2014:83,85). In this study there was no interaction of the researcher with study subjects as this was a retrospective study based on existing medical records. Therefore, the possibility of harm being inflicted during the study was remote.

The next important principle stated in the Belmont Report is about the respect for human dignity. This principle entails the researcher to respect confidences of research subjects, and recognize that they have the right to decide for themselves (Zaidi 2014:75). It means they have the right to information about the study and the right to decide to participate in a study or end it anytime they feel. The researcher cannot influence their decision in any way. This is ensured through the implementation of informed consent whenever it applies (Jacobsen 2011:154). In this study, once again, since there was no interaction between study subjects and investigator or data collector, there was no need for informed consent. (LoBiondo-Wood & Haber 2010:250)

The last major principle in the Belmont Report is the principle of justice. This principle deals with the right to fair treatment and the right to privacy (Zaidi 2014:75). In this study the privacy of patients' information was kept in confidence. Access to patients' medical records (including electronic records) was limited to data clerks assigned and already working in the facility. Medical records are kept in medical record room under normal circumstances which is secured and this was maintained throughout the study. Since random selection of patients' records was implemented, all eligible patients had equal opportunity to be studied. (LoBiondo-Wood & Haber 2010:251).

3.5.1 External review

It is important for every researcher to make a risk-benefit analysis for participants when conducting a research. However, there may be bias in balancing risk and benefit as a result of the researcher being over enthusiastic to conduct a high quality study or as a result of benefit one may get out of the findings of a study. For this reason, this judgment should not be left for the researcher alone and it is important for external body to review the protocol of a study (Jacobsen 2011:161; Polit & Beck 2012:165; Shaughnessy et al 2012:61). For this study, ethical approval was sought from UNISA College of Human Sciences, National Research Ethics Review Committee of Ethiopia, ethics committee of Southern Nations Nationalities and People's Regional Health Bureau of Ethiopia, and the directors of each hospital where the HIV clinics were being used for the purpose of this study.

3.6   SCOPE AND LIMITATIONS

The scope of this study was limited in many ways.

- The study focused on patients in ART care in Southern Nations Nationalities and People's Region and no other regions in Ethiopia.
- The study was done among patients in selected hospitals and not health centres.
- Site selection was not random.
- Existing patient medical records were used as data source with all its limitations.

3.7   CONCLUSION

This chapter provided an in-depth discussion of the research methodology that was applied in this study. The details of research design and research methods were covered. The next chapter will cover the results of the study.

CHAPTER 4

PRESENTATION AND DESCRIPTION OF THE RESEARCH FINDINGS

4.1   INTRODUCTION

Chapter three addressed the research methodology used in this study. This included the research design, research methods, data management and design, data and design quality, and ethical considerations. This chapter is dedicated to present and discuss the research findings. The aim of the study was to describe the prevalence of immunologic failure and identify its determinants among people living with HIV in selected hospitals.

The specific objectives included:

- To assess the pattern of immunologic monitoring in selected hospitals in southern Ethiopia
- To assess the pattern of immunologic response of patients on HAART in selected hospitals in southern Ethiopia
- To describe immunologic treatment failure and identify its predictors among patients on HAART in selected hospitals in southern Ethiopia

4.2   DATA MANAGEMENT AND ANALYSIS

In this section data management are described.

4.2.1   Data management

A self-designed document checklist was used for the purpose of data collection. The checklist consisted three sections: demographic data, clinical data, and immunologic data.

The documents of people living with HIV on antiretroviral therapy enrolled and started on ART from September 1, 2005 to December 31, 2012, those aged five or more years at ART initiation, those with two or more CD4 cell count tests, and those with at least six

months of follow-up were included in the study. Documents of patients transferred-in from other facilities after starting HAART were excluded from the study. Follow-up information was abstracted for all patients until end of August 2013.

After applying the eligibility criteria, there were 1,321 patient records. Since the study sample was 1,304, the researcher used all the available patient records for analysis. Data cleaning was done with Epi Info^{TM} 3.5.1 statistical software (United States Centers for Disease Control and Prevention 2007). Data on patient follow-up weight was missing in 43% of cases for which reason the variable was excluded during analysis. The data was analysed using Stata/IC 12.0 (StataCorp LP) statistical software. The results of analysis are presented in the next section.

4.3   RESEARCH RESULTS

The results of the study are presented in this section. Both descriptive and inferential statistics are presented as follows.

4.3.1   Sample characteristics

In the following section, the study sample is described with respect to demographic, clinical characteristics, follow-up time, and follow-up status.

4.3.1.1   Demographic characteristics

Demographic data collected in this study include age and gender which are presented as follows.

4.3.1.1.1   Age

The age distribution characteristic of patients in the study sample is displayed in table 4.1 and 4.2. The mean age for both male and female patients was 32 years with a standard deviation of 9.6. The median age was 30 years. The minimum and maximum ages of those in the study sample were five and 71 years, respectively. Out of the total patients in the study (N=1,321), paediatric patients between five and 14 years of age accounted for 3.94% (n=52), while adults accounted for 96.06% (n=1,269) of it (see

table 4.2). This is very close to the national figure for the proportion of patients under 15 years of age on antiretroviral therapy, which is 4.8% (FHAPCO & FMOH 2012:33).

TABLE 4.1   DESCRIPTIVE STATISTICS FOR CONTINOUS VARIABLES OF PATIENTS ON ANTI-RETROVIRAL THERAPY (N=1321)

Variable	Number	Mean	Standard deviation	Minimum	Median	Maximum
Age	1321	32	9.6	5	30	71
Baseline CD4 cell count	1321	163	100	1	156	1221
Follow-up CD4 cell count	5241	415	234	5	372	2024

4.3.1.1.2   Gender

The gender distribution of the study sample is presented in table 4.2. Female patients accounted for the majority of those in the study sample at 62.91% (n=831) while males made up 37.09% (n=490) of the sample. This is in line with the proportion of women with HIV in Ethiopia which, according the FHAPCO and FMOH (2012:32), is estimated at 60% of the total population in 2011.

TABLE 4.2   CHARCTERISTICSOF   PATIENTS   ON   ANTI-RETROVIRAL   THERAPY, (N=1,321)

Variable	Category	Frequency, n	Per cent,%
Age in years	5-14	52	3.94
	>14	1269	96.06
Gender	Female	831	62.91
	Male	490	37.09
WHO Stage	I or II	535	40.50
	III or IV	786	59.50
Baseline CD4 (cell count/mm^3)	<100	378	28.61
	100-199	547	41.41
	200-349	359	27.18
	>349	37	2.80
Tuberculosis	No	1086	82.21
	Yes	235	17.79
Adherence	Good	1080	81.76
	Poor	241	18.24
Final follow-up status	Active	960	72.67
	Dead	44	3.33
	Drop	62	4.69
	Lost	4	0.30
	Stop	2	0.15
	Transferred out	249	18.85

4.3.1.2   Clinical information

This section provides data on clinical characteristics of those in the study sample.

4.3.1.2.1 Baseline WHO Stage

Out of the total number of patients' files examined in this study, those patients at WHO Stages I or II during initiation of anti-retroviral therapy accounted for 40.5% (n=535) while those at advanced disease stages (WHO Stages III or IV) accounted for 59.5% (n=786) (see table 4.2). This is similar to what was reported in the SNNP region by Mulissa et al (2010:online) who reported that the proportion of patients who started antiretroviral therapy who were at WHO stages III or IV at HAART initiation being 62.5% (n=1,369) at one hospital in the SNNP. However, it was different from what Tsegaye and Worku (2011:104) reported. In their study, 81.2% (n=4,580) of patients in WHO stages III or IV had started ARVs in eight hospitals in the SNNP region. This finding is higher than that reported in this study.
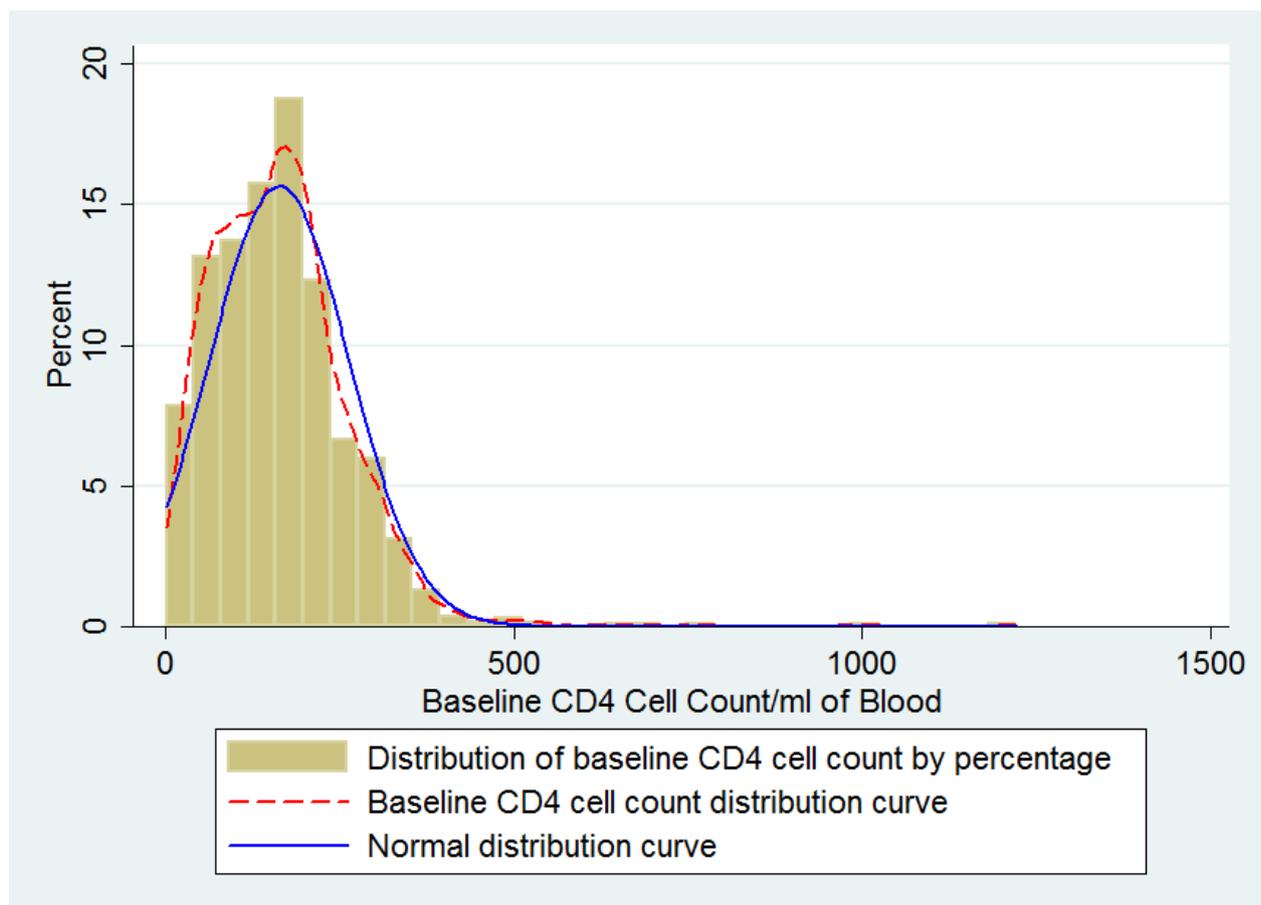
Figure 4.1   Baseline CD4 cell count distribution for the study sample (N=1,321)

4.3.1.2.2 Baseline CD4 cell count

The mean CD4 cell count at the point of initiation of anti-retroviral therapy was 163 cells/mm^3, while the standard deviation was 100. The median CD4 cell count at initiation of anti-retroviral therapy was 156 cells/mm^3. The minimum and maximum baseline CD4 cell counts were one and 1,221 cells/mm^3 respectively (see table 4.5). The distribution of baseline CD4 cell counts as well as the expected normal distribution for those in the study sample is shown in figure 4.1. These findings show a distribution skewed to the right. Table 4.2 shows the percentage of patients with a baseline CD4 cell count between 100 and 199 which accounts for the highest proportion of 41.41% (n=547), followed by those below 100, 28.61% (n=378), and those between 200 and 349, 27.18% (n=359). Of the total sample only 2.8% (n=37) had a CD4 cell count of 350 and above. The proportion of patients with a baseline CD4 cell count of ≥200 was similar to what was reported by Tsegaye and Worku (2011:104) who found that 29.2%of a total sample of 5,181 had CD4 cell count above that (n=1,516).

4.3.1.2.3   Diagnosis of tuberculosis during follow-up

Out of 1,321 patients, 235 were diagnosed as having tuberculosis during and after starting anti-retroviral therapy (see table 4.2). This put the prevalence of tuberculosis after initiation of anti-retroviral therapy at 17.79%. Reported prevalence of tuberculosis varied among those on HAART in different studies done in Ethiopia. Alene, Nega and Taye (2013:online) report a higher prevalence in their study namely that 28% of a sample of 470 patients on ART followed for a maximum of five years in northern Ethiopia, while it was 21% in a multisite study in southern Ethiopia involving 6,230 patients by Yirdaw, Jerene, Gashu, Edginton, Kumar, Letamo, Feleke, Teklu, Zewdu, Weiss and Ruff (2014:online). In yet another study done by Ali and Klotz (2012:200) who followed patients for two years, it was 14% (n=143). In the latter study, the prevalence was smaller probably because of the short duration of follow-up. The prevalence of tuberculosis was even smaller (3.3%) in another study in SNNP among185 patients followed up for three to 68 weeks (Jerene, Næss & Lindtjørn 2006:online).

4.3.1.2.4   Adherence to medication refill

Of 1,321 patients, 18.24% (n=241) exhibited poor adherence to anti-retroviral therapy, the measure for adherence being adherence to appointment for their medication refill.

Patients who had used up all their medication, and had not reported to the clinic for a refill in a timely fashion, were declared to have poor adherence to treatment. For this study cohort, 81.76% (n=1,080) had good adherence to picking up their medication refill on time (see table 4.2). This is less than what was reported by Chalker et al (2010:online), who found adherence to medication refill (termed as adherence to appointment date in their study) to be 86.3%. This higher percentage could, however, be attributable to the shorter duration of follow-up in that study, which was one year.

4.3.1.2.5 Final follow-up status

The final follow-up status, that is, whether patients were still in follow-up or not at the time of the last observation, is presented in this section with respect to demographic and baseline clinical characteristics. In general, as shown in Table 4.2, there were 72.67% (n=960) patients who were still on follow-up at the time of the last observation. This was higher than what was reported by Mulissa et al (2010:online) which found it to be 63.1% (n=901). The percentage of patients transferred for follow-up to other facilities was 18.85% (n=249). The percentage of patients who died, dropped out, were lost from follow-up, or stopped treatment was 8.48% (n=112) (see table 4.2).

TABLE 4.3   FINAL FOLLOW-UP STATUS BY DEMOGRAPHIC AND BASELINE CHARACTERISTICS OF STUDY PATIENTS ON ANTI-RETROVIRAL THERAPY (N=1,321)

Variable	Category	Total, n	Number Active (%)	Pearson Chi Square	P- value
Age in years	5-14	52	38 (73.08)	0.0045	0.947
	>14	1269	922 (72.66)		
Gender	Female	831	599 (72.08)	0.3932	0.531
	Male	490	361 (73.67)		
WHO Stage	I or II	535	392 (73.27)	0.1623	0.687
	III or IV	786	568 (72.26)		
Baseline CD4 (cell count/mm^3)	<100	378	270 (71.43)	2.8564	0.414
	100-199	547	409 (74.77)		
	200-349	359	257 (71.59)		
	>349	37	24 (64.86)		

The following section presents findings concerning the comparison of the final follow-up status with respect to demographic and baseline clinical features.

4.3.1.2.5.1 Final follow-up status by age group

The percentage of patients actively on follow-up ages 5-14 years was 73.08% (n=38), while that for those more than 14 years of age was 72.06% (n=922) (see table 4.3). This indicates that there was no difference in the percentage of patients who were actively on follow-up based on age group (p-value > 0.05).

4.3.1.2.5.2 Final follow-up status by gender

The percentage of patients actively on follow-up was 72.08% (n=599) for females while for males it was 73.67% (n=361) (see table 4.3). This indicates that there was no significant difference in the percentage of patients who were actively on follow-up based on gender (p-value > 0.05).

4.3.1.2.5.3 Final follow-up status by baseline WHO Stage

The percentage of patients actively on follow-up, for patients at baseline WHO Stages I or II, was 73.27% (n=392), while for those at baseline WHO Stages III or IV was 72.26% (n=568) (see table 4.3). This indicates that there was no significant difference in the percentage of patients who were actively on follow-up based on baseline WHO Stages (p-value > 0.05).

4.3.1.2.5.4 Final follow-up status by baseline CD4 cell count

The highest percentage of patients actively on follow-up was recorded for patients with a baseline CD4 cell count between 100 and 199, 74.77% (n=409), while the lowest percentage was for patients with baseline CD4 cell counts of more than 349, at 64.86% (n=24). Of patients receiving the treatment, those with baseline CD4 cell counts below 100 and baseline CD4 cell counts between 200 and 249, the percentages of those actively on follow-up was comparable 71.43% (n=270) and 71.59% (n=257) respectively. Overall, the percentages of patients active on follow-up from the different categories of baseline CD4 cell count just described was comparable (p-value > 0.05) (see table 4.3).

4.3.1.2.6 Follow-up time

All in all, patients were observed for a minimum time of 0.51 years and a maximum of 8.36 years. The mean and median duration of follow-up were 4.06 and 3.89 years respectively. Patients were observed for a collective time of 5,366 years from ART initiation to last visit.

4.3.2 Objective 1: assess the pattern of immunologic (CD4 cell count) monitoring in selected hospitals in southern Ethiopia

The pattern of timely CD4 cell count monitoring for this study sample is depicted graphically in figure 4.2.The 'expected CD4 cell count tests' represents the number of follow-up CD4 cell count tests that should have been determined had the national monitoring and evaluation guidance for determination of CD4 cell count been implemented correctly as described in the methods section. This number is seen to get smaller with increase in follow-up time as there were progressively fewer patients on follow-up. For example, the number of patients active on follow-up for seven years or 84 months was 136, while there were 1,262 patients who were active for at least one year (see table 4.4).
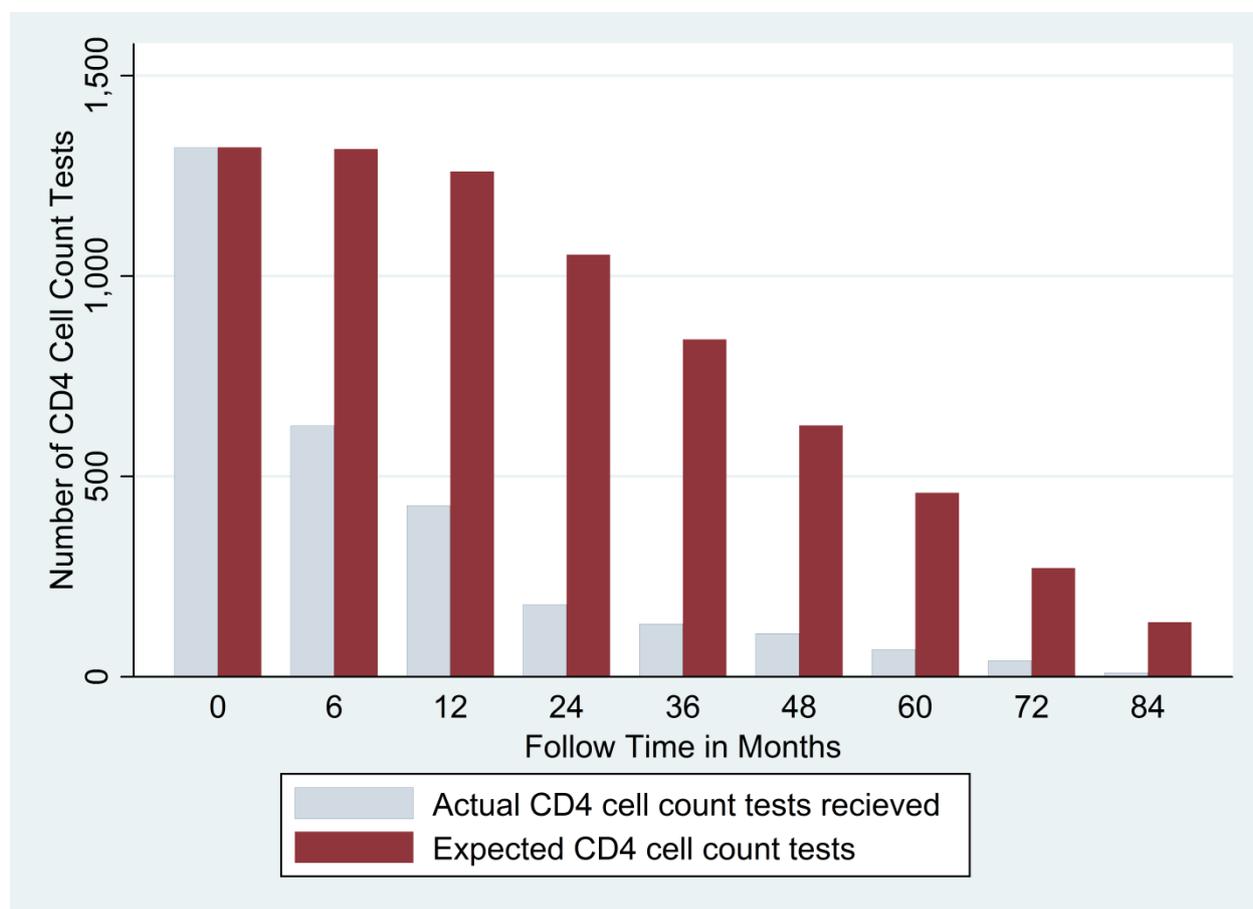
Figure 4.2   **Pattern of CD4 cell count monitoring by follow-up time (N=1,321)**

The 'actual CD4 cell count tests received' represents the actual number of CD4 cell count tests determined at designated follow-up times.

TABLE 4.4   DETERMINATION OF TIMELY FOLLOW-UP CD4 TESTING BY FOLLOW-UP PERIOD IN MONTHS AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (N=1,321)

Follow-up Month	Expected number of CD4 cell count tests	Number of follow-up CD4 cell count tests provided	Per cent (%) CD4 cell count tests provided
0	1321	1321	100.00
6	1317	610	46.31
12	1262	417	33.04
24	1053	179	17.00
36	842	132	15.68
48	628	108	17.20
60	459	68	14.81
72	271	40	14.76
84	136	10	7.35
Total follow-up CD4 cell count tests	7289	2885	40.58

Although both the number of patient's on follow-up (or expected number of CD4 cell count tests) as well as the number of timely follow-up CD4 cell count tests determined decreased progressively, the proportion of timely follow-up CD4 cell count tests provided decreased much faster. The actual percentage of follow-up CD4 cell count tests provided is shown in table 4.4. It can be seen that all patients had baseline CD4 cell count testing at month zero. The number of timely CD4 cell count tests performed decreased to 46.31% (n=610) at six months of follow-up while it was 33.04% (n=417) at one year after ART initiation. It decreased and reached 7.35% (n=10) for patients who were in follow-up for seven years. Overall, of the expected 7,285 baseline and follow-up CD4 cell count tests, only 2,885 tests were done in a timely fashion. This was 40.58%of the total expected, which is very low.

Berhan (2011b:199) reports low follow-up CD4 cell count monitoring as well among patients studied in southern Ethiopia. According to that study, at six and 24 months after initiating HAART, follow-up CD4 cell count monitoring was done for 68% and 37% of a total sample of 1,163 patients. The findings from the current study indicate that the level of monitoring for the same follow-up time interval from ART initiation was much lower, at 46.31% (n=610) and 17% (n=179) respectively. In a recent study in Malawi (Palchaudhuri, Tweya & Hosseinipour 2014:43), only 5,361 ART patients out of 17,737

(30.2%) on follow-up received follow-up CD4 cell count testing. And out of 15,924 patients who were eligible for two tests, only 1,006 (6.3%) received testing as per guideline. The reasons for poor follow-up CD4 cell count testing could be multifactorial: providers not requesting tests, patients not coming for testing, lack of awareness from the patients' side, or a breakdown of machines or lack of reagents at facilities. This is consistent with the opinions of Were et al (2011:152) and Zachariah et al (2011:39).

The following sections present an analysis on the pattern of follow-up CD4 cell count monitoring with respect to whether there were more or less timely follow-up CD4 cell count tests based on demographic and baseline clinical characteristics.

4.3.2.1 *Pattern of CD4 cell count monitoring by age*

Table 4.5 shows a comparison of CD4 cell count monitoring by age group. Among patients ages 5-14 years, follow-up CD4 cell count tests were done for 35.97% (n=100), and for those patients older than 14 years of age, 39.73% (n=2,786) had follow-up tests (p value>0.05). This shows that there was no substantial difference in provision of timely follow-up CD4 cell count testing by age group.

TABLE 4.5 **DETERMINATION OF TIMELY FOLLOW-UP CD4 CELL COUNT TESTING BY DEMOGRAPHIC AND BASELINE TREATMENT VARIABLES AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (N=1,321)**

Variable	Category	Total expected number of CD4 cell count tests	Number with timely follow-up CD4 cell count test	Per cent timely follow-up CD4 cell count test done	Chi square	P value
Age in years	5-14	278	100	35.97	1.5812	0.209
	>14	7012	2786	39.73		
Gender	Female	4578	1806	39.45	0.0993	0.753
	Male	2712	1080	39.82		
WHO Stage	I or II	2897	1150	39.70	0.0233	0.879
	III or IV	4393	1736	39.52		
Baseline CD4 (cell count/mm^3)	<100	2163	860	39.76	0.2358	0.972
	100-199	3089	1220	39.49		
	200-349	1843	726	39.39		
	>349	195	80	41.03		

4.3.2.2 *Pattern of CD4 cell count monitoring by gender*

Table 4.5 shows a comparison of CD4 cell count monitoring by gender. Among female patients, 39.45% (n=1,806) of expected follow-up CD4 cell count tests were done, while

it was done for 39.82% (n=1,080) of male patients (p value>0.05). This shows that there was no significant difference in provision of timely follow-up CD4 cell count testing by gender.

4.3.2.3 Pattern of CD4 cell count monitoring by baseline WHO Stage

Table 4.5 shows a comparison of CD4 cell count monitoring by baseline WHO Stage groups. Follow-up CD4 cell count tests were done for 39.7% (n=1,150) of patients at baseline WHO Stage I or II. It was almost the same for those with baseline WHO Stage III or IV. This shows that there was no difference in provision of timely follow-up CD4 cell count testing by baseline WHO Stage (p value>0.05).

4.3.2.4 Pattern of CD4 cell count monitoring by baseline CD4 cell count

Table 4.5 shows a comparison of CD4 cell count monitoring by baseline CD4 cell count groups. Patients with a baseline CD4 cell count of more than 349 had the highest percentage of follow-up CD4 cell count testing namely 41.03% (n=80), while for the other groups, it was between 39% and 40% (n=860, 1,220, and 726 for those with baseline CD4 cell count<100, between 100 and 199, and between 200 and 349 respectively). Overall, there was no association between follow-up CD4 cell count testing and baseline CD4 cell count (p value>0.05).

4.3.3 Objective 2: assess the pattern of immunologic (CD4 cell count) response for patients on HAART in selected hospitals in southern Ethiopia

Figure 4.3 shows the pattern of immunologic (CD4 cell count) response after the initiation of anti-retroviral therapy (N=1,321). The total number of follow-up CD4 count tests done was 5,241 over the study period of eight years. It can be seen that the median CD4 cell count for those who made up the study sample showed a rapid increase over the first six months. CD4 cell count recovery continued after that, but with slight subsequent change, followed by a levelling off after 60 months (five years) of follow-up. This is similar to what was described by Tsegaye and Worku (2011:107), Reda et al (2013:online), Kassa et al (2013:online), and more recently by Mulu, Liebert and Maier (2014:online).

The pattern of immunologic response was further analysed by demographic (age and gender) and baseline clinical characteristics (baseline WHO Stage and baseline CD4 cell count) presented in the following sections taking as an outcome the percentage of those patients in whom the follow-up CD4 cell count achieved was more than or equal to 350 (see table 4.7). The median follow-up CD4 cell count was used as guide to decide what cut off to use. Since the median follow-up CD4 cell count was 371, the researcher chose 350 as an approximation for ease of interpretation and communication of results.
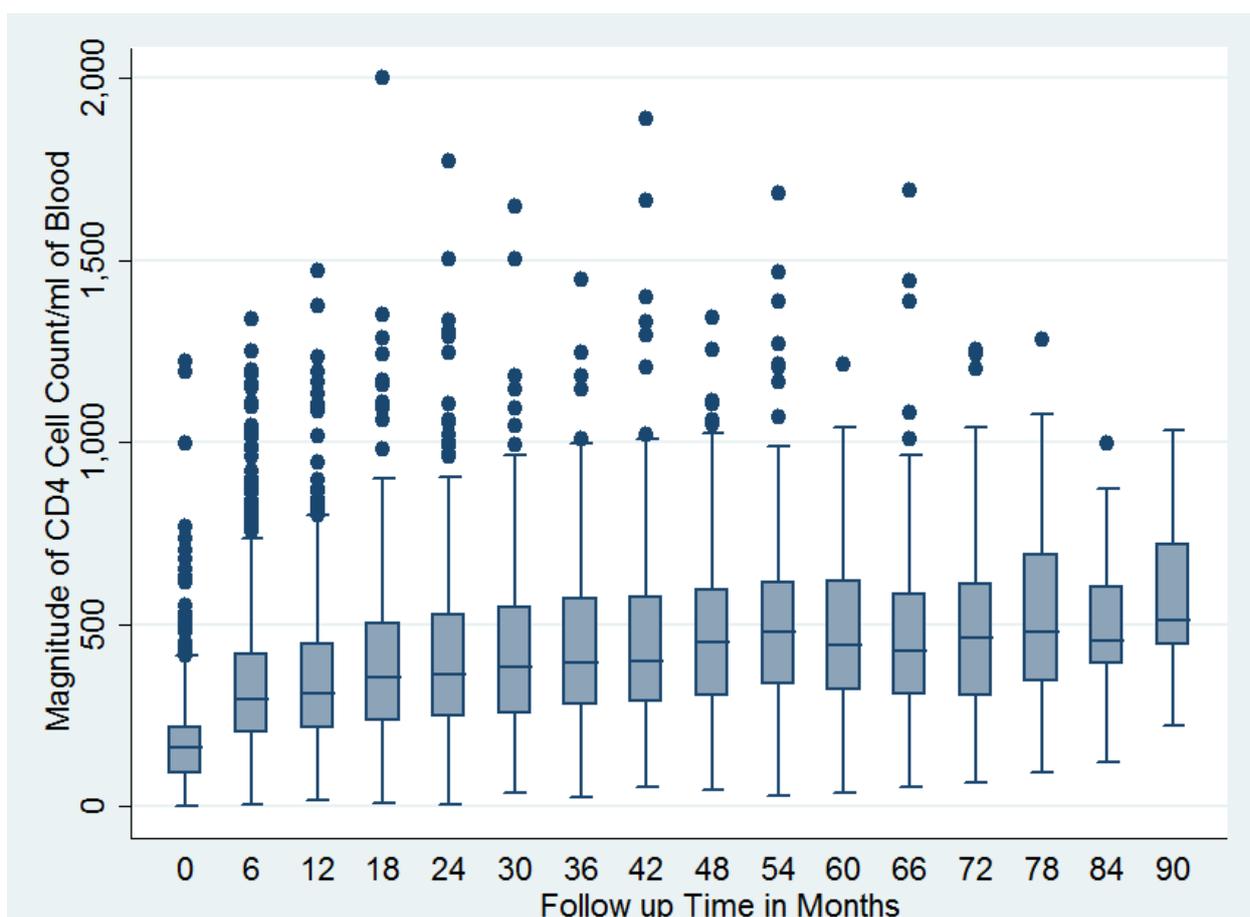
Figure 4.3   Box plot for magnitude of baseline and follow-up CD4 cell count among cohort of patients on anti-retroviral therapy by follow-up month (N=1,321)

4.3.3.1   Pattern of CD4 cell count response by age

In table 4.6 the follow-up CD4 cell count tests amounted to 5,241 for the total study sample of 1,321 patients during the study period.

The percentage of patients whose age was between five and fourteen inclusive and who had a follow-up CD4 cell count of more than or equal to 350 was 88.48% (n=146).

This was in contrast to the percentage of those whose age was more than 14 years, among whom 53.49% (n=2,715) had such a follow-up CD4 cell count. This finding suggests that the percentage of those whose follow-up CD4 cell count values was more than or equal to 350was greater for those ages 5-14 years (p value<0.05) (see table 4.6).

TABLE 4.6   MAGNITUDE OF FOLLOW-UP CD4 CELL COUNT BY DEMOGRAPHIC AND BASELINE TREATMENT VARIABLES AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (N=1,321)

Variable	Category	Total, n	Follow-up CD4 cell count ≥350	Per cent with follow-up CD4 cell count ≥350	Chi square	P value
Age in years	5-14	165	146	88.48	78.9601	0.000
	>14	5076	2715	53.49		
Gender	Female	3330	2006	60.24	117.6648	0.000
	Male	1911	855	44.74		
WHO Stage	I or II	2079	1193	57.38	10.8558	0.001
	III or IV	3162	1668	52.75		
Baseline CD4 (cell count/mm^3)	<100	1569	512	32.63	568.0106	0.000
	100-199	2224	1253	56.34		
	200-349	1300	983	75.62		
	>349	148	113	76.35		

4.3.3.2   Pattern of CD4 cell count response by gender

The percentage of female patients with a follow-up CD4 cell count more than or equal to 350 was 60.24% (n=2,006). This was in contrast to male patients, for whom the percentage of follow-up CD4 cell count test result of more than or equal to 350 was 44.74% (n=855). This finding suggests that the percentage of patients with higher follow-up CD4 cell count values was greater for female patients (p value<0.05) (see table 4.6). This finding is consistent with the findings of Kassa et al (2013:online) who report a steeper increase in follow-up CD4 cell counts results for female patients than those for males.

4.3.3.3   Pattern of CD4 cell count response by baseline WHO Stage

Table 4.6providesa comparison by percentage of follow-up CD4 cell count levels of ≥350 by baseline WHO Stage groups. The percentage of patients whose baseline WHO Stage was I or II who had follow-up CD4 cell counts of more than or equal to 350 was 57.38% (n=1,193). This was in contrast to those whose baseline WHO stage was III or IV, 52.75% (n=1,668) of whom had follow-upCD4 cell count ≥350. This finding suggests

that the percentage of those whose follow-up CD4 cell count values was higher was greater for those at baseline WHO Stage I or II (p value<0.05).

4.3.3.4   Pattern of CD4 cell count response by baseline CD4 cell count

Table 4.6 also provides a comparison by percentage of those with follow-up CD4 cell counts by baseline CD4 cell count groups. Patients with baseline CD4 cell count below 100 had the lowest percentage of follow-up CD4 cell count values ≥350, 32.63% (n=512). The next lowest percentage was for patients with a baseline CD4 cell count between 100 and 199, among whom 56.34% (n=1,253) had follow-up CD4 cell count ≥350. The other two baseline CD4 cell count groups had comparable percentages with at around 76% each for those with a baseline CD4 cell count of 200-349 (n=983) and >349 (n=113) respectively. Overall, the percentage of patients with follow-up CD4 cell count tests more than or equal to 350 was statistically different among the different baseline CD4 cell count groups (p value<0.05). This finding is supported by Kassa et al (2013:online) who report better immune restoration among patients with baseline CD4 cell counts higher than 200 as compared to those with counts less than 200. Kanters et al (2013:online) also report the baseline CD4 cell count to be a strong predictor of immunological response, stating that only those with CD4 cell counts above 200 were able to achieve follow-up CD4 cell counts of more than 500.

In conclusion, the association between demographic and baseline clinical characteristics with magnitude of follow-up CD4 cell count was evident in this study. In a study done in eastern Ethiopia by Reda et al (2013:online), there was no such association evident between the variables stated above and CD4 cell count increment. The findings were different, however, probably because different outcome parameters were used in the form of CD4 cell count increment in the above mentioned study in contrast to achieving a follow-up CD4 cell count of greater than or equal to 350 used in the current study. In a more recent study in northern Ethiopia by Mulu et al (2014:online) that used a CD4 cell count of 200 as cut off amongst 100 adults and 100 children, no association was found in contrast to this study. The findings of the current study are, however, comparable to a study done in Uganda among 325 patients on ART (Crawford, Wakabi, Magala, Kibuuka, Liu & Hamm 2014:online) where they found out that age was inversely related to CD4 cell count recovery and female patients had higher baseline CD4 cell count as well as higher recovery during follow-up.

4.3.4 Objective 3: describe immunologic treatment failure and identify its predictors among patients on HAART in selected hospitals in southern Ethiopia

Of the 1,321 patients' medical records reviewed, 2.95% (n=39) discontinued their treatment for considerable time. Such patients would have needed additional follow-up before treatment failure diagnosis could be made, for which reason they were not evaluated for treatment failure (see table 4.7).

TABLE 4.7   IMMUNOLOGIC TREATMENT FAILUREAMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (N=1,321)

Variable	Category	Frequency, n	Per cent,%
Immunologic failure (first instance)	No	1056	79.94
	Yes	226	17.11
	Not Evaluated	39	2.95
Immunologic failure (at last immunologic evaluation)	No	1135	85.92
	Yes	147	11.13
	Not Evaluated	39	2.95

4.3.4.1   Prevalence of immunologic failure

After excluding those not evaluated, the prevalence of patients that ever experienced immunologic treatment failure was found to be 17.6% (95% confidence interval: 15.6%-19.8%) (n=226). The prevalence of immunologic failure at the last immunologic evaluation was 11.5% (95% confidence interval: 9.8%-13.3%) (n=147). This is almost comparable to what was reported in Ethiopia by Bacha et al (2012:online), a percentage of immunologic failure of 8.2% (n=79), as well as Workneh et al (2009:78), who reported immunologic failure percentage of11.5% (n=11). Direct comparison is not possible, though; since both studies focused on paediatric patients with a mean age of six years, and follow-up duration was shorter in the second study (a mean follow-up duration of 13.7 months). In other sub-Saharan African countries, studies point to a higher prevalence of immunologic failure, at a range of 10%-32%, even when the follow-up duration was shorter compared to that of this study (El-Khatib et al 2011:online; Jaka et al 2009:6; Rawizza et al 2011:1286; Reynolds et al 2009:3).

4.3.4.2   Immunologic failure by criteria of diagnosis

Table 4.8 provides information on the type of criteria that was met to diagnose immunologic failure. Among 226 patients with immunologic failure, looking further into the criteria that led to the diagnosis indicates that the majority namely 57.96% (n=131) were diagnosed with failure based on the criteria 'drop by 50% of follow-up CD4 cell count from peak value'. Diagnosis by the criteria 'drop to or below baseline CD4 cell count' accounted for 34.51% (n=78) of patients. The criteria used for the diagnosis of immunologic failure for the remaining 7.52% (n=17) of cases was a follow-up CD4 cell count persistently below 100 CD4 cells/mm^3 of blood.

TABLE 4.8   DIAGNOSTIC CRITERIA FOR FIRST INSTANCE IMMUNOLOGIC FAILURE AMONG STUDY PATIENTS ON ANTI-RETROVIRAL THERAPY IN (n=226)

Diagnostic criteria	Frequency	Per cent
Follow-up CD4 cell count dropped by at least 50% from peak value	131	57.96
CD4 cell count persistently below 100/mm^3	17	7.52
Follow-up CD4 cell count dropped to or below baseline	78	34.51
Total	226	100.00

4.3.4.3   Immunologic failure at the final immunologic evaluation (n=226)

Table 4.9 shows that, among patients with first instance immunologic failure (that is patients who ever experienced immunologic failure), follow-up immunologic evaluation was not done for 23.89% (n=54) of the patients. Of the remaining patients, immunologic treatment failure diagnosis persisted and was recorded at last evaluation in 54.07% (n=93), while 45.93% (n=79) of patients did not fulfil the immunologic failure criteria anymore. This may be due to improvement in adherence to medication after the first treatment failure diagnosis. This statement is consistent with the views expressed by the WHO (2013:183).

TABLE 4.9   FOLLOW-UP IMMUNOLOGIC EVALUATION AND FINAL IMMUNOLOGIC STATUS OF PATIENTS WHO EVER HAD IMMUNOLOGIC FAILURE (n=226)

Follow-up CD4 cell count testing	Immunologic failure at last evaluation		Total, number (%[*])
	Yes, number (%[*])	No, number (%[*])	
No	54 (100.00)	0 (0)	54 (100%)
Yes	93 (54.07)	79 (45.93)	172 (100%)
Total	147 (65.04)	79 (34.96)	226 (100%)

[*] All percentages are row percentage.

4.3.4.4 Patients on second line HAART among those with confirmed immunologic failure

Of the 93 patients with confirmed immunologic failure, 7.53% (n=7) were put on second line anti-retroviral therapy (see table 4.10). The rest (92.47% and n=86) remained on first line therapy for as long as they were studied. Among all patients who were followed (N=1,321), the prevalence of patients on second line HAART treatment was 1.67% (95% confidence interval: 1.0% - 2.4%) (n=22).

This finding is comparable to what was reported by Assefa et al (2011:online), which in their study found that among 7,451 patients, 2.13% (95% confidence interval: 1.8% to 2.5%) at the second year of HAART treatment were on second line HAART.

TABLE 4.10   FREQUENCY DISTIBUTION OF PATIENTS ON SECOND LINE TREATMENT AMONG THOSE WITH CONFIRMED IMMUNOLOGIC FAILURE (n=93)

On second line HAART	Frequency, n	Per cent, %
No	86	92.47
Yes	7	7.53
Total	93	100.00

4.3.4.5 Predictors of immunologic failure

Predictors of immunologic failure were examined with survival analysis. Specifically, the time from onset of treatment to first instance immunologic failure was examined. Firstly, descriptive survival analysis was done. Then, Kaplan-Meier survival curves were examined in univariable analysis for effect of independent variables on immunologic treatment failure. The log-rank test was used to statistically compare different groups. This was then followed by multivariable analysis using Cox proportional hazards regression modelling. All results were adjusted for site level effects by stratification. Patients not evaluated for treatment failure were excluded (n=39). This left97% (n=1,282) of patients to be included in survival analysis.

4.3.4.5.1 Event free survival

Follow-up time for patients included in the study from start of observation to diagnosis of immunologic failure or censoring ranged between 0.51 and 8.36 years; the mean and

median duration of follow-up time were 3.61 and 3.42 years respectively. The patient data scrutinized amounted to a total of 4,634 person-years at censoring or at diagnosis of first instance of immunologic failure (see table 4.11).Table 4.12 and figure 4.4 summarize event free survival, the event being the first instance of immunologic treatment failure. It can be deduced that there had been a more or less steady drop in event free survival with time. At the end of the second year of follow-up, the probability of being free of immunologic failure was 0.89 (95% CI: 0.87 – 0.91), while at end of five years it was 0.80 (95% CI: 0.77 - 0.82). The confidence interval widens after this, as the number of patients who had reached more than five years of follow-up progressively decreased, leading to more uncertainty of estimates.

TABLE 4.11   DESCRIPTION OF FOLLOW-UP TIME IN YEARS FOR STUDY PATIENTS ON ANTI-RETROVIRAL THERAPY I (n=1,282)

	No. of subjects	Total time at risk	Mean	Minimum	Median	Maximum
Failures	226	543.08	2.40	0.51	1.80	7.92
Censored	1056	4091.23	3.87	0.51	3.72	8.36
Total	1282	4634.32	3.61	0.51	3.42	8.36

TABLE 4.12   LIFE TABLE FOR IMMUNOLOGIC FAILURE AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (n=1,282)

Follow-up Interval in Years	Number of Patients at Beginning of Interval	Immunologic Failure	Censored	Event free Survival	Standard Error	95% Confidence Interval
0-1	1282	67	55	0.9466	0.0063	0.9326 - 0.9577
1-2	1160	60	184	0.8934	0.0090	0.8744 - 0.9097
2-3	916	33	172	0.8579	0.0105	0.8358 - 0.8772
3-4	711	21	179	0.8289	0.0119	0.8041 - 0.8509
4-5	511	18	143	0.7950	0.0139	0.7662 - 0.8206
5-6	350	16	140	0.7495	0.0171	0.7141 - 0.7812
6-7	194	8	93	0.7089	0.0214	0.6646 - 0.7484
7-8	93	3	71	0.6719	0.0290	0.6114 - 0.7251
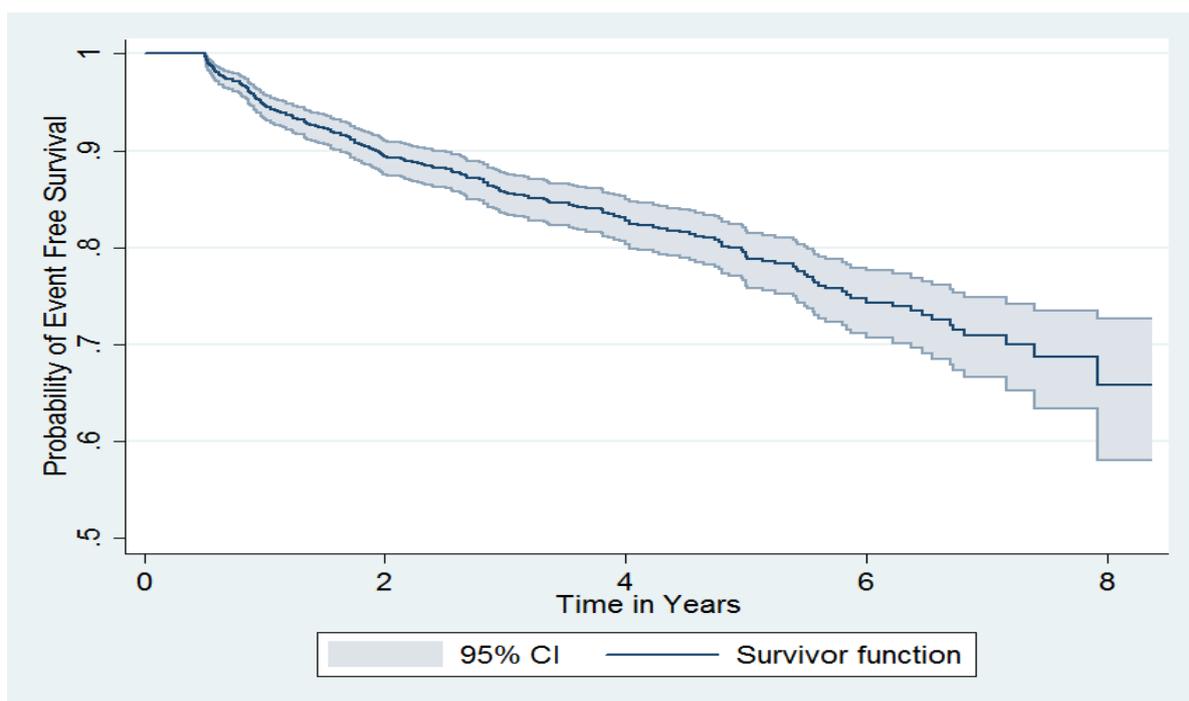
Figure 4.4   **Kaplan-Meier survival functions for survival free of immunologic treatment failure (n=1,282)**

4.3.4.5.2   Univariable analysis

In this section, results of univariable survival analysis are presented.

TABLE 4.13   STRATIFIED LOG-RANK TEST FOR EQUALITY OF FAILURE FUNCTIONS BY COVARIATES AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (n=1,282)

Variable	Category	Events Observed	Events Expected	Chi square	P value
Age(in years)	5-14	10	7.59	0.79	0.3742
	>14	216	218.41		
Gender	Female	133	142.33	1.65	0.1989
	Male	93	83.67		
WHO Stage	I or II	77	91.20	3.71	0.0540
	III or IV	149	134.80		
Baseline CD4 (cell count/mm^3)	<100	60	69.79	51.19	0.0000
	100-199	78	98.03		
	201-349	71	54.04		
	>349	17	4.14		
Tuberculosis	No	223	222.87	0.01	0.9358
	Yes	3	3.13		
Adherence	Good	187	186.20	0.02	0.8896
	Poor	39	39.80		
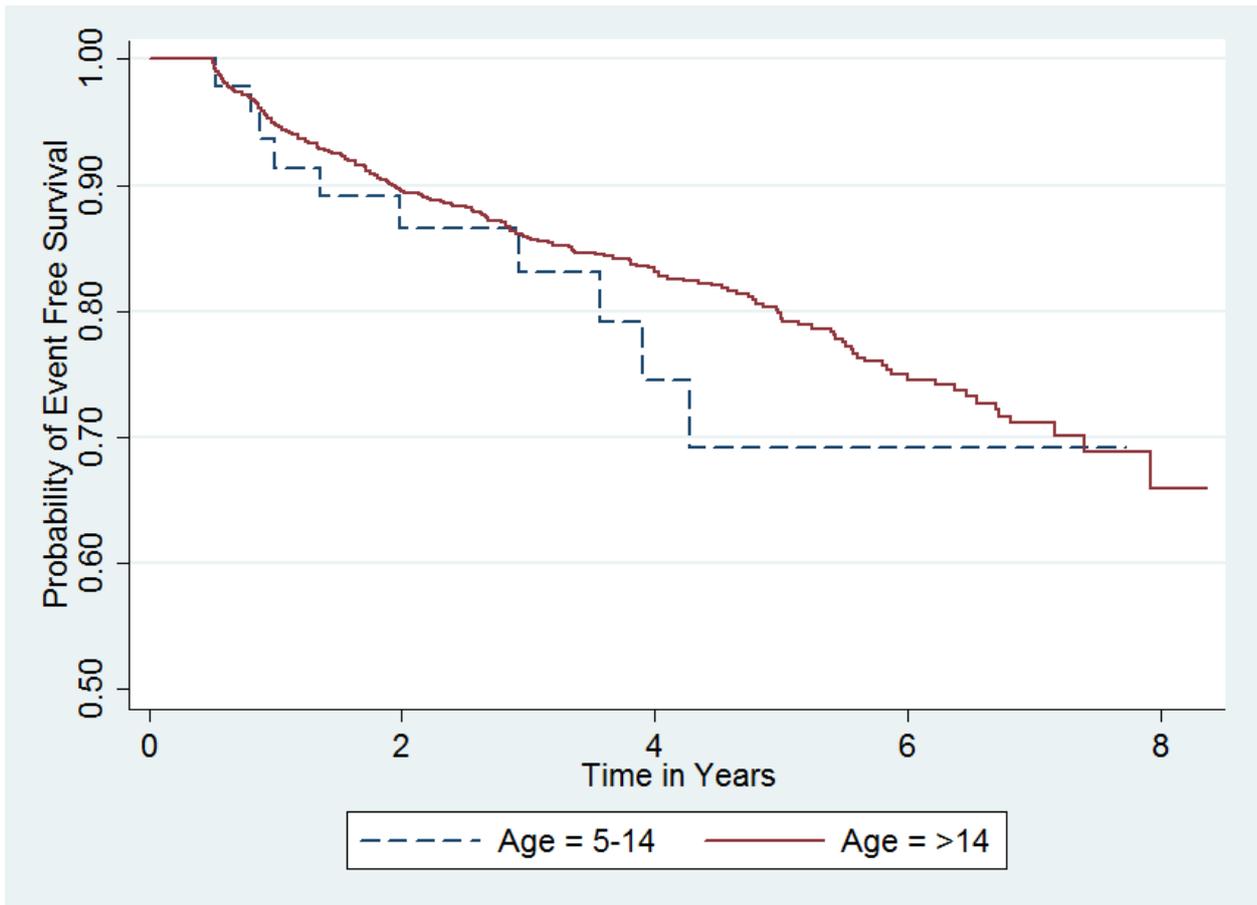
Figure 4.5   Kaplan-Meier survival functions by age group (n=1,282)

4.3.4.5.2.1   Age and immunologic failure

Figure 4.5 shows the Kaplan-Meier survival function by age. The probability of event free survival was similar for the age groups under comparison since their survival curves remained close to one another. The log rank test for equality of these functions adjusted for site indicated that the two age groups under comparison had a similar probability of event free survival (p value>0.05) as shown in table 4.13.

4.3.4.5.2.2   Gender and immunologic failure

Figure 4.6 shows the Kaplan-Meier survival function by gender. The probability of event free survival for was different, especially after two years. The probability of event free survival was higher for female patients after two years, reaching around 0.68 at year eight as compared to 0.63 for male patients. However, overall, the log rank test of equality of these functions adjusted for site indicated that the difference in probability of

event free survival was statistically not significant (p value >0.05) as shown in table 4.13.
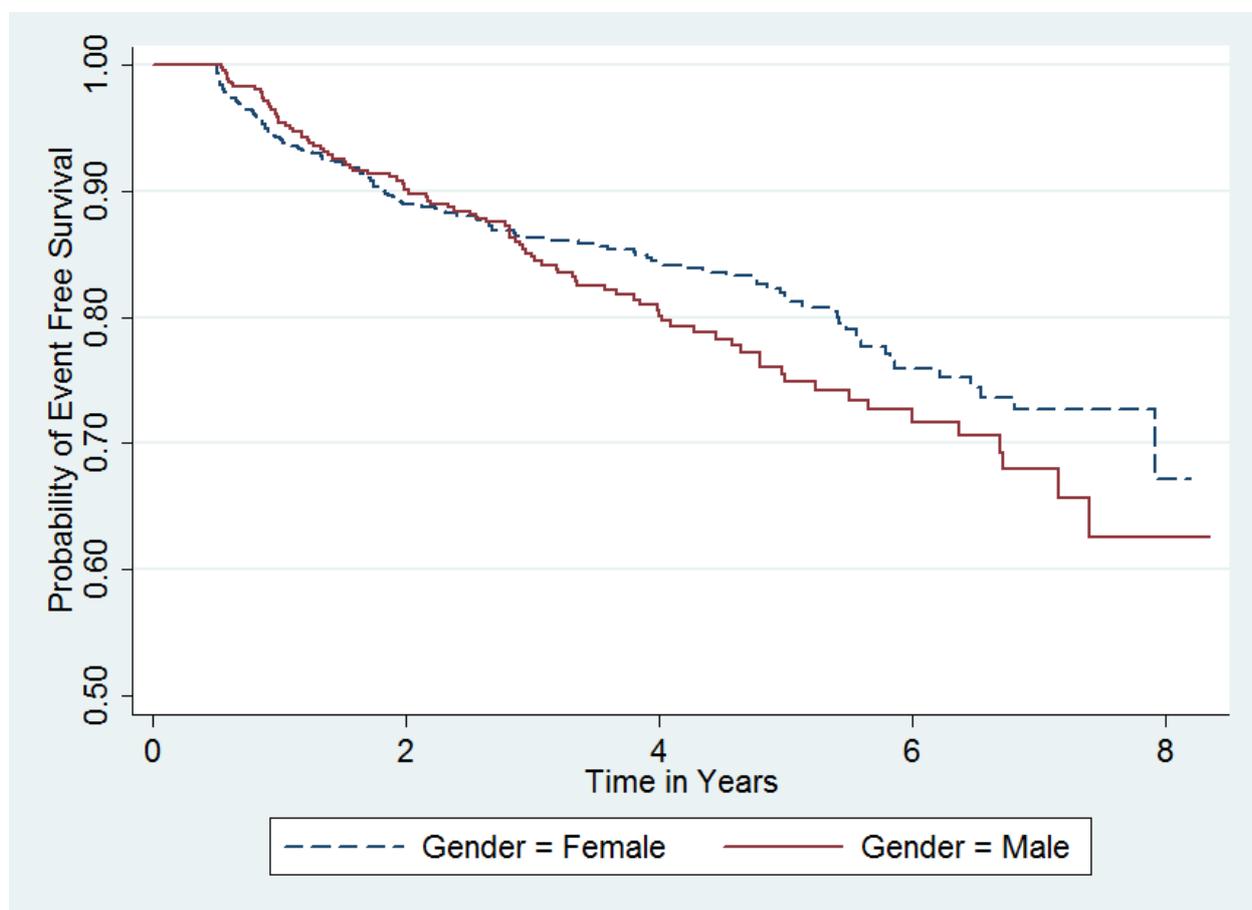
Figure 4.6   Kaplan-Meier survival functions by gender (n=1,282)

4.3.4.5.2.3   Baseline WHO Stage and immunologic failure

Figure 4.7 shows Kaplan-Meier survival function by baseline WHO stage. The probability of event free survival for patients at WHO stage III or IV was lower throughout follow-up as compared to those at WHO stages I or II reaching around 0.65 at year eight for the former group in contrast to slightly above that for the latter group. The log rank test of equality of these functions adjusted for site indicates that the difference in probability of event free survival was statistically not significant at the 5% level (p value= 0.054), but was significant at the 10% level, as shown in table 4.13.
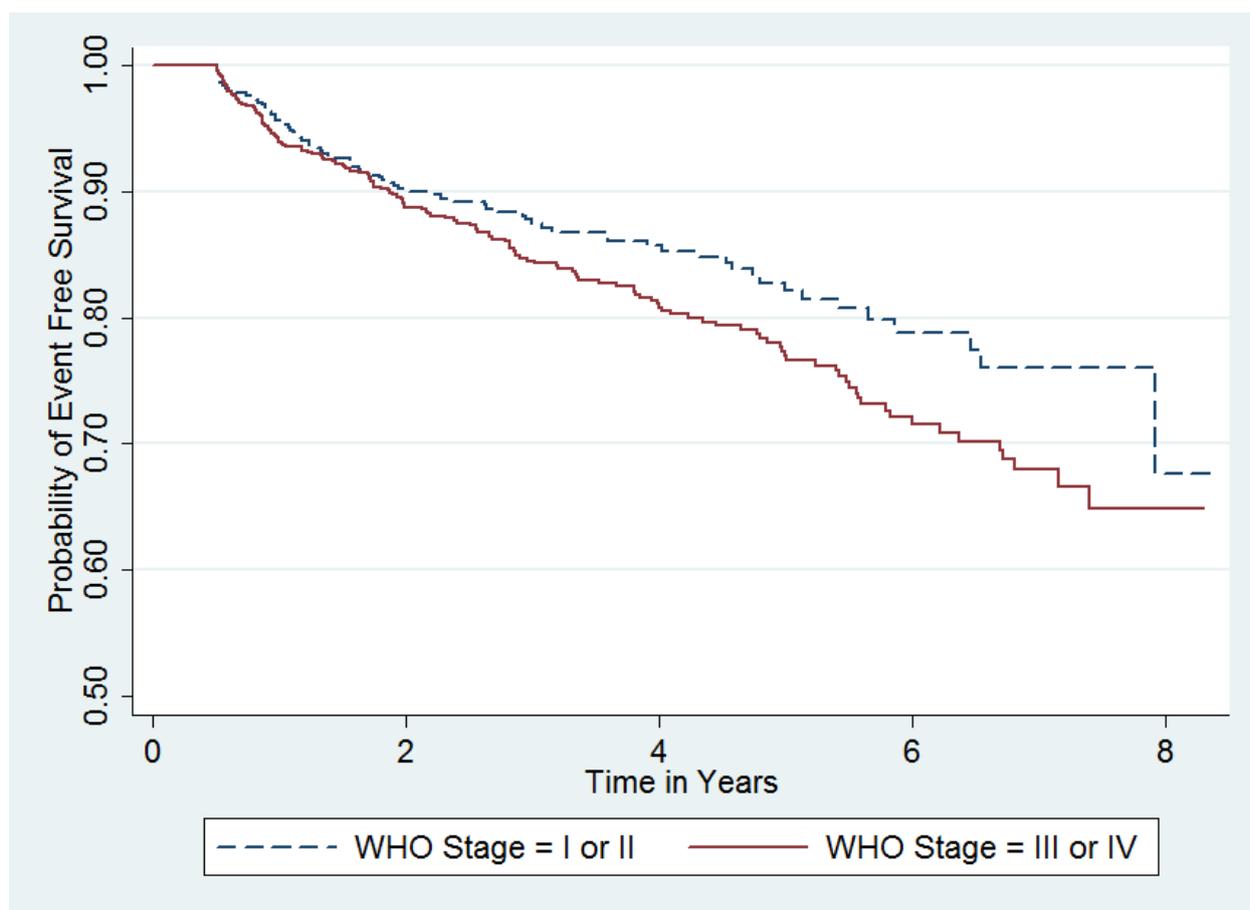
Figure 4.7   Kaplan-Meier survival functions by WHO Stage (n=1,282)

4.3.4.5.2.4   Baseline CD4 cell count and immunologic failure

Figure 4.8 shows the Kaplan-Meier survival function by baseline CD4 cell count group. The probability of event free survival for patients with a baseline CD4 cell count ≥350 was higher, followed by those whose baseline CD4 cell count was <100 or 200-349. Patients with a baseline CD4 cell count between 100 and199 had a curve slightly higher than the rest. The log rank test for equality of these functions adjusted for site indicates that the difference in probability of event free survival was statistically significant for those in the various baseline CD4 cell count groups (p value < 0.05), as shown in table 4.13. The log-rank test for trend was also statistically significant (p value < 0.05).
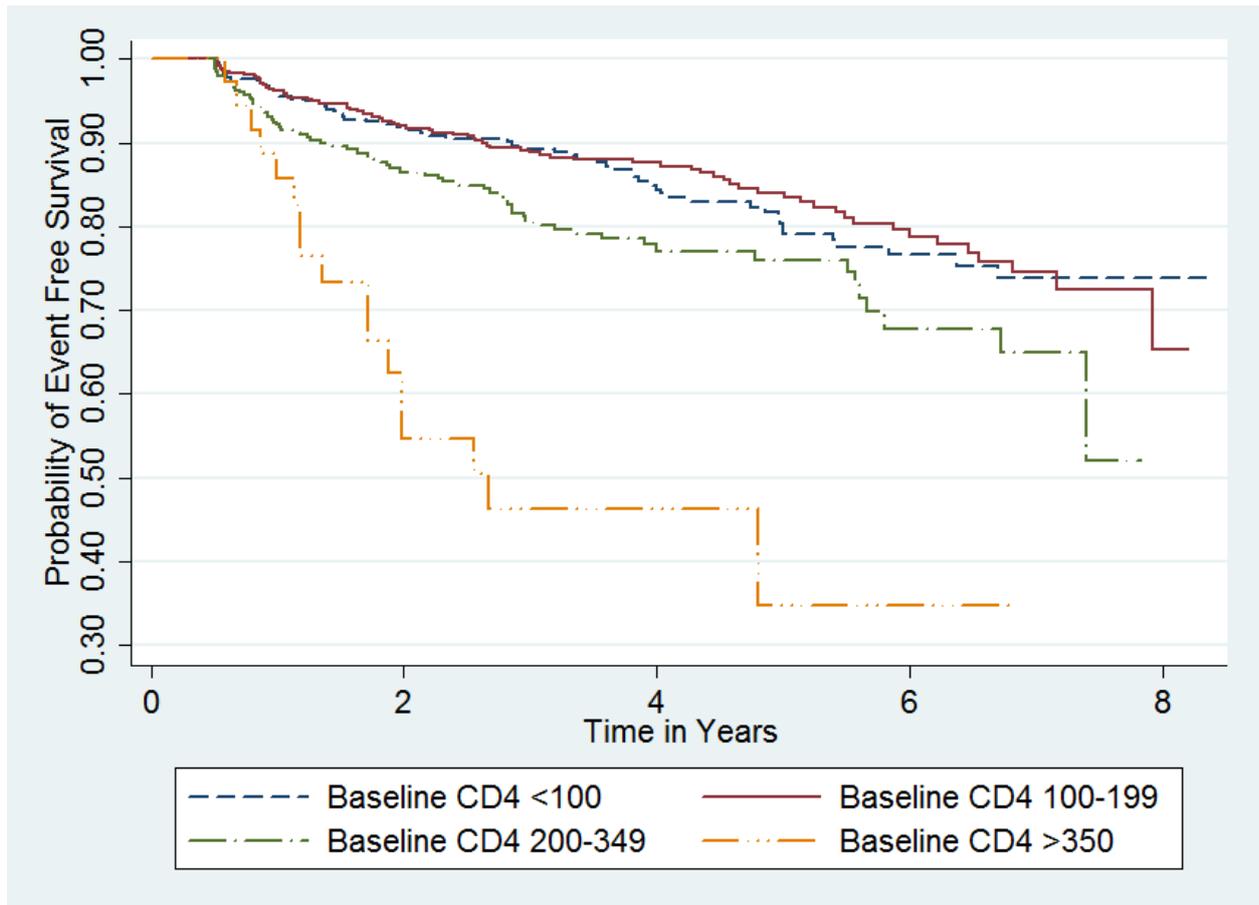
Figure 4.8   Kaplan-Meier survival functions by baseline CD4 cell count (n=1,282)

4.3.4.5.2.5   Tuberculosis disease and immunologic failure

Figure 4.9 shows Kaplan-Meier survival function by diagnosis of tuberculosis disease after the first six months on HAART. The probability of event free survival for those diagnosed with tuberculosis as compared to those without tuberculosis was very similar. The log rank test of equality of these functions adjusted for site indicates that the difference in probability of event free survival was not statistically significant (p value <0.05) as shown in table 4.13. But in general, there were far too few events of immunological failure recorded among patients with tuberculosis, so generalization should be made cautiously.
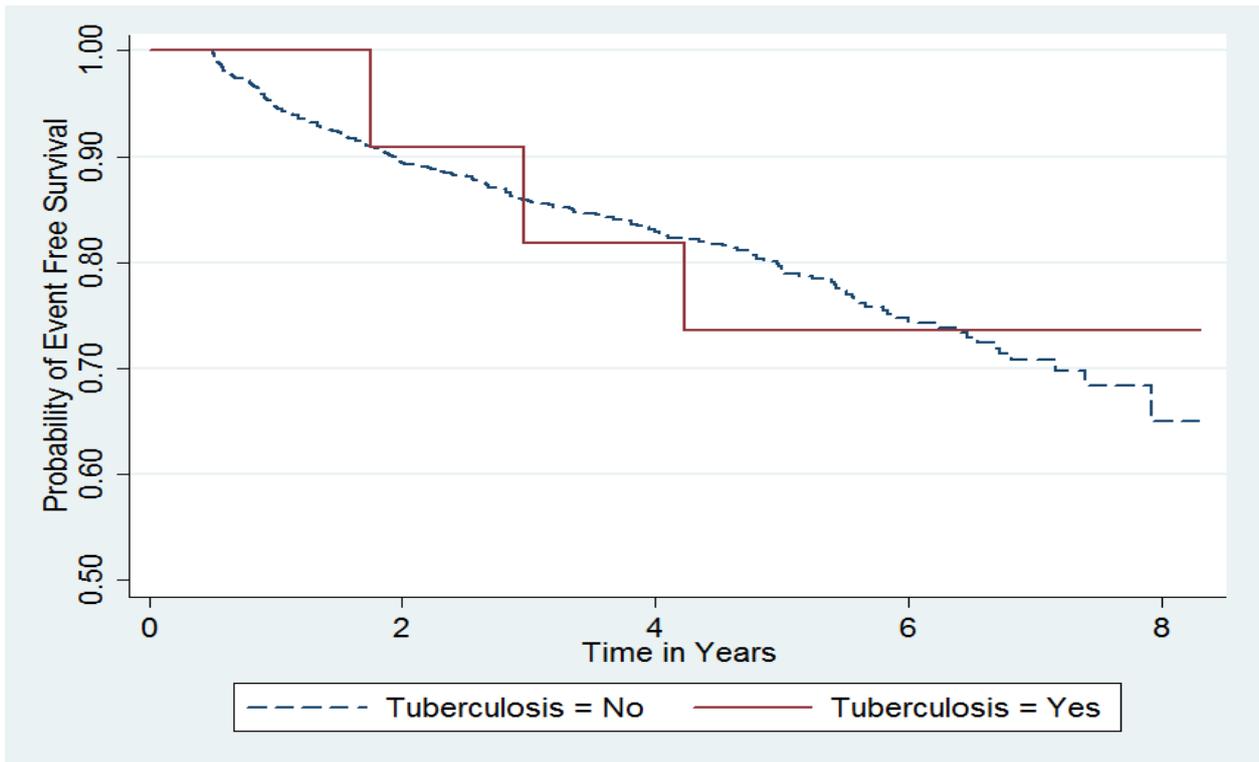
Figure 4.9   Kaplan-Meier survival functions by diagnosis of tuberculosis disease (n=1,282)

4.3.4.5.2.6   Adherence to medication refill and immunologic failure

Figure 4.10 shows the Kaplan-Meier survival function by adherence to medication refill.
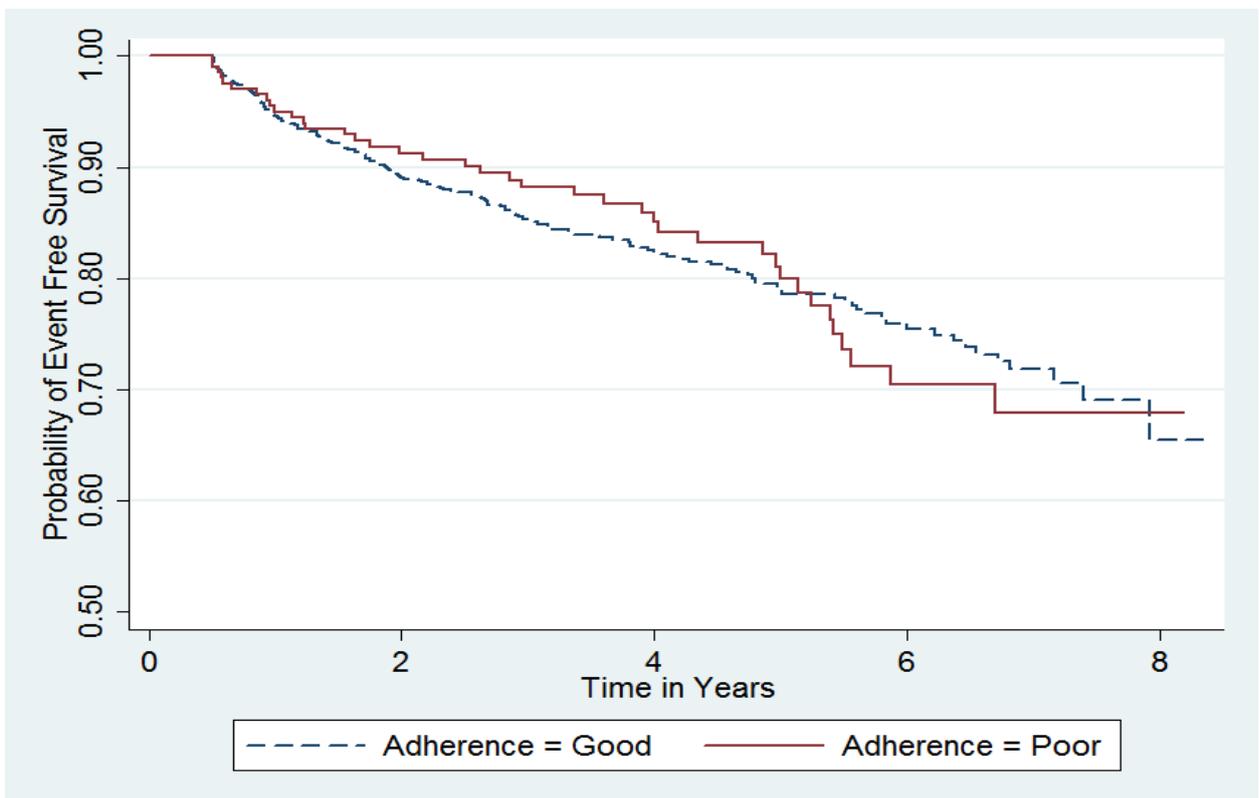
Figure 4.10   Kaplan-Meier survival functions by adherence (n=1,282)

The probability of event free survival for those with good and poor adherence to medication refill remains close to one another. The log rank test of equality of these functions adjusted for site indicates that the difference in probability of event free survival was not statistically significant (p value <0.05) as shown in table 4.13.

4.3.4.5.3 Multivariable analysis with Cox proportional hazards regression modelling

First, results from individual regression analysis are presented in table 4.14. Age and baseline CD4 cell count were treated as continuous variables throughout the regression modelling. It can be seen that baseline CD4 cell count and baseline WHO Stage were significant at 5% level of significance at individual level.

TABLE 4.14   INDIVIDUAL COVARIATE COX PROPORTIONAL HAZARDS REGRESSION ANALYSIS FOR PREDICTORS OF IMMUNOLOGIC FAILURE AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (n=1,282)

Covariate	Coefficient	Standard error	Wald test	Wald P value	95% confidence interval
Age	0.0058159	0.0070915	0.82	0.412	-0.0080832 - 0.0197150
Gender	0.1542121	0.1355448	1.14	0.255	-0.1114507 - 0.4198749
Stage	0.2957729	0.1416056	2.09	0.037	0.0182310 - 0.5733148
Baseline CD4 cell count	0.0029675	0.0004540	6.54	0.000	0.0020776 - 0.0038574
Tuberculosis	-0.0216643	0.5867462	-0.04	0.971	-1.1716660 - 1.1283370
Adherence*	0.1125241	0.4546164	0.25	0.805	-0.7785077 - 1.0035560

* Adherence to medication refill

4.3.4.5.3.1   Step 1

In this step, a multivariable model containing all variables significant in the individual analysis at the 20-25 % level were included, as shown in table 4.15.

TABLE 4.15   INITIAL MULTIVARIABLE MODEL FOR PREDICTORS OF IMMUNOLOGIC FAILURE AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (n=1,282)

Covariate	Coefficient	Standard error	Wald test	Wald P value	95% confidence interval
WHO Stage	0.2827662	0.1419603	1.99	0.046	0.0045292 - .5611118
Baseline CD4 cell count	0.0028975	0.0004497	6.44	0.000	0.0020161 - .0037790

4.3.4.5.3.2   Step 2

TABLE 4.16   PARTIAL LIKELIHOOD RATIO TEST FOR THE EFFECT OF REMOVAL OF COVARIATES ONE AT A TIME FROM THE INITIAL MULTIVARIABLE MODEL (n=1,282)

Models	-2 x log likelihood for initial model	-2 x log likelihood for model without covariate	Degree of freedom	Likelihood ratio Chi square	P value
WHO Stage	2660.15	2664.22	1	4.07	0.0436
Baseline CD4 cell count	2660.15	2688.58	1	28.43	0.0000

In this step, p-values from the Wald tests of the individual coefficients were used to identify covariates that might be deleted from the model. The deletion was made one variable at a time. The p-value of the partial likelihood ratio test was used to determine whether or not the deleted covariate made any significant change in the model. The finding is as shown in table 4.16. Deletion of both variables results in statistically significant change, for which reason both were kept in the model.

4.3.4.5.3.3   Step 3

Since no variable was removed during Step 2, checking for the effect of confounding was not made at this stage.

4.3.4.5.3.4   Step 4

In this step, variables left out from inclusion in the initial multivariable model were added one at a time and checked to see if that made any significant change in the model or changed the coefficients in the model. The partial likelihood ratio test revealed that the addition, one at time, of the above mentioned covariates didn't result in significant change at p value of 0.05 (see table 4.17),nor did any covariate added affect the coefficient of variables in the model by more than 20%. For this reason, all added variables were left out (see tables 4.18 and 4.19). So, the preliminary main effects model consisted of only the WHO Stage and the baseline CD4 count.

TABLE 4.17 **PARTIAL LIKELIHOOD RATIO TEST FOR THE EFFECT OF THE ADDITION OF COVARIATES NOT INCLUDED IN STEP 1, ONE AT A TIME, TO THE INITIAL MULTIVARIABLE MODEL (n=1,282)**

Models	-2 x log likelihood for initial model	-2 x log likelihood for model with covariate	Degree of freedom	Likelihood ratio Chi square	P value
Age	2660.15	2658.37	1	1.78	0.1826
Gender	2660.15	2658.24	1	1.91	0.1671
Tuberculosis	2660.15	2660.14	1	0.01	0.9030
Adherence*	2660.15	2660.08	1	0.07	0.7935

* Adherence to medication refill

TABLE 4.18 **CHANGES IN THE COEFFICIENT OF WHO STAGE DURING ADDITION OF COVARIATES EXCLUDED DURING STEP 1 OF MODEL BUILDING (n=1,282)**

Models	Coefficient in Step 1	Coefficient in Step 4	Percentage change of coefficient
Age	0.2827662	0.2644602	-6.47%
Gender	0.2827662	0.2577927	-8.83%
Tuberculosis	0.2827662	0.2820564	-0.25%
Adherence*	0.2827662	0.2801304	-0.93%

* Adherence to medication refill

TABLE 4.19 **CHANGES IN THE COEFFICIENT OF BASELINE CD4 CELL COUNT DURING ADDITION OF COVARIATES EXCLUDED DURINGSTEP 1 OF MODEL BUILDING (n=1,282)**

Models	Coefficient in Step 1	Coefficient in Step 4	Percentage change of coefficient
Age	0.0028975	0.0030015	3.59%
Gender	0.0028975	0.0029719	2.57%
Tuberculosis	0.0028975	0.0029002	0.09%
Adherence*	0.0028975	0.0029016	0.14%

* Adherence to medication refill

4.3.4.5.3.5 Step 5

In this step, the scale of continuous covariates found in the model was checked. There was only one continuous variable, namely the baseline CD4 cell count. The scale of this variable was checked graphically by plotting Martingale residuals from a model excluding the baseline CD4 cell count against it. The Lowess curve, as shown in figure 4.11, indicates that it was linear after a CD4 cell count value of 200 but slightly curved upwards for the lowest CD4 cell count values. This suggests its scale may be non-linear. For this reason, fractional polynomials analysis was carried out.
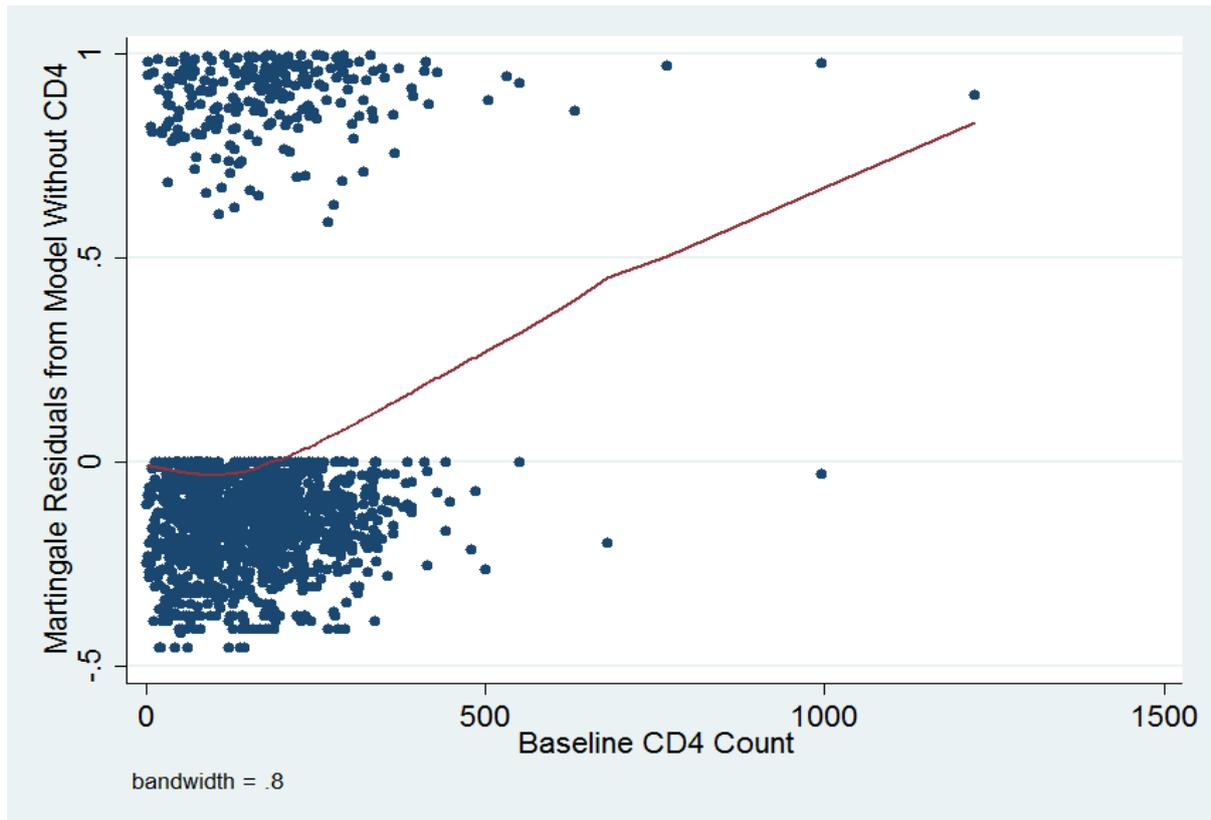
Figure 4.11   Martingale residual vs baseline CD4 cell count (n=1,282)

TABLE 4.20   SUMMARY OF A FRACTIONAL POLYNOMIALS ANALYSIS OF THE BASELINE CD4 CELL COUNT (n=1,282)

Baseline CD4 cell Count	Degree of freedom	Deviance	Deviance difference	P value [*]	Powers
Not in model	0	2993.500	33.177	0.000	
Linear	1	2965.176	4.853	0.183	1
M=1	2	2965.176	4.853	0.088	1
M=2	4	2960.323	--	--	2,3

[*] P-value from deviance difference comparing reported model with m = 2 mode

Table 4.20 shows findings from fractional polynomials analysis. The findings suggest that a two term model did not differ statistically from the linear model. In figure 4.12, it can be seen that there are differences in the two graphs for CD4 cell count values below 200 and above 500. Had the two term model been a better fit, the Grambsch, Therneau and Fleming smoothed plot and fractional polynomial function would have aligned better. For this reason, the baseline CD4 cell count was used as a linear variable without transformation.
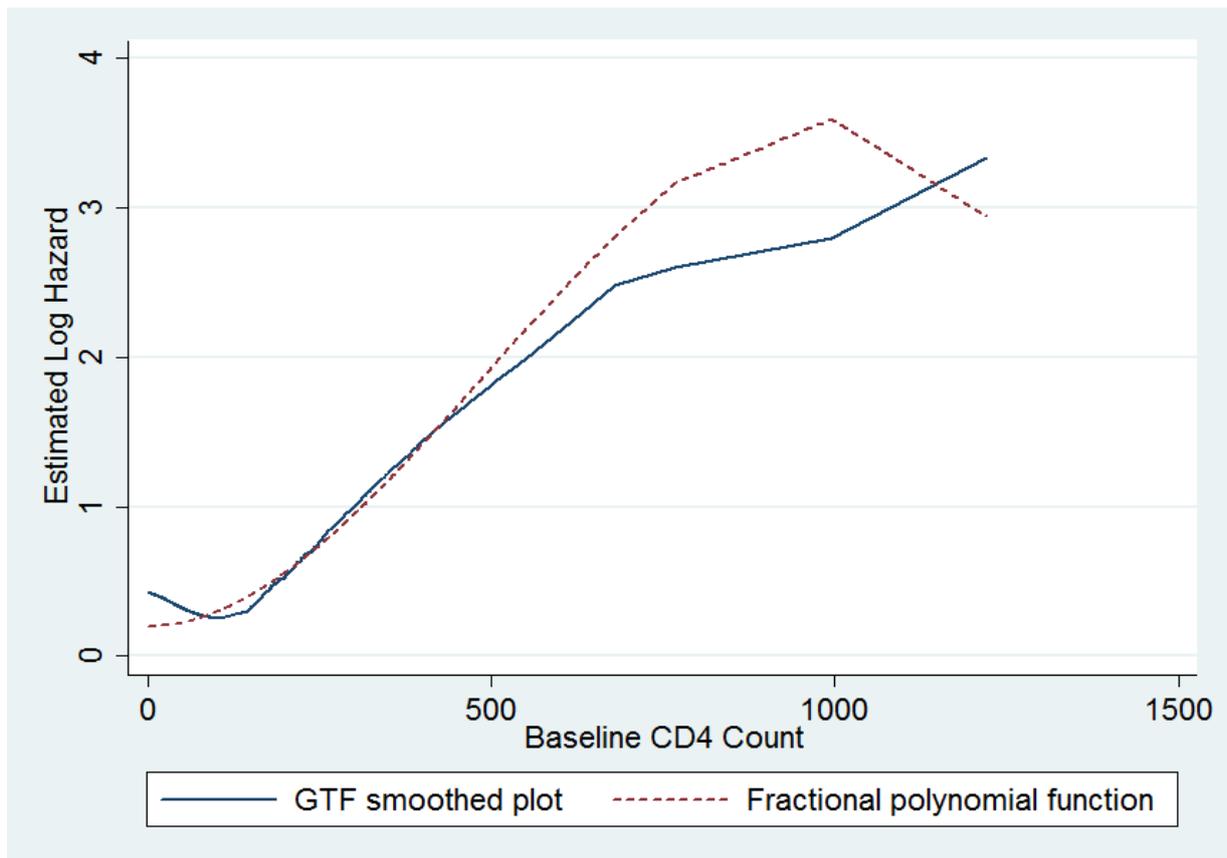
Figure 4.12   Plot of the Grambsch, Therneau and Fleming smooth and fitted two-term (2, 3) fractional polynomial model (n=1,282)

4.3.4.5.3.6   Step 6

In this step, interaction between the baseline CD4 cell count and the WHO stage was checked in the final model. As shown in table 4.21, the interaction term failed to make any statistically significant change in the model. For this reason, it was not included in the preliminary final model.

TABLE 4.21   PARTIAL LIKELIHOOD RATIO TEST FOR THE ADDITION OF INTERACTION TERM BETWEEN THE BASELINE CD4 CELL COUNT AND THE WHO STAGE TO THE PRELIMINARY MULTIVARIABLE MODEL (n=1,282)

Interaction	-2 x log likelihood for initial model	-2 x log likelihood for model with interaction term	Degree of freedom	Likelihood ratio Chi square	P value
WHO Stage, Baseline CD4 cell count	2660.15	2660.09	1	0.06	0.8052

4.3.4.5.3.7   Step 7

In this step, the preliminary final model containing the baseline CD4 cell count and the WHO stage was checked for collinearity between covariates, fulfilment of the

proportional hazards assumption, and regarding the existence of influential records, as well as the goodness-of-fit test.

4.3.4.5.3.7.1 Collinearity between covariates

TABLE 4.22   COLLINEARITY BETWEEN COVARIATES IN THE FINAL MODEL (n=1,282)

Variables	WHO Stage	Baseline CD4 cell count
WHO Stage	1.0000	
Baseline CD4 cell count	-0.0047	1.0000

The correlation between the Baseline CD4 cell count and the WHO Stage was -0.0047. There was no significant correlation.

4.3.4.5.3.7.2 Proportional hazards assumption

TABLE 4.23   TEST OF THE PROPORTIONAL HAZARDS ASSUMPTION OF THE PRELIMINARY MULTIVARIABLE MODEL (n=1,282)

Variables	rho	Chi square	Degree of freedom	P value
WHO Stage	0.04934	0.55	1	0.4575
Baseline CD4 cell count	-0.02933	0.18	1	0.6749
Global test		0.69	2	0.7095
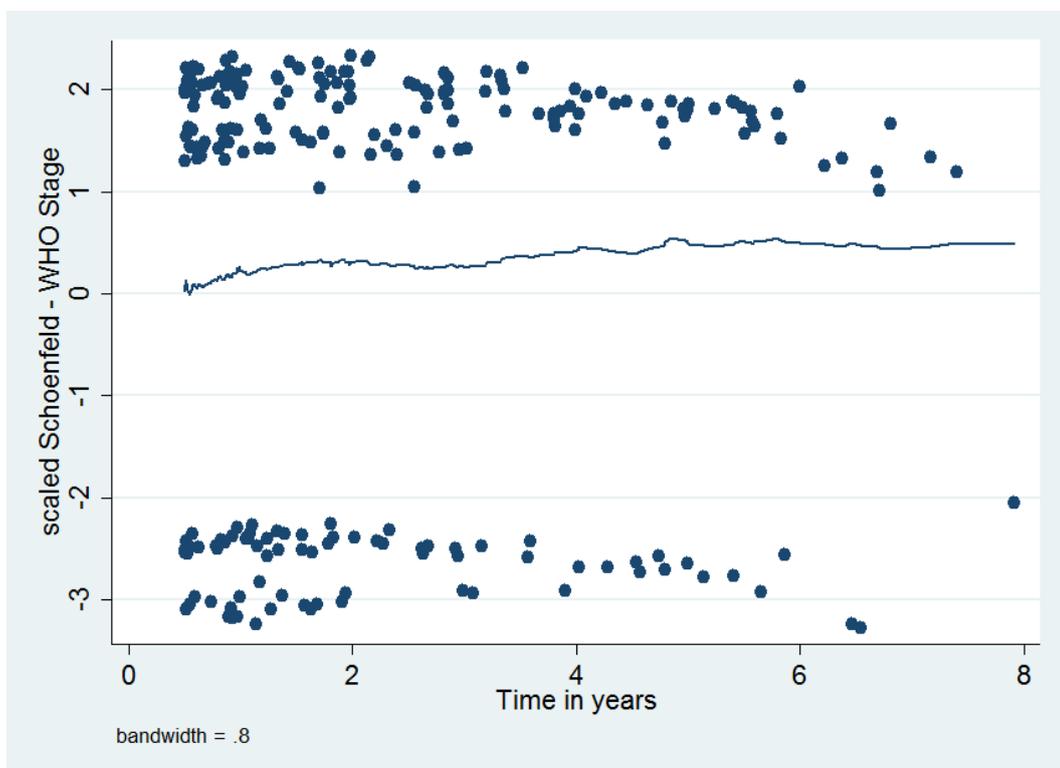
Figure 4.13   Graphs of the scaled Schoenfeld residuals and their Lowess smooth curves for the WHO Stage (n=1,282)
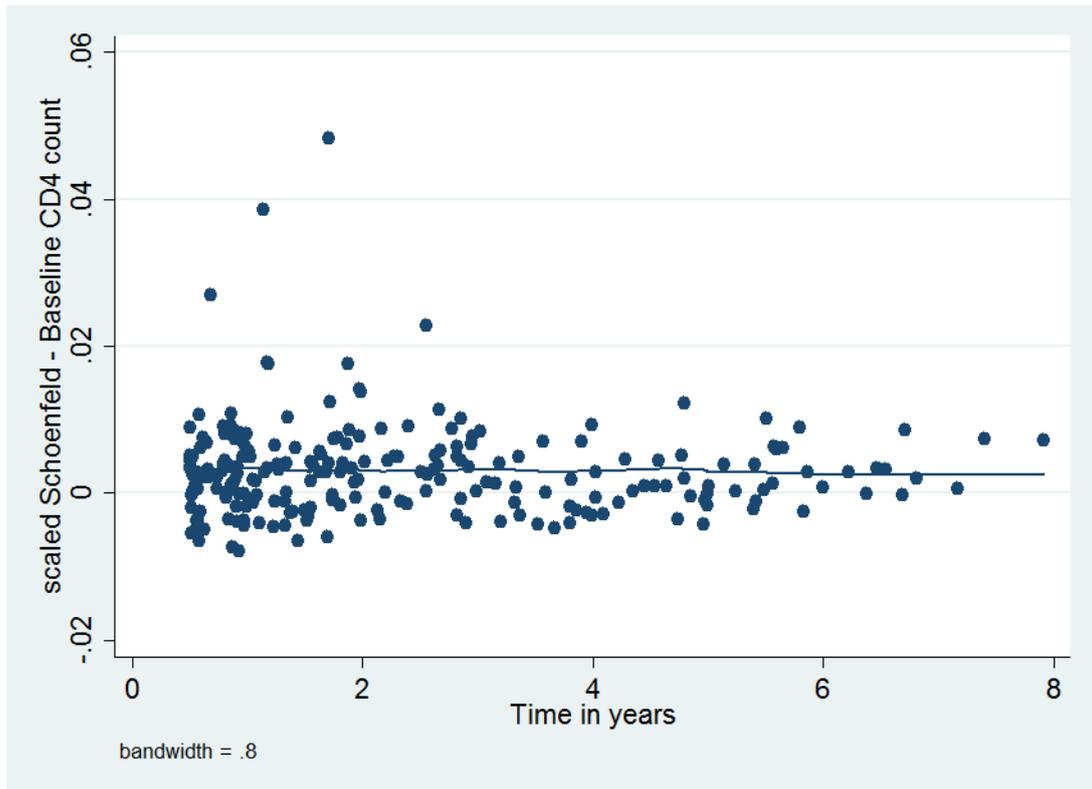
Figure 4.14 **Graphs of the scaled Schoenfeld residuals and their Lowess smooth curves for the baseline CD4 cell count (n=1,282)**

Global and individual covariate tests of the proportional hazards assumption indicated there was no evidence that would lead to rejecting the assumption as shown in table 4.20 (p value>0.05). This evidence was supported by the smoothed scaled Schoenfeld residuals plots (see figures 4.13 and 4.14) that show randomly scattered residuals as well as smoothed curves with slope very close to zero for both covariates.

4.3.4.5.3.7.3 Checking influential records

In this step, regression diagnostic statistics were examined to assess whether there were outliers, and if there were, what their influence on estimates of parameters, and their influence on the fit of the model would be. Figure 4.15 depicts that there were four records with results that were located apart from the rest. Table 4.24 shows all their values. Baseline CD4 cell and WHO Stage values for these records were analysed from clinical care point of view and were found to be normal. Delta-beta ($\Delta\beta$) values and the percentage change of the respective standard errors are displayed in table 4.25a and 4.25b for the five highest differences. It can be seen that none of these records had a $\Delta\beta$ greater than the standard error of the respective parameter. This indicates that there was no influential record affecting the coefficient of covariates in the model.

Figure 4.15   Graph of the score residuals for baseline CD4 cell count (n=1,282)

TABLE 4.24   EXAMINING EXTREME VALUES FOR POTENTIALLY INFLUENTIAL VARIABLES (n=1,282)

Score	Baseline CD4 cell count	WHO Stage	Age	Id
-1262.357	1221	III or IV	20	20541
-350.3844	680	III or IV	38	10976
302.6999	996	I or II	25	20458
447.6042	768	III or IV	25	21418

TABLE 4.25a   HIGHEST DIFFERENCES IN PARAMETER ESTIMATES OF VARIABLES INCLUDED IN THE PRELIMINARY MODEL, APPROXIMATE DELTA-BETAS FOR THE BASELINE CD4 (n=1,282)

ID number of deleted observation	Δβ	Standard error for Baseline CD4 cell count	% change of Δβ of the standard error
11242	-0.0149249	0.1419612	11%
10518	-0.0146230	0.1419612	10%
10492	-0.0146215	0.1419612	10%
11018	-0.0145682	0.1419612	10%
10505	-0.0145401	0.1419612	10%

TABLE 4.25b HIGHEST DIFFERENCES IN PARAMETER ESTIMATES OF VARIABLES INCLUDED IN THE PRELIMINARY MODEL, APPROXIMATE DELTA-BETAS FOR THE WHO STAGE (n=1,282)

Id number of deleted observation	Δβ	Standard error for WHO Stage	% Change of Δβ of the standard error
20541	-0.0002530	0.0004497	56%
10976	-0.0000700	0.0004497	16%
20824	-0.0000460	0.0004497	10%
20160	-0.0000443	0.0004497	10%
20358	-0.0000439	0.0004497	10%

4.3.4.5.3.7.4 Goodness-of-fit test

The fit of the model was evaluated by using Cox-Snell residuals. It can be seen that the two curves fit well for small values but match poorly at large values. This may be because of the large number of censored records present in the data Collett (2003:240), which was around 83% for this study (n=1056).
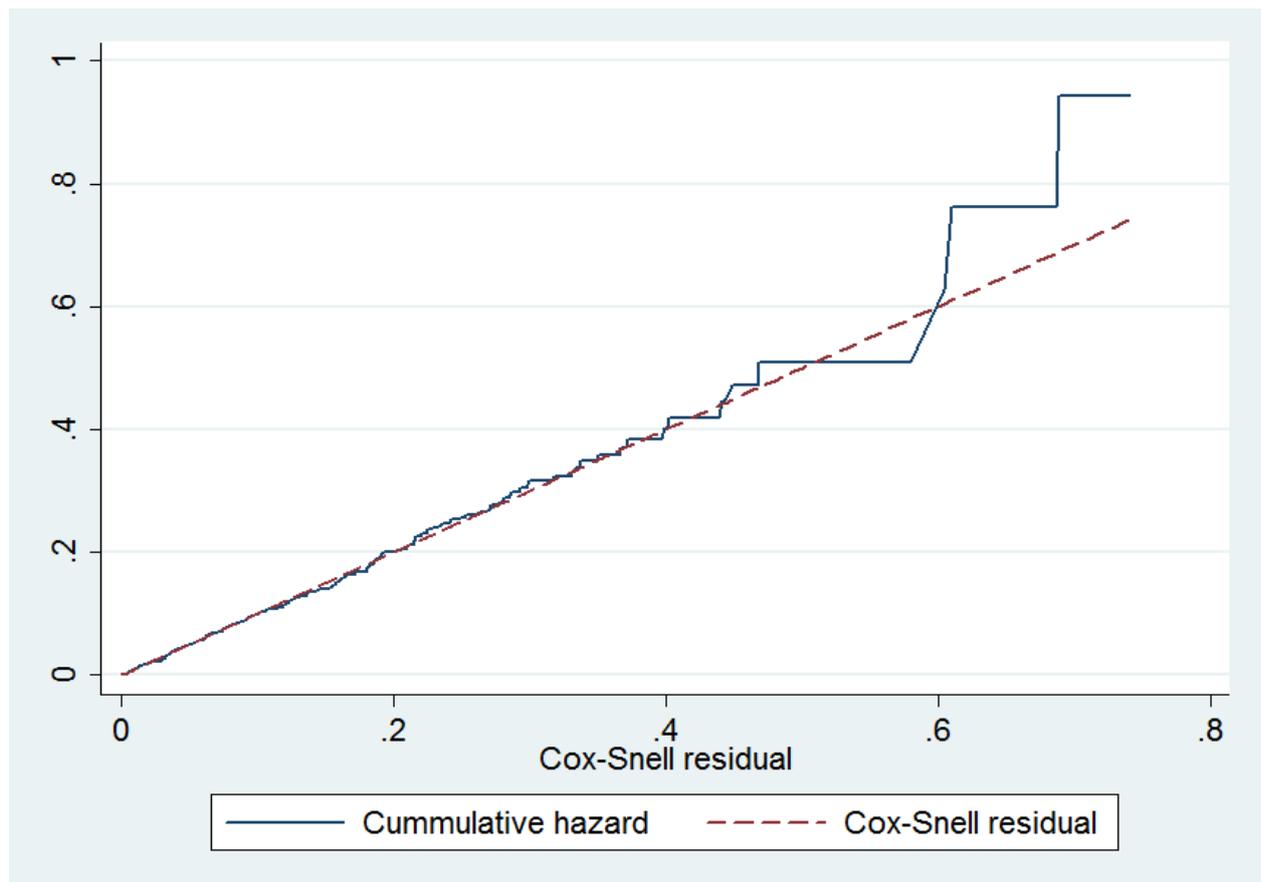
Figure 4.16   Cumulative hazard plot of the Cox-Snell residual (n=1,282)

4.3.4.5.3.8 Summary of regression analysis to predict immunologic failure (n=1,282)

The final model is shown in tables 4.26a and 4.26b. Only the WHO Stage and baseline CD4 cell count remained in the model. Their coefficients are shown in the first table while their hazard ratios are shown in the second one. The WHO Stage is a categorical variable, the reference in the model being WHO Stage I or II. Hence, patients at WHO Stage III or IV had a hazard function 33% higher than the reference. Baseline CD4 cell count was treated as a continuous variable. Hence, it means a single unit increase in baseline CD4 cell count increased the hazard of immunologic failure by 0.3% while an increase by 100 increased the hazard of immunologic failure by 33.6% ($2.718^{(100 \times 0.0028975}$). Age, gender, tuberculosis status, and adherence to medication refill appointment were not included in the model since they failed to predict immunologic treatment failure.

TABLE 4.26a   COEFFICIENTS OF VARIABLES IN FINAL MULTIVARIABLE MODEL FOR PREDICTORS OF IMMUNOLOGIC FAILURE AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (n=1,282)

Covariate	Coefficient	Standard error	Wald test	Wald P value	95% confidence interval
WHO Stage	0.2827662	0.1419603	1.99	0.046	0.0045292 - 0.5611118
Baseline CD4 cell count	0.0028975	0.0004497	6.44	0.000	0.0020161 - 0.0037790

TABLE 4.26b   HAZARD RATIO OF VARIABLES IN FINAL MULTIVARIABLE MODEL FOR PREDICTORS OF IMMUNOLOGIC FAILURE AMONG PATIENTS ON ANTI-RETROVIRAL THERAPY (n=1,282)

Covariate	Hazard ratio	Standard error	Wald test	Wald P value	95% confidence interval
WHO Stage	1.326937	0.1883735	1.99	0.046	1.004645 - 1.752620
Baseline CD4 cell count	1.002902	0.0004510	6.44	0.000	1.002018 - 1.003786

After an extensive search of the PubMed database, only one published study in northern Ethiopia by Assefa, Gelaw, Getnet and Yitayew (2014:online) was found that described prevalence and predictors of immunologic failure using the WHO (2010a:50) immunologic failure criteria to define immunologic failure. According to that study, the prevalence of immunologic failure was 22.2% (n=89) which is slightly higher than the current study. It identified CD4 cell count <100, male gender and tuberculosis as predictors of immunologic failure. A number of issues make it difficult to make direct comparison with this study. Follow-up CD4 cell count values not falling within two months of follow-up months that are multiples of six, for example, 6, 12, 18, etc., were excluded during their analysis. There is no mention of the number of CD4 cell counts excluded. This may bias the findings of the study because the WHO criteria doesn't put

such limitations on the timing of follow-up CD4 cell count testing apart from the fact that immunologic treatment failure assessment couldn't be made before having ART for at least six months. In addition, 122 charts were excluded because of missing and incomplete information that may result in selection bias.There is no mention of control to make sure that tuberculosis cases diagnosed prior to occurrence of immunologic failure were considered during regression analysis to predict immunologic failure. In fact, in that study, the median time for the diagnosis of immunologic failure for those patients with TB was six months as compared to 9.5 months for the occurrence of incident TB. This clearly shows that at least a considerable number of TB cases occurred after occurrence of treatment failure and can only be taken as consequence of immunologic failure rather than cause for it. This is in direct violation of the Bradford Hill criteria (Hill 1965:297) that stipulates that temporal relationship must hold in order to consider cause-effect relationship between two variables; i.e., exposure must always precede outcome. There are other studies that assessed predictors of immunologic failure but comparison was not possible because different immunologic failure criteria were used. For instance, Kassa et al (2013:online) used the following criteria to define immunologic failure: increase of CD4 cell count by fewer than 50cells/μl at month six, and the criteria of less than 100 cells/μl at months 18 and 24 of HAART. Anude et al (2013:online), used the criteria of a fall of follow-up CD4 cell count to baseline or below, or an increase in the CD4 cell count of less than 50 cells/mm^3 at one year after HAART initiation. All these criteria are different from that stated by the WHO.

4.4   OVERVIEW OF RESEARCH FINDINGS

A total of 1,321 documents of patients which had had two or more CD4 cell count tests and at least six months of follow-up were reviewed. The study was conducted at two hospitals in Ethiopia in the time frame September 1, 2005 to August 31, 2013.

The findings show that the median age of those in the study sample was 30 years (a range of 5-71 years). Females accounted for 63% (n=831) of patients. Sixty per cent of patients (n=786) were in the WHO stage III or IV at ART initiation. The median baseline CD4 cell count at ART initiation was 156 cells per mm^3 (a range of 1-1,221 cells mm^3). After the initiation of HAART, tuberculosis was diagnosed in 17.79% (n=235) of patients. Eighteen per cent (n=241) of patients showed poor adherence as they could not adhere to their appointments for medication refill. By the end of the follow-up, 73%

(n=960) of patients were still on HAART in the same facility where treatment was initiated. There was no difference with respect to being actively on follow-up with regard to baseline covariates.

Taking into account only the time patients were on follow-up, the pattern of timely immunologic monitoring was, in general, inadequate. There was no predilection for determination of timely follow-up CD4 cell count test by age, gender, baseline WHO stage or baseline CD4 cell count (p value > 0.05 for all).

Immunologic (CD4 cell count) recovery was observed among patients on antiretroviral therapy. The magnitude of follow-up CD4 cell count increase was rapid in the first few years of follow-up after starting antiretroviral therapy and reached a plateau thereafter. Children of less than 14 years of age, female patients, those who were at baseline WHO clinical stages I or II, as well as patients with a higher baseline CD4 cell count were able to achieve a higher proportion of follow-up CD4 cell count greater than or equal to 350 than their respective counterparts (p value < 0.05 for all).

The prevalence of patients who experienced immunologic failure at least once was 17.6% (n=226) (95% confidence interval: 15.6%-19.8%).But, with further evaluation and follow-up CD4 cell count determination this percentage dropped to 11.5% (n=147) (95% confidence interval: 9.8%-13.3%). Having a follow-up immunologic evaluation resulted in a decrease of the number of patients who fulfilled immunologic failure criteria, with almost 46% of those with follow-up CD4 cell count evaluation not fulfilling the criteria anymore. Diagnosis of treatment failure was mostly based on the criteria 'drop in follow-up CD4 cell count by 50% from follow-up peak' (58%, n=131) followed by the criteria 'drop of follow-up CD4 cell count to or below baseline CD4 cell count' (35%, n=78). There were very few patients on second line HAART among those with confirmed immunologic failure (7.53%, n=7) even though there were a total of93patients who were confirmed to have had immunologic treatment failure.

The median duration of follow-up from HAART initiation to diagnosis of first instance immunologic failure was 1.8 years (range: 0.51-7.92). For those who never experienced treatment failure, the median time of follow-up was 3.72 years (0.51-8.36).

During univariable as well as multivariable analysis, being at WHO Stage III or IV or having a higher CD4 cell count at baseline were associated with being at level of increased hazard for immunologic failure. None of the other covariates (age, gender, tuberculosis, adherence to picking up medication refill on time) predicted immunologic treatment failure.

4.5   CONCLUSION

In this chapter, analysis techniques, and results of the study were presented. Comparison of findings with other studies was made as much as possible but was limited to few a few studies because of lack of similarity in variable selection, study design, and study setting.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 INTRODUCTION

Chapter 4 addressed data analysis, presentation and description of the research findings. In this chapter interpretation of the findings, conclusions, recommendations and limitations of the study are presented.

The aim of the study was to describe the prevalence of immunologic failure and identify its determinants among people living with HIV receiving treatment at selected hospitals.

The objectives of this study were to:

- Assess the pattern of immunologic (CD4 cell count) monitoring in selected hospitals in southern Ethiopia
- Assess the pattern of immunologic (CD4 cell count) response for patients on HAART in selected hospitals in southern Ethiopia
- Describe immunologic treatment failure and identify its predictors among patients on HAART in selected hospitals in southern Ethiopia

5.2 RESEARCH DESIGN AND METHOD

A self-designed document checklist was used for the purpose of data collection. The checklist consisted of three sections: demographic data, clinical data and immunologic data in the form of CD4 cell count tests.

The documents of people living with HIV on antiretroviral therapy enrolled and started on ART between September 1st, 2005 and December 31st, 2012 in the selected study sites, those with age five or older at ART initiation, those with two or more CD4 cell count tests and those with at least six months of follow-up were included in the study. Documents of patients transferred into the study sites from other facilities after starting

HAART were excluded from the study. Follow-up information was abstracted for all eligible patients until August 31st, 2013.

After applying the eligibility criteria, there were 1,321 patient records to draw data from. Since the ideal for the study sample was 1,304, the researcher used all available patient records for analysis. Data on follow-up weight was missing in a significant number of records, for which reason, that factor was excluded from analysis. Data cleaning was done with Epi Info^{TM} 3.5.1 statistical software. The data was analysed using Stata/IC 12.0 (StataCorp LP). Descriptive statistics were presented in tables and graphs. The Chi Square test and Cox proportional hazards regression were used to analyse data. The P value was set at 0.05.

5.3   CONCLUSIONS

This section contains the conclusions of the study in relation to the study's objectives.

5.3.1 Objective 1: assess the pattern of immunologic (CD4 cell count) monitoring in selected hospitals in southern Ethiopia

5.3.1.1   *Pattern of immunologic monitoring*

The pattern of timely immunologic monitoring was, in general, inadequate. There was a correlation between the length of follow-up and the inadequacy of CD4 cell count determination: at the 6th month of follow-up, 46% (n=610) of patients still under care had their CD4 cell count determined, while at one year, only 33% (n=417) did so; it went below 20% after two years of follow-up and below 10% (n=10) at the seventh year of follow-up. There was no preference for determining whether there was follow-up CD4 cell count testing in a timely fashion of those studied by age, gender, baseline WHO stage or baseline cell CD4 cell count (p value>0.05 for all).

5.3.2 Objective 2: assess the pattern of immunologic (CD4 cell count) response for patients on HAART in selected hospitals in southern Ethiopia

5.3.2.1   *Pattern of immunologic response*

Immunologic recovery was observed among patients on treatment. The magnitude of follow-up CD4 cell count increased progressively in the first few years of follow-up after starting antiretroviral therapy and reached a plateau thereafter. Children of less than 14 years of age, female patients, those at baseline WHO stage I or II, as well as patients with higher baseline CD4 cell count were able to achieve a higher proportion of follow-up CD4 cell count greater than or equal to 350 than their respective counterparts (p value <0.05 for all). This signifies that earlier initiation of HAART would result in better immunological recovery in the form of an increased CD4 cell count reaching the normal range soon after treatment initiation.

5.3.3 Objective 3: describe immunologic treatment failure and identify its predictors among patients on HAART in selected hospitals in southern Ethiopia

5.3.3.1   Prevalence and predictors of immunologic failure

Despite the adequacy of group level immunologic recovery, immunologic failure was observed at an individual level. The proportion of patients who ever experienced immunologic failure was 17.6% (95% confidence interval: 15.6%-19.8%).However with further evaluation and follow-up CD4 cell count determination, it dropped to 11.5% (n=147) (95% confidence interval: 9.8%-13.3%). Since a significant number of patients who were re-evaluated after immunologic failure didn't fulfil immunologic failure criteria, it could be possible that the actual percentage of patients with immunologic failure would have been even lower than this, had there been proper follow-up immunologic evaluation. This also means that it may not be wise to rush to change antiretroviral medication to second line antiretroviral medication before proper evaluation or follow-up has been made. This could be done by repeating the CD4 cell count, addressing adherence issues, or utilizing more sensitive measures of treatment failure like viral load testing.

During multivariable analysis, two factors were found to be important in predicting immunologic failure: the baseline WHO Stage and the baseline CD4 cell count. In general, the findings were that the higher the baseline CD4 cells count, the higher the risk or hazard of immunologic failure; the same was true for baseline WHO stage. This means, patients fulfilling these criteria should be prioritized for immunologic

assessment. With the recent changes in the eligibility criteria for the initiation of antiretroviral treatment for adults and adolescents raising the CD4 cell count cut off to below 500 (FMOH 2013:2), the proportion of patients who experience a diagnosis of immunologic failure could become higher.

5.4 RECOMMENDATIONS

Immunologic treatment failure was prevalent in this study setting. Since immunologic monitoring was at the same time inadequate, the true level of immunologic failure could not be determined, though it was expected to be lower since with on-going evaluation less number of patients fulfilled the immunologic failure criteria. Also, since there are variables collected in routine care that are significant predictors of immunologic failure, they should be incorporated in a treatment plan that identifies and manages such patients. The following section discusses such plan that could be used by health care providers to screen and manage patients with presumptive immunologic failure based on the literature review and findings of the study.

5.4.1 Treatment plan to identify and manage patients with immunologic failure

Table 5.1 is a sample documentation tool to screen for and manage patients with respect to immunologic failure. This tool would be placed in each patient's medical document. Risk assessment will be done once at enrolment since the predictors identified are determined at baseline. The cut-off to identify those at greater and lower risk for immunologic failure using baseline CD4 cell count as criteria has been made 350 cells/mm^3 in order to simplify the tool. This is based on findings displayed in table 4.12 and figure 4.9 that show a greater risk of immunologic failure for patients with baseline CD4 counts of more than or equal to 350. Patients with a baseline CD4 cell count between 200 and 349 were at risk as well, compared to those with a count below 200, but the magnitude of difference was small as can be seen in figure 4.9. For baseline WHO staging, it is clear that those at WHO stages III or IV are at greater risk.

Those patients who have a baseline CD4 cell count more than or equal to 350 and/or those at baseline WHO stages III or IV are at risk of immunologic failure and would need prioritization for assessment as well management. Assessment for immunologic failure would be conducted at time of every follow-up CD4 cell count determination.

TABLE 5.1   IMMUNOLOGIC FAILURE RISK ASSESSMENT AND MANAGEMENT TOOL

IMMUNOLOGIC FAILURE RISK ASSESSMENT AND MANAGEMENT DOCUMENTATION TOOL						
Immunologic failure risk at baseline:			1. Baseline CD4 cell count ≥350/mm^3?	Yes No	High risk for immunologic treatment failure if	
			2. Baseline WHO Stage III or IV?	Yes No	'Yes' to either question	
Follow-up date	Month on ART	Adherence (G, F, P)	Antiretroviral medication regimen	CD4 cell count/mm^3	Immunologic failure (Y or N)	Decision (Use if there is immunologic failure) 0 = Re-evaluate with CD4 cell count 1 = Re-enforce adherence 2 = Re-evaluate with viral load 3 = Regimen change to second line
	0					
	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	13					
	14					
	15					
	16					
	17					
	18					

*Adherence= G (Good) if missed doses<3 per month; F (Fair) if missed doses 3-5 and 3-9 for 30 and 60 doses regimen per month respectively; P (Poor) if missed doses >5 or >9 for 30 and 60 doses regimen per month respectively. (FMOH 2008a:72)

**Immunologic failure criteria: 1. 50% below maximum follow-up CD4 cell count; 2. at or below baseline CD4 cell count; 3. persistently below 200. The presence of any one of these criteria in patients who have been taking antiretroviral treatment for at least six months and is still on treatment is used to diagnose it. (FMOH 2008a:159)

There would be a reminder to give a follow-up CD4 cell count test emphasized by shaded rows at months 0, 6, 12, 18, 24, and so on at and after the initiation of ART based on the current WHO recommendation (2013:132). Assessment for antiretroviral medication intake needs to be carried out as well, since it is one of the reasons for CD4 cell count decline. If a patient fulfills immunologic failure criteria while on the medication and after at least six months of treatment, then that would be documented on a specified column and a decision would be made on what to do next. The decision could be to repeat the CD4 cell count, to address adherence issues, to confirm treatment failure with viral load testing, or to change the treatment regimen to second line antiretroviral medication if failure is confirmed. Adherence issues could be addressed by proper counseling on the consequences of poor adherence for the patient, and the risk of transmission to others, as well as the means to handle it.

5.4.2 Recommendations to health care providers

In order to identify patients with immunologic failure or to know the true magnitude of the problem, proper CD4 cell count monitoring has to be done. This study indicates that immunologic monitoring has been poor in the study facilities. Hence, health care providers need to assess their CD4 cell count determination practices at facility level and take corrective measures whenever possible. In addition to that, the low proportion of patients on second line HAART indicates that, even in patients who had adequate CD4 cell count monitoring and fulfilled immunologic failure criteria, providers failed to manage them by shifting their antiretroviral regimen to a second line medication. Again, heath care providers need to work more on the proper implementation of treatment guidelines. In the case of resource constraints in the form of limited capacity to do CD4 cell count monitoring, health care providers should give priority to those at a high risk of immunologic failure as outlined in this study and to those with immunologic failure. Regimen changes based on immunologic criteria alone should be thought out carefully as a number of factors including adherence could easily affect it. There was no predictive effect of adherence to medication pick-upon immunologic treatment failure. Hence, providers should not rely on it to assess the risk of immunologic failure.

5.4.3 Recommendations to program managers

The reported magnitude of immunologic failure reasserts the need for the continuation and scaling up of the ongoing effort to address development of drug resistance to antiretroviral medications and transmission of drug resistance. Since immunologic monitoring is one of the most important elements of this process, the reason its implementation is suboptimal and how it can be corrected should be evaluated.

5.4.4 Recommendations for further research

This study merely identified the problem of poor immunologic determination. Therefore, further studies need be made to address barriers to proper CD4 cell count determination. In the face of confirmed immunologic failure, very few patients were managed by shifting antiretroviral treatment regimen to second line. Hence, providers' knowledge, attitude, and practice to managing patients with immunologic failure, which could be one determinant, should be evaluated in order to design an intervention to address observed gap. Also, the proposed tool to monitor and manage immunologic criteria needs to be validated and piloted before implementation.

5.5   CONTRIBUTIONS OF THE STUDY

The study may contribute to patients and providers at the study sites and beyond since it identified gaps in the implementation of immunologic monitoring and management of immunologic failure and potential interventions to alleviate the challenges. Providers in study facilities will be made aware of the prevalence of immunologic failure among patients under their care in addition to the gap in immunologic monitoring. Due to a large number of records of patients and difficulty in reviewing immunologic monitoring, an immunologic failure screening tool will be provided for health care providers to adapt and conduct proper CD4 cell count monitoring and screening of patients for immunologic failure.

For the wider health care system, the estimate of the prevalence of immunologic failure for children older than five years, adolescents, and adults provides an estimate for the specified age groups for Ethiopia using the WHO immunologic failure diagnostic criteria. This would help in planning for better management of patients on HAART as it provides an estimate of the magnitude of the problem. Most importantly it gives some idea as to the proportion of patients who may require advanced treatment monitoring in the form of

viral load testing. Finally, this study is an example of how data collected from routine program monitoring can be used to shed light on important questions that may in turn help to improve the quality of care.

5.6   LIMITATIONS OF THE STUDY

Despite the overall positive contributions of this study for clinical practice and program implementation, it has important limitations. Since this was a retrospective study based on existing medical records, the effect of possibly erroneous entries as well as incomplete entries of data cannot be underestimated. For instance, these reasons have led the researcher to drop the variable weight. In addition, the study could not assess the effect of clinical disease progression on immunologic failure except for tuberculosis as treatment staging was not well implemented.

Not all patients were active on follow-up at last observation because of treatment interruption or transfer to other facility for follow-up. For this reason, immunologic failure could only be measured for as long as patients were on follow-up. This effect was accounted for during survival analysis, which is specifically, designed to analyze such data. Moreover, there was no association between baseline covariates and being active on follow-up at last observation making the effect of incomplete observations minimal.

Finally, since only two hospitals were selected for convenience, findings couldn't be generalized for the whole region.

5.7   CONCLUDING REMARKS

This chapter concludes the study. The most important finding is that immunologic failure was found to be prevalent in the study setting. A number of recommendations were made in order to better implement identification and management of immunologic failure among patients on antiretroviral therapy. Proactive implementation of these and other existing measures should be made timely so that patients benefit sooner than later.

BIBLIOGRAPHY

Aber, V, Aboulker, JP, Babiker, AG, Bragman, K, Breckenridge, AM, Carbon, C, Charreau, I, Chene, G, Collis, P, Cooper, D, Darbyshire, JH, Dormont, J, Fiddian, P, Flepp, P, Gazzard, B, Goebel, FD, Hooker, M, Lange, J, Luthy, R, Peto, TEA, Reiss, P, Seligmann, M, Stone, AB, Thomis, J, Vella, S, Walckenaer, G, Warrell, D, Weller, IVD, Wilber, R, Yeni, P, Yeo, J & Withnall, R. 1996. Delta: a randomised double-blind controlled trial comparing combinations of zidovudine plus didanosine or zalcitabine with zidovudine alone in HIV-infected individuals. *Lancet* 348: 283-291.

Alemayehu, YK, Bushen, O & Muluneh, AT. 2009. Evaluation of HIV/AIDS clinical care quality: the case of a referral hospital in North West Ethiopia. *International Journal for Quality in Health Care* 21(5):356–362.

Alemu, AW & San Sebastián, M. 2010. Determinants of survival in adult HIV patients on antiretroviral therapy in Oromiyaa, Ethiopia. *Global Health Action* 3: 5398.
From: www.globalhealthaction.net/index.php/gha/rt/printerFriendly/5398/6222
(accessed on January 2013).

Alene, KW, Nega, A & Taye, BW. 2013. Incidence and predictors of tuberculosis among adult people living with human immunodeficiency virus at the University of Gondar referral hospital, Northwest Ethiopia. *Biomedical Central Infectious Diseases* 13:292.

Ali, K & Klotz, SA. 2012. The immune reconstitution inflammatory syndrome with tuberculosis: a common problem in Ethiopian HIV-infected patients beginning antiretroviral therapy. *Journal of International Association of Physicians AIDS Care* 11(3):198-202.

Althoff, KN, Justice, AC, Gange, SJ, Deeks, SG, Saag, MS, Silverberg, MJ, Gill, MJ, Lau, B, Napravnik, S, Tedaldi, E, Klein, MB & Gebo, KA. 2010. Virologic and immunologic response to HAART, by age and regimen class. *AIDS* 24:2469–2479.

Anude, CJ, Eze, E, Onyegbutulem, HC, Charurat, M, Etiebet, M, Ajayi, S, Dakum, P, Akinwande, O, Beyrer, C, Abimiku, A & Blattner, W. 2013.Immuno-virologic outcomes

and immunovirologic discordance among adults alive and on anti-retroviral therapy at 12 months in Nigeria. *Biomedical Central Infectious Diseases* 13:113.

Assefa, A, Gelaw, B, Getnet, G & Yitayew, G. 2014. The effect of incident tuberculosis on immunological response of HIV patients on highly active anti-retroviral therapy at the University of Gondar hospital, northwest Ethiopia: a retrospective follow-up study. *Biomedical Central Infectious Diseases*14:468.

Assefa, Y, Kiflie, A, Tesfaye, D, Haile-Mariam, D, Kloos, H, Edwin, W, Laga, M & Van Damme, W. 2011. Outcomes of antiretroviral treatment program in Ethiopia: retention of patients in care is a major challenge and varies across health facilities. *Biomedical Central Health Services Research* 11:81. doi: 10.1186/1472-6963-11-81. From: http://www.biomedcentral.com/content/pdf/1472-6963-11-81.pdf (accessed on 25 February 2013).

Azmeraw, D & Wasie, B. 2012. Factors associated with adherence to highly active antiretroviral therapy among children in two referral hospitals, northwest Ethiopia. *Ethiopian Medical Journal* 50(2):115-124.

Azzoni, L, Foulkes, AS, Liu, Y, Li, X, Johnson, M, Smith, C, Kamarulzaman, A, Montaner, J, Mounzer, K, Saag, M, Cahn, P, Cesar, C, Krolewiecki, A, Sanne, I & Montaner, LJ. 2012. Prioritizing CD4 count monitoring in response to ART in resource-constrained settings: a retrospective application of prediction-based classification. *Public Library of Science Medicine* 9(4): e1001207. From: http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1001207 (accessed 26 February 2013).

Bacha, T, Tilahun, B & Worku, A. 2012. Predictors of treatment failure and time to detection and switching in HIV-infected Ethiopian children receiving first line anti-retroviral therapy. *Biomedical Central Infectious Diseases* 12:197. From: http://www.biomedcentral.com/1471-2334/12/197 (accessed 25 February 2013).

Bärnighausen, T, Chaiyachati, K, Chimbindi, N, Peoples, A, Haberer, J & Newell, ML. 2011. Interventions to increase antiretroviral adherence in sub-Saharan Africa: a systematic review of evaluation studies. *Lancet Infectious Diseases* 11: 942-951.

Barré-Sinoussi, F, Chermann, JC, Rey, F, Nugeyre, MT, Chamaret, S, Gruest, J, Dauguet, C, Axler-Blin, C, Vézinet-Brun,F, Rouzioux, C, Rozenbaum, W & Montagnier, L. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science*220(4599):868-71.

Bélec, L & Bonn, JP. 2011. Challenges in implementing HIV laboratory monitoring in resource-constrained settings: how to do more with less. *Future Microbiology* 6(11):1251-1260.

Berhan, Y. 2011a. Change in absolute CD4 count with antiretroviral treatment in resource limited setting: a retrospective cohort study in children in Ethiopia. *Ethiopian Medical Journal* 49(4):289-298.

Berhan, Y. 2011b. Evaluation of adherence to national guideline for clinical follow-up of HIV infected children using the proxy hematological, biochemical and anthropometric indicators of care. *Ethiopian Medical Journal* 49(3):199-209.

Beyene, KA, Gedif, T, Gebre-Mariam, T & Engidawork, E. 2009.Highly active antiretroviral therapy adherence and its determinants in selected hospitals from South and Central Ethiopia. *Pharmacoepidemiology and Drug Safety* 18(11):1007-1115.

Boruett, P, Kagai, D, Njogo, S, Nguhiu, P, Awuor, C, Gitau, L, Chalker, L, Ross-Degnan, D, Wahlström, R & Tomson, G. 2013. Facility-level intervention to improve attendance and adherence among patients on anti-retroviral treatment in Kenya: a quasi-experimental study using time series analysis. *Biomedical Central Health Services Research* 13:242.

Bruce, N, Pope, D & Stanistreet, D. 2008.Quantitative methods for health research: a practical interactive guide to epidemiology and statistics. Chichester: John Wiley & Sons.

Burns, N & Grove, SK. 2009.*The practice of nursing research: appraisal, synthesis, and generation of evidence.* 6th edition.St Louis: Elsevier/Saunders.

Chalker, JC, Andualem, T, Gitau, LN, Ntaganira, J, Obua, C, Tadeg, H, Waako, P, Ross-Degnan, D &International Network for the Rational Use of Drugs Initiative on Adherence to Antiretrovirals. 2010. Measuring adherence to antiretroviral treatment in resource-poor settings: the feasibility of collecting routine data for key indicators. *Biomedical Central Health Services Research* 10:43. From: http://www.biomedcentral.com/1472-6963/10/43 (accessed February 1 2013).

Chernick, MR. 2011. *The essentials of biostatistics for physicians, nurses, and clinicians.* New Jersey: John Wiley & Sons.

Chun, T & Fauci, AS. 2012. HIV reservoirs: pathogenesis and obstacles to viral eradication and cure. *AIDS* 26:1261-1268.

Clavel, F, Mansinho, K, Chamaret, S, Guetard, D, Favier, V, Nina, J, Santos-Ferreira, M, Champalimaud, J & Montagnier, L. 1987. Human Immunodeficiency Virus Type 2 Infection Associated with AIDS in West Africa. *New England Journal of Medicine* 316:1180-1185.

Cohen, CJ, Meyers, JL & Davis, KL. 2013. Association between daily antiretroviral pill burden and treatment adherence, hospitalisation risk, and other healthcare utilisation and costs in a US Medicaid population with HIV. *British Medical Journal Open* 3:e003028. From: http://bmjopen.bmj.com/content/3/8/e003028.long (accessed August 15 2013).

Collett, D. 2003. *Modelling survival data in medical research.* 2nd edition. London: Chapman & Hall.

Crawford, KW, Wakabi, S, Magala, F, Kibuuka, H, Liu, M& Hamm, TE. 2014. Evaluation of treatment outcomes for patients on first-line regimens in US President's Emergency Plan for AIDS Relief clinics in Uganda: predictors of virological and immunological response from RV288 analyses. *HIV Medicine* doi: 10.1111/hiv.12177.

Dagnew, YW. 2009. Factors influencing anti-retroviral therapy adherence in Ethiopia. Unpublished Master of Public Health dissertation. Pretoria: University of South Africa

Daniel, WW. 2009. *Biostatistics: a foundation for analysis in the health sciences.*9th edition. New York: John Wiley & Sons.

Department of Health and Human Services [USA]. 2013. *Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents.* New York: Department of Health and Human Services.

Egger, M, May, M, Chêne, G, Phillips, AN, Ledergerber, B, Dabis, F, Costagliola, D, D'Arminio Monforte, A, De Wolf, F, Reiss, P, Lundgren, JD, Justice, AC, Staszewski, S, Leport, C, Hogg, RS, Sabin, CA, Gill, MJ, Salzberger, B & Sterne, JA. 2002. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 360(9327):119-129.

EHNRI & FMOH. See Ethiopian Health and Nutrition Research Institute & Federal Ministry of Health.

El-Khatib, Z, Katzenstein, D, Marrone, G, Laher, F & Mohapi, L. 2011. Adherence to drug-refill is a useful early warning indicator of virologic and immunologic failure among HIV patients on first-line ART in South Africa. *Public Library of Science* 6(3):e17518.

Ethiopian Health and Nutrition Research Institute & Federal Ministry of Health. 2012. *HIV Related Estimates and Projections for Ethiopia – 2012.* Ethiopia.

Eze, EU & Uyagu, O. 2009. Prevalence of anti-retroviral treatment failure and salvage therapy in University of Benin teaching hospital Edo state, Nigeria. *Annals of Biomedical Science* 8(2):40-46.

Fawibe, AE, Olafimihan, KO, Salami, AK, Desalu, OO & Odeigah, LO. 2010. A retrospective evaluation of pretreatment and follow-up monitoring of HIV-infected adult patients in a resource-poor setting. *Journal of International Association of Physicians AIDS Care* 9(5):289-291.

Federal HIV/AIDS Prevention and Control Office & Federal Ministry of Health. 2007. *Guidelines for management of opportunistic infections and antiretroviral treatment in adolescents and adults in Ethiopia.* Addis Ababa, Ethiopia.

Federal HIV/AIDS Prevention and Control Office & Federal Ministry of Health. 2008a. *Participant manual for the basic chronic HIV care, antiretroviral therapy and prevention*. Addis Ababa, Ethiopia.

Federal HIV/AIDS Prevention and Control Office & Federal Ministry of Health. 2008b.*Guidelines for Paediatric HIV/AIDS Care and Treatment in Ethiopia.* Addis Ababa, Ethiopia.

Federal HIV/AIDS Prevention and Control Office & Federal Ministry of Health. 2012. *Country Progress Report on HIV/AIDS Response.* Ethiopia.

Federal Ministry of Health. 2010a. *Health management information system (HMIS) participants' handout & instruments.* Ethiopia.

Federal Ministry of Health. 2010b. *National training package provider-initiated HIV testing and counselling participant's manual*. Ethiopia.

Federal Ministry of Health. 2013. *Technical updates on adopted recommendations on adolescent and adult anti-retroviral treatment.* Ethiopia.

Fischl, MA, Richman, DD, Grieco, MH, Gottlieb, MS, Volberding, PA, Laskin, OL, Leedom, JM, Groopman, JE, Mildvan, D, Schooley, RT, Jackson, GG, Durack, DT, King, D and the AZT collaborative working group. 1987. The efficacy of azidothymidine (AZT) in the treatment of participants with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. *New England Journal of Medicine* 317:185-191.

FHAPCO. See Federal HIV/AIDS Prevention and Control Office.

FHAPCO & FMOH. See Federal HIV/AIDS Prevention and Control Office & Federal Ministry of Health.

FMOH. See Federal Ministry of Health.

Gianotti, N, Galli, L, Bocchiola, B, Cahua, T, Panzini, P, Zandonà, D, Salpietro, S, Maillard, M, Danise, A, Pazzi, A, Lazzarin, A & Castagna, A. 2012. Number of daily pills, dosing schedule, self-reported adherence and health status in 2010: a large cross-sectional study of HIV-infected patients on antiretroviral therapy. *HIV Medicine* 14(3):153-160.

Giday, A & Shiferaw, W. 2010. Factors affecting adherence of antiretroviral treatment among AIDS patients in an Ethiopian tertiary university teaching hospital. *Ethiopian Medical Journal* 48(3):187-94.

Glynn, M & Drake, WM. 2012.*Hutchison's clinical methods: an integrated approach to clinical practice*. 23rd edition. China: Saunders Elsevier.

Grossman, Z, Meier-Schellersheim, M, Paul, WE & Picker, LJ. 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nature Medicine* 12:289– 295.

Heeney, JL, Dalgleish, AG & Weiss, RA. 2006. Origins of HIV and the evolution of resistance to AIDS. *Science*313: 462–66.

Hill, AB. 1965. The environment and disease: association or causation? *Proceedings of the Royal Society of Medicine* 58:295-300.

Hosmer, DW, Lemeshow, S and May,S. 2008. *Applied survival analysis regression modeling of time-to-event data*. 2nd edition. New Jersey: John Wiley & Sons, Inc.

Jaka, HM, Mshana, SE, Liwa, AC, Peck, R & Kalluvya, S. 2009. Prevalence of immunological failure and durability of first line antiretroviral therapy at Bugando Hospital Mwanza, Tanzania. *Tanzania Medical Journal* 24(2): 5-8.

Jacobsen, KH. 2011. *Introduction to health research methods: a practical guide.* Subury: Jones &Bartlett.

Jerene, D, Næss, A & Lindtjørn, B. 2006.Antiretroviral therapy at a district hospital in Ethiopia prevents death and tuberculosis in a cohort of HIV patients. *AIDS Research and Therapy* 3(10):10.

Joint United Nations Programme on HIV/AIDS. 2012. *Together we will end AIDS.* From:
http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/JC2296_UNAIDS_TogetherReport_2012_en.pdf (accessed 18 August 2012).

Joint United Nations Programme on HIV/AIDS. 2013a. *Aidsinfo epidemiological status.* From: http://www.unaids.org/en/dataanalysis/datatools/aidsinfo (accessed 13 November 2013).

Joint United Nations Programme on HIV/AIDS. 2013b. *Global report: UNAIDS report on the global AIDS epidemic 2013.* From:
http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf (accessed 3 October 2013).

Joint United Nations Programme on HIV/AIDS. 2014. *The gap report.* From: http://www.unaids.org/en/resources/campaigns/2014/2014gapreport/gapreport/ (accessed 20September 2014).

Kanters, S, Nachega, J, Funk, A, Mukasa, B, Montaner, JS, Ford, N, Bucher, HC & Mills, EJ. 2013. CD4 T-Cell recovery after initiation of antiretroviral therapy in a resource-limited setting: a prospective cohort analysis. *Antiviral Therapy* doi: 10.3851/IMP2670. From: http://www.unboundmedicine.com/medline/citation/23963170/CD4_T_cell_recovery_after_initiation_of_antiretroviral_therapy_in_a_resource_limited_setting:_a_prospective_cohort_analysis_ (accessed August 31 2013).

Kassa, D, Gebremichael, G, Alemayehu, Y, Wolday, D, Messele, T &Van Baarle, D. 2013. Virologic and immunologic outcome of HAART in Human Immunodeficiency Virus (HIV)-1 infected patients with and without tuberculosis (TB) and latent TB infection

(LTBI) in Addis Ababa, Ethiopia. *AIDS Research and Therapy* 10:18. From: http://www.aidsrestherapy.com/content/10/1/18 (accessed July 24 2013).

Katlama, C, Deeks, SG, Autran, B, Martinez-Picado, J, Van Lunzen, J, Rouzioux, C, Miller, M, Vella, S, Schmitz, JE, Ahlers, J, Richman, DD & Sekaly, RP. 2013. Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs. *Lancet* 381: 2109-2117.

Kebede, A & Wabe, NT. 2012. Medication adherence and its determinants among patients on concomitant tuberculosis and antiretroviral therapy in South West Ethiopia. *North American Journal of Medical Sciences* 4:67-71.

Keiser, O, MacPhail, P, Boulle, A, Wood, R, Schechter, M, Dabis, F, Sprinz, E & Egger, M. 2009.Accuracy of WHO CD4 cell count criteria for virological failure of antiretroviral therapy. *Tropical Medicine and International Health* 14(10): 1220–1225.

Keiser, O, Tweya, H, Braitstein, P, Dabis, F, MacPhail, P & Boulle, A. 2010. Mortality after failure of antiretroviral therapy in sub-Saharan Africa. *Tropical Medicine and International Health* 15(2): 251-258.

Kestenbaum, B. 2009.*Epidemiology and biostatistics: an introduction to clinical research,* edited by Adeney, KL, Weiss. New York: Springer.

Le, T, Wright, EJ, Smith, DM, He, W,Catano, G, Okulicz, JF, Young, JA, Clark, RA, Richman, DD, Little, SJ & Ahuja, SK. 2013.Enhanced CD4+ T-Cell recovery with earlier HIV-1 antiretroviral therapy. *New England Journal of Medicine* 368(3): 218–230.

Lederman, MM, Calabrese, L, Funderburg, NT, Clagett, B, Medvik, K, Bonilla, H, Gripshover, B, Salata, RA, Taege, A, Lisgaris, M, McComsey, GA, Kirchner, E, Baum, J, Shive, C, Asaad, R, Kalayjian, RC, Sieg, SF & Rodriguez, B. 2011. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *The Journal of Infectious Diseases* 204(8):1217–26.

Levy, JA. 2009. HIV pathogenesis: 25 years of progress and persistent challenges. *AIDS* 23:147–160.

Lifson, AR, Krantz, EM, Eberly, LE, Dolan, MJ, Marconi, VC, Weintrob, AC, Crum-Cianflone, NC, Ganesan, A, Grambsch, PL & Agan, BK. 2011. Long-term CD4+ lymphocyte response following HAART initiation in a U.S. Military prospective cohort. *AIDS Research and Therapy* 8:2. From: http://www.aidsrestherapy.com/content/8/1/2/comments (accessed July 24 2013).

LoBiondo-Wood, G & Haber, J. 2010.*Nursing research: methods and critical appraisal for evidence-based practice.* 7th edition. Louisiana: Mosby.

Longo, DL, Fauci, AS, Kasper, DL, Hauser, SL, Jameson, JL & Loscalzo, J. 2012.*Harrison's principles of internal medicine.*18th edition. New York: McGraw-Hill.

Macmillan Dictionary Online. 2013. Sv "adherence" "HIV" "patient" "predict" "treatment". New York: Macmillan Publisher.
From: http://www.macmillandictionary.com/dictionary/british/ (accessed 1 August 2013).

Machin, D, Cheung, MB & Parmar, MKB. 2006. *Survival analysis: a practical approach.* 2nd edition. West Sussex: John Wiley & Sons.

Maskew, M, Fox, MP, Van Cutsem, G, Chu, K, MacPhail, P, Boulle, A & Egger, M. 2013. Treatment Response and Mortality among Patients Starting Antiretroviral Therapy with and without Kaposi Sarcoma: A Cohort Study. *Public Library of Science* 8(6): e64392. From: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0064392 (accessed June 19 2013).

Mghamba, FW, Minzi, OMS, Massawe, A & Sasi, P. 2013. Adherence to antiretroviral therapy among HIV infected children measured by caretaker report, medication return, and drug level in Dar Es Salaam, Tanzania. *Biomedical Central Paediatrics* 13(1):95. From: http://www.biomedcentral.com/1471-2431/13/95 (accessed July 1 2013).

Mulissa, Z, Jerene, D & Lindtjørn, B. 2010. Patients present earlier and survival has improved, but Pre-ART attrition is high in a six-year HIV cohort data from Ethiopia. *Public Library of Science ONE* 5(10): e13268. doi:10.1371/journal.pone.0013268. From:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952597/pdf/pone.0013268.pdf
(accessed 4 March 2013).

Mulu, A, Liebert, UG&Maier, M. 2014. Virological efficacy and immunological recovery among Ethiopian HIV-1 infected adults and children. *Biomedical Central Infectious Diseases* 14:28.

Murray, JS, Elashoff, MR, Iacono-Connors, LC, Cvetkovich, TA & Struble, KA. 1999. The use of plasma HIV RNA as a study endpoint in efficacy trials of antiretroviral drugs. *AIDS* 13:797–804.

Nakanjako, D, Ssewanyana, I, Nabatanzi, R, Kiragga, A, Kamya, MR, Cao, H & Mayanja-Kizza, H. 2013. Impaired T-cell proliferation among HAART treated adults with suboptimal CD4 recovery in an African cohort. *Biomedical Central Immunology* 14:26. From: http://www.biomedcentral.com/1471-2172/14/26(accessed 31 August 2013).

Ortegoa, C, Huedo-Medinab, TB, Vejoa, J & Llorcac, FJ. 2011. Adherence to highly active antiretroviral therapy in Spain. A meta-analysis. *Gac Sanit* 25(4):282–289.

Oxford Dictionary Online. 2013. Sv "HAART" "failure" "prevalence". Oxford: Oxford University Press.
From: http://www.oxforddictionaries.com) (accessed 1 August 2013)

Palchaudhuri, S, Tweya, H & Hosseinipour, M. 2014. Assessment of laboratory test utilization for HIV/AIDS care in urban ART clinics of Lilongwe, Malawi. *Malawi Medical Journal* 26 (2) 42-44.

Phillips, JC, Webel, A, Rose, CW, Corless, IB, Sullivan, CM, Voss, J, Wantland, D, Nokes, K, Brion, J, Chen, W, Lipinge, S, Eller, LS, Tyer-Viola, L, Rivero-Méndez, M, Nicholas, PK, Johnson, MO, Maryland, M, Kemppainen, J, Portillo, CM, Chaiphibalsarisdi, P, Kirksey, KM, Sefcik, E, Reid, P, Cuca Y, Huang, E & Holzemer, WL. 2013. Associations between the legal context of HIV, perceived social capital, and HIV antiretroviral adherence in North America. *Biomedical Central Public Health* 13:736. From: http://www.biomedcentral.com/1471-2458/13/736 (accessed 5 August 2013).

Podlekareva, DN, Reekie, J, Mocroft, A, Losso, M, Rakhmanova, AG, Bakowska, E, Karpov, IA, Lazarus, JV, Gatell, J, Lundgren, JD & Kirk, O. 2012. Benchmarking HIV health care: from individual patient care to health care evaluation. An example from the EuroSIDA study. *Biomedical Central Infectious Diseases* 12:229. From: www.biomedcentral.com/1471-2334/12/229 (accessed 27 August 2013).

Polit, DF & Beck, CT. 2012.*Nursing research: generating and assessing evidence for nursing practice.* 9th edition. Philadelphia: Lippincott Williams and Wilkins.

Pujades-Rodriguez, M, O'Brien, D, Humblet, P & Calmy, A. 2008. Second-line antiretroviral therapy in resource-limited settings: the experience of Medecins Sans Frontieres. *AIDS* 22(11):1305-1312.

Rawizza, HE, Chaplin, B, Meloni, ST, Eisen, G, Rao, T, Sankale´, JL, Dieng-Sarr, A, Agbaji, O, Onwujekwe, DI, Gashau, W, Nkado, R, Ekong, E, Okonkwo, P, Murphy, RL & Kanki, PJ. 2011. Immunologic criteria are poor predictors of virologic outcome: implications for HIV treatment monitoring in resource-limited settings.*HIV/AIDS*. 53: 1283-1290.

Reda, AA, Biadgilign, S, Deribew, A, Gebre, B & Deribe, K. 2013. Predictors of Change in CD4 Lymphocyte Count and Weight among HIV Infected Patients on Anti-Retroviral Treatment in Ethiopia: A Retrospective Longitudinal Study. *Public Library of Science ONE* 8(4): e58595.
From: www.plosone.org/article/info:doi/10.1371/journal.pone.0058595 (accessed 20 May 2013).

Reynolds, SJ, Nakigozi, G, Newell, K, Ndyanabo, A, Galiwongo, R, Boaz, I, Quinn, TC, Gray, R, Wawer, M&Serwadda, D. 2009.Failure of immunologic criteria to appropriately identify antiretroviral treatment failure in Uganda. *AIDS* 23(6): 697–700.

Roux, P, Kouanfack, C, Cohen, J, Marcellin, F, Boyer, S, Delaporte, E, Carrieri, P, Laurent, C & Spire, B. 2011. Adherence to antiretroviral treatment in HIV-positive patients in the Cameroon context: promoting the use of medication reminder methods. *Journal of Acquired Immune Deficiency Syndrome* 57 Supplement 1:S40-43.

Sabin, CA & Lundgren, JD. 2013. The natural history of HIV infection. Current Opinion in HIV and AIDS 8(4):311-317.

Seligmann, M, Warrell, DA, Aboulker, JP, Carbon, C, Darbyshire, JH, Dormont, J, Eschwege, E, Girling, DJ, James, DR, Levy, JP, Peto, TEA, Schwarz, D, Stone, AB, Weller, IVD, Withnall, R, Gelmon, K, Lafon, E, Swart, AM, Aber, VR, Babiker, AG, Lhoro, S, Nunn, AJ & Vray, M. 1994. *Lancet* 343(8902):871-881.

Shaughnessy, JJ, Zechmeister, EB & Zechmeister, JS. 2012. *Research methods in psychology.*9th edition. New York: McGraw-Hill.

Sodora DL & Silvestri G. 2008.Immune activation and AIDS pathogenesis. *AIDS* 22: 436–446.

Tiyou, A, Belachew, T, Alemseged, F & Biadgilign, S. 2010. Predictors of adherence to antiretroviral therapy among people living with HIV/AIDS in resource-limited setting of Southwest Ethiopia. *AIDS Research and Therapy* 7:39. From: http://www.aidsrestherapy.com/content/pdf/1742-6405-7-39.pdf (accessed 21 February 2013).

Touloumi, G, Pantazis, N, Chaix, M-L, Bucher, HC, Zangerle, R, Kran, AB, Thiebaut, R, Masquelier, B, Kucherer, C, Monforte, AA & Meyer, L. 2013.Virologic and Immunologic Response to cART by HIV-1 Subtype in the CASCADE Collaboration. *Public Library of Science ONE* 8(7): e71174. From: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0071174 (accessed August 13 2013).

Tsegaye, E & Worku, A. 2012.Assessment of antiretroviral treatment outcome in public hospitals, South Nations Nationalities and Peoples Region, Ethiopia. *Ethiopian Journal of Health Development* 25(2):102-109.

Turner, BJ. 2002. Adherence to Antiretroviral Therapy by Human Immunodeficiency Virus—Infected Patients. *Journal of Infectious Diseases* 185 (Supplement 2): S143-S151. doi: 10.1086/340197.

From: http://jid.oxfordjournals.org/content/185/Supplement_2/S143.full.pdf (accessed February 21, 2013)

UNAIDS. See Joint United Nations Programme on HIV/AIDS.

United Nations. 2012. *The Millennium Development Goals Report 2012.* New York: United Nations publishers.

Webb, P, & Bain, C. 2011. *Essential epidemiology: an introduction for students and health professionals.* 2[nd]ed. New York, NY: Cambridge University Press.

Were, MC, Shen, C, Tierney, WM, Mamlin, JJ, Biondich, PG, Li, X, Kimaiyo, S & Mamlin, BW. 2011. Evaluation of computer-generated reminders to improve CD4 laboratory monitoring in sub-Saharan Africa: a prospective comparative study. *Journal of American Medical Informatics Association* 18:150-155.

Weiss, RA. 2008. 25 years of HIV research: successes and challenges. *Clinical and Experimental Immunology* 152(2): 201-210.

WHO. See World Health Organisation.

Workneh, N, Girma, T & Woldie, M. 2009.Immunologic and Clinical outcomes of Children on HAART: a retrospective cohort analysis at Jimma university specialized hospital. *Ethiopian Journal of Health Science* 19(2):75-82.

World Health Organization. 2003. *Adherence to long term therapy: evidence for action.* Geneva: World Health Organization.

World Health Organization. 2008. *Task shifting: global recommendations and guidelines.* Geneva: World Health Organization.

World Health Organization. 2009. *Towards universal access scaling up priority HIV/AIDS interventions in the health sector: progress report.* Geneva: World Health Organization.

World Health Organization. 2010a. *Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach– 2010 revision.* Geneva: World Health Organization.

World Health Organization.2010b. *WHO recommendations on the diagnosis of HIV infection in infants and children.* Geneva: World Health Organization.

World Health Organization. 2012a. *Service delivery approaches to HIV testing and counselling (HTC): a strategic HTC policy framework.* Geneva: World Health Organization.

World Health Organization. 2012b. *WHO HIV drug resistance report 2012.* Geneva: World Health Organization.

World Health Organization. 2013. *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach.* Geneva: World Health Organization.

Yirdaw, KD, Jerene, D, Gashu, Z, Edginton, ME, Kumar, AMV, Letamo, Y, Feleke, B, Teklu, AM, Zewdu, S, Weiss, B & Ruff, A. 2014. Beneficial effect of isoniazid preventive therapy and antiretroviral therapy on the incidence of tuberculosis in people living with HIV in Ethiopia. *Public Library of Science ONE* 9(8): e104557.

Zachariah, R, Reid, SD, Chaillet, P, Massaquoi, M, Schouten, EJ & Harries, AD. 2011. Why do we need a point-of-care CD4 test for low-income countries? *Tropical Medicine and International Health* 16(1):37–41.

Zaidi, SH. 2014. *Ethics in medicine.* Switzerland: Springer.

Zuckerman, AJ, Banatvala, JE, Griffiths, P, Schoub, B, & Mortimer, P. 2009. *Principles and practice of clinical virology.*6th edition.London: John Wiley & Sons.

ANNEXURE

Annexure A: Document checklist

ID		Site Code		
A. Socio demographic data				
A.1. Age		Category	Tick one	Code 1
		<15		
		≥15		
A.2. Gender		Category	Tick one	Code 2
		Female		
		Male		
B. Clinical data				
B.1. Baseline WHO Stage		Category	Tick one	Code 3
		I or II		
		III or IV		
B.2. Baseline CD4 Count		Value		
B.3.1 Tuberculosis diagnosed?		Category	Tick one	Code 4
		No		
		Yes		
B.3.2 If 'Yes', date Tuberculosis diagnosed: Day: _____ Month: _____ Year:_____				
B.4.1 Adherence		Category	Tick one	Code 5
		Good		
		Poor		
B.4.2. If 'Poor', month and year ARV pick up was missed		Month: _____ Year:_____		
		Month: _____ Year:_____		
		Month: _____ Year:_____		
		Month: _____ Year:_____		

	Value	
B.5.1. Weight at last follow-up visit		
B.5.2. Weight six months prior last follow-up visit		

B.5.3. Weight loss in the last six months	Category	Tick one	Code 6
	Weight loss>10% in the last six months		
	Weight loss 5%-10% in the last six months		
	Weight loss <5% in the last six months		

B.6. Date ART started: Day: _____ Month: _____ Year:_____

B.7. Last follow-up status	Category	Tick one	Code 7
	Active		
	Lost		
	Drop		
	Stop		
	Dead		
	Transferred out		

B.8. Last visit date: Day: _____ Month: _____ Year:_____

C. Immunologic data

C.1. Immunologic monitoring

CD4 tests determined within one month of designated follow-up month? Tick on cells that apply

0	6	12	24	36	48	60	72	84	96	108

C.2. Immunologic response

Enter CD4 count value that was determined within two months of designated follow-up month

0	6	12	18	24	30	36	42	48	54	60

66	72	78	84	90	96	102	108

C.3. Immunologic treatment failure			
C.3.1. Immunologic treatment failure diagnosed? (If 'No', go to item C.3.6)	Category	Tick one	Code 8
	No		
	Yes		
C.3.2. Date first immunologic treatment failure diagnosed: Day: _____ Month: _____ Year:_____			
C.3.3. Immunologic treatment failure criteria used to make diagnosis	Category	Tick one	Code 9
	Follow-up CD4 Persistently <100		
	<50% of peak follow-up CD4 value		
	below baseline CD4 value		
C.3.4. Follow-up CD4 present after first immunologic treatment failure diagnosis? (If 'No', go to item C.3.6)	Category	Tick one	Code 10
	No		
	Yes		
C.3.5. Immunologic treatment failure at last CD4 evaluation present?	Category	Tick one	Code 11
	No		
	Yes		
C.3.6. Last treatment regimen containing lopinavir/ritonavir?	Category	Tick one	Code 12
	No		
	Yes		

Annexure B   University of South Africa ethics clearance letter

UNISA | university of south africa

UNIVERSITY OF SOUTH AFRICA
Health Studies Higher Degrees Committee
College of Human Sciences
ETHICAL CLEARANCE CERTIFICATE

HSHDC/218/2013

Date:	16 October 2013	Student No:	4836-405-3

Project Title: Prevalence and predictors of immunologic failure among HIV patients on HAART in Southern Ethiopia.

Researcher: Kesetebirhan Delele Yirdaw

Degree: Masters in Public Health   Code: DLMPH95

Supervisor: Prof SP Hattingh
Qualification: D Litt et Phil
Joint Supervisor: –

DECISION OF COMMITTEE

Approved   [√]   Conditionally Approved   [ ]

Prof L Roets
CHAIRPERSON: HEALTH STUDIES HIGHER DEGREES COMMITTEE

Prof MM Moleki
ACADEMIC CHAIRPERSON: DEPARTMENT OF HEALTH STUDIES

PLEASE QUOTE THE PROJECT NUMBER IN ALL ENQUIRES

Annexure C   Application to conduct study sent to Ethiopian National Research Ethics Review Committee

Date: February 27, 2014

From: Kesetebirhan Delele
       Student number: 48364053
       Mobile:+251910212658
       Hawassa
       E-mail: 48364053@mylife.unisa.ac.za

To: Science and Technology Health Research Ethics Unit, Addis Ababa

Subject: **REQUEST TO CONDUCT A STUDY**

Dear Sir/Madam:

I am undertaking my masters in public health (MPH) studies at university of South Africa (UNISA). As fulfilment for that I am required to complete a research project. Thus, I intend to do a study on **PREVALENCE AND PREDICTORS OF IMMUNOLOGIC FAILURE AMONG HIV PATIENTS ON HAART IN SOUTHERN ETHIOPIA.**

The proposed study requires the following variables: demographic, baseline WHO clinical stage, baseline CD4, date ART initiated, follow up CD4, follow up regimen, follow up weight, and date tuberculosis diagnosed among patients enrolled and started with antiretroviral therapy (ART) before 01/05/2005 (Ethiopian calendar). Data source is existing medical records. The study takes place in two public hospitals of Southern Nations Nationalities and People's Regional State: Butajira and Hosanna hospitals.

Therefore, I am requesting for permission to conduct the study. This study is quite significant not only for academic qualification but also for the estimation of immunologic monitoring, estimation of prevalence of immunologic treatment failure among patients on ART, and identification of factors related with it.

The issue of ethics has been seriously considered and covered in the attached proposal. All data will be handled confidentially and will be de-identified from patient identifiers before it is transferred outside the treating facility.

Let me assure you that if permission is granted and the study is completed, findings will be disseminated to federal and regional health bureau and supporting partners. Should you require further information you can contact me with the email or phone listed below.

Sincerely

Kesetebirhan Delele (MD)
Tel: +251910212658
Email: 48364053@mylife.unisa.ac.za
HAWASSA

Annexure:-
- Permission from UNISA Ethical Committee
- Proposal and checklist

Annexure D Ethics clearance letterfromthe Ethiopian National Research Ethics Review Committee

በኢትዮጵያ ፌዴራላዊ ዴሞክራሲያዊ ሪፐብሊክ
የሳይንስና ቴክኖሎጂ ሚኒስቴር

The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

ቁጥር *3.10/649/06*
Ref. No.

ቀን *22/08/06*
Date

To: UNISA
Addis Ababa

Re: Prevalence and Predictors of Immunologic Failure among HIV Patients on HAART in Southern Ethiopia

Dear sir/Mr./s/Dr.

The National Research Ethics Review committee (NRERC) has reviewed the aforementioned project protocol in an expedited manner. We are writing to advise you that NRERC has granted

Full Approval

To the above named project, for a period of **one year (May 1, 2014- April 30, 2015)**. All your most recently submitted documents have been approved for use in this study. The study should comply with the standard international and national scientific and ethical guidelines. Any change to the approved protocol or consent material must be reviewed and approved through the amendment process prior to its implementation. In addition, any adverse or unanticipated events should be reported within 24-48 hours to the NRERC. Please ensure that you submit progress report once in a four month and annual renewal application 30 days prior to the expiry date.

We, therefore, request your esteemed organization to ensure the commencement and conduct of the study accordingly and wish for the successful completion of the project.

With regards,

Yohannes Sitotaw

Secretary of NRERC

Cc _ SNNP Regional State

_ Dr Kesetebirhan Delele (PI)

ማንኛውም ቢያስፈልግዎ
You may Contact

ፖ.ሳ.ቁ. አዲስ አበባ ኢትዮጵያ ስልክ ፋክስ
P.O.Box 2490 Addis Ababa, Ethiopia Tel. 251-011-4-674353 Fax +251-011-4-66 02 41
E-mail most@ethionet.et Web site:-http://www.most.gov.et

Annexure E   Southern Nations Nationalities and Peoples' Regional State Health Bureau support letter

የደቡብ ብሄሮች ብሔረሰቦችና ሕዝቦች ክልላዊ
መንግስት ጤና ቢሮ
South Nations Nationalities and People's Regional
State Health Bureau

ቁጥር ___ ዎለ]-]|13941
Ref. No
ቀን ___ 11|9| 06
Date

To: NigistEleni Hospital
        Hosanna
To: Butajira Hospital
        Butajira

Subject: Cooperation for a research project

As mentioned above Dr Kesetebirhan Delele who is a student at University of South Africa is conducting a study titled 'Prevalence and Predictors of Immunologic failure among HIV patients on HAART in Southern Ethiopia'. The proposal of the study has been approved by the National Ethics Review Committee of the Ministry of Science and Technology. Therefore, this study could provide relevant input for the fulfillment of HIV related health goals in the region. SNNP regional health bureau kindly requests you to support the investigators.

Not a single mom should experience maternal mortality!

Cc/

- Health Research and Technology Transfer Process Owner
- Dr Kesetebirhan Delele

Annexure F   Nigist Eleni Mohamod Memorial (Hosanna) hospital support letter

Southern Nations Nationalities and peoples Regional state
Hadiya zone Nigst Eleni Mohammed Memorial General Hospital

To whom it may concern

NEMM Hospital has received a letter from SNNP regional health bureau stating that Dr Kesetebirhan Delele is conducting a study titled Prevalence and predictors of immunologic failure among patients on HAART in Southern Ethiopia' and that he has full approval from the National Ethics Review committee, So, I am writing this letter on behalf of NEMM Hospital in the capacity of medical director for all concerned facility staff to cooperate with Dr Kasetebirhan for the success of this study since the finding may provide crucial information to improve quality of clinical service delivery in our hospital as well as the region.

Not a single mom should experience maternal mortality

Cc

➤ To Chief clinical Officer.

Adane Desta Gonemo(M.D)

Clinical Service Officer

☎ 046 –555- 43-79   (046 - 555- 43- 78   ✉ P.O.BOX   22 fax 22 93

Annexure G   Butajira hospital support letter

በ/ደ/ብ/ብ/ህ/ክ/መ/በን-ራ-ጌ ሞን ጤና መምሪያ የቡታ-ጀራ ሆስፒታል
Southern Nation Nationality & peoples Region Government.
Gurage Zone Health Department Butajira Hospital

ቁጥር   Ref.No   በ/ህ/86/ጸ77ፍ
ቀን   Date   12 - 9 - 2ዐዐ6

To Whom It May Concerned

Dr Kesetebirhan Delele, a master student at UNISA is conducting a study titled 'Prevalence and predictors of immunologic failure among patients on HAART in Soutern Ethiopia'. He has produced documents includingethics approval certificate from UNISA. approval from the National Research Ethics Review Committee. and support leteer from SNNP regional health bureau. The study is pertinent to our Hospital as well clients we serve. For this reason. I am writing this letter on behalf of Butajira Hospital in the capacity of medical director for all concerned facility staff to assist Dr Kesetebirhan for the success of his project.

No woman should die while giving birth!

Dr Daniel
ም ሕክምና አገልግሎት ኃላፊ
Chief Clinical Service Officer

☎ 0461150098/0461150202   ፖ.ሣ. 46   Fax 0461151152   E-mail butagenehospital@gmail.com