FACTORS AFFECTING RESPONSE TO ANTIRETROVIRAL AGENTS AT ONE YEAR IN AN HIV COHORT AT ROMA HOSPITAL, LESOTHO

BY

ADEBANJO ADEFOLARIN BABAFEMI

Submitted in partial fulfilment of the requirements for the degree

MSc (Clinical Epidemiology)

at the School of Health Systems and Public Health

at the University of Pretoria

2012

Supervisor: Prof. Paul Rheeder

I, **Adefolarin Babafemi ADEBANJO**, hereby declare that the work which I hereby submit as partial fulfilment for the degree **MSc (Clinical Epidemiology),** on which this thesis is based, is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been submitted, or is being submitted, for another degree at this or any other university.

Signed: Date: 1st April 2012

ABSTRACT

Objective: The objective of this retrospective cohort study is to assess whether demographic and anthropometric parameters, laboratory tests, co-morbidity, co-infection, treatment regimen, IRIS and adherence predict response to HAART as measured by CD4 count, weight gain and functional status in a cohort of patients in Roma, the Kingdom of Lesotho.

Method: Data were collected from a computerised database of the Antiretroviral Centre of the hospital. A cohort of 300 subjects was identified from hospital records from January 2007. Each of these subjects was followed up over a period of 12 months with data obtained for at least two visits within the 12-month span. Data were obtained on weight and CD4 at baseline, three months and also at six and 12 months, and data for haemoglobin were obtained only at 12 months. Variables that may be potential confounders were identified and univariate and multivariate logistic regression analyses were carried out to establish differences independent of confounding factors for the combined endpoints, as well as for each endpoint separately.

Results: Three-hundred patient records were analysed. Approximately 70% of the patients had a CD4 increase of at least 150 cells over baseline values at the end of the review period and in 52.3% of the patients an increase in weight of 10% over baseline measurements was seen. Seventy-nine patients (26.3%) had a haemoglobin level of at least 14g/dL at 12 months, regardless of baseline values or gender. The inclusion of Zidovudine (AZT) in treatment regimens was found in 73% of the patients and in multivariate analysis AZT was associated with not having anaemia at the end of the review period. However there was a slight reduction in haemoglobin level in the first two to three months of therapy in comparison with both Stavudine (d4T) and Tenofovir (TDF) but not significant enough to result in clinical anaemia. Baseline CD4 values were similar for all treatments options but dissimilar in other outcome variables and continued to vary significantly throughout the review period.

The outcomes of multivariate analyses suggest that the male gender appears to have better response to HAART as seen in each of the multivariate models. The most important determinant of haemoglobin response was baseline haemoglobin values. In the haemoglobin-associated multivariate model, HAART is associated with an increase in haemoglobin over baseline values. A history of TB prior to HAART was a major factor in weight response and it is thought to be as a result of IRIS, which is the unmasking of latent infections as the immune system reconstitutes. CD4 values have no direct influence on weight however, but an increase in weight was observed in all therapy groups.

Conclusion: Clinical and immunological parameters can be used to monitor response to HAART and predict treatment outcomes. These parameters can be organised into monitoring tools that will be useful in resource-limited areas. This study suggests that AZT-containing regimens appear not to result in anaemia and that symptomatic anaemia might need additional investigation. Treatment with TDF appeared to have shown the best possible response pattern more but patients on TDF therapy will have to be included in the study to justify this observation.

KEY WORDS: HAART, RESPONSE, ZIDOVUDINE, TENOFOVIR, ANAEMIA, STAVUDINE, RESOURCE-LIMITED, HAEMOGLOBIN, WEIGHT, CD4

DEDICATION.

This work is dedicated to God Almighty for His Grace; my father, Professor Afolabi Adebanjo, my queen Adetoun and my princess Folabomi.

ACKNOWLEDGMENT.

The following people have been extraordinary in their support and encouragement for me not only on the academic front but on all fronts: My heartfelt appreciation goes to my wife, Toun for her love, support and prayers while I was working on this research and for all the times I travelled far and late in very bad weather. I also appreciate the unwavering love and support of my father, Professor Afolabi Adebanjo, and my mother-in-law, Mrs. Bisi Toriola. I would also like to thank my supervisor, Professor Paul Rheeder for the mentorship and the demand of nothing but the best from me. I also appreciate Bukola Olateju for her very many supports and for reminding me that tough times don't ever last. I would also like to say a big thank you to the very wonderful patients of St. Joseph's Hospital, Roma, Lesotho. It was an honor and a great privilege to have served them. Lastly, I would like to appreciate my princess and my sunshine, Bomi, who has made life more meaningful to me. Thank you.

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Abbreviations

ACTG	AIDS Clinical Trial Group
AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
ARV	Antiretroviral
AZT	Zidovudine
CEO	Chief Executive Officer
ddC	Zalcitabine
d4T	Stavudine
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid
GRID	Gay-Related Immune Deficiency
HAART	Highly Active Antiretroviral Treatment
HIV	Human Immune Deficiency Virus
HLTV	Human T-Lymphotrophic Virus
IRIS	Immune Reconstitution Inflammatory Syndrome
LAV	Lymphadenopathy-Associated Virus
MTCT	Mother-To-Child Transmission
NGO	Non-governmental Organisation
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OIP	Opportunistic Infection Prophylaxis
PCP	*Pneumocystis* Pneumonia
PHC	Primary Health Care
PJP	*Pneumocystis jiroveci* Pneumonia
PMTCT	Prevention of Mother-To-Child Transmission
RNA	Ribonucleic Acid
TDF	Tenofovir
UNAIDS	United Nations Programme on HIV/AIDS
VCT	Voluntary Counselling and Testing
WHO	World Health Organization

CHAPTER 1

BACKGROUND

The burden that the HIV/AIDS pandemic has put on the economies of countries, particularly on those of developing countries, has necessitated the development of various interventions of which antiretroviral agents are principal. Worst-hit countries of the world are still grappling with effective coverage of their various sub-regions and have developed several programmes to scale up the supply of antiretroviral agents, manage complications effectively, and train various staff to perform these functions. However, it is not just enough to have indiscriminate roll-out of these agents to eligible individuals and have efforts made at ensuring proper adherence and adequate follow-up. Similar efforts are needed to predict the possible outcomes of therapy prior to its commencement in eligible individuals, to determine the factors that drive response in some patients, while similar responses are not seen in others. These efforts will improve overall outcome of patients placed on antiretroviral agents.

This study evaluated an HIV cohort in a district hospital in Roma, a semi-urban region of Lesotho.

LITERATURE REVIEW

Human immunodeficiency virus (HIV) is a retrovirus that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail and which leads to greater susceptibility to life-threatening opportunistic infections. Previous names for the virus include human T-lymphotropic virus-III (HLTV-III), lymphadenopathy-associated virus (LAV), or AIDS-associated retrovirus (ARV).[1, 2]

Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The three major routes of transmission are unprotected sexual intercourse, contaminated needles, and transmission from an infected mother to her baby at birth, or through breast milk. Screening of blood

products for HIV in the developed world has largely eliminated transmission through blood transfusions or infected blood products in these countries.

HIV infection in humans is now pandemic. As of January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) estimated that AIDS has killed more than 25 million people since it was first recognised in 1981, making it one of the most destructive pandemics in recorded history. In 2005 alone, AIDS claimed an estimated 2.4 to 3.3 million lives, of which more than 570,000 were those of children. It is estimated that about 0.6% of the world's living population is infected with HIV.[3] A third of these deaths are occurring in sub-Saharan Africa, retarding economic growth and increasing poverty.[4] According to current estimates, HIV is set to infect 90 million people in Africa, resulting in a minimum estimate of 18 million orphans.[5] Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection, but routine access to antiretroviral medication is not available in all countries in Africa.[6]

HIV primarily infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through three main mechanisms: firstly, direct viral killing of infected cells; secondly, increased rates of apoptosis in infected cells; and thirdly, killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognise infected cells. When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. If untreated, eventually most HIV-infected individuals develop AIDS and die; however about one in ten remains healthy for many years, with no noticeable symptoms.[7] Treatment with antiretrovirals, where these are available, increases the life expectancy of people infected with HIV. It is hoped that current and future treatments may allow HIV-infected individuals to achieve a life expectancy approaching that of the general public.

EPIDEMIOLOGY

Globally, between 33.4 and 46 million people currently live with HIV.[3] In 2005, between 3.4 and 6.2 million people were newly infected and between 2.4 and 3.3 million people with AIDS died, an increased from 2004 and the highest number since 1981.

Sub-Saharan Africa remains by far the worst-affected region, with an estimated 21.6 to 27.4 million people currently living with HIV. Two million (1.5 to 3.0 million) of them are children younger than 15 years of age. More than 64% of all people living with HIV are in sub-Saharan Africa, as are more than three quarters of all women living with HIV. In 2005, there were 12.0 million (10.6 to 13.6 million) AIDS orphans living in sub-Saharan Africa 2005.[3] South & South East Asia are the second worst-affected with an estimated 15% of the orphans coming from these regions. Since discovery AIDS have accounted for the death of 500,000 children in these regions. Two-thirds of HIV/AIDS infections in Asia occur in India, with an estimated 5.7 million infections (estimated 3.4 to 9.4 million) (0.9% of population), surpassing South Africa's estimated 5.5 million (4.9 to 6.1 million) (11.9% of population) infections, making India the country with the highest number of HIV infections in the world.[8] In the 35 African nations with the highest prevalence, the average life expectancy is 48.3 years, which is 6.5 years less than it would be without the disease.[9]

The latest evaluation report of the World Bank Operations Evaluation Department assesses the development effectiveness of the World Bank's country-level HIV/AIDS defined as policy dialogue, analytic work, and lending with the explicit objective of reducing the scope or impact of the AIDS pandemic.[10] This is the first comprehensive evaluation of the World Bank's HIV/AIDS support to countries, from the beginning of the pandemic through mid-2004. Because the Bank aims to assist in implementation of national government programmes, their experience provides important insights on how national AIDS programmes can be made more effective.

The development of Highly Active Antiretroviral Agents (HAART) as effective therapy for HIV infection and AIDS has substantially reduced the death rate from this disease in those areas where these drugs are widely available. This has created the misperception that the disease has vanished. In fact, as the life expectancy of people

with AIDS has increased in countries where HAART is widely used, the number of people living with AIDS has increased substantially. In the United States, the number of people with AIDS increased from about 35,000 in 1988 to over 220,000 in 1996.[11]

In Africa, the number of MTCT and the prevalence of AIDS are beginning to reverse decades of steady progress in child survival. Countries such as Uganda are attempting to curb the MTCT pandemic by offering voluntary counselling and testing (VCT), prevention of mother-to-child transmission (PMTCT) and ante-natal care (ANC) services, which include the distribution of antiretroviral therapy.

TREATMENT

There is currently no vaccine or cure for HIV or AIDS. The only known method of prevention is avoiding exposure to the virus. However, an antiretroviral treatment, known as “post-exposure prophylaxis” is believed to reduce the risk of infection if begun directly after exposure.[12] Current treatment for HIV infection consists of HAART.[13] This has been highly beneficial to many HIV-infected individuals since its introduction in 1996, when the protease inhibitor-based HAART initially became available.[14] Current HAART options are combinations (or "cocktails") consisting of at least three drugs belonging to at least two types, or "classes," of antiretroviral agents. Typically, these classes are two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI). Because AIDS progression in children is more rapid and less predictable than in adults, particularly in young infants, more aggressive treatment is recommended for children than adults.[15] In developed countries where HAART is available, doctors assess their patients thoroughly – measuring the viral load, how fast CD4 declines, and patient readiness. Doctors then decide when to recommend starting treatment.[16]

The optimal time to initiate antiretroviral therapy in adults who are infected with human immunodeficiency virus HIV) remains uncertain. There have been no randomized trials to determine the optimal time to start antiretroviral therapy in adults who have CD4+ T-cell counts that are greater than 200 and less than 350 per cubic millimeter. Furthermore, there are few data on the optimal time to start antiretroviral

therapy in persons who live in locations with limited resources, where high rates of tuberculosis, malnutrition, and co-infection with tropical diseases may alter the natural history of HIV disease and the optimal time to initiate therapy. Therefore, international guidelines differ on when to start antiretroviral therapy.[17-22] In Haiti, for instance, following World Health Organization (WHO) guidelines, the first-line regimen of antiretroviral therapy, which consists of Zidovudine, Lamivudine and Efavirenz, is initiated when the CD4+ T-cell count in a patient with HIV type 1 (HIV-1) infection is 200 per cubic millimeter or less or when clinical acquired immunodeficiency syndrome (AIDS) develops.[17,18] Among patients who are treated according to this standard strategy for the initiation of antiretroviral therapy, approximately 80% are alive at 5 years.[23,24]

MONITORING TREATMENT

HAART allows the stabilisation of the patient's symptoms and viraemia, but it neither cures the patient nor alleviates the symptoms. High levels of HIV-1, often HAART resistant, return once treatment is stopped.[25, 26] Moreover, it would take more than a lifetime for HIV infection to be cleared using HAART.[27] Despite this, many HIV-infected individuals have experienced remarkable improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed world.[14, 28, 29] A computer-based study in 2006 projected that following the 2004 United States treatment guidelines gave the average life expectancy of an HIV-infected individual as 32.1 years from the time of infection if treatment was started when the CD4 count was 350/µL.[30] This study was limited as it did not take into account possible future treatments and the projection has not been confirmed within a clinical cohort setting. In the absence of HAART, progression from HIV infection to AIDS has been observed to occur at a median of between nine to ten years and the median survival time after developing AIDS is only 9.2 months.[31] However, HAART sometimes achieves far less than optimal results, in some circumstances being effective in less than 50% of patients. This observation is due to a variety of reasons such as medication intolerance/side effects, prior ineffective antiretroviral therapy and infection with a drug-resistant strain of HIV. However, non-adherence and non-persistence with antiretroviral therapy are the major reasons for failure to benefit from HAART.[32] The reasons for non-adherence

and non-persistence with HAART are varied and overlapping. Major psychosocial issues, such as poor access to medical care, inadequate social support, psychiatric disease and drug abuse contribute to non-adherence. The complexity of HAART regimens – whether due to pill number, dosing frequency, meal restrictions or other issues along with side effects that create intentional non-adherence also contribute to this problem,[33, 34, 35] The side effects include lipodystrophy, dyslipidaemia, insulin resistance, an increase in cardiovascular risks and birth defects.[36, 37]

The timing for starting HIV treatment is still debated. There is no question that treatment should be started before the patient's CD4 count falls below 200, and most national guidelines recommend initiation of treatment once the CD4 count falls below 350; but there is some evidence from cohort studies that treatment should be started before the CD4 count falls below 350.[38,28] There is also evidence that treatment should be started before the CD4 percentage falls below 15%.[39] In those countries where CD4 counts are not available, patients with WHO stage III or IV disease[40] should be offered treatment.

Anti-retroviral drugs are expensive, and the majority of the world's infected individuals do not have access to medication and treatments for HIV and AIDS.[41] Research to improve current treatments includes decreasing the side effects of current drugs, further simplifying drug regimens to improve adherence, and determining the best sequence of regimens to manage drug resistance. Unfortunately, only a vaccine is thought to be able to halt the pandemic. This is because a vaccine would cost less, thus being affordable for developing countries, and would not require daily treatment.[41] However, after over 20 years of research, HIV-1 remains a difficult target for a vaccine.[41] In February 2007, The National Institute of Allergy and Infectious Diseases published a report that gave details of a potential region on HIV's surface that is a potential target for a vaccine.[42]

PREDICTING RESPONSE TO TREATMENT

The ability to use clinical or laboratory findings to predict antiretroviral success or failure is the most vital component of HIV care and management. Two developments have spurred on this field of research. The first is the "death" of the large clinical

endpoint trial. The second is the advances in developing surrogate markers of response.

The definition of antiretroviral response is usually based upon clinical endpoints (i.e., infections, death, and quality of life) or surrogate markers (i.e., predictors of the clinical endpoints). For years CD4+ lymphocyte counts have been used as a surrogate marker. More recently, HIV-1 RNA tissue and serum levels have been employed as surrogate markers. Currently, many new markers related to the host, the virus, or the drugs are being evaluated for correlation between clinical status and progress, and response to therapy.

Katzenstein, from Stanford University in the United States reviewed the predictive markers of clinical endpoints in a nested case-cohort study of AIDS Clinical Trials Group (ACTG)-175. This was a trial of zidovudine versus zidovudine plus ddI versus zidovudine plus zalcitabine (ddC) versus ddI in asymptomatic patients with CD4+ lymphocyte counts of between 200 to 500 cells/cu mm. Comparison of 245 patients who progressed (i.e., experienced either an AIDS-defining illness or death) versus 212 controls showed that both CD4+ lymphocyte count and HIV-1 RNA levels at week eight were predictive of outcome.[43]

A 74% reduction in risk of progression was observed in those who had a 1 log decline in serum HIV-1 RNA levels after eight weeks of therapy. The researchers were able to create a table that predicted outcomes. For example, 95% of those who had a CD4+ count > 300 cells/cu mm and a serum HIV-1 RNA level < 1,000 were AIDS free after 30 months. In contrast, no patient with a week eight serum HIV-1 RNA level > 10,000 and a CD4+ lymphocyte count < 200 cells/cu mm remained AIDS free. Katzenstein emphasised that HIV-1 RNA levels remained predictive at each year of measurement. His suggested application of this trend was that more aggressive therapy may be delayed in persons with a CD4+ lymphocyte count > 200 cells/cu mm and an HIV-1 RNA < 10,000 copies/mL.

This suggestion is in contrast to what many experts recommend: that the very first regimen should be designed to maximise suppression of HIV-1 replication in order to circumvent the development of mutations that may lessen the benefit of antiretroviral therapy in the future.[43]

MEASURING RESPONSE

Regular monitoring of CD4 count and viral load is critical in identifying poor adherence to therapy or treatment failure early. The CD4 count should be performed every three to six months. The viral load should be done six to eight weeks after commencing antiretroviral therapy and then every three to six months together with the CD4 count. The purpose of an early viral load test is to detect an adequate viral load response (more than 1 log reduction). These tests should not be done following vaccination or if an intercurrent infection is present, as this will increase the viral load and give a falsely low value of the CD4 count.[44]

With HAART, at least a ten-fold (1 log) drop in the viral load can be expected within eight weeks and the viral load should be undetectable after 16 to 24 weeks of therapy. The viral load is the most important test for monitoring response to therapy.[44]

The CD4 count rises rapidly within four weeks on starting HAART and then rises more gradually. The average rise in CD4 is about 75 in the first six months, 150 in the first year, and 80 per annum thereafter, but this rise is extremely variable. In some patients (about 10% to 20%) the CD4 fails to rise despite a suppressed viral load but changing their HAART regimens will not influence CD4 levels.[44]

Clinical monitoring is also important, including of general well-being and sustained weight gain. Changes to therapy should not be based only on laboratory results. It is important to note that an intercurrent clinical event should not be an indication for changing therapy if the viral load is suppressed. Furthermore, clinical deterioration and CD4 decline both occur after many months of virological failure. Thus the main criterion for changing initial HAART regimen is virological failure.

STUDY DESIGN INFLUENCES

Case definition of response

Regensberg and Whitelaw (2007) examined the trend and patterns in the viral load at 8, 16, and 24 weeks of therapy and their findings influence the defining of clinical virological and immunological case response with differing extents. According to the

authors, there appears to be no gold standard for the definition of response, and studies have reported widely varying degrees.[44] For this reason the current study attempted to combine certain immunological and clinical parameters to act as a benchmark for the determination of response, particularly in resource-poor settings.

MOTIVATION FOR AND AIM OF THE STUDY

Antiretroviral therapy is available free of charge in most parts of Lesotho and all government-owned as well as religious institutions of health provide HIV- allied services such as counselling and support, health education, laboratory investigations, and treatment of opportunistic infections free of charge. Despite all these measures, however, not all patients respond equally to antiretroviral agents and it is the thinking of most lay people and especially HIV-infected persons that once antiretroviral agents have been commenced the patient is on his way to full recovery.

Unfortunately, health workers in the field of HIV/AIDS who sometimes inappropriately initiate patients on treatment sometimes echo this sentiment. Despite good measures of community-based motivation and extensive education on the concept of antiretroviral agents, there still remains that erroneous impression that whatever time a patient is started on therapy and regardless of duration or extent of illness, the patient is bound to recover fully. The study attempted to show who will respond and who will not. In addition this type of study has never been done in this country.

CHAPTER 2

METHODS

Setting

This study was conducted in a district hospital in Roma, a semi-urban region of the Kingdom of Lesotho. This means that health facilities located in such areas bridge the gap between clients that have access to better health care and those who don't. It is the responsibility of such health facilities to serve clients from the very rural parts. Often these clients are without basic amenities such as food shelter clothing and access to health care. The subject comprised a cohort of 300 adults initiated on antiretroviral therapy from January 2007 with each of these subjects followed up at regular three monthly intervals over a period of one year. Subjects in this study are essentially cases detected in the out-patient department and those identified during routine community-based screening exercises. The rest are patients screened for HIV after being admitted for non-HIV-related conditions.

Bias and confounding

Selection bias is a systematic error that may occur in studies where subjects are selected by using a procedure or by factors that may influence the participation in a study.[45] This type of bias comes about when the association between exposure and disease differs between those who participate and those who do not participate in the study. However, the use of computer-generated case file numbers to select participants into the study and incorporating this system in the study design reduces to a large extent selection bias due to the inappropriate selection of cases. The misclassification of subjects can lead to information bias if the information collected from study subjects is erroneous.[45] When using a categorical scale, a person may be placed in the incorrect category or misclassified as a result of this error. Attempts to reduce bias were made throughout this study. Firstly, the selection of patient files for the study was done randomly via computer-generated numbers, which prevented the selection of patients with poorer being compared with patients who had better care. Secondly, one and the same person audited the patient records and entered data

into the collection sheets at baseline. Entry of data at the other point periods was done by different people. Observer bias was therefore limited.

All doctors attending to patients in the clinic were blinded to which patients were selected for the study.

Study design choice

In the absence of randomised controlled trials (RCTs) with clinical endpoints relevant to the individual patient, non-experimental studies have been applied to evaluate response and the pattern of differences of therapy with HAART. Prospective and retrospective cohort studies have been used, as well as case-control designs. Of these, the prospective cohort study has consistently been regarded as the strongest design.[45] Retrospective studies are usually more economical, especially when medical records have to be reviewed to retrieve valid information on large numbers of people. By using this study design, one can assess the effectiveness of HAART on endpoints such as changes in CD4 pattern weight and haemoglobin. There are advantages of retrospective studies over RCTs, including lower costs, enhanced timelines, larger power, and improved generalisability.[45]

Study design

A retrospective cohort study design was used. Data were collected from a cohort of patients with at least 12 months follow up and with at least two visits in the 12 months. The cohort began in January 2007 with patients that met all eligibility criteria for starting HAART. They were started on treatment and followed up for at least 12 months.

Aim:

To study the response and the factors predictive of response to antiretrovirals in a cohort over a 12 month period.

Objective:

To assess whether anthropometric parameters such as weight change, laboratory tests, co-infection, treatment regimen, iris which has occurred as a result of

unmasking of latent infections and adherence affect response to HAART as measured by CD4 count, weight gain and haemoglobin level.

Inclusion criteria

- HIV positive
- On antiretroviral therapy
- A CD4 count of 200 or less
- On treatment for at least 12 months and have at least two clinic visits during the study period

Exclusion criteria

- Patients with CD 4 count of > 200
- Patients not initiated on treatment within the study period
- Defaulters of treatment (did not have treatment for at least six months)
- Patients who develop TB during the review period. TB was assessed only at baseline

Subjects in the cohort qualified as cases if they were eligible and already started on HAART as at the time the study began and continued on treatment regardless of hospital admission or therapy for co-morbidities during the period from January 2007 to January 2008, as confirmed by hospital Primary Health Care (PHC) claims data. The subjects became responders when the respective outcomes of interest were demonstrable from data obtained from them. Patients were identified as defaulters of treatment if they died or did not have at least two follow-up visits during the course of this study and were not eligible to become cases. The ART centre database was used to identify patients who died or defaulted during this period.

The outcome measures

A responder was defined as one with an increase in CD4 cell count of 150 or more above baseline measurement, a 10% rise in body weight from baseline values and a haemoglobin of 14g/dl or more at 12 months irrespective of baseline levels.

Response was measured only at 12 months.

Ethics

Approval for this study was granted by the medical director of St. Joseph's Hospital, Roma Lesotho, The Ministry of Health and Social Welfare Lesotho, and the Academic Advisory Committee of the University of Pretoria.

MEASUREMENT OF COVARIATES

The following data were retrieved from the membership, authorisation for medicine and hospitalisation claims databases for each of the cases:

Demographic covariates

- Age (measured on 1 January 2007 in years)
- Gender (male or female)

Covariates indicating opportunistic-infection conditions

These covariate data were determined from chronic medication authorisations and database and are non-mutually exclusive (0 = condition not registered; 1 = condition registered):

1. Kaposis sarcoma
2. Cryptococcal meningitis
3. Atypical pneumonias
4. Herpes zoster infections
5. Other opportunistic infections-determined from the WHO clinical staging criteria

Covariates related to therapy

The covariates here refer to the treatment modality adopted for each subject. The outcomes of interest are a direct function of the member of the NRTI group of HAART included in the patient's medication.

Data management

All patient data were captured on a form designed on Microsoft Access and this program was subsequently used to produce a data spreadsheet on Microsoft Excel, where all data cleansing and editing were done. Thereafter, data was transferred to STATA version 10 statistical computer package via STAT-TRANSFER version 7, for analysis.

Sample size

300+ sufficient for regression analysis[46]

DATA ANALYSIS

Descriptive analysis with STATA 10 software was done.[47]

To determine which explanatory variables to use in the multivariable model univariate logistic regression was done and it was used to explore the relationship between sex, age, TB history, ART regimen and opportunistic infection history (all being explanatory variables) and CD4 count, weight estimation and changes in haemoglobin concentration (being the outcome variables) at one year. Sex, TB history and opportunistic infection history were modelled as dichotomous variables. The variable “age” was modelled first as a continuous variable and then as a categorical variable with three categories corresponding to the tertiles for the ages. All treatment groups were included but treatment group 5 was dropped automatically by the software for most of the analysis largely because of the small numbers in these groups. Variables with p-value < 0.25 were then put into a multivariable model when manual stepwise elimination was done based on p-values in the model. Variables were dropped if the likelihood ratio test was non-significant. Initial model fit was checked using Pearson’s goodness of fit test. This test was employed because it is a more reliable test in general especially if the number of covariates is not many. The test is based on the chi square distribution and if the number of covariate patterns is large the expected numbers in some of the cells are likely to be small, usually less than five, making the Hosmer-Lemeshow goodness of fit test the preferred option with respect to reliability. The confusion matrix or classification tables give the sensitivity and specificity of models as a ‘test’ of whether the outcome

is a success or a failure. It gives the predictive values and percentages of outcomes that are correctly predicted by the model. Whether age was linear in the logit was checked with grouping and the use of dummy variables. To determine the calibration of the final model, ROC analysis was done with calculation of the c-statistic. A *p*-value of < 0.05 was regarded as statistically significant.

CHAPTER 3

Results of the study

The results of this study will be reported in the following sequence.

- Patient selection
- Demographic characteristics
- Outcome measures in the following order-CD4 count, weight response and finally haemoglobin.

The reporting of the outcome measures will be done at baseline and then at 12 months

Patient enrollment

Patients randomly selected into the AZT arm	151
Patients that died during the review period	4*
Patients that did not have at least two follow-up visits	11**
Patients that developed TB during therapy	11**

*Excluded from study

**Excluded and replaced in study

Patient demographics

Table 3.1 shows patients' demographic characteristics at baseline.

Treatment: All patients were placed on treatment in accordance with the national HAART guidelines, which stipulate triple therapy. All patients had two NRTIs and one NNRTI. Representation by AZT 3TC and EFV was highest at 130 (43.3% of subjects in the study), followed by AZT 3TC and NVP with 88 subjects representing 29.3% of patients. Representation by d4T 3TC and EFV/NVP was equal at 36 subjects (12%) each. TDF 3TC and EFV had 10 subjects (3.33%).

Age: The age was normally distributed and, when stratified into groups, the 25-44 years group had the most representation, with 179 subjects (59.67) (p = 0.05).

Gender: Most patients in the study were females representing 68% of the study population (204).

Table 3.1: Patients' demographics at baseline

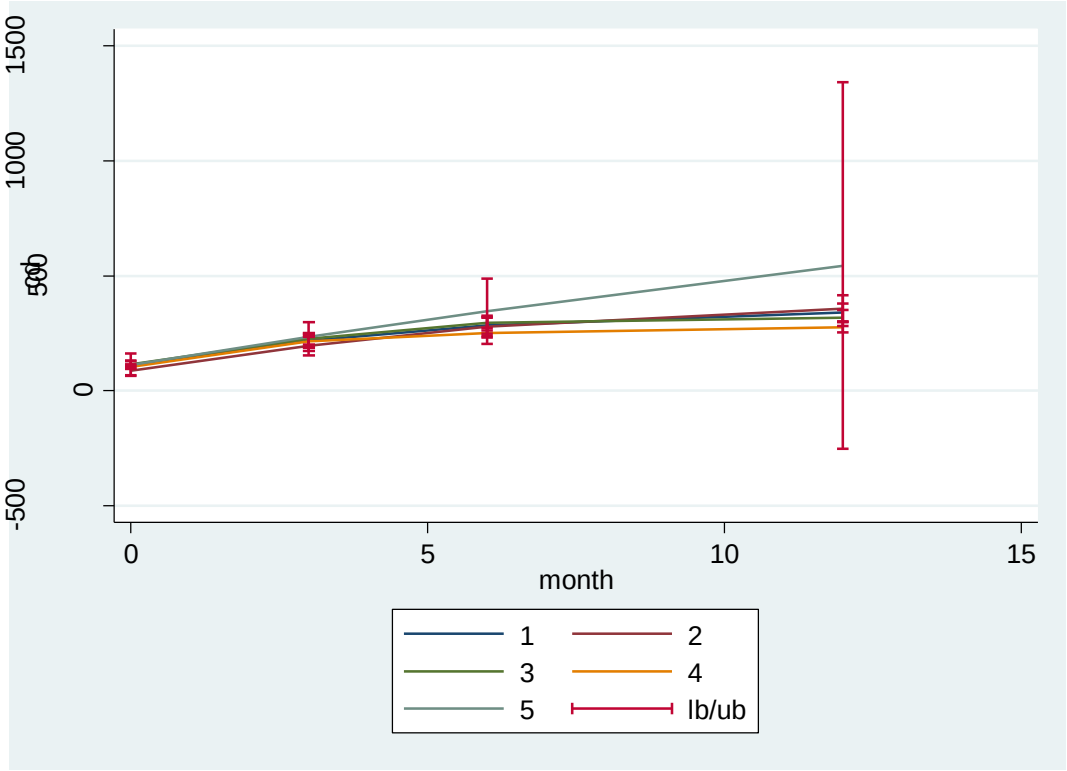
Variable	Baseline
CD4 Count *	111.5 (60,155.5)
Weight (kg) **	55.28 (11.99)
Haemoglobin **	11.78 (2.06)
Age category 1: 19-24yrs n=14 Age category 2**: 25-44yrs n=179 Age category 3**: 45+yrs n=107**	22.21 (1.19) 34.75 (5.33) 53.40 (6.42)
Regimen 1: d4T+3TC+NVP (n/%)	36/12
Regimen 2: d4T+3TC+EFV (n/%)	36/12
Regimen 3: AZT+3TC+NVP (n/%)	88/29.3
Regimen 4: AZT+3TC+EFV (n/%)	130/43.3
Regimen 5: TDF+3TC+EFV (n/%)	10/3.33
Females (n/%)	204/68
Opportunistic infection present (n/%)	48/16
TB history present	74/24.7

*Median (25^{th}, 75^{th} percentile), **Mean (*SD*)

Table 3.2: Distribution of responders and pattern of response

Responders	ART Regimen 1	ART Regimen 2	ART Regimen 3	ART Regimen 4	ART Regimen 5	Total % responders	*p-value*
CD4 count (% of total responders)	30	29	59	81	10	209	0.008
Weight % (% of total responders)	20	21	49	59	8	157	0.162
Haemoglobin% (% of total responders)	14	14	40	45	7	120	0.99

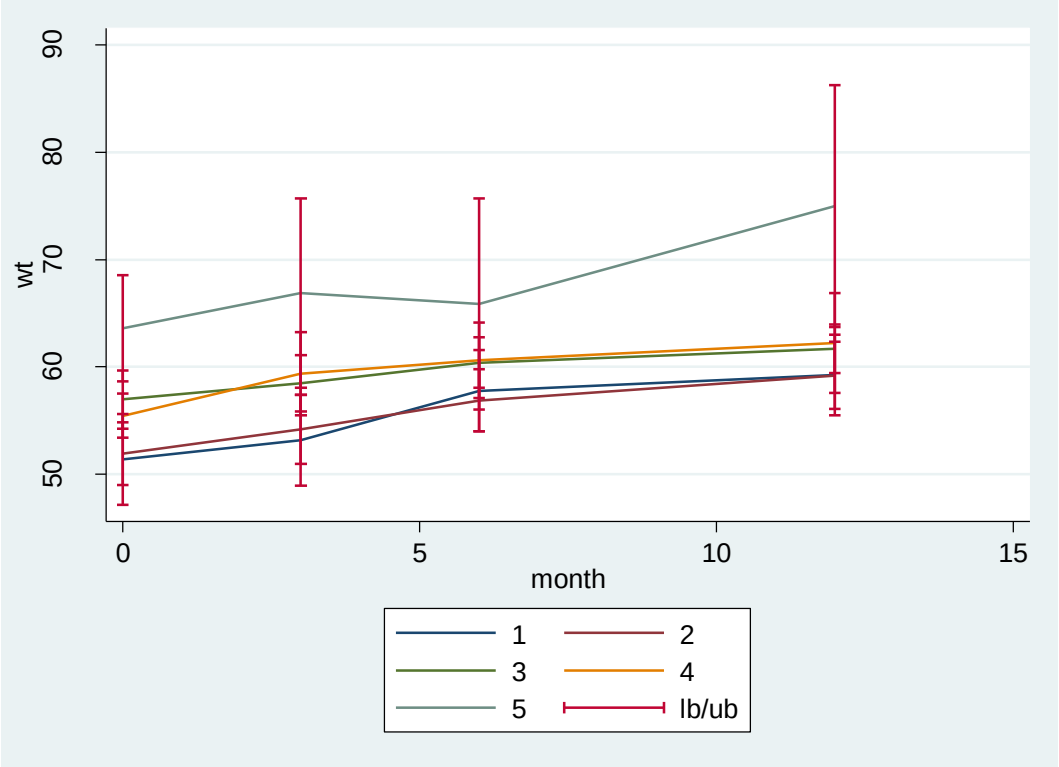
Variations of mean values of response of outcome variables over 12 months by ART regimens.



1=d4T+3TC+NVP; **2**=d4T+3TC+EFV; **3**=AZT+3TC+NVP; **4**=AZT+3TC+EFV; **5**=TDF+3TC+EFV

Figure 1: CD4 count (Medians and IQR) over 12 months by treatment group

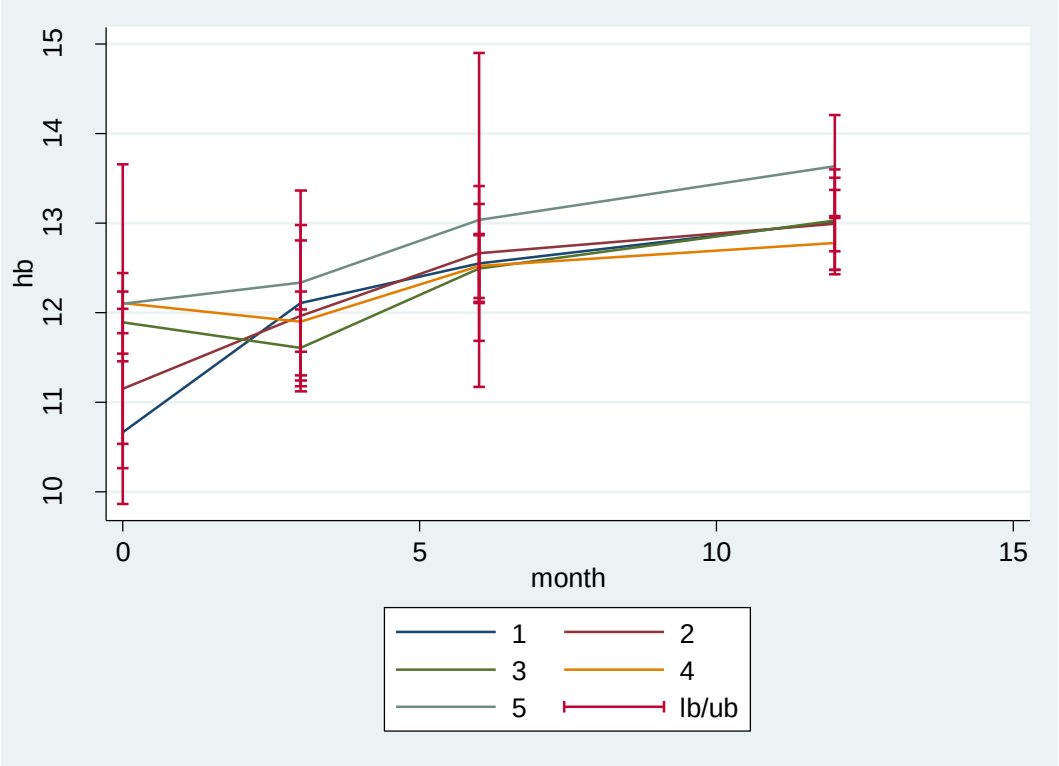
Variations of mean values of response of outcome variables over 12 months by ART regimens by weight



1=d4T+3TC+NVP; **2**=d4T+3TC+EFV; **3**=AZT+3TC+NVP; **4**=AZT+3TC+EFV; **5**=TDF+3TC+EFV

Figure 2: Weight (Medians and IQR) over 12 months by treatment group

Variations of mean values of response of outcome variables over 12 months by ART regimens by haemoglobin



1=d4T+3TC+NVP; **2**=d4T+3TC+EFV; **3**=AZT+3TC+NVP; **4**=AZT+3TC+EFV; **5**=TDF+3TC+EFV

Figure 3: Haemoglobin level (Medians and IQR) over 12 months by treatment group

UNIVARIATE ANALYSIS

The purpose of performing a univariate analysis in this study was to establish if at the end of review period there was any statistically significant difference between variables and to identify potential confounding factors that may influence the results.

Firstly, it was established which variables varied significantly across the review period in this study. Tables 3.5, 3.6 and 3.7 describe the univariate analyses in the study during the review period. Variables with a statistically significant difference (*p*-value ≤ 0.05) could have been potential confounders in a statistical model if they were also associated with the outcomes of interest.

OUTCOME MEASURE OF CD4 COUNT

Table 3.3: Univariate analysis (crude odds ratios) of factors associated with a CD4 response at 12 months

n (explanation)	Description	Odds Ratio	95% CI
		CD4 RESPONSE AT 12 MONTHS OF THERAPY	
300 (males=96)	Participants' gender (m)	0.35	0.21-0.59
300 (present=74)	History of TB at baseline	0.95	0.54-1.68
300 (present=48)	History of opportunistic infections at baseline	0.76	0.40-1.45
300	Baseline CD4	0.99	0.99-0.99
300 (3 centiles) 18-30 years 30-39 years 39-49 years 49-99 years	Participants' age in groups group 1 group 2 group 3 group 4	 Ref 0.99 0.59 0.37	 Ref 0.47-2.11 0.29-1.20 0.18-0.76
300 (5 groups) Regimen 1 Regimen 2 Regimen 3 Regimen 4	 d4T+3TC+NVP d4T+3TC+EFV AZT+3TC+NVP AZT+3TC+EFV	 Ref 0.83 0.41 0.33	 Ref 0.25-2.76 0.15-1.09 0.13-0.85

The variable CD4 was categorised for analysis based on documentation in literature. An estimated 12.5 cells increase per month totalling 150 cells' increase in the first year of HAART and 80 cells increase annually subsequently. This is widely variable and formed the basis of analysis and age, being categorical, was organised into centiles and ART regimen grouped based on the regimen patients were put on. ART regimen 5 which was initially part of the univariate analysis was subsequently dropped as it did not reflect the outcome of interest and therefore was not included in the final analysis.

The variable "age" was shown to be linear in the logit and the respective outputs were parsimonious.

Following stepwise backwards binary logistic regression, the following variables were retained in the explanatory model:

Table 3.4: Multivariate analysis (adjusted odds ratios) of factors associated with a CD4 response at 12 months (n = 300)

n (explanation)	Description	Odds Ratio	95% CI
(males=96)	Participant's gender (m)	0.37	0.21 – 0.63
300 (3 centiles) Age group 1 Age group 2 Age group 3 Age group 4	Participants' age in groups	Ref 1.03 0.68 0.43	Ref 0.47-2.22 0.33-1.46 0.20-0.91

This implies that if gender changes from male to female, the log-odds of CD4 responses at twelve months are expected to decrease by one. This means that the odds of CD4 response for males are 0.37 times that of females in any particular age group. The odds of CD4 response for age group 2 (0 – 12 years) is 1.03 times the odds of response for age group 1, (72 – 100 years). The 95% confidence interval includes 1 which suggests that there is no statistically significant difference between the odds of the two age groups. The odds of CD4 response for age group 4 (0 – 12 years) is 0.43 times the odds of response for age group 1, (72 – 100 years). The 95% confidence interval, (0.20-0.91) excludes 1 which suggests that there is a statistically significant difference between the odds of the two age groups. Age group one is more likely to get a CD4 response.

Post regression Pearson's Goodness of fit test was statistically non-significant (p = 0.70) indicating satisfactory goodness of fit. The number of covariate pattern of 8 annuls the need to conduct the Hosmer and Lemeshow's Goodness of fit test. A confusion matrix test shows a sensitivity of 93 per cent and a specificity of 16.5 per cent, thus with the correctly classified value of 70 per cent, the model appears to be appropriate. Variables "age group" and "ART regimen", which are categorical were analysed together in their respective categories and were found to be statistically significant. They were subsequently retained in the final model. The area under the ROC curve was 0.67, and the p-value for the full model was < 0.001.

OUTCOME MEASURE OF WEIGHT

Table 3.5: Univariate analysis (crude odds ratios) of factors associated with a weight response at 12 months.

		WEIGHT RESPONSE AT 12 MONTHS OF THERAPY	
n (explanation)	**Description**	**Odds Ratio**	**95% CI**
300 (males=96)	Participant's gender (m)	0.60	0.37-0.98
300 (present=74)	History of TB at baseline	1.70	0.99-2.91
300 (present=48)	History of opportunistic infections at baseline	1.21	0.65-2.25
300	Baseline weight	0.92	0.89-0.94
300 (3 centiles)	Participant's age in groups		
18-30 years	group 1	Ref	Ref
30-39 years	group 2	0.61	0.32-1.16
39-49 years	group 3	0.51	0.26-0.97
49-99 years	group 4	0.59	0.31-1.16
300 (5 groups)	ART Regimen		
Regimen 1	d4T+3TC+NVP	Ref	Ref
Regimen 2	d4T+3TC+EFV	1.12	0.44-2.85
Regimen 3	AZT+3TC+NVP	1.00	0.46-2.19
Regimen 4	AZT+3TC+EFV	0.66	0.32-1.40
Regimen 5	TDF+3TC+EFV	3.20	0.59-17.2

Table 3.6: Multivariate analysis (adjusted odds ratios) of factors associated with a weight response at 12 months

n (explanation)	Description	Odds Ratio	95% CI
(present=74)	History of TB at baseline	1.53	0.83-2.80
300	Participants' baseline weight	0.91	0.89-0.94
300 (males=96)	Participants' gender (m)	0.74	0.43-1.29
300 (5 groups)	ART Regimen		
Regimen 2	d4T+3TC+EFV	1.30	0.46-3.61
Regimen 3	AZT+3TC+NVP	1.72	0.71-4.19
Regimen 4	AZT+3TC+EFV	0.89	0.38-2.07
Regimen 5	TDF+3TC+EFV	10.61	1.74-64.5

This can be translated to mean that for every unit increase in baseline weight, the log-odds of response in weight at twelve months increases by a value of negative 0.0911976 while holding all other variables constant. Also, if gender changes from male to female, the log-odds of weight response at twelve months is expected to increase by a value of negative 0.2993518 holding the other independent variables constant. Likewise for every one-unit increase in tb history, that is for every subject with a past history of TB, the log-odds of weight response at twelve months is expected to increase by a value of 0.4234483. Similarly, for every one-unit increase in age in the categories "art_regimen2", "art_regimen3", "art_regimen4" and "art_regimen5", the log-odds of weight response at twelve months is expected to respectively increase by values of 0.2591987, 0.5442423, -0.1195394 and 2.361929.

The Hosmer and Lemeshow's Goodness of fit test was preferred over the Pearson's Goodness of fit test for this model. The latter test had a *p*-value of 0.08 which not

only approached statistical significance but also a large number of covariate pattern (208). With a *p*-value of 0.63 when conducted for twelve, there was no need to expect five or more successes between the observed and the predicted outcomes in each covariate group. Thus, the Hosmer and Lemeshow test appeared to be more reliable. The area under the curve was 0.76 showing good fit and the *p*-value for the full model was < 0.001. A confusion matrix test shows a sensitivity of 73 per cent and a specificity of 63 per cent, and a correctly classified value of 68 per cent.

OUTCOME MEASURE OF HAEMOGLOBIN

Table 3.7: Univariate analysis (crude odds ratios) of factors associated with a haemoglobin response at 12 months

n (explanation)	Description	Odds Ratio	95% CI
		HB RESPONSE AT 12 MONTHS OF THERAPY	
300 (males=96)	Participants' gender (m)	0.49	0.27-0.90
300 (present=74)	History of TB at baseline	1.62	0.92-2.88
300 (present=48)	History of opportunistic infections at baseline	0.92	0.45-1.87
300	Baseline haemoglobin	0.30	0.23-0.41
300 (3 centiles) 18-30 years 30-39 years 39-49 years 49-99 years	Participants' age in groups group 1 group 2 group 3 group 4	 Ref 0.87 0.91 0.74	 Ref 0.49-1.52 0.50-1.66 0.39-1.41
300 (5 groups) Regimen 1 Regimen 2 Regimen 3 Regimen 4 Regimen 5	ART Regimen d4T+3TC+NVP d4T+3TC+EFV AZT+3TC+NVP AZT+3TC+EFV TDF+3TC+EFV	 Ref 1.00 0.39 0.27 0.54	 Ref 0.39-2.53 0.17-0.89 0.12-0.60 0.11-2.41

Table 3.8: Multivariate analysis (adjusted odds ratios) of factors associated with a haemoglobin response at 12 months.

n (explanation)	Description	Odds Ratio	95% CI
(males=96)	Participants' gender (m)	2.96	1.26-6.98
300	Baseline haemoglobin	0.27	0.19-0.37

Therefore, for every unit increase in baseline haemoglobin, the log-odds of response in haemoglobin at twelve months increases by a value of negative-1.318302 while holding all other variables constant. Changing from male to female, the log-odds of haemoglobin response at twelve months is expected to increase by a value of 1.086733 holding the other independent variables constant.

Just like it was seen in the analysis for variable "weight" above, the large number of covariate patterns negates the need to adopt the results of a Pearson's Goodness of fit test owing to reliability. The p-value of the Hosmer and Lemeshow's Goodness of fit test was 0.62 when conducted for ten and perfectly predicted success between the observed and expected in the various groups. The area under the curve was 0.90, and the confusion matrix test revealed a sensitivity of 61 per cent and a specificity of 92 per cent. There was a correctly classified value of 84 per cent. The *p*-value for the full analysis was < 0.001.

CHAPTER 4

Discussion

This study was carried out to ascertain the pattern of clinical and immunological response as well as factors influencing these patterns in patients taking different first-line HAART regimens.

The assessment of subjects for TB and opportunistic infections was done only at baseline in this study to prevent effect modification during statistical analysis. Subjects with TB or other opportunistic infections diagnosed before the start of this study were included in the final analysis regardless of the stage or completeness of therapy and those who developed any of these conditions during the review period were excluded. Eleven cases of TB were reported, accounting for 3.7% of the study population. These cases were perceived to have occurred as a result of unmasking of latent infections and contributory to the immune reconstitution inflammatory syndrome. On the other hand, however, there were more cases reported in the general population of patients on the pre-HAART programme and also among defaulters of treatment with HAART.

Response to treatment across the study population followed the expected pattern. The aim of therapy is to achieve optimal response and this study showed that the use of HAART was associated with an increase, in haemoglobin weight and CD4 count during the 12-month follow up.

Subjects were similar at baseline for CD4 assessment. Rapid and sustained increase was seen with all treatment regimens during the first six months of therapy, after which an increase was significantly less rapid. The data demonstrated that TDF-containing treatment regimens recorded the highest rise in CD4 cells, while the lowest rise was seen in the AZT-containing regimens.

Weight and haemoglobin values differed significantly at baseline across treatment populations (figures 2 and 3). In the initial three months of treatment, haemoglobin levels appear to drop slightly below baseline levels for subject on AZT-containing regimens but then rapidly rise to levels above baseline values over the next two to

three months and then matching haemoglobin levels of other treatment options during that interval. Subsequently response pattern and continual rise are similar to those for other treatment regimens. A re-run of the analyses using baseline weight and haemoglobin values altered the coefficients.

From this study it seems that the role of age is vital if the expected response to treatment is to be achieved. Patients in the age range 32 to 39 had the least response with respect to CD4 when compared to other groups in the study. Odds ratio was 1.60 (95% CI 0.92 - 3.03).

However, an important observation was in the TDF-containing regimen where haemoglobin response failed to show concordance with respect to therapy, odds ratio 2.56 (95% CI 0.70 – 9.35). Judging by the wide confidence interval the low number of patients in that category who satisfied the concept of response as defined in the study is believed to be the reason for failure of haemoglobin to concordance.

POSSIBLE LIMITATIONS OF THE STUDY

The unique terrain of the country makes it extremely difficult to achieve good coverage with respect to HIV care. In addition to this, the proportion of people with health insurance in the country is negligible, making holistic and comprehensive health care including HIV care almost non-existent. The study was done in a population of Basothos who are natives of the Kingdom of Lesotho, a country completely embedded within South Africa and with a relatively constant GDP and very little economic growth over the last four years. A GDP of USD1500 effectively places the country among the poorest nations in the world. This population differ substantially from their South African counterparts with respect to education level, economic status and other socio-economic factors. The gold standard monitoring tool for patients on HAART is the viral load. However CD4 count may be used to monitor response to therapy and alongside proper monitoring of some clinical parameters, an effective monitoring of patients can be achieved particularly in resource-poor settings.[44]

In ideal settings, with regard to the study design a prospective randomised controlled trial with more enrolled patients and viral load assay would have been better,

although the cost and manpower would have been difficult to reach with the resources that were available for this study. Furthermore, this is a non-randomised observational study where random allocation of treatments is done. Propensity scoring can provide means for adjusting for selection bias in observational studies such as this, making causal inference in comparing exposure especially when there is several information on how exposures were selected. Admittedly, adjustment for covariates can be made during selection but increased cost and complexity, in addition to issues of data quality and completeness are limiting factors. Multivariate analysis is another way of evaluating data from this study. Multivariate analysis deals with the statistical analysis of the data collected on more than one variable.[45] These variables may be correlated with each other, and their statistical dependence is often taken into account when analyzing such data. In fact, this consideration of statistical dependence makes multivariate analysis somewhat different in approach and considerably more complex than the corresponding univariate analysis, when there is only one response variable under consideration. A significant downside to multivariate analysis, however, is the multivariate normal distribution. It is one of the most frequently made distributional assumptions for the analysis of multivariate data.

STUDY RESULTS IN RELATION TO OTHER STUDIES

Process measures

Data from the baseline figures of this study compare well to those of studies carried out elsewhere in the world.[47] What is clearly different in these other studies is that dual HIV therapy was used in some and a combination of three NRTIs was used in some of the others to provide triple therapy with respect to AZT.

On therapy with TDF, however, there is paucity of information regarding therapy with this NRTI. A study done in the United States compared responses between AZT 3TC and EFV with TDF FTC (Emtricitabine) and EFV. Two observations were made from these studies-first, no therapy with NVP and viral load assay was an integral aspect of monitoring and final evaluation. Furthermore, a CD4 cut off was never established at entry into the study by participants as any CD4 level was acceptable at baseline.

Viral load of greater than 10 000 copies per ml of blood was the acceptable viral load level for entry.[48,49]

OUTCOME MEASURES

Generally, the results of the outcomes of this study compared very favourably with their American counterparts, where resources abound. Also, even though the time frame for preliminary assessments differed, the end results were quite similar.

QUESTIONS ARISING FROM THIS STUDY FOR FURTHER STUDY.

The first question arising is: how best should the database be managed in order to be able to generalise the findings from this study?

Secondly, with the perennial problems associated with bone marrow toxicity leading to poor adherence to AZT-containing treatments, has this study been able to show that the incidence of AZT-induced anaemia is negligible, especially given the fact that there was no reported case among the participants in this study. In the same vein how possible is it to determine patients that may go on to develop anaemia secondary to AZT administration prior to commencement of treatment?

Thirdly, what will happen to the patients that cannot even afford a hospital visit more than once or twice in a year owing to transportation difficulties? Can the findings from this study be employed in their care?

CONCLUSION

In conclusion this study succeeded in providing evidence that clinical and immunological parameters can be combined into an effective toolkit for the monitoring of patients and assessment of response particularly in resource-poor settings. Treatment with HAART demonstrated improvement in laboratory and anthropometric parameters over baseline values. A slight reduction in haemoglobin was observed with therapy with AZT but not enough to cause significant anaemia necessitating investigation for other causes of anaemia when present. Stavudine and Lamivudine elicited response patterns similar to those of Zidovudine. While treatment with TDF appeared to have shown the best possible response pattern

more patients on TDF therapy will have to be included in the study to justify this observation.

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ADDENDUM 1

Data-collection form

DATA COLLECTION SHEET				
Parameter measured	**Baseline**	**3 months**	**6 months**	**1 year**
CD4				
Haemoglobin				
WHO clinical stage				
Weight				
Function: Working Ambulatory Bed-ridden				
HAART regimen				
TB history: Pulmonary Extra-pulmonary Past treatment On treatment None				
Co-morbidity				
Opportunistic-infection history: PCP Cryptococcus Others				
Bacrim prophylaxis				
History of malignancy: Kaposi Lymphoma				
Adherence				
IRIS				

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YUNIBESITHI YA PRETORIA
Faculty of Health Sciences
School of Health Systems and Public Health

30 June 2008

Dr AB Adebanjo
27411011
MSc (Clin Epi)

Dear Dr Adebanjo

Approval Academic Advisory Committee

This serves to confirm that your protocol was served and approved at the Academic Advisory Committee on 24 June 2008.

Please note that your title was approved:

Factors associated with response to antiretroviral agents in an HIV cohort at Roma Hospital, Lesotho

Please contact your supervisor, Prof Rheeder, in connection with some minor editorial changes before finalising your protocol for ethics submission.

Sincerely

Prof C de Jager
Chairperson
SHSPH Academic Advisory Committee

P O Box 667, PRETORIA, 0001, RSA
5th Floor, HW Snyman North, 31 Bophelo Road, Gezina
Tel: +27 12 354 1472
http://shsph.up.ac.za

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