The use of haemoglobin and body mass index as predictors of mortality in HIV patients newly initiated on highly active antiretroviral therapy

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Declaration

I, Abraham Tesfay, declare that this thesis is my own work. It is being submitted for the degree of Masters of Science in the field of Epidemiology and Biostatistics in the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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25th of March, 2013

I. Abstract

Background

More than 33 million people are estimated to be living with HIV worldwide. Sub-Saharan Africa bears a disproportionate share of the global HIV burden. An estimated 15 million people living with HIV in low and middle income countries were in need of (HAART) in December 2009. HAART services require advanced laboratory technologies to monitor disease progression and therapeutic response, which are scarce in developing countries. Several simple and widely available markers have been proposed for use in low income countries including total lymphocyte count (TLC), haemoglobin and body mass index.

Methodology

This study is a secondary data analysis of prospectively collected cohort data from HIV positive adults. The study measured the effect of exposure variables of haemoglobin (Hb) and body mass index (BMI). All cause mortality was the outcome of interest. Crude estimates of mortality were made with Kaplan-Meier mortality curves. Cox proportional hazards models were used to estimate adjusted hazard ratios. Exposure status was considered at initiation period. Outcomes were measured from two weeks post initiation of treatment to a maximum of two years of follow-up period. A composite score was developed to estimate the overall risk of mortality.

Results

A total of 11,884 patients who satisfied the inclusion criteria were included in the analysis. A total of 1,305 deaths were observed during the follow-up period, representing 10.2% of the cohort at baseline. Most of the deaths were observed during the first four months of follow-up period. Patients with moderated to severe anaemia experienced 2.6 (HR = 2.6, 95% CI 1.8 - 3.6) times greater hazard of mortality adjusted for possible confounders. Patients with very

low BMI experienced twice (HR=2.0, 95% CI 1.6, -2.5) greater hazard of mortality adjusted for a list of predictors. Race, age at initiation, employment status, smoking, alcohol consumption, baseline TB and baseline WHO stage did not show significant effect in the multivariate cox regression model.

A composite score was developed to estimate the overall risk of mortality in patients based on measurements of baseline BMI and haemoglobin. Cox regression model adjusted for CD4 cell count shows high risk patients experienced 4.7 (HR = 4.7, 95% CI 2.9 – 7.6) times greater hazard of mortality compared to patients in the low risk group. Patients in the medium risk group experienced 3.4 (HR = 2.6, 95% CI 2.6 – 4.4) times greater hazard of mortality as opposed to patients in the low risk group.

Conclusion

Haemoglobin and body mass index provide excellent prognostic information independent of CD4 cell count in HIV positive patients newly initiated on HAART. They can be used to reliably predict mortality. Combining measurements of haemoglobin and BMI through composite scoring improves their predictive ability. They can have good clinical application in rural and remote facilities to screen patients for clinical and diagnostic services.

II. Acknowledgement

I am heartily thankful to my supervisor, Dr. Mhairi Maskew, for her indispensable encouragement, guidance and support from the initial to the final level of this project. I have come to understand the essence of doing a research because of her extensive input towards this project.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

Abraham Tesfay

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IV. Nomenclature

AIDS: Acquired immunodeficiency syndrome, a condition in which the body's weakened immune system is unable to fight off opportunistic infections. These infections can, if untreated, result in death.

ART: Anti-retroviral therapy is when a combination of drugs is given that delays HIV replication and immune system deterioration and allows for increased survival and better quality of life.

ARVs: Anti-retrovirals are compounds that inhibit the replication of HIV and make up ART. These are the special drugs being used to treat people with HIV and prevent further immune suppression.

BMI: Body Mass Index is an anthropometric measure calculated by dividing an individual's weight to height squared

CD4: A protein present on T-helper cells in the body. Counting the level of CD4 proteins via a laboratory test can help practitioners learn how strong a person's immune system is. This test is used in combination with the viral load test, which measures the amount of HIV in the blood.

HAART: Highly active anti-retroviral therapy is the name given to treatment regimens that aggressively suppress HIV replication and progression of HIV disease. The usual HAART regimen combines three or more anti-HIV drugs.

HIV: Human immunodeficiency virus, technically classified as a retrovirus, is the causative agent of AIDS.

OI: Opportunistic infection (as related to AIDS) refers to an infection caused by organisms that do not affect a person with a healthy immune system.

STI: Sexually transmitted infection is an infection that spread by the transfer of organisms from person to person during sexual contact.

Side effects: Refers to unpleasant reactions that the body has as a result of ART. Often side effects cause people to stop taking their ARVs.

Viral load: The number of copies of the virus in the body per unit of blood. This is used in combination with the CD4 level, which is a protein present on T-helper cells.

CHAPTER 1

INTRODUCTION

This section highlights the latest figures on the epidemiology of HIV worldwide and in South Africa and efforts made toward expanding access to highly active antiretroviral therapy (HAART) services in low and middle income countries. It describes the challenge of providing and monitoring HAART in resource limited settings. Finally it outlines the relevance of the study to the South African context.

1.1 Background information

Data from the UNAIDS global report on the AIDS epidemic shows that an estimated 33.3 million people were living with HIV worldwide by the end of 2009 [1]. With an estimated 22.5 million people living with HIV, sub-Saharan Africa still bears a disproportionate share of the global HIV burden [1]. The growths of the global HIV epidemic seem to be stabilizing. Overall, the annual number of new HIV infections has been on a steady decline since the late 1990s [1]. Although number of new HIV infections has been falling, the prevalence level remains staggeringly high [1]. This coupled with significant reduction in mortality has led to an overall increase in the number of people living with HIV and AIDS worldwide. South Africa is experiencing the largest HIV epidemic in the world with an estimated 5.6 million people living with HIV at the end of 2009 [2]. An estimated 15 million people living with HIV in low and middle income countries were in need of highly active antiretroviral treatment (HAART) in December 2009, yet only a third of them were receiving this potentially life-saving therapy [1, 2]. The number of HIV patients receiving HAART services in South Africa has surpassed one million.

There has been a concerted effort to expand access to antiretroviral therapy in low and middle income countries [3]. This, and the emergence of generic antiretroviral (ARV) drugs, has led

to substantial reduction in the cost of ARVs and increased usage of highly active antiretroviral therapy in developing countries [3]. However, monitoring HAART as recommended in National and International guidelines requires sophisticated laboratory technology and specialized skill. This monitoring system is not widely available in resource limited settings and has been one of the greatest impediments for the scale up of HAART services [3].

On average, CD4 test costs around $10 per individuals test [5]. The test requires that a venous blood sample be processed by a laboratory. In South Africa, a blood sample drawn from HIV patients is sent to NLHS laboratory for processing. Patients are sometimes referred for blood venous collection if a particular facility does not take blood samples. Depending on laboratory capacity, results are typically available between 2 and 14 days after the patient has provided the sample [5]. Patients are asked to return to the health care facility to receive their CD4 results. This process is associated with significant loss of HIV patients from the HIV treatment and care as many patients do not return to collect their results. Haemoglobin, BMI and total lymphocyte count can be done at point of care and do not involve costly laboratory service [5].

1.2 Problem statement

Significant progress has been made in expanding access to HAART in middle and low income countries. The number of patients receiving HAART has increased substantially since the launch of the WHO strategy "3 by 5"in 2003 [2]. The delivery of HAART service requires well developed laboratory technologies which are often not accessible in resource limited settings. The standard reference markers used in monitoring HIV infection and therapeutic response include measurement of CD4+ T lymphocyte counts and plasma viral loads [4]. These markers assist clinicians in determining disease stage and progression, and in decisions regarding when to start or change antiretroviral therapy [3]. Such reference assays

are not widely available in resource-limited countries as they require sophisticated equipment and specialized skills [5, 6]. Moreover, middle and low income countries are in the process of decentralizing HAART service delivery to make these services accessible in primary health care facilities as tertiary institutions can no longer keep pace with the growing demand [5]. These facilities frequently operate under difficult conditions with limited staff and infrastructure and are sometimes not equipped to handle these laboratory technologies [6].

1.3 Justification

The South African health care system is facing the challenge of providing quality HAART service to millions of HIV infected patients. The number of individuals needing ARV has reached close to 2 million and is expected to grow substantially as the people currently living with HIV progress to the final stage of the infection requiring the life sustaining medicines [2]. The primary health care facilities in South Africa do not have the capacity and required personnel to monitor HIV patients with standard assays such as CD4 cell count and viral load at each visits [6]. Hence, there is a need for simple and widely accessible alternative predictors that can be applied in resource limited settings for initiating treatment and monitoring therapeutic response.

CHAPTER 2

LITERATURE REVIEW AND RESEARCH OBJECTIVES

This section summarizes existing literature on the benefits of HAART service and its application in resource limited settings. It highlights the lack of access to standard monitoring tools in resource limited settings and the need for alternative markers to initiate treatment monitor response. It summarizes alternative predictors of mortality that can be applied in resource limited settings to screen high risk patients. It summarises the application of haemoglobin, body mass index and total lymphocyte count as alternative tools.

2.1 Effectiveness of Antiretroviral Treatment

Highly active antiretroviral therapy (HAART) is a combination treatment with three or four antiretroviral drugs [2]. It is currently the most important component of managing HIV infection [7]. Combination therapy has shown greater benefit both in clinical outcomes and surrogate markers [8] and treatment with HAART resulted in the greatest benefit compared to mono-therapy and dual therapy [9- 11].

There is now considerable evidence on the short term clinical and laboratory benefits of HAART [9-11]. There was, however, some concern over the long term health benefits of HAART. Studies have consistently showed that HIV patients on HAART experience persistently low progression rates compared to increasing progression rates for patients who remain untreated [11- 13]. Before widespread use of HAART, duration since seroconversion and age at seroconversion were the major determinants of survival and development of AIDS [13]. The widespread use of HAART is credited with substantial improvement in the prognosis of HIV-1 infected patients across Europe and North America [11, 14]. The use of HAART led to a substantial reduction in death rates across Europe among patients infected with HIV-1 [15].

Although the benefits of highly active antiretroviral therapy for the treatment of HIV infection is well documented [16], access to treatment remains very limited in sub-Saharan countries that bear the greatest burden of the epidemic [2, 17]. The recent decreases in prices of ARV drugs as a result of generic manufacturing and increased commitment of funds from donor countries have opened the possibility of expanding access to treatment in resource poor countries [2, 18]. There have been, however, some controversies on the feasibility of HIV treatment programmes in countries with poor health care infrastructure [19]. Some voiced doubts if the successes observed in the developed countries could be replicated in resource poor countries [20].

Several studies have demonstrated that antiretroviral therapy is both feasible and effective in low income countries [21, 22]. A meta-analysis of 10 observational studies carried out in resource poor settings demonstrated that HAART was as effective for HIV infected individuals in resource limited setting as it is in resource rich settings with comparable virological and immunological responses [23]. Evidence from South Africa suggests HAART can be provided in resource limited settings with good patient retention and clinical outcomes [20, 24]. Studies carried out in Cote d'Ivoire, Ethiopia, Malawi and Uganda showed that providing HAART in resource poor settings is both feasible and beneficial for long term survival [21, 25, 26].

Therefore, there is now overwhelming evidence that use of HAART does improve quality of life and prolong survival of HIV patients in both resource rich and resource poor settings. However, an effective cure is yet to be developed [27]. Decades of scientific research have not delivered medicines that can suppress the presence of HIV completely. Some latently infected leukocytes persist in the lymphatic tissues even after prolonged combination therapy and can cause immune complication at a later stage [28]. The main aim of HAART remains the maintenance of the plasma viral load below detection level and the restoration of the

immune system with the ultimate goals of reducing HIV related morbidity and improving quality of life [2, 29]. Hence, patients are expected to demonstrate considerable degree of adherence to the treatment program to be eligible as it requires lifelong commitment.

These lifesaving medicines are administered with vigilant monitoring as their use is associated with adverse drug reactions that can compromise the health of the patients and lead to serious complications or death [29]. Hence, several laboratory markers are needed to meticulously monitor their effect. Unlike many infectious diseases with fairly predictable prognostic patterns, there is wide variation in the rate of progression to full blown AIDS in HIV patients [30, 31]. Hence, the timing of initiating antiretroviral medications is largely determined by clinical features and certain laboratory markers [32].

2.2 HAART Monitoring

Some laboratory markers have been developed to assist clinicians determine the likelihood of progressing to AIDS for each individual patient [33]. The number of circulating CD4+ lymphocytes was initially established to be the single best predictor of progression to AIDS [34]. Studies later demonstrated that plasma viral load also provided independent prognostic information [35] and may, in fact, be a better predictor of prognosis in patients who are taking HAART. Patients with viral load measurements of 30000 copies/mL were 75% more likely to experience fast disease progression compared to patients with viral load measurements of less than 500 copies/mL [36]. Measurement of both viral load and CD4+ lymphocyte counts at once provides excellent prognostic information on the risk of progression to AIDS and death [32]. Combined use of these prognostic markers is useful in the planning and evaluation of HAART regimen for individual patients [32].

The standard monitoring of HIV infection includes routine clinical assessment and measurement of CD4+ T lymphocyte counts and plasma viral load. These indicators are used

to determine disease progression assist in decisions regarding when to start or change antiretroviral therapy, and assess treatment response. [32, 33, 34]. In addition, drug sensitivity testing for detection of existing drug resistant strains of the virus is increasingly becoming available and is used to guide the choice of antiretroviral treatment [4].

2.3 Alternative markers and their predictive ability of mortality

Several simple and widely available markers have been proposed for use in low income countries that have limited access to standard monitoring system. These include total lymphocyte count (TLC), haemoglobin body mass Index, serum albumin and p24 antigen. This study will focus on haemoglobin and body mass index as they are widely accessible in most resource limited settings.

2.3.1 Haemoglobin

Though the precise mechanism by which HIV causes anaemia is not yet clear, it is the most common haematological complication observed in HIV infected patients and constitutes an important part of the WHO staging classification [44]. Anaemia is associated with increased risk of progression to AIDS and death in all age categories [45, 46]. A prospective cohort study involving 6725 HIV patients in Europe found that low haemoglobin level was associated with rapid disease progression [15]. The study also found that haemoglobin level was an independent predictor of death among patients with similar CD4 cell count and viral load. HIV related anaemia is reversible when patients are treated with HAART and may be used to monitor therapeutic response [47].

2.3.2 Weight

Wasting syndrome is an AIDS defining illness according to the WHO Clinical staging of HIV/AIDS [37]. Unexplained weight loss of between 5% and 10% has been demonstrated to

be predictive of decreased survival and increased risk of developing AIDS defining opportunistic infections [48].

Body mass index (BMI) is a simple index of weight-for-height commonly used to classify degree of weight loss. It is a sensitive anthropometric measure that can be used as alternative surrogate marker to monitor HIV infection and response to HAART [49]. BMI categorized according to WHO threshold can provide reliable predictive information on disease progression and survival [50].

Data from a cohort study that followed 2376 HIV patients for a median period of 43 months in Southern France shows that BMI has a predictive value independent of CD4 cell count in terms of disease progression and survival [51].

2.3.3 Total lymphocyte count:

In 2002, WHO endorsed the use of TLC of less than 1200 cells/mm as an alternative indication for initiating HAART in resource limited settings where there is limited access to standard assays [29]. A study conducted in the US among 828 Air Force HIV patients found that 98% of those who had a TLC below 1000 cells/mm also had a CD4 cell count bellow 200cells/mm [38]. An observational study conducted in Cape Town among 831 out-patients on ART found that TLC<1250 cells/mm and CD4count <200 cell/mm had similar prognostic values in predicting the likelihood of disease progression [39].

Other observational work has shown a high degree of correlation between paired TLC and CD4 cell counts [40, 41] suggesting that TLC can be used as an alternative marker for initiating prophylaxis for opportunistic infections in low income countries.

There is some concern regarding the usefulness of TLC as an alternative surrogate marker for CD4 cell count due to its inconsistent sensitivity and specificity according to the TLC cut off point [42]. Sensitivity refers to the ability of a test to correctly identify patients with a

condition while specificity refers to the ability of a test to correctly identify those who do not have the condition. A study conducted among 2,777 HIV patients in South Africa indicated that the sensitivity and specificity of TLC in predicting CD4 cell count were not sufficient enough to replace CD4 cell count [43]. Poor sensitivity and specificity of TLC would lead to significant misclassification of patients with serious therapeutic implications.

These alternate markers do not require sophisticated technology and specialized skill to perform compared to the standard assays. They are widely available in resources limited settings such as primary health care facilities [6]. Measuring haemoglobin, body mass index and TLC at once can provide excellent prognostic information [52].

In summary, existing literature suggests that HAART is having beneficial effect in the management of HIV infection in resource limited countries. Access to standard monitoring system and the necessary skill to operate these tools remains one of the major challenges in the scale-up treatment program in resource-limited settings. There is a need to develop a simple alternative strategy that can be applied in resource limited facilities. This study attempts to evaluate the usefulness of two possible alternative markers (haemoglobin and body mass index) in predicting the likelihood of mortality in HIV patients newly initiated on therapy and its application as a screening tool to identify patients at greater risk of mortality. .

2.4 Aim

The aim of this study is to determine the usefulness of haemoglobin and body mass index in predicting mortality in HIV patients being initiated on HAART.

2.4 Specific Objectives

- To determine the relationship between independent variables of haemoglobine and body mass index and the outcome variable of mortality.

- To determine how predictive haemoglobin and body mass index are in reliably predicting the outcome of mortality in HIV patients newly initiated on HAART.

CHAPTER 3

METHODOLOGY

This section describes the study design, the study population, the inclusion and exclusion criteria and the sampling technique used to select the cohort of patients included in this study. It highlights the data source, defines exposure and outcome variables and expounds on how exposure and outcome variables are measured. Finally, it highlights on data processing methods and describes in detail the statistical analysis carried out.

3.1 Study design

This study is a secondary data analysis of prospectively collected cohort data from HIV positive adults.

3.2 Study population

The study was conducted among HIV infected patients receiving HAART services from Themba Lethu Clinic, a Right to care supported HIV care and treatment facility. The clinic is based in Johannesburg, South Africa. Since the launch of the South African Department of Health rollout of antiretroviral treatment in 2004, the clinic has provided HAART services to over 20,000 patients. Over 12,000 of these individuals have received antiretroviral treatment according to the Department of Health guidelines on Rollout of antiretroviral therapy [54]. The study population consists of all HIV positive adults enrolled at the Themba Lethu Clinic since its inception on 1st of April 2004. Themba Lethu Clinic maintains an electronic patient record of all patients receiving HAART services.

3.3 Sample

Inclusion criteria

The study sample consisted of male and female adults initiated on HAART at the Themba Lethu Clinic between 1st of April 2004 and 31st of March 2009. Patients were selected based on the following inclusion criteria:

- Patients older than 18 years of age at the time of enrolment in the clinic
- HAART naïve prior to the initiation visit at the clinic

Exclusion criteria:

- Patients transferred in from other clinics or rollout sites already initiated on HAART.
- Pregnant women were also excluded from the study due to the effect of pregnancy on measurements of the exposure variables especially haemoglobin and BMI.

Sample size: All patients meeting the study inclusion criteria were included in the analysis. As such no sampling was done.

3.4 Measurement and data sources

The Themba Lethu clinic records both demographic and clinical patient information on an electronic patient management and decision support system called TherapyEdge-HIV^{TM}. Data are entered directly into the system by clinical staff during the patient visit. The patients' demographic and contact details are recorded at the initiation visit on the "ARV Initiation Form". At every visit the patient's vitals and weight are recorded as well as any symptoms and new diagnoses made on the "Follow-up Visit Form". Additionally, blood tests for CD4 count, HIV viral load and full blood counts are measured at each scheduled visit (four months after initiation and six monthly thereafter) and entered onto the database. All data used in the study were obtained from variables already captured on this electronic database.

The study analysed measurements made at the initiation visit and at each subsequent visit up to a maximum of two years of follow-up.

Exposure variables: the study measured the effect of exposure variables haemoglobin (Hb) and body mass index (BMI). Body mass index was computed from the weight and height of each patient. BMI was categorized based on WHO thresholds [49]. BMI greater than or equal to 18.5 kg/m^2 and less than 30 kg/m^2 was considered as the normal value against which the effect of low BMI was measured. Exposure status was classified into three categories based on severeity. Patients with BMI 17.00 - 18.49 kg/m^2 were classified as mildly underweight. These patients will be described as "Low BMI" category in this report. BMI measurement of 16.00 - 16.99 kg/m^2 was classified as moderately underweight and BMI less than 16.00 - 12kg/m^2 was classified as severely underweight. Moderate and severe BMI was collapsed into one category as their effect was similar. Patients with moderate to severe BMI will be identified as "Very low BMI" in this report.

Haemoglobin (Hb) level was classified into three categories based on Harrison's principles of Internal Medicine classification [53]. Hb level greater than 14g/dl and 12 g/dl was classified as the normal values for males and females respectively; Hb level of 8–14 g/dl and 8–12 g/dl was classified as mild anaemia for male and female patients respectively and Hb level less than 8 g/dl was classified as severe anaemia for both males and females. Patients with normal Hb level were used as a reference group against which the effect of low Hb level was compared.

Outcome variables: All cause mortality was the outcome of interest. Death notifications are provided to the clinic through family members and hospital reports and vital status is also verified with the National Death registry.

Data on CD4 cell count, age, gender, duration of treatment, baseline WHO stage, baseline TB and treatment regimen was obtained from the dataset.

3.5 Data processing and analysis

The data was provided in multiple relational datasets, each containing detailed measurements on specific exposure or outcome variable. A total of 13 data sets were obtained. Patient ID was used as a unique identifier to link various measurements across the datasets.

The data was checked for completeness and error using the STATA 11 statistical software. The extent of gaps in the dataset was determined and values that fall outside acceptable range were replaced with missing values. Generally, there was rich data at baseline for most variables of interest. There was little gap on patient's final status during the follow-up period.

A descriptive statistical analysis was carried out for the exposure and outcome variables. The values of nominal variables were summarized using frequencies and proportions and presented in a table for ease of comparison between the exposure and reference groups. Continuous variables were summarized using means and standard deviations. They were also categorized into different level of exposure status such as mild, moderate and severe with corresponding frequencies.

Crude estimates of mortality stratified by the exposure variables were made with Kaplan-Meier mortality curves. Differences between the curves were assessed using the log rank test. Cox proportional hazards models were used to estimate hazard ratios of haemoglobin and body mass index adjusted for the effect of potential confounders. Predictors of mortality in the regression analysis were selected using backward stepwise selection method. Variables that did not show any significant effect at 95% confidence interval were dropped while those with significant effect were kept. Confidence interval was used to determine statistical

significance. Exposure status was considered at initiation period. Outcomes were measured from two weeks post initiation of treatment to a maximum of two years of follow-up period.

Sensitivity and specificity tests were conducted to determine how well the values of alternative markers performed against the existing standard monitoring tools. Two by two tables were constructed and patients were classified based on measurements of CD4 cell count and alternative markers. ROC curves were used to determine the optimal cut-off value for haemoglobine and body mass index. Various discrimination thresholds were compared and the one that best discriminates the positives and negatives was selected. Measurements made at initiation period were analysed to determine the usefulness of alternative markets in identifying patients at greater risk of mortality clinical decision related to the timing of ART initiation. Measurements made at initiation were analysed to determine clinical use of haemoglobine and BMI in predicting short term clinical outcomes. Sensitivity and specificity of haemoglobine and BMI were computed against the standard CD4 cell count.

A composite score was developed to estimate the overall risk of mortality in patients based on measurements of baseline BMI and haemoglobin. Patients were assigned score of 0, 1, 2 or 3 based on their baseline BMI and haemoglobin measurements. A composite score of 0 was assigned to patients with normal BMI and haemoglobin measurements at initiation. A composite score 3 was assigned to patients with moderate to severe anaemia and a very low BMI measurement at initiation. The composite scores were used to classify patients in to four risk groups. Those with normal baseline BMI and Hb measurements were classified as a reference group. Patients with either low BMI or mild anaemia or both were classified as low risk groups. Those with moderate to either severe anaemia or moderate to severe underweight were classified as medium risk groups and those with both severe anaemia and moderate to severe underweight were classified as high risk groups.

CHAPTER 4

RESULTS

The results of statistical analysis are presented in great detail in this chapter. Results are displayed in tables and figures with corresponding narrative summary. A brief description of clinical and demographic attributes of the cohort is provided in the first section. This chapter also highlights the outcome variable. It provides description of baseline and follow-up haemoglobin, body mass index and CD4 cell count measurements and their effect on mortality. The result of composite score index, sensitivity analysis and kappa statistics is presented in the last section of this chapter.

4.1 Description of the study sample

Patients were selected based on the inclusion and exclusion criteria outlined in the methodology section. A total of 11,884 patients who satisfied the inclusion criteria were included in the analysis. These individuals were followed for a mean period of 1.6 years [standard deviation 0.68 years] after being initiated on therapy. Nearly seventy percent of the patients were alive and in care by the end of the follow-up period. The cohort contributed a total of 18,977 person years at risk.

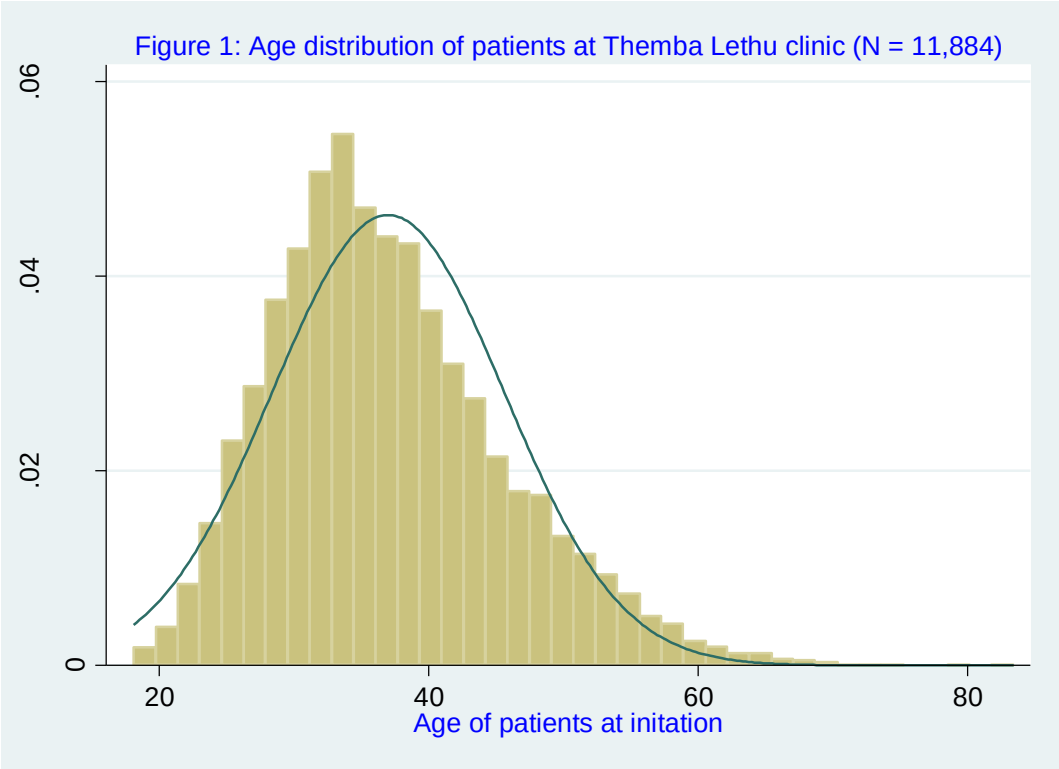
Sixty five percent of the study sample was female patients. Most of the patients were adults in their thirties with a mean age of 37.0 years and an standard deviation of 8.6. As depicted in Figure 1, age was approximately normally distributed. The majority (95%, n=11,884) of the cohort was classified as blacks and 53.5% of them were unemployed. Nearly 10% of the patients included in this study were classified as current smokers at date of initiation while a further 4.5% of them had a history of smoking in the past. About 10% of the patients were classified as current consumers of alcohol while 7% of them had a history of alcohol use in the past. Over three quarter (78.3%) of the participants were initiated on the standard

government first-line regimen (d4T_3TC_EFV) while 7.4% were initiated on d4T_3TC_NVP and 6.3% were initiated on d4T_3TC_LPVr. One third of the patients presented with a WHO clinical stage III [29] at initiation, while 7.6% of the cohort had already progressed to stage IV at initiation. Nearly 15% of the patients had TB co-infection at initiation and 10% of them had a history of TB infection in the past. One fifth of the patients had low baseline body mass index measurements and 10% of them had very low BMI at initiation. Nearly three quarter of the patients had low haemoglobin measurements at initiation. About 90% of patients had below 200 baseline CD4 cell counts. Nearly 40% of them had less than 50 CD4 counts at initiation. Baseline figures are summarized in table 1 below.

Table 1: Baseline values of demographic and clinical variable of adults initiating HAART at TLC

Baseline value	**Frequency (Percentage of total)**
Gender of Patients	
Male	4,205 (35%)
Female	7,679 (65%)
Race of patients	
Black	11,293 (95%)
Other race	591 (5%)
Employment status	
Unemployed	6,357 (53%)
Employed	5,130 (45%)
Smoking Status	
Non-smokers	9,305 (85%)
History of smoking	538 (5%)
Current smokers	1,162 (11%)
Alcohol consumption	
Non-alcohol users	8,917 (81%)
History of alcohol use	872 (8%)
Current alcohol use	1,239 (11%)
HAART Regimen initiated	
d4T_3TC_EFV	9,256 (78%)
d4T_3TC_LPVr	750 (6%)
d4T_3TC_NVP	864 (7%)

Other regimen	960 (8%)
Baseline WHO stage	
Stage I	3,948 (46%)
Stage II	1,322 (15%)
Stage III	2,689 (30%)
Stage IV	904 (10%)
TB infection	
TB co-infection at initiation	1,727 (14.5%)
History of TB infection	1,190 (10%)



4.2 Mortality during the first 2 years of follow-up period

The outcome of interest was all cause mortality confirmed by hospital report or through death notifications provided to the clinic through family members or by verifying the National Death registry. A total of 1,305 deaths were observed during the follow-up period, representing 10.2% of the cohort at baseline. Seven patients died before starting HAART and 67 patients died within 2 weeks of starting HAART. A total of 1,082 patients died in the

subsequent 2 years of follow-up. Patients who died after two weeks of therapy and within two years of follow-up period were included in the cohort analysis.

Most of the deaths were observed during the first four months of follow-up period. Almost 4% of patients initiated on therapy died during the first four months of follow-up period. Having survived the first four months, the likelihood of mortality was much lower in subsequent follow-up periods. Overall, the cohort experienced 68.4 deaths per 1000 person-years at risk. Number of deaths, number of patients who are not in care at Themba Lethu clinic and probability of survival at each follow-up period are summarized in table 2 below.

Table 2: Attrition of patients due to death during the first two years of follow-up period

Follow-up period	No at risk at start of interval	Number of deaths	Probability of survival	[95% Conf. Int.]
0 to 4 Months	11,128	481 (4.3%)	0.9593	0.9555 0.9627
4 to 10 months	9,386	328 (3.5%)	0.9284	0.9234 0.9330
10 to 16 months	8,770	142 (1.6%)	0.9140	0.9086 0.9191
16 to 24 months	8,191	132 (1.6%)	0.8999	0.8940 0.9054

4.3 Measurements of haemoglobin and its effect on mortality

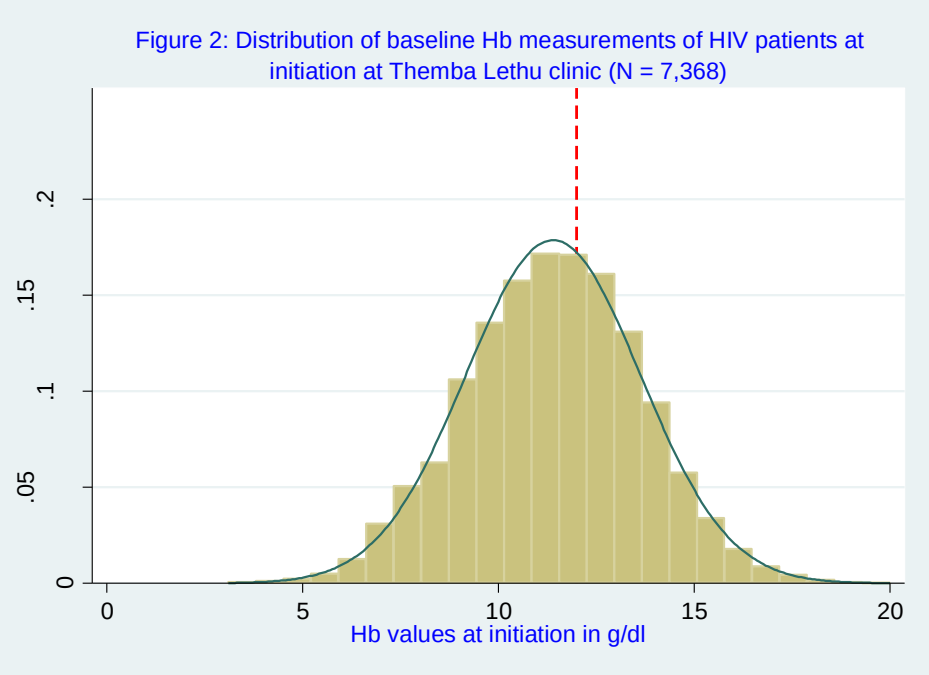
4.3.1 Baseline and follow-up haemoglobin measurements

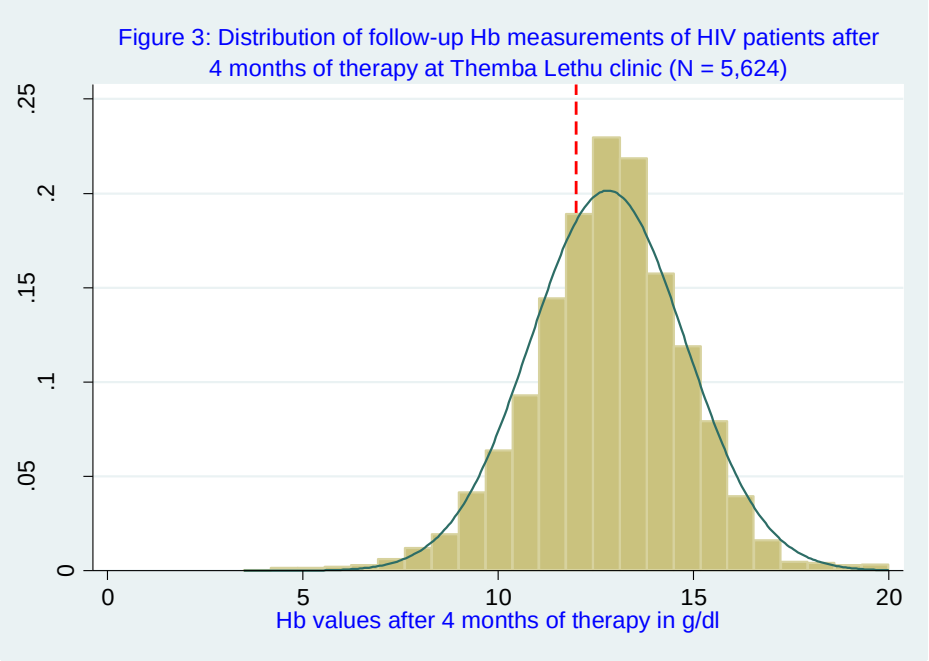
Baseline haemoglobin level was measured within 30 days before starting ART or up to 7 days after initiation. Measurements below 3g/dl and above 20g/dl were considered outliers as such extreme measurements are not biologically plausible. These measurements are probably the results of errors at measurement and data entry level. Hence, they were replaced with missing values to limit their effect on the estimates. A total of 7,368 patients had a recorded baseline haemoglobin values within the plausible range. Figure 2 shows that baseline haemoglobin

measurements were approximately normally distributed with a mean value of 10.4g/dl. Haemoglobin measurements of 14 to 20 g/dl and 12 to 20 g/dl were considered the normal values for male and female patients respectively. 69.5% of the study participants had baseline haemoglobin measurements below normal levels. Haemoglobin measurements of less than 8g/dl were considered moderate to severe anaemia for male and female patients.

The first follow-up haemoglobin level was measured at first follow-up visit after four months of treatment. Measurements made up to 30 days prior to or 30 days after a four months of follow-up period were included in this analysis. Measurements that fall outside the biological range were replaced with missing values. A total of 9,386 patients were alive and in care at the end of the first four months of therapy and 5,561 of them had a follow-up haemoglobin measurement done at this stage. About 40% of the patients who survived and remained in care did not have a recorded haemoglobin measurement at the end of four months of therapy. Figure 3 shows that the first follow-up haemoglobin measurement was approximately normally distributed with a mean value of 12.77g/dl. About 40% of the patients had low haemoglobin measurement after four months of therapy.

Mean haemoglobin value improved from 10.4g/dl at baseline to 12.77g/dl after four months of therapy. The proportion of patients with normal baseline haemoglobin measurements was just 30%. Yet, almost 60% of patients had normal haemoglobin measurements after four months of therapy. This difference can also be noted from a shift of the histogram towards the right in figure 3.





The second follow-up haemoglobin was measured at second follow-up visit after 10 months of therapy. Measurements made within 30 days prior to or after a 10 months follow-up period were included in this analysis. The third follow-up haemoglobin was measured at the third follow-up visit after 16 months of ARV therapy. Measurements made within 30 days prior to or after a 16 months follow-up period were included in this dataset. Lastly, the fourth follow-up haemoglobin was measured at fourth follow-up visit after two years of treatment.

Measurements made 30 days prior to or after a two year follow-up period were included in this analysis. All follow-up measurements that fall outside the acceptable range were replaced with missing values.

The level of missing values increases in subsequent follow-up periods. There were only 3,084 recorded values for the second follow-up haemoglobin measurement and just 1,558 values for the third follow-up haemoglobin measurement. 1,364 patients had a record for haemoglobin measurement after two years of treatment.

Table 3: Summary of haemoglobin measurements at initiation and subsequent follow-up visits

Hb Measurements	No. Of Observations	Mean value	Std. Dev.
Hb at initiation	7,368	11.40183	2.23
Hb at first follow-up visit *(after 4 months of therapy)*	5,624	12.81115	1.98
Hb at 2nd follow-up visit *(after 10 months of therapy)*	3,084	13.46807	1.94
Hb at 3rd follow-up visit *(after 16 months of therapy)*	1,558	13.48006	2.00
Hb at 4th follow-up visit *(after 2 years of therapy)*	1,364	13.7279	1.89

4.3.2 Distribution of baseline haemoglobin measurements in the sub-groups

Test of homogeneity was done to investigate the distribution of haemoglobin in the subgroups. Female patients had higher proportion of patients with anaemia. The proportion of patients with anaemia was much lower in patients who smoke and those who consume alcohol compared to the non-smokers and those who do not consume alcohol. Underweight patients were more likely to experience moderate so severe anaemia compared to patients with normal body mass index measurement. Patients with low baseline CD4 count, those in advanced stage of AIDS and those co-infected with TB experienced higher percentage of anaemia. Table 4 below presents the distribution of anaemia in the subgroups.

Table 4: Distribution of baseline haemoglobin measurements in the subgroup population

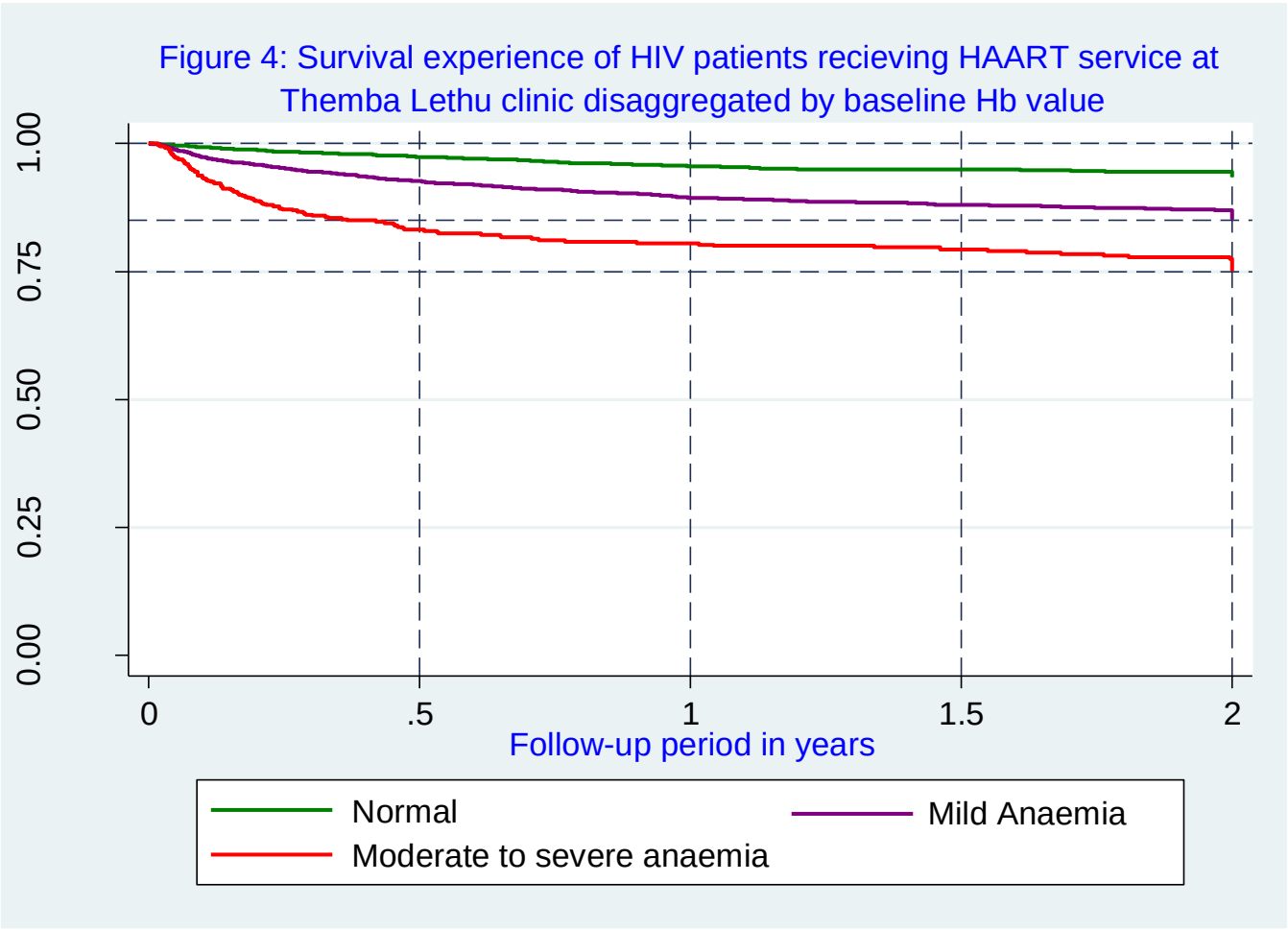
Baseline value	Baseline Hemoglobin Measurements			
	Frequency	% with normal Hb	% with mild anemia	% with moderate to severe anemia
Gender of Patients				
Male	2,653	24.2	70.3	5.5
Female	4,646	34.0	58.6	7.4
Race of patients				
Black	6,950	30.0	63.3	6.7
Other race	349	39.5	53.0	7.5
Employment status				
Employed	3,188	33.4	61.4	5.2
Unemployed	3,881	28.0	64.1	7.9
Smoking Status				
Non-smokers	5,797	30.2	63.0	6.7
Current smokers	666	36.6	59.8	3.6
Alcohol consumption				
Non-alcohol users	5,479	29.9	63.3	6.8
Current alcohol use	762	39.0	58.5	2.5
Baseline BMI				
Normal BMI	4,707	35.0	60.4	4.6
Mild underweight	677	15.7	74.0	10.3
Moderate to severe underweight	622	10.3	74.3	15.4
Baseline CD4				
> 200	725	54.8	41.4	3.9
100 to 199	1,521	40.3	55.4	4.3
50 to 99	1,192	27.6	65.4	7.0
< 50	2,499	17.3	73.2	9.5
Baseline WHO stage				
Stage I	3,976	40.5	58.8	0.8
Stage II	113	38.1	61.1	0.9
Stage III	2,641	18.5	66.4	15.1
Stage IV	569	14.2	75.0	10.7
TB infection				
No TB co-infection at initiation	6,276	33.3	60.7	6.1
TB co-infection at initiation	1,020	13.1	76.3	10.6

4.3.3 The effect of anaemia on mortality

Of the cohort of patients initiated on ART at Themba Lethu clinic, 11.6% died during the first two years of follow-up. Less than 6% deaths were observed in patients with normal baseline haemoglobin measurement in the first two years of follow-up while 13.2% of patients with mild anaemia died in the same follow-up period. Patients with moderate to severe anaemia experienced the highest mortality standing at 22.4%.

A greater proportion of patients with moderate to severe anaemia at initiation (n=108, 22%) died compared to those with mild anaemia (n=606, 13%) and those with normal haemoglobin level (6%). Kaplan Maier analysis for post initiation mortality censored for transfer outs and lost to follow-up showed significantly better 2-years survival in patients with normal baseline haemoglobin measurements as opposed to patients with mild anaemia. Patients with moderate to severe anaemia showed poor survival compared to patients with normal haemoglobin and those with mild anaemia. Patients with moderate to severe anaemia showed worst survival in the first four months of therapy. A log-rank test for equality of survivor functions shows statistically significant difference in the survival probability of patients across the exposure groups (p value < 0.0001).

Crude estimates from cox regression models show that the hazard of mortality was highest in patients with moderate to severe anaemia. Patients with mild anaemia at initiation experienced 2.4 (HR = 2.4, 95% CI 2.0 - 2.9) times greater hazard of mortality compared to patients with normal baseline haemoglobin level. Patients with moderate to severe anaemia experienced 4.6 (95% CI 3.6 - 5.0) times greater hazard of mortality compared to patients with normal baseline haemoglobin measurement.



Multivariate cox regression analysis was carried out to estimate the effect of anaemia adjusted for a list of significant predictors of mortality. Patients with mild anaemia experienced 1.6 (HR = 1.6, 95% CI 1.2 - 2.0) times greater hazard of mortality adjusted for BMI, CD4 cell count, gender and year initiated compared to patients with normal haemoglobin measurement. Similarly, patients with moderated to severe anaemia experienced 2.6 (HR = 2.6, 95% CI 1.8 - 3.6) times greater hazard of mortality adjusted for these predictors. Race, age at initiation, employment status, smoking, alcohol consumption, baseline TB and baseline WHO stage did not show significant effect in a multivariate cox regression model.

Table 5: Hazard of mortality adjusted for BMI, CD4 cell count, gender and year initiated

Predictors of Mortality	Number	% died in first 2 years	Adjusted HR (95% CI)
Anaemia			
No Anaemia	2,222	5.9	1
Mild Anaemia	4,587	13.2	1.6 (1.2, 2.0)
Moderate to severe anaemia	490	22.0	2.6 (1.8, 3.6)
Body Mass Index			
Normal BMI	6,797	8.5	1
Low BMI	1016	14.5	1.4 (1.1, 1.7)
Very low BMI	870	22.3	2.0 (1.6, 2.5)
Body Mass Index			
CD4 200 +	922	5.1	1
CD4 100 - 199	2,178	7.9	1.2 (0.8, 1.8)
CD4 50 - 99	1,513	10.7	1.4 (0.9, 2.1)
CD4 < 50	2,951	17.6	2.2 (1.5, 3.3)
Gender			
Female	7,679	9.6	1
Male	4,205	13.5	1.2 (1.0, 1.4)
Year Initiated			
Initiated after Jan 1, 2007	5,642	8.8	1
Initiated before Jan 1, 2007	6,241	12.9	1.4 (1.1, 1.6)

HR= hazard ratio from Cox proportional hazard model

CI – confidence interval

4.4 Measurements of Body mass index and its effect

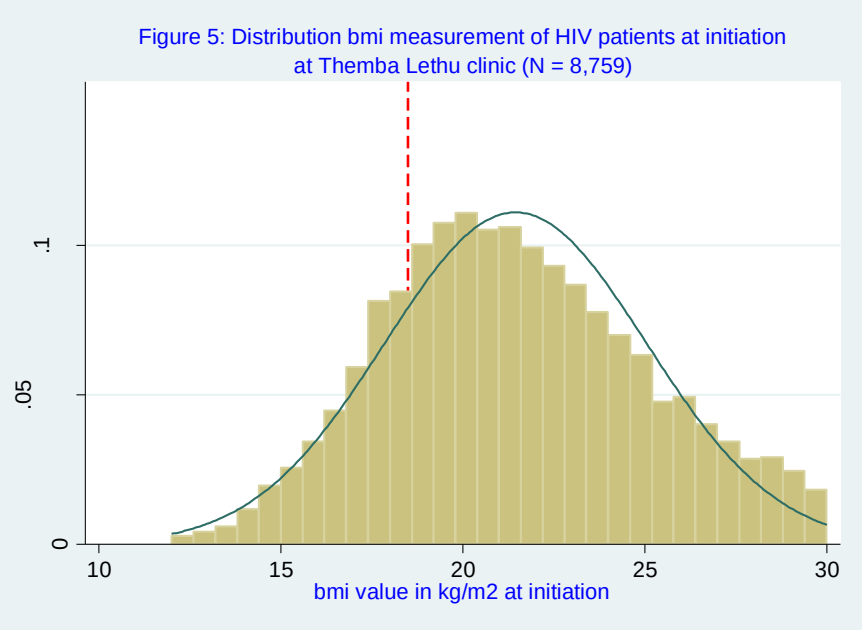
4.4.1 Measurements of baseline and follow-up BMI

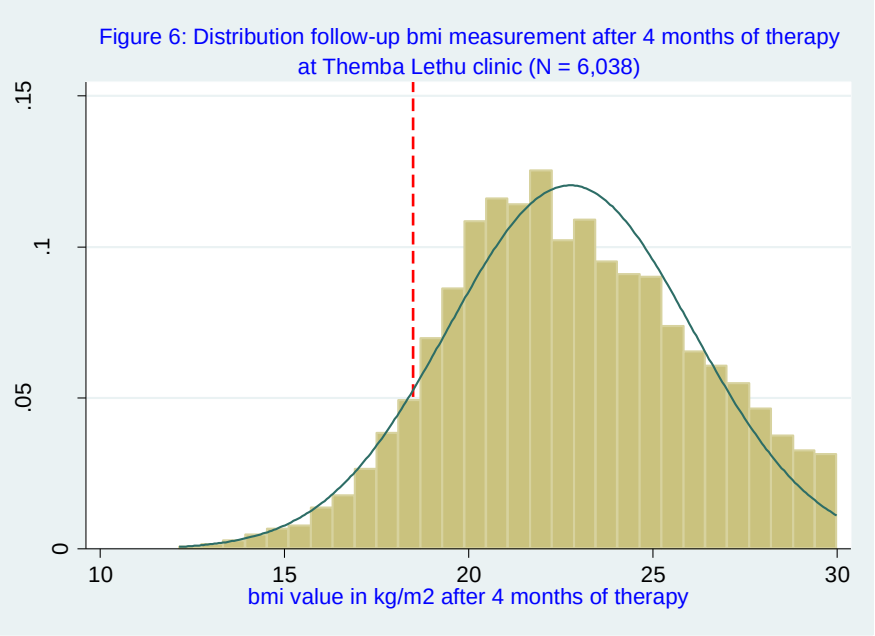
Body mass index was computed from the weight and height of patients by dividing kilograms of weight by height square (m^2). Baseline body mass index was measured within 30 days before starting ART or upto seven days post initiation. Seventy two patients had a recorded baseline body mass index value of below 12kg/m^2 and 16 patients had a recorded baseline

body mass index value of above 50kg/m^2 with some records as large as 260 kg/m^2. These values lay outside the biologically plausible range. They were considered as missing values as the process of verifying them with source document was not feasible. A total of 9,457 patients had a baseline BMI values within the plausible range and 698 patients had a BMI measurements above 30, which is the cut off point for obesity. As can be observed from figure 5, baseline BMI was approximately normally distributed with a mean value of 22.5kg/m^2. Nearly 20% of the study participants had below the normal baseline BMI measurements.

The first follow-up body mass index was measured at first follow-up visit after four months of treatment. Measurements made within 30 days prior to or 30 days after a four months follow-up period were included in this analysis. Measurements that fall outside the acceptable range were replaced with missing values. A total of 6,764 out of a total of 9,386 patients who survived and remained in care at the end of the first four months of therapy had a recorded follow-up BMI values. Nearly 30% of the patients had a missing BMI value for this follow-up visit. First follow-up BMI was approximately normally distributed with a mean value of 23.8kg/m^2. About 8% of the patients had below the normal first follow-up BMI measurement.

There was notable improvement in BMI measurements between baseline and first follow-up measurement. Mean BMI value improved from 22.5kg/m^2 at baseline to 23.8kg/m^2 after 4 months of therapy, an improvement of 1.3%. Almost 20% of patients had low BMI measurement at baseline. This was reduced to 8% after the four months of therapy. The rightward shift in the histogram also indicates an overall improvement in BMI measurement.





The second follow-up body mass index was measured at second follow-up visit after 10 months of treatment. Measurements made within 30 days prior to or 30 days after a 10 months follow-up period were included in this analysis. The third follow-up BMI was measured at the third follow-up visit after 16 months of treatment. Measurements made within 30 days prior to or 30 days after a 16 months follow-up period were included in this analysis. Lastly, the fourth follow-up BMI was measured at a visit made after two years of treatment. Measurements made within 30 days prior to or 30 days after a two year follow-up period were

included in this dataset. All measurements that fall outside the acceptable range were replaced with missing values.

There were only 5,172 recorded BMI measurements out of 9,386 patients alive and in care after 10 months of therapy and just 3,880 BMI measurements were recorded for the third follow-up visit out of 8,770 patients alive and in care at this point and 3,052 patients had a recorded BMI value at two years of follow-up visit out of 8,191 patients still alive and in care at this point.

Table 6: Summary of BMI measurements at initiation and subsequent follow-up visits

BMI Measurements	No. Of Observations	Mean value	Std. Dev.
BMI at initiation	9,457	22.36	4.83
BMI at first follow-up visit *(after 4 months of therapy)*	6,764	23.94	4.78
BMI at 2nd follow-up visit *(after 10 months of therapy)*	5,172	24.88	4.86
BMI at 3rd follow-up visit *(after 16 months of therapy)*	3,880	24.81	4.92
BMI at 4th follow-up visit *(after 2 years of therapy)*	3,052	24.84	4.95

4.4.2 Distribution of baseline BMI measurements in the subgroups

Test of homogeneity was conducted to investigate the distribution of baseline body mass index in the subgroups. The proportion of patients with moderate to severe underweight was higher in black and male patient. The proportion of patients with underweight was also much higher in patients with anaemia and those in advanced stage of AIDS. Patients with low baseline CD4 count and those co-infected with TB experienced higher percentage of underweight. Table 7 below presents the distribution of underweight in the subgroups.

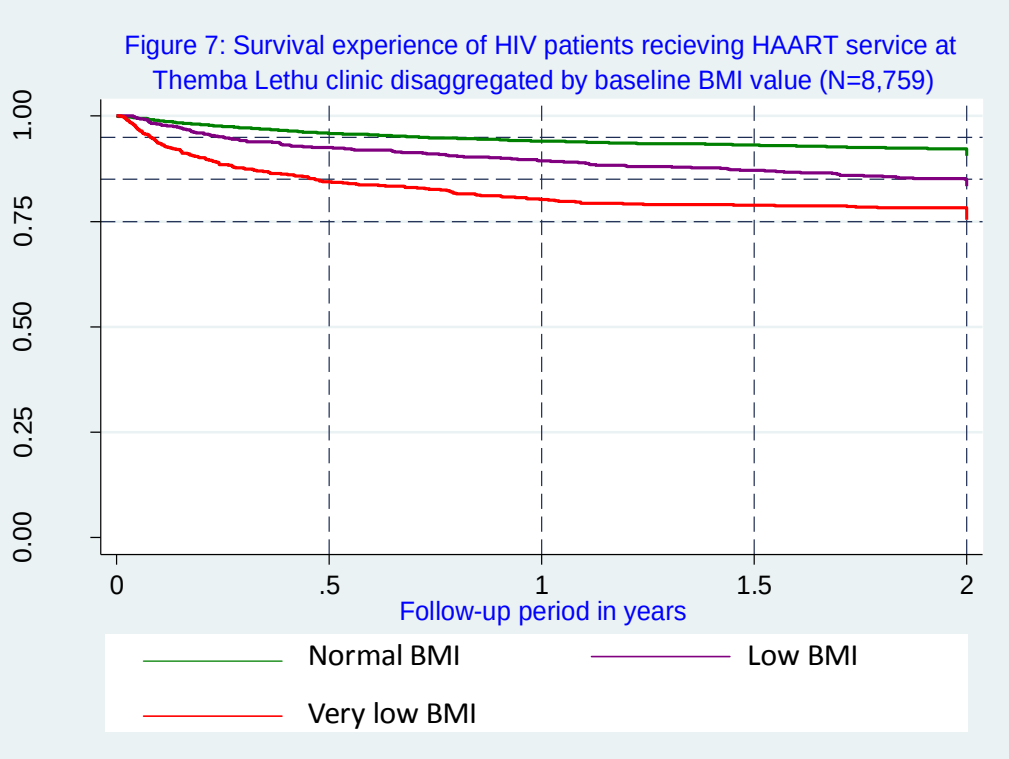
Table 7: Distribution of baseline BMI measurements in the sub-group population

Baseline value	Frequency	Baseline Body Mass Index Measurements		
		% with normal BMI	% with mild underweight	% with moderate to severe underweight
Gender of Patients				
Male	3,369	73.2	15.0	11.9
Female	6,004	83.6	8.5	7.8
Race of patients				
Black	451	74.3	12.4	13.3
Other race	8,922	80.2	10.8	9.1
Employment status				
Employed	4,197	83.5	9.4	7.1
Unemployed	4,928	77.0	11.8	11.1
Smoking Status				
Non-smokers	7,498	81.0	10.0	9.0
Current smokers	916	75.4	15.2	9.4
Alcohol consumption				
Non-alcohol users	7,162	80.3	10.3	9.4
Current alcohol use	989	80.8	11.8	7.4
Baseline Hb Measurement				
Normal Hb	1,819	90.7	5.8	3.5
Mild Anaemia	3,805	74.7	13.2	12.1
Moderate to severe anaemia	382	56.5	18.3	25.1
Baseline CD4 Measurement				
> 200	637	90.3	5.8	3.9
100 to 199	1,800	86.5	8.1	5.4
50 to 99	1,292	81.3	10.5	8.2
< 50	2,454	69.2	14.8	16.1
Baseline WHO stage				
Stage I	5,057	87.3	7.6	5.1
Stage II	164	87.2	8.5	4.3
Stage III	3,429	71.9	14.4	13.7
Stage IV	723	64.0	17.4	18.5
TB co-infection at initiation				
No TB co-infection	7,947	81.9	10.1	7.9
TB co-infection	1,424	68.3	14.9	16.8

4.4.3 The effect of low BMI on mortality

A greater proportion of patients with very low BMI at initiation (n=194, 22%) died in the first two years of follow-up period compared to those with low BMI (n=147, 14%) and those with normal BMI measurement (8.5%).

Kaplan Maier analysis for post initiation mortality censored for transfer outs and lost to follow-up showed significantly superior 2-year survival in patients with normal baseline BMI measurements as opposed to patients with low BMI. Patients with very low baseline BMI showed poor survival compared to patients with normal BMI and those with low BMI. Patients with very low BMI showed worst survival in the first four months of therapy. A log-rank test for equality of survivor functions shows statistically significant difference in the survival probability of patients across the exposure groups (p value < 0.0001).



Crude cox regression model shows that mortality was higher in patients with very low BMI. Patients with low BMI at initiation experienced 1.9 times greater hazard of mortality

compared to patients with normal baseline BMI. Patients with very low BMI experienced 3.1 times greater hazard of mortality compared to patients with normal BMI.

In a multivariate cox regression model, patients with low baseline BMI experienced 1.4 (HR=1.4, 95% CI 1.1- 1.7) times greater hazard of mortality adjusted for haemoglobin, CD4 cell count, gender and year initiated compared to patients with normal BMI measurement. Similarly, patients with very low BMI experienced twice (HR=2.0, 95% CI 1.6-2.5) greater hazard of mortality adjusted for these predictors. Race, age at initiation, employment status, smoking, alcohol consumption, baseline TB and baseline WHO stage did not show significant effect in the multivariate cox regression model.

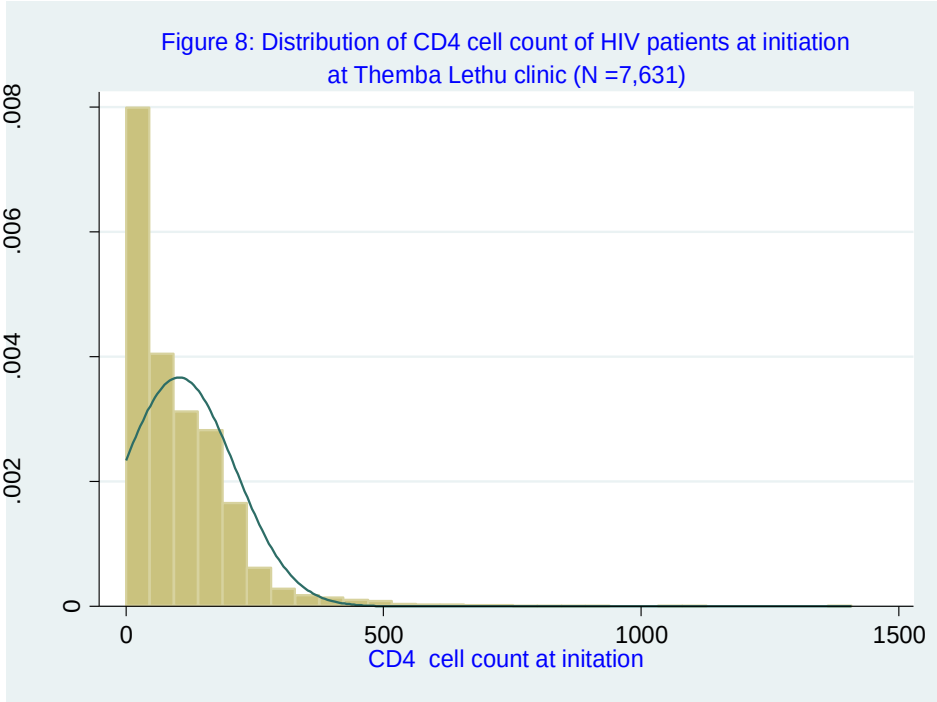
4.5 CD4 cell count and its effect on mortality

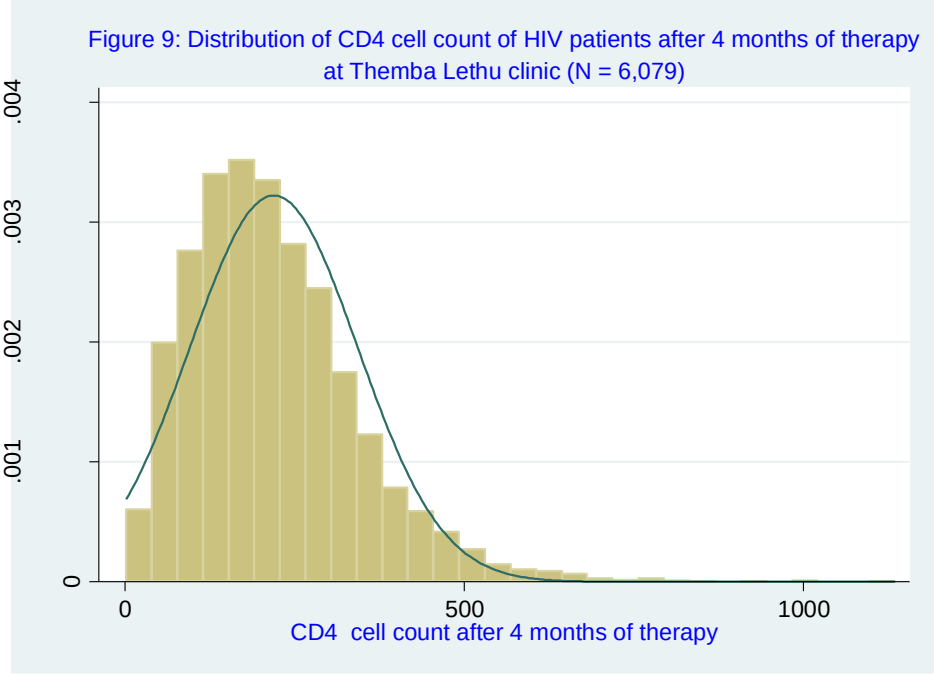
4.5.1 Measurements of CD4 cell count at baseline and follow-up period

The window period for CD4 cell measurement at initiation and follow-up visits was expanded to minimize the extent of missing values. All CD4 cell count measurements made 42 days prior to the date of initiation and 7 days after being initiated on therapy were included in the analysis. A total of 7,631 (64.2% of the sample) patients had a record of baseline CD4 cell count. Nearly 90% of these patients had a baseline CD4 cell count measurements below 200/mm^3 and 59% of the patients had baseline CD4 cell measurement of less than 100/mm^3. Almost 40% of the patients had merely less than 50/mm^3 baseline CD4 cell count. The distribution of baseline CD4 cell count measurements was skewed to the right with a median CD4 cell count of just 77/mm^3.

First follow-up CD4 cell count was measured after four months of treatment with HAART. Measurements of CD4 cell count made within a window period of 60 days (30 days prior to or 30 days) after four months of follow-up were considered for the first follow-up CD4 cell count. 6,079 recorded measurements were identified, which represents 64.8% of patients alive

and in care at four months follow-up. 49.6% these patients had a first follow-up CD4 cell count measurement below 200/mm^3 and 15.6% of the patients had first follow-up CD4 cell count of less than 100/mm^3. 3.7% of the patients had less than 50/mm^3 first follow-up CD4 cell count. As can be noted from figure 9 below, first follow-up CD4 cell count measurements were approximately normally distributed with a median CD4 cell count of 202/mm^3





There was notable improvement in CD4 cell measurements between the baseline and four months follow-up period. 87% of patients at baseline had CD4 cell measurement of less than 200 compared to only 49.6% after four months of therapy. The difference in the distribution CD4 cell measurements in figure 8 and figure 9 also suggests an overall improvement.

There were considerable missing values on follow-up CD4 cell counts especially after 10 months of follow-up period. CD4 cell count for the 2^{nd}, 3^{rd} and 4^{th} follow-up visits were measured up to 42 days prior to or 42 days after the follow-up date to minimize the extent of missing values. 3,705, representing 39.5% of patients alive and in care had measurement of CD4 cell at 10 months follow-up period. 3,674 patients, which represent 41.9% of patients alive and in care, had a recorded CD4 cell count at 16 months of follow-up period. Finally, 3,421 patients had a CD4 cell count measurement after 2 years of follow-up period, which represents 41.8% of patients alive and in care at two years of follow-up.

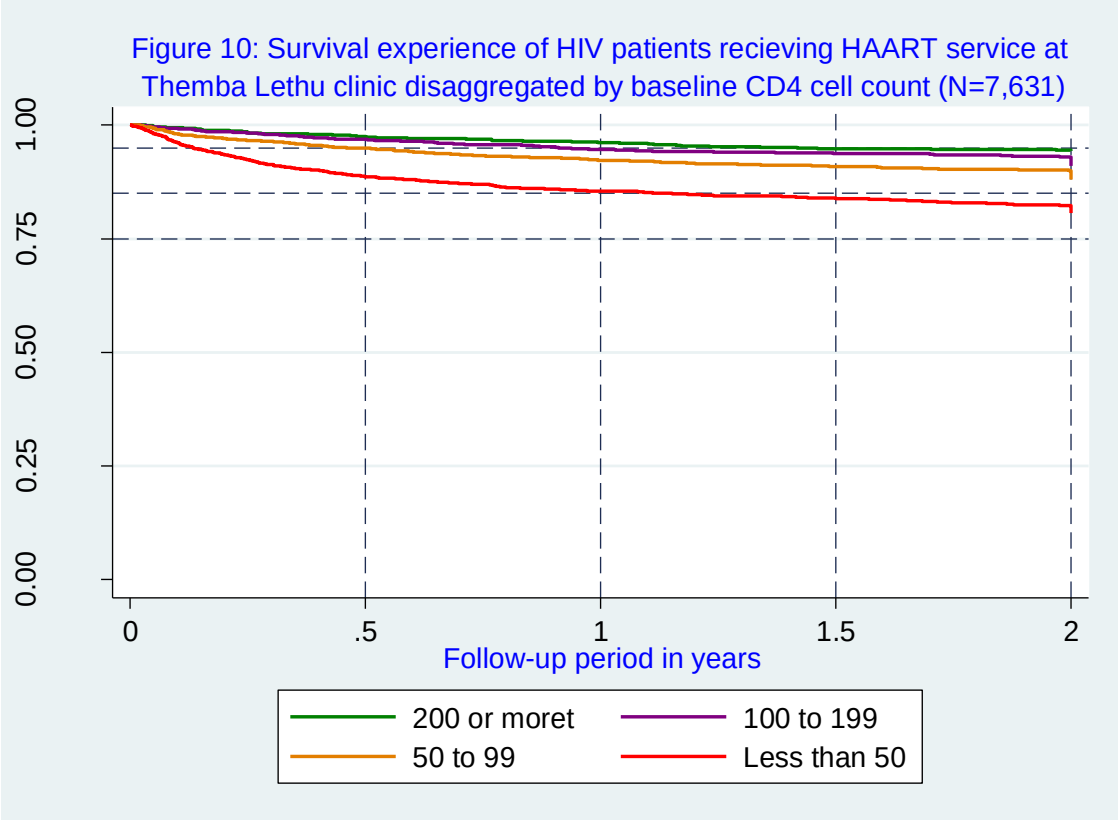
Table 8: Summary of CD4 cell counts at baseline and subsequent follow-up visits

CD4 cell count	No. Of Observations	Mean value	Std. Dev.
Baseline CD4 cell count	7,631	103	108.7
CD4count at first follow-up visit *(after 4 months of therapy)*	6,079	219	123.7
CD4count at 2nd follow-up visit *(after 10 months of therapy)*	3,705	277	139.7
CD4count at 3rd follow-up visit *(after 16 months of therapy)*	3,674	342	383.9
CD4count at 4th follow-up visit *(after 2 years of therapy)*	3,421	384	176.7

4.5.2 The effect of CD4 cell count on mortality

Most patients were initiated on therapy with very low baseline CD4 cell count. Nearly 90% of the patients had a baseline CD4 cell count of less than 200 cells/dl and more than a third of them had below 50 cell/dl baseline CD4 cell count measurement. Patients with less than 50 CD4 cell count experienced the highest rate of mortality compared to patients who had a baseline CD4 cell count of above 50 cells/dl. Kaplan Mair analysis for post initiation mortality censored for transfer outs and lost to follow-up showed significantly superior 2-

years survival in patients with a CD4 cell count greater than 200 as opposed to patients with CD4 cell count of less than 200. Patients with a baseline CD4 cell count of less than 50 showed poor survival compared to patients with higher CD4 cell count. A log-rank test for equality of survivor function shows significant difference in survival probability of patients with different CD4 cell measurements (p value < 0.0001). There was, however, no statistically significant difference in the survival experience between patients in the exposure group of 100 to 200 and 200 and above as can be seen from figure 10 below.



4.6 Composite score and risk grouping

A composite score was developed to estimate the overall risk of mortality in patients based on measurements of baseline BMI and haemoglobin. Patients were assigned score of 0, 1, 2 or 3 based on their baseline BMI and haemoglobin measurements as can be noted from table 9 below. A composite score of 0 was assigned to patients with normal BMI and haemoglobin measurements at initiation. A composite score 3 was assigned to patients with moderate to

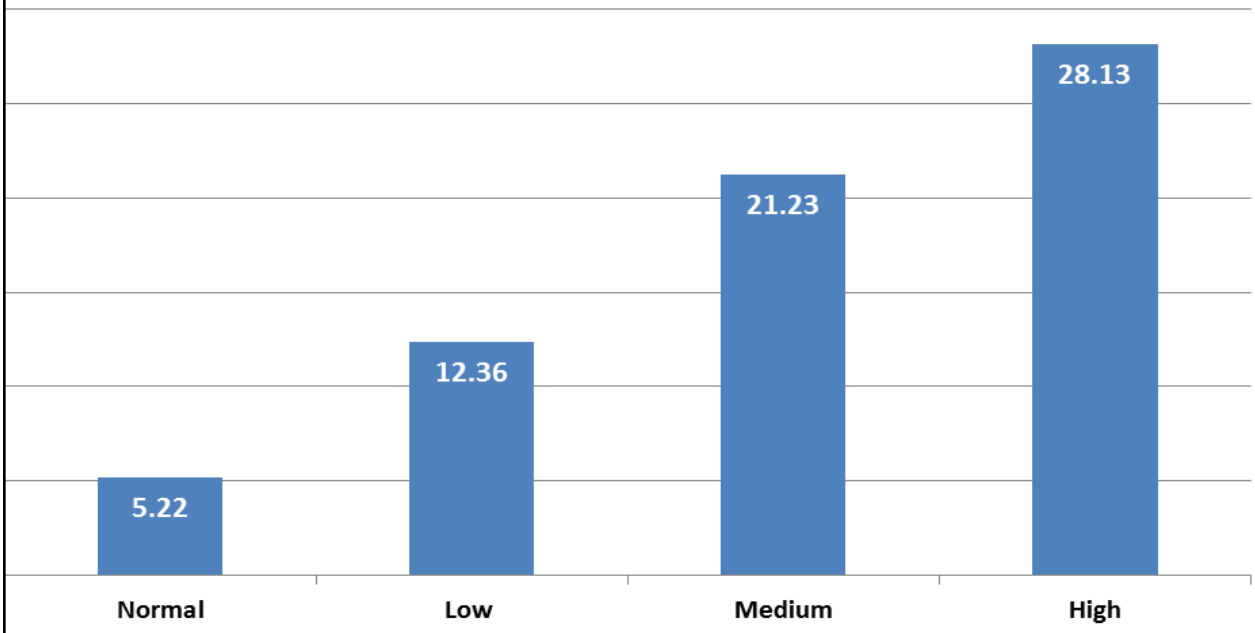
severe anaemia and a very low BMI measurement at initiation. The composite scores were used to classify patients in to four risk groups. Those with normal baseline BMI and Hb measurements were classified as a reference group. Patients with either low BMI or mild anaemia or both were classified as low risk groups. Those with moderate to either severe anaemia or moderate to severe underweight were classified as medium risk groups and those with both severe anaemia and moderate to severe underweight were classified as high risk groups.

Table 9: Classification of patients into risk groups based on baseline measurements of Hb and BMI

Low BMI / Anaemia	Normal BMI	Mild underweight	Moderate to severe underweight
Normal Hb	**0**	**1**	**2**
Mild Anaemia	**1**	**1**	**2**
Moderate to severe anaemia	**2**	**2**	**3**

The reference group with normal BMI and Hb measurements at initiation experienced merely 5.2% mortality. Patients classified as low risk groups experienced 12% of mortality while those in medium risk group experienced about 21% of mortality during the two year follow-up period. Patients in the high risk group experienced more than 28% of mortality.

Figure 11: Percentage of deaths observed in low, medium and high risk patients based on baseline BMI and Hb



Cox regression model adjusted for CD4 cell count shows high risk patients experienced 4.7 (HR = 4.7, 95% CI 2.9 – 7.6) times greater hazard of mortality compared to patients in the reference group. Patients in the medium risk group experienced 3.4 (HR = 3.4, 95% CI 2.6 – 4.4) greater hazard of mortality as opposed to patients in the reference group.

4.7 Effect of missing data on observed relationship

All baseline exposure measurements included in this analysis were within biologically plausible range. About 30% of patients with valid baseline exposure measurements were not followed-up to the end of the study due to causes other than death. Baseline exposure status was done disaggregated by censoring to determine the effect of missing data in the observed relationship. There was significant difference in the distribution of exposure variables between those who were followed-up and those who were not followed-up. Patients who were not followed-up had significantly lower baseline measurements of haemoglobin and body mass index than those who were followed-up. Table 10 below presents baseline exposure values for patients who were followed-up and those who were not followed-up

Table 10: Baseline exposure status of patients who were followed-up and those who were not followed-up

Baseline Exposure Status	Followed-up till death or end of study		Not followed-up till death or end of study	
	No.	**%**	**No.**	**%**
Normal Hb	1671	33.93	437	26.98
Mild Anaemia	3018	61.28	1024	63.21
Moderate to severe anaemia	236	**4.79**	159	**9.81**
Normal BMI	5,418	83.01	1,593	76.96
Mild Underweight	628	9.62	261	12.61
Moderate to severe underweight	481	**7.37**	216	**10.43**

4.9 Sensitivity and specificity of Hb and BMI against CD4 cell count at initiation

Sensitivity analysis was carried out for measurements made at initiation to determine how well haemoglobine and BMI discriminates positives and negatives against CD4 cell count. Optimal cut-off point that best discriminates between positive and negative test was determined using ROC curve as indicated in table 15 and figure 12 below. CD4 cell count of 200 was the cut-off point used at initiation as it also served as an indication for initiating HAART. Haemoglobine measurements of 12 and 14 were found to be the optimal cut-off points for female and male patients respectively. BMI measurement of 21 was the cut-off point determined using ROC curve for both female and male patients. Two by two tables were then constructed to classify patients based on standard test (CD4 cell count) and the alternative tests. Sensitivity and specificity were calculated from the two by two tables using the simple formula of a/(a + b) and d/(b + d), respectively.

Table 11: Area under the ROC curve for different cut-off points of haemoglobine test

Hb Cutoff point (Female, Male)	Sensitivity	1 - Specificity	Area under ROC curve
(13, 15)	0.88	0.63	0.617
(12, 14)	0.75	0.44	0.642
(11,13)	0.57	0.29	0.641
(10,12)	0.38	0.17	0.608
(9, 11)	0.25	0.11	0.573

Figure 12: ROC graph for varied cut-off points of BMI test

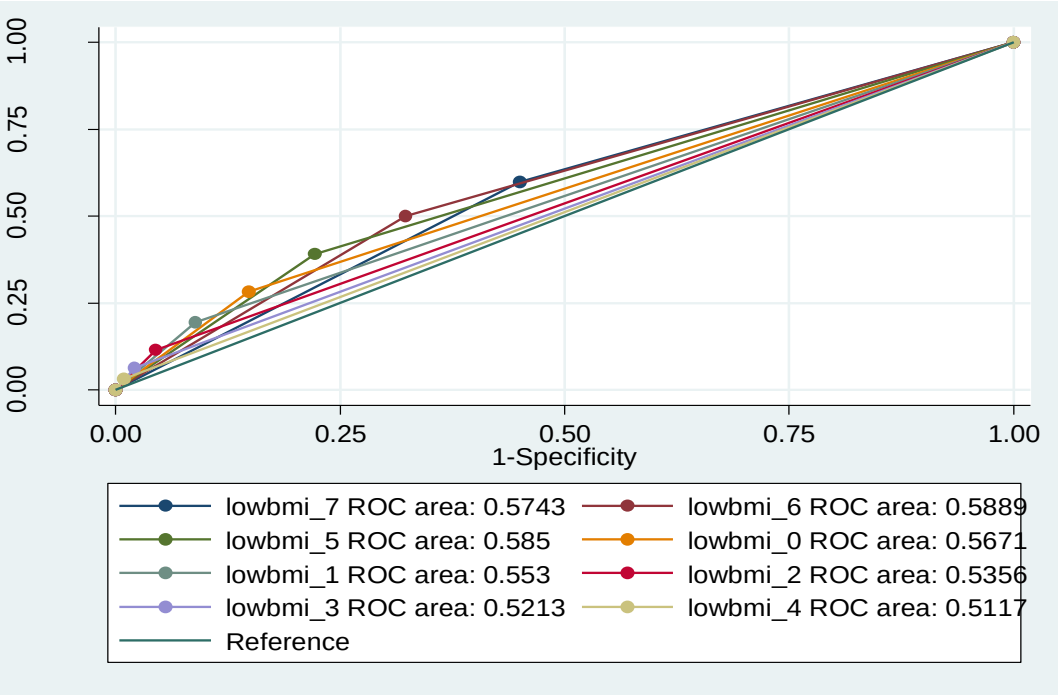


Table 12: Classification of patients based on CD4 cell count and haemoglobine at initiation

Haemoglobine below 12 and 14 for female and male patients	Standard test (CD4 cell count < 200 at initiation)		
	Positive	Negative	Total
Positive	397	328	725
Negative	1,372	3,835	5,207
Total	1,769	4,163	5,932

Table 13: Classification of patients based on CD4 cell count and BMI tests at initiation

BMI less than 21	Standard test (CD4 cell count < 200 at initiation)		
	Positive	Negative	Total
Positive	379	181	560
Negative	2,582	2,592	5,174
Total	2,961	2,773	5,734

Haemoglobin measurements at initiation had a sensitivity of 75% in correctly identifying patients with CD4 cell count of less than 200 cells/dl and a specificity of 56%. Body mass index measurements had 50% of sensitivity and 68% of specificity at initiation.

CHAPTER 5

DISCUSSION

This section discusses findings of the analysis in the context of relevant literatures and existing body of knowledge. The prognostic values of haemoglobin and body mass index are interpreted given the health care infrastructure in South Africa. Limitation of the study in terms of missing values, bias and generalizability to other settings are also highlighted.

An estimated 34 million people are living with HIV and AIDS worldwide. Africa, particularly Sub-Saharan Africa (SSA), remains the epicentre of the epidemic with two third of global cases although it is home to only 12% of the world population [55]. Over the last decade, the epidemic has stabilized in most sub-Saharan countries, albeit at unacceptably high level. South Africa is experiencing a generalized HIV epidemic and has more people living with HIV and AIDS than any other country in the world, estimated to number around 5.6 million [56]. South Africa currently ranks the third highest in the world in terms of the TB burden. South Africa has one of the highest TB/HIV co-infection rates with an HIV prevalence of almost 75% among people with incident tuberculosis [57].

The advent of HAART and other interventions have enabled HIV to be managed as a chronic condition requiring a lifelong care [8]. However, treatment coverage has been very low in most of the countries hardest hit by the epidemic. Large scale counselling and testing programs were encouraged to bridge the treatment gap. In 2010, the government of South Africa scaled up its response to HIV with a massive campaign to mobilize all South Africans to get tested and know their HIV status [58]. South Africa's policy on voluntary counselling and testing was expanded to provider-initiated HIV Counselling and Testing (HCT) that places an obligation on the health care worker to explain to patients the importance of

knowing one's HIV status [59]. Over 13 million individuals were tested during the campaign and more than 2 million newly diagnosed HIV positive persons were identified (NDOH HCT campaign update, January 2012). The Government of South Africa is currently intensifying a second round of HCT campaign. The revised HIV treatment guideline has also raised the CD4 thresholds for accessing treatment to350 cells/dl from its previous level of 200. The newly diagnosed HIV positive persons will eventually need to be initiated on HAART and this will dramatically increase the treatment burden. This will put enormous strain on the public health systems [60]. The public health sector will have to undergo unprecedented level of reorganisation and integration of services to accommodate this scale of increase.

Sub-Saharan Africa suffers from the world's most pronounced crisis in human resources for health. These shortages intensify--and are intensified by--the HIV/AIDS pandemic; HIV has substantially increased the burden of disease and stretched the human resources of the public health sector beyond the limit. HIV also takes a direct toll on the health care work force through illness and death [61]. One response to this shortage has been to de-professionalise HIV care and reassign clinical roles by shifting tasks to different cadres of health workers such as involving nurses in initiating ART and prescribing drugs [62].

Reengineering of primary health care facilities and shifting much of the treatment and care burden down to clinic and health centre level is a strategy employed in South Africa. HAART programs are being decentralized and integrated into the primary health care systems to achieve universal coverage and avoid a situation where few referral facilities are stretched beyond capacity [63]. There is evidence that provision of HAART in rural communities is feasible, given appropriate resources and infrastructure [64, 65]. The South African government has made a policy decision to expand access to HIV care and treatment by capacitating all health care facilities in the country to provide treatment services through a nurse initiated management of HAART as there are few doctors in the public sector to

follow-up millions of patients. Theses primary health care facilities operate at low capacity and are, most of the time, not equipped to handle advanced laboratory technologies that are currently used in monitoring HIV infection and therapeutic response to HAART [6].

Measurement of CD4 cell counts and plasma viral loads are the standard reference markers currently used in monitoring progression of HIV infection and therapeutic response to HAART [4]. There is limited access to these monitoring tools in most primary health care facilities [5, 6]. The public health sector has to adopt an alternative markers that can help in reliably identifying patients at greater risk of mortality in resource limited settings such as rural clinics and health centres so as to fast track treatment initiation.

Anaemia is the most common haematological complication observed in HIV infected patients [44] and is associated with increased risk of progression to AIDS and death [45, 46]. HIV related anaemia is also reversible when patients are treated with HAART, hence it can have good application in identifying patients at greater risk of mortality and measuring therapeutic response [47]. Most of the patients initiated at Themba Lethu clinic had low baseline haemoglobin measurements that correspond with low baseline CD4 cell count. Measurements of both Hb and CD4 cell count improved in subsequent follow-up visits, a positive response to HAART.

The data from Themba Lethu clinic shows that anaemia was the strongest predictor of mortality post initiation. Patients with mild anaemia at initiation experienced 2.4 (95% CI, 2.0 - 2.9) times greater hazard of mortality compared to patients with normal baseline haemoglobin level. Patients with moderate to severe anaemia experienced 4.6 (95% CI, 3.6 - 5.0) times greater hazard of mortality compared to patients with normal baseline haemoglobin measurement. The hazard of mortality associated with moderate to severe anaemia adjusted for the effects of BMI and CD4 was 2.6 (HR = 2.6, 95% CI 1.8 - 3.6). Low

haemoglobin measurement maintained significant effect on mortality in the subgroups of baseline WHO stage, CD4 count and BMI.

Stratified risk analysis shows that patients with normal measurements of haemoglobin, BMI and CD4 cell count experienced the lowest risk of mortality standing at just 17 per 1000 person years at risk. The risk increases to 134 deaths per 1000 person years at risk in patients with moderate to severe baseline haemoglobin, keeping measurements of BMI and CD4 constant. The stratified and adjusted hazards indicate that haemoglobin was the strongest independent predictor of mortality compared to the standard reference markers of CD4 cell count and viral load.

Measurements of haemoglobin and CD4 cell count do not correlate very well despite having independent prognostic values. Generally there exists positive but very weak degree of correlation between the two measurements. Haemoglobin test has a sensitivity of 75% in correctly identifying patients with a CD4 cell count of less than 200. About 75% of patients with low haemoglobin measurements also had a CD4 cell count of less than 200. However, the specificity of haemoglobin test is only 66%. This would mean 34% of patients who are tested negative would actually have a CD4 cell count of below 200.

Perhaps there is no linear relationship between the new markers and CD4 cell count and non-parametric analysis could have shown strong correlation. Non-parametric analysis is beyond the scope of this small research work.

Unexplained weight loss as little as 5% to 10% has been demonstrated to be predictive of decreased survival and increased risk of developing AIDS defining opportunistic infections [48]. Body mass index (BMI) is a sensitive anthropometric measure that may assist in therapeutic decision making [49].

Based on WHO weight classification about one fifth of the patients at Themba Lethu clinic were underweight at initiation and 8.9% of them were classified as having very low baseline BMI measurement. Hence, the majority of patients had a normal or above the normal BMI measurement. This may not be a true reflection of weight change due to progression of AIDS. Obesity is rampant in South Africa especially among African women [66]. Hence, a single weight measurement at initiation would be less informative of weight loss due to HIV infection. Weight loss in the period leading to the date of initiation could be a better indicator of disease progression [48].

The data from Themba Lethu clinic shows that body mass index was excellent predictor of mortality after haemoglobin. Patients with very low BMI measurement at initiation experienced 3.1 times greater hazard of mortality compared to patients with normal baseline BMI in a uni-variable analysis. When adjusted for the effects of haemoglobin, CD4 cell count, gender and year initiated, patients with very low BMI experienced twice (95% CI, 1.6, 2.5) greater hazard of mortality. Patients with low baseline BMI measurement experienced 1.4 (95% CI, 1.1, 1.7) times greater hazard of mortality adjusted for compared to patients with normal BMI measurement at initiation. Patients who were exposed to moderate to severe anaemia, very low BMI and with a CD4 cell count of less than 50 experienced exceptionally high risk of mortality standing at 269 deaths per 1000 person-years at risk.

Patients classified as low risk groups based on their baseline BMI and haemoglobin measurements experienced merely 8.5% of mortality while those in medium risk group experienced about 19% of mortality during the two year follow-up period. Patients in the high risk group experienced more than 28% of mortality.

Patients were reclassified into low, medium and high risk groups based on the composite score from haemoglobin and body mass index measurements. The score seem to improve prediction of overall risk of mortality. Cox regression model adjusted for CD4 cell count

shows high risk patients experienced 4.7 (HR = 4.7, 95% CI 2.9 – 7.6) times greater hazard of mortality compared to patients in the low risk group. Patients in the medium risk group experienced 3.4 (HR = 2.6, 95% CI 2.6 – 4.4) times greater hazard of mortality as opposed to patients in the low risk group.

Generally, there exists positive but weak correlation between measurements of BMI and CD4 cell count. BMI has very low sensitivity (50%) in correctly identifying patients with a CD4 cell count of less than 200 at initiation and a specificity of 68%. The inter-rater agreement between measurements of BMI and CD4 cell count at initiation is only 19.7%. The inter-rater agreement at first and second follow-up visits is 50% and 67.2% respectively. Combining measurement of haemoglobin and BMI does not seem to improve the sensitivity and specificity of alternative tests in discriminating patients eligible for HAART services.

5.1 Limitations of the study

Missing data: the dataset required for this study was obtained from the existing database of HIV patients at Themba Lethu Clinic. The researcher did not have control on the quality of data in terms of its validity and completeness. However, Themba Lethu clinic employs qualified staff members with relevant training background and skill and the latest technology to manage their data. There were specified window periods for which measurements were considered at initiation and follow-up visits. This was done intentionally to ensure consistency of measurements. Some measurements were not included in the analysis because they were not performed within the defined window period. This increased the magnitude of missing values that might have had some effect in the observed statistical relationship between the exposure and outcome variables. Missing data might be the result of missed clinic appointments or unconfirmed outcomes. While censoring by the end of the study is expected to be independent of the outcome, the analysis shows that right censoring may not be independent of the outcome. Considering the large size of the sample, the extent of

missing values for the independent variables was not so pronounced. Hence, it is unlikely that missing values could bias the observed relationship.

Confounding: The distribution of potential confounders across the exposure groups was assessed using chi square analysis. There was uneven distribution of potential confounders across the exposure groups. Stratified analysis was carried out for variable that showed significant effect. Cox regression analysis was carried out to adjust for the effect of potential confounders. Therefore, confounding effects of risk factors was controlled and it unlikely that the observed effects could be explained by confounding.

Bias: This research is based on retrospective analysis of cohort data at Themba Lethu clinic. Hence, it is susceptible to follow-up bias. The researcher could not determine the true status of patients who are lost to follow-up. Some individuals might have dropped-off from the treatment program due to severe illness; others might have been lost-to-follow-up due to death yet their true status may not have been ascertained correctly; individuals who were initiated on this facility might have changed their residence and continued their follow-up in other facility. Follow-up bias might occur if there was systematic difference between the exposure groups in the degree of lost to follow-up. However, Themba Lethu clinic has a team of staff dedicated to tracing patients who are lost to follow-up and ascertaining their true status. Patient retention was also very high. Less than 20% of patients who were initiated at the clinic were either lost to follow-up or continue their care in other facility. The observed effects are less likely to be due to bias considering the large sample size and low attrition during follow-up period.

Generalizability: the data has got limitation in terms of being representative of the general HIV infected people in South Africa. The database of Themba Lethu Clinic contains information of a small fraction of the entire HIV infected patients in South Africa and may not be representative in terms of age, gender, race, socio-economic factors and other

variables. Themba Lethu Clinic provides free HAART services to HIV patients residing around Johannesburg metropolitan area and does not have good representation of rural patients and patients receiving their care from private facilities. The outcomes of this study may not be directly generalized to affluent patients who receive their treatment services from well-resourced private health care facilities as they may not be comparable with respect to other risk factors. Children and pregnant women were excluded from the study. Hence, the results of this study cannot be generalized to these population groups.

5.2 Ethical Considerations

Ethical clearance was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. Approval to use the data set was obtained from Themba Lethu Clinic and the Clinical HIV Research Unit. The researcher strictly followed the standard operating procedures of the unit. The identity of patient records was concealed to protect their privacy and ensure confidentiality. The information was never disclosed to any unauthorized party and the data was kept in a pass word protected folders.

5.3 Conclusion

Haemoglobin and body mass index provide excellent prognostic information independent of CD4 cell count in HIV positive patients newly initiated on HAART. They can be used to reliably predict mortality. Combining measurements of haemoglobin and BMI through composite scoring improves their predictive ability. They can have good clinical application in rural and remote facilities to screen patients at greater risk of mortality and fast track them for clinical and diagnostic services.

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Addendum 1: Scatter plot for log transformed Hb and CD4 measurements

Addendum 2: Ethical clearance from the University of Witwatersrand, School of Public Health

Addendum 3: Permission to use confidential clinical data from Themba Lethu Clinic