Incidence of and risk factors for hepatotoxicity following antiretroviral initiation in patients attending Themba Lethu Clinic, Johannesburg

A Research Report Presented

by

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CANDIDATE'S DECLARATION

I, Munamato Mirira (student number 304786) am a post-graduate student registered for the degree MSc Epidemiology (in the field of Biostatistics and Epidemiology) in the School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg

I am submitting written work for the research report component of the afore-mentioned
degree.

I hereby declare the following:

- I confirm that the report I am submitting is my own work, except where I have stated otherwise.
- The work has not been submitted before for any degree or examination at this or any other University.
- I have followed the required conventions in referencing the thoughts and ideas of others

Signed: Munamato Mirira
This 7th Day of October 2011

DEDICATION

This work is dedicated to my parents Vengesayi and Juliet Mirira, my wife Linda who stood firm during the course of the past year and to our children Tawananyasha and Juliet for their patience and endurance while I was away from home.

ABSTRACT

Background and Objectives

The advent of Highly Active Antiretroviral Therapy (HAART) has resulted in a significant reduction in HIV/AIDS related morbidity and mortality in sub-Saharan Africa. However, toxicities due to HAART continue to pose challenges to the success of different regimens. Severe hepatotoxicity is one of the significant adverse events occurring in patients on HAART. Information on the incidence and risk factors for severe hepatotoxicity in cohorts from resource poor settings is limited. It is against this background that we undertook the study to determine the incidence and explore factors associated with severe hepatotoxicity following HAART initiation in a South African cohort.

Materials and Methods

Secondary data analysis of a prospective cohort 9764 HIV-infected adult patients initiated on HAART at the Themba Lethu clinic antiretroviral rollout facility in Johannesburg, South Africa between 1st April 2004 and 30th June 2009 was conducted. Severe hepatotoxicity cases were identified within the first 12 months of initiating HAART as grade 3 or 4 elevation in baseline ALT levels. The incidence rate of severe hepatotoxicity was calculated and potential socio-demographic and clinical predictors were explored using Cox proportional hazard regression modelling.

Results

At baseline, 91.8% of patients were commenced on an efavirenz-based regimen while only 8.2% were on a nevirapine-based regimen. The median CD4 count at

initiation of HAART for this cohort was 80 cells/ mm^3, a figure lower than the Department of Health (DoH) CD4 cut off for initiating HAART of 200 cells/ mm^3.

The overall incidence rate of severe hepatotoxicity was 10.7 (95% CI: 8.7 – 13.1) cases per 1000 p-yrs of follow-up. The period with the highest risk of severe hepatotoxicity was within 2 months of initiating HAART. Incidence of severe hepatotoxicity was 21.1(95% CI: 12.7 – 34.9) cases per 1000 p-yrs among patients on a nevirapine-based regimen and 9.7 (95% CI: 7.8 – 12.1) cases per 1000 p-yrs in those on an efavirenz-based one.

The hazard for severe hepatotoxicity within the first year of initiating HAART was 2.17 times higher in individuals on a nevirapine-based regimen compared to those on an efavirenz-based regimen after adjusting for baseline ALT, baseline CD4, age and gender (HR = 2.17; 95%CI = 1.18 – 3.97; p = 0.013). Though imprecise, the estimate for baseline ALT category suggested an increased risk for severe hepatotoxicity in individuals with a baseline ALT more than 40 I.U/L compared to those with a baseline ALT of less than 40 I.U/L (HR = 1.63; 95%CI = 1.00 – 2.67; p = 0.050).

Conclusion

The results of the study suggest that severe hepatotoxicity following initiation of HAART in this cohort is low compared to other previously studied cohorts. The high incidence rate of severe hepatotoxicity in the first two months of initiating HAART necessitates the need for more frequent and careful monitoring of ALT levels early during therapy. Patients on a nevirapine-based regimen have a higher risk of developing severe hepatotoxicity when compared to their counterparts on an efavirenz-based regimen, a result consistent with findings from previous studies.

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NOMENCLATURE

ACTG	AIDS Clinical Trials Group
ALT	alanine aminotransferase enzyme
HIV	human immunodeficiency virus
AIDS	Acquired Immunodeficiency Syndrome
HAART	Highly Active Antiretroviral Therapy
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organisation
DoH	Department of Health (South Africa)
BMI	body mass index
HBV	hepatitis B virus
HCV	hepatitis C virus

CHAPTER ONE

INTRODUCTION

Introduction: *The significance of Highly Active Antiretroviral Therapy (HAART) programs in Sub-Saharan Africa, a region severely affected by HIV/AIDS, is discussed in this chapter. Hepatotoxicity in patients on HAART is reviewed. A discussion on published literature of factors associated with hepatotoxicity in patients on HAART is outlined. The chapter ends with the study's aims and objectives outlined in the report.*

Background

The introduction of highly active antiretroviral therapy (HAART) has improved survival and life expectancy in HIV-infected patients [1-5]. . However, this success has also resulted in the emergence of adverse events, some of which might interrupt antiretroviral therapy intake or adherence. Anaemia, skin rash, fat re-distribution syndrome, peripheral neuropathy and hepatotoxicity are among the most common adverse events following initiation of antiretroviral therapy by HIV-infected patients [3, 6-7].

Hepatotoxicity is one of the common adverse events in patients on antiretroviral therapy. It can result in interruption of therapy, clinical hepatitis and even death [8]. All antiretroviral classes are associated with hepatotoxicity, though this is more commonly seen with the Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) [8-9]. The South African Department of Health (DoH) estimates that hepatotoxicity

occurs in 8-18% of patients on antiretroviral drugs and in some cases the event may even be fatal [10]. It is for this reason that the liver function of patients initiated on antiretroviral therapy is assessed by checking levels of liver enzymes regularly.

While several retrospective and prospective clinic-based cohort studies in resource-rich settings have examined associations between specific antiretroviral regimens, socio-demographic and clinical factors and the development of hepatotoxicity in patients initiated on antiretroviral therapy [3, 8, 11-18], the incidence and risk factors of antiretroviral-associated hepatotoxicity in resource poor settings has been described in a limited number of studies [19-22].

Problem Statement

The rapid scale up of HAART programmes in sub-Saharan Africa, a region at the epicentre of the HIV/AIDS epidemic, suggests that the incidence of antiretroviral associated hepatotoxicity may also rise. The serious complications of developing hepatotoxicity (clinical hepatitis or death) make the public health impact of hepatotoxicity of particular concern in our setting. While broad patterns of hepatotoxicity have emerged [13, 23], differing duration of clinical monitoring and lack of standardised definitions of hepatotoxicity in previous studies makes comparisons across studies difficult.

Justification for study

Aside from the association with high morbidity and mortality, hepatotoxicity may also lead to interruption of and poor adherence to antiretroviral treatment [3]. It is therefore important to understand and explore possible factors associated with

hepatotoxicity in patients initiated on antiretroviral therapy. This information will assist policy makers to modify current guidelines to reduce the number of patients developing hepatotoxicity following initiation of antiretroviral therapy. Previous work in this field has largely been carried out in resource-rich settings where patient characteristics and antiretroviral regimens are different from resource-limited settings and hence their findings might not be applicable to this setting.

Literature Review

Scale of the HIV epidemic

The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that 1.9 million people in Sub-Saharan Africa became newly infected with HIV in 2008 bringing the total number of people living with HIV in this region to 22.4 million [5] . Treatment scale-up programmes in Sub-Saharan Africa have significantly reduced HIV-related mortality and orphan-hood [5]. In Botswana, where antiretroviral therapy coverage exceeds 80%, there has been a more than 50% decline of the annual number of AIDS-related deaths between 2003 and 2007, and a 40% decline in children newly orphaned by AIDS [24]. However, HAART use has also been associated with a number of adverse events, which include anaemia, skin rashes, peripheral neuropathy, fat re-distribution syndrome and hepatotoxicity [3, 6-8]. Severe hepatotoxicity is one of the frequently described life threatening adverse events encountered by patients on HAART [8]. This has necessitated the need for close patient monitoring during treatment.

Definitions of hepatotoxicity

According to the AIDS Clinical Trials Group (ACTG), liver enzyme elevations are categorised into four grades according to severity. The grades are based on elevation of Alanine aminotransferase (ALT) from the upper limit of normal (ULN): grade 0, <1.25 x ULN; grade 1, 1.25-2.5 x ULN; grade 2, 2.6-5 x ULN; grade 3, 5.1-10 x ULN; and grade 4, > 10 x ULN [25]. Several different definitions of hepatotoxicity have been adopted across studies to date. Some studies have used grade 3 and 4 elevation of liver enzymes to define hepatotoxicity [8, 12-13, 15, 17, 22, 26-27], whereas others have considered grade 1 elevation in liver enzymes [20, 28] or grade 2 elevation in liver enzymes [29] to be evidence of hepatotoxicity.
For the purposes of this study, severe hepatotoxicity is defined as ACTG grade 3 or 4 elevation in ALT blood levels.

Incidence of severe hepatotoxicity

There is limited information on the incidence of severe hepatotoxicity in a general HAART roll-out clinic in South Africa. An incidence rate of 77 cases per 1000 person-years (p-yrs) of follow-up time has been reported in a study conducted on a South African cohort. However, the study was conducted in a mining environment where the participants were mainly male and a high proportion of individuals were on anti-tuberculous drugs at the time of initiating HAART [26].

An Italian study reported an incidence rate of severe hepatotoxicity of 177.1 per 1000 p-yrs of follow-up time [30]. This study was done on a cohort that has different first-line HAART regimens from ours. 52% of the clients in this cohort were on a protease inhibitor and 48% were on an NNRTI-based regimen. All the participants in

this study were also co-infected with Hepatitis C virus (HCV) which is rare in sub-Saharan Africa [31]. It is therefore difficult to extrapolate these results to our setting.

Risk factors for development of hepatotoxicity

Hepatotoxicity is a well described component of adverse events seen in patients on antiretroviral therapy and is seen with almost all classes of antiretroviral drugs [11-13]. However, a number of studies have shown higher frequencies of hepatotoxicity in patients on nevirapine-based regimens (4-18%) compared to those on efavirenz-based regimens (1-8%) [8, 14-15, 28].

HIV and hepatitis B virus (HBV) co-infection is more common in some parts of sub-Saharan Africa than it is in resource-rich countries [20, 32]. Co-infection with HBV has been identified as an independent risk factor for severe hepatotoxicity in patients on antiretroviral therapy in South African cohorts [26, 33]. Similar results have also been noted in resource rich settings in Europe and China where patients co-infected with HBV are more likely to develop severe hepatotoxicity while on HAART compared to patients who are not [12-13, 28].

Although HCV infection is not common in our setting [31], available evidence has consistently demonstrated that co-infection with HCV significantly increases the risk of HAART associated hepatotoxicity [34-35]. Several mechanisms may account for hepatotoxicity among patients with HIV/HCV co-infection. Firstly, HCV infection leads to significant underlying liver damage in an individual thereby increasing the susceptibility to potential hepatotoxic HAART drugs. Furthermore, HCV/HIV co-infection has been demonstrated to result in accumulation of functional HIV-specific T-cells in the liver thereby resulting in accelerated progression of liver disease [36].

Conflicting evidence exists as to whether a high CD4 count is a risk factor for hepatotoxicity in patients on a nevirapine-based HAART regimen. Female patients with CD4 counts greater than 250 cells/mm^3 who are initiated on a nevirapine based regimen have been demonstrated to have a 12-fold increased risk of developing severe hepatotoxicity [11], while their male counterparts have a 5-fold risk of severe hepatotoxicity if their CD4 counts exceed 500 cells/mm^3 [8]. However, some studies failed to demonstrate an association between a high CD4 count and the development of severe hepatotoxicity in female patients [37-38].This could have been due to different study populations as the studies which demonstrated an association were mainly done in populations with a high HIV/HCV co-infection. This relationship is of important significance in sub-Saharan Africa since most antiretroviral programs use nevirapine-based regimens.

High HIV infection rates and weak health-care systems in sub-Saharan Africa have been noted to be driving the tuberculosis epidemic [19, 39]. The fact that tuberculosis is the commonest opportunistic infection in our setting is of great significance as co-administration of tuberculosis treatment and HAART is inevitable. Studies conducted in South Africa have demonstrated that co-administration of anti-tuberculous drugs in patients on HAART increases the risk hepatotoxicity [26, 40].

Higher levels of baseline Alanine aminotransferase (ALT) have been associated with the development of severe hepatotoxicity in patients on HAART [11, 21, 29, 37]. The lack of an association between baseline ALT levels and development of

hepatotoxicity in other studies [16-17] could be due to methodological inadequacies such as bias and poor sample size.

Heavy alcohol consumption has been demonstrated to increase the risk of hepatotoxicity in patients initiated on antiretroviral therapy [16, 41]. Paucity of information on alcohol as a possible risk factor in many studies could be attributed to the crude measure of alcohol use by investigators. It may also be due to the fact that fewer studies collected this type data.

Advancing age has also been associated with increased risk of hepatotoxicity in patients on antiretroviral treatment [16].

Rapid CD4 count increases while on HAART have been postulated to increase the risk of developing hepatotoxicity in some studies. A prospective cohort study in the United States of America (USA) demonstrated that a CD4 count increase of more than 50 cells/mm^3 in a 25 week follow-up period increased the risk of hepatotoxicity by 3.6 times [18].

The debate over potential gender differences in risk of developing hepatotoxicity while on HAART is ongoing and the results are conflicting. Some studies have demonstrated an increased risk of hepatotoxicity in female patients compared to their male counterparts [42-43].The association was stronger in females with a body mass index (BMI) of less than 18.5 [22]. Some studies failed to demonstrate such association [44-45]. A common weakness of these studies is that they included a relatively small number of women participants and hence were not sufficiently powered to detect sex-based differences.

Some independent viral, biochemical and haematological risk factors for HAART-induced hepatotoxicity have been described in a South African randomised, double-blinded multicentre trial. These factors included a protein serum level of less than 35g/L, a mean corpuscular volume greater than 85fL, a plasma HIV-1 RNA load of less than 2000copies/ml and a lactate dehydrogenase level of less than 164 IU/L [22].

Limitations of previous studies

Besides the lack of a standard definition of hepatotoxicity, most of the studies looking at the factors associated with hepatotoxicity in patients on HAART were done in resource rich settings on European cohorts [3, 8, 11-18]. A few studies have looked at African cohorts in resource poor settings [19-22]; however, these settings face challenges of limited regimen options and poor laboratory facilities to monitor patients adequately.

Different patient follow-up time to the development of hepatotoxicity also makes it difficult to extrapolate different study findings to our setting. Not all studies considered the same possible predictors of hepatotoxicity and hence confounding could have played a factor in some of the observed associations or lack of it.

There is limited data from resource limited cohorts especially in Sub-Saharan Africa. Hence this research project to investigate the incidence of and risk factors for hepatotoxicity in patients initiated on HAART at an antiretroviral rollout-clinic in Johannesburg, South Africa.

Definition of terms

Alanine aminotransferase (ALT): A liver enzyme that generally indicates liver damage (hepatotoxicity) when found in blood in elevated quantities. Normal levels are usually less than or equal to 40 International Units per litre (I.U/L).

Highly Active Antiretroviral Therapy (HAART): The use of three or more anti-HIV drugs in order to decrease viral multiplication and progress of HIV disease.

Efavirenz-based HAART: Use of stavudine, lamivudine and efavirenz drugs for managing HIV/AIDS

Nevirapine-based HAART: Use of stavudine, lamivudine and nevirapine drugs for managing HIV/AIDS

Body Mass Index (BMI): It is an anthropometric measure which is calculated by dividing an individual's weight (in kilograms) by the square of height (in metres)

Study Objectives

General Objective

To determine the incidence and factors associated with severe hepatotoxicity following initiation of antiretroviral therapy in a South African cohort

Specific objectives

- To describe the baseline characteristics of patients at initiation of antiretroviral therapy
- To determine the incidence of severe hepatotoxicity within 12 months of initiating antiretroviral therapy
- To investigate factors associated with incident hepatotoxicity in patients initiated on antiretroviral therapy

CHAPTER TWO

METHODOLOGY

Introduction: *This chapter outlines the study design and methods used in the report. The study population and selection of participants is described. Details of data collection and management are described. Variables used for analyses are outlined. The definition of severe hepatotoxicity is explained and the chapter concludes with an outline of the data analysis plan and ethical considerations.*

Study Design

The study design is a cohort study. Secondary analysis of prospectively collected cohort data among patients initiating HAART at Themba Lethu Clinic between 1 April 2004 and 30 June 2009 was done.

Study Site

Themba Lethu clinic is one of the largest urban antiretroviral sites annexed to Helen Joseph Hospital, a teaching public hospital situated in Johannesburg, South Africa. It started operating in April 2004 following the antiretroviral treatment roll-out program initiated by the South African government. From the time of its inception, the clinics' enrolment has been increasing considerably. Currently, the clinic has an enrolment of over 25000 patients in care and provides HAART to over 16000 of these patients according to the National Department of Health (DoH) guidelines [46]. Even though

the clinic enrols and follow-up patients from Gauteng Province and beyond, the majority of its clients are mainly of urban origin.

Study Population

The study population consisted of all HIV positive individuals started on HAART at Themba Lethu Clinic from 1 April 2004 to 30 June 2009.

Study Sample

No sampling was done. 9764 HIV positive adults initiated on HAART at Themba Lethu Clinic between 1 April 2004 and 30 June 2009 meeting the following criteria were included in the analysis:

Inclusion criteria

- Patients started on first line HAART at Themba Lethu Clinic between 1 April 2004 and 30 June 2009.
- Patients with ALT results at baseline and at least once after initiating HAART
- HAART naïve patients
- Adults aged 18 years and older at time of HAART initiation

Exclusion Criteria

- Patients with no baseline ALT levels
- Pregnant women because they are initiated on HAART according to different guidelines and on different regimens. The haemodilution effect of pregnancy can affect laboratory results.
- Baseline ALT ≥ 104 I.U/L
- Viral load < 400 copies/mm^3

- Clients aged less than 18 years
- Clients on other HAART regimens other than the 6 possible first line combinations

Data sources

The data used in the study was recorded at the patient's initial and subsequent clinic visits. Data is stored on an electronic patient management and decision support system called Therapy Edge-HIV ^{TM}. The database is managed and maintained by the non-profit organisation, Right to Care (RTC). Data are entered directly into the system by clinical staff during patient visits. Demographic and contact details of clients are recorded at the initiation visit. Patients' vital measurements, weight and any symptoms or new diagnoses made on each subsequent visit to the clinic are also recorded and entered into Therapy Edge-HIV ^{TM}. Additionally, results of blood tests for ALT, CD4 count, haemoglobin and other laboratory tests are measured at each scheduled clinic visit and entered into the database.

The data used in analysis was obtained from variables already captured on Therapy Edge-HIV^{TM} database. The names of patients were removed from the data set and replaced with unique study numbers before the data was provided for analysis.

Study variables

Outcome variable

The risk of developing severe hepatotoxicity while on HAART with different potential risk factors (exposure variables) is estimated. Severe hepatotoxicity (the primary study outcome) was defined as either grade 3 or 4 elevation in ALT level within the

first twelve months of initiating antiretroviral treatment in patients with normal baseline ALT levels.

We followed study participants for 12 months from the day of initiating the first line HAART regimen. According to the National Department of Health (DoH) guidelines, patients are usually started on one of the following first line regimens: stavudine+lamuvidine+efavirenz, stavudine+lamuvidine+nevirapine, zidovudine+lamuvidine+efavirenz and zidovudine+lamuvidine+nevirapine [46]. Following initiation of therapy, liver function was assessed two weekly for the first month, at 8 weeks then 6 monthly for those clients on nevirapine, whereas clients on EFV had their liver function assessed at one month, and thereafter every six months following HAART initiation. A patient was regarded as having developed the event of interest if ALT was found to be elevated at these or any other clinically indicated visits.

Exposure variables

Socio-demographic variables

- Age,
- Gender
- alcohol intake status
- smoking status

Clinical variables

- baseline haemoglobin (Hb) level
- clinical HIV Stage
- HAART regimen

- diagnosis of tuberculosis at time of initiating HAART
- baseline body mass index (BMI)
- baseline CD4 count,
- baseline ALT levels

Data management and cleaning

Observations where individuals were pregnant, under 18 years of age or on other regiments than the standard first line HAART were dropped from the data set.
The de-identified nature of the dataset, made it impractical to verify values which appeared unrealistic and therefore these values were set to missing. The following variables had biologically implausible values in the dataset. The action taken was that implausible values were excluded.

- BMI (values between 15 kg/m^2 and 50 kg/m^2 were taken as plausible)
- Haemoglobin (values between 1g/dL and 18g/dL were taken as plausible)
- Age at initiation (values between 18 years and 90 years were considered)

New variables were generated; data was coded and recorded to allow for appropriate analysis in order to meet the study objectives.

Data processing methods and data analysis

Statistical analysis was done using STATA version 11.0 (STATA corporation, college station, Texas, USA).

Descriptive statistics (Table 1) were used to summarize the baseline characteristics of the cohort overall and by presence and absence of severe hepatotoxicity.

A comparison of the characteristics of the overall cohort and individuals excluded from the study on the basis of absent baseline ALT results is given in Table 2.

The overall incidence rate of severe hepatotoxicity was calculated. Incidence rates at specific time periods were determined and given in Table 3. Incidence rates by HAART regimen and baseline ALT category were also calculated and presented.

Time-to-event analysis was performed using survival techniques, including Kaplan-Meier estimates, log rank test and Cox proportional hazards models.

Univariate Cox proportional hazard models were built to determine the crude estimates between potential risk factors and severe hepatotoxicity.
Biological plausibility and change in estimate method were used to select variables for the multivariate model. Factors known to be biologically associated with severe hepatotoxicity post-HAART initiation were chosen *a priori,* together with the factor with the most significant estimate in univariate analysis to be the initial model. A possible risk factor which changed the estimate in the initial model by more than 10% was selected for the final adjusted model.
Final model adequacy and assumptions were tested for and presented in the appendix section. Interactions between exposure variables was tested for and reported.
The 5% significance level was used for all statistical significance tests in the report.

Sensitivity and specificity of clinical diagnosis of severe hepatotoxicity (with laboratory diagnosis as the gold standard) was calculated. Correlation between

clinical diagnosis of severe hepatotoxicity and biochemical diagnosis was ascertained by calculating the kappa (κ) statistic.

Ethical considerations

The study was conducted according to the Standard Operation Procedure (SOP) of the Clinical HIV Research Unit governing the analysis of data from the Themba Lethu Clinical Cohort (*appendix A)* which includes obtaining the approval of the research protocol by the University of the Witwatersrand Committee for Research on Human Subjects (Medical) *(appendix B)* and permission to conduct the study from the Chief Executive Officer of Helen Joseph Hospital where the Themba Lethu Clinic is based *(appendix C).* Names of patients were removed from the dataset and replaced by unique identifiers by personnel at the site before analysis. This was done to respect the privacy of the patients who provided the information

CHAPTER THREE

RESULTS

Introduction: *In this chapter, the results of the research report are presented by first describing how the study sample was obtained. The overall baseline characteristics of the study participants and characteristics stratified by severe hepatotoxicity post-HAART are outlined next. Incidence rates of severe hepatotoxicity are considered. Factors associated with the development of severe hepatotoxicity in this cohort are investigated and presented. The chapter concludes by investigating the correlation between clinicians' and laboratory diagnosis of severe hepatotoxicity.*

Study participants

Since its inception, Themba Lethu Clinic has enrolled 27 941 patients in care. A total of 13 983 of these patients were started on HAART between 1 April 2004 and 30 June 2009. The remainder of the enrolled patients were either not on HAART or were on HAART but enrolled outside the study period. Of the 13 983 patients who started HAART during the study period, 9764 eligible patients were included in the analysis according to the flow diagram in Figure 1.

Determination of study sample and clients at the end of the study period

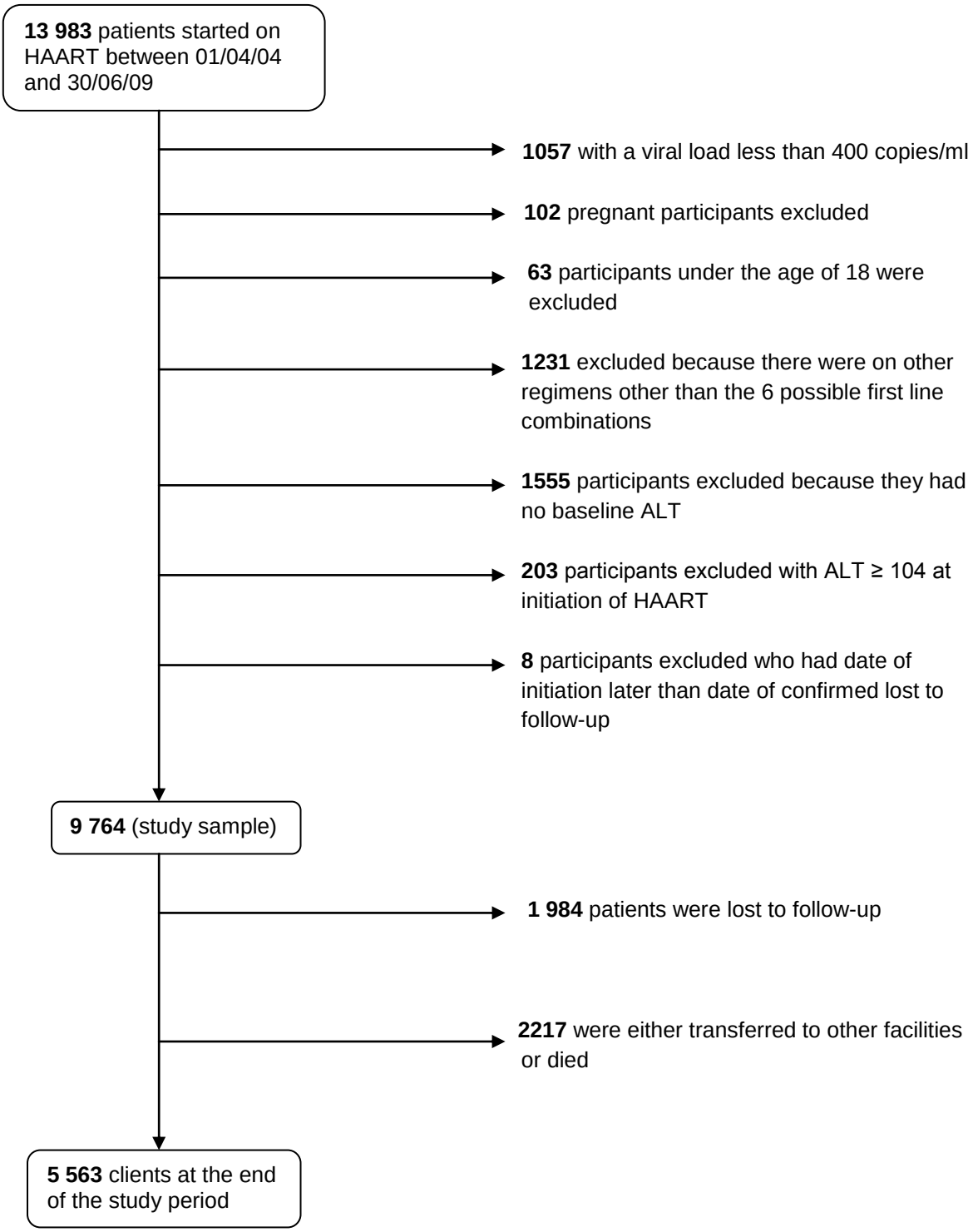


Figure 1: Flow chart showing selection of study participants and patients remaining on study at the end of the study period

After applying the inclusion and exclusion criteria *(see Figure 1 above),* 9 764 patients were left for analysis. Out of the 9 764 patients, 1 984 (20.3 %) were lost to follow-up and 2 217 (22.7 %) were either transferred to other facilities or died during the follow-up period.

Baseline characteristics of study participants

The baseline characteristics (demographic, clinical and social) of the overall cohort, as well as the baseline characteristics compared by severe hepatotoxicity post HAART initiation are presented in Table 1 below. The categorisation of severe hepatotoxicity was based on the development or absence of new hepatic disease following HAART initiation.

Table 1: Baseline characteristics of the Themba Lethu Clinic cohort

			Severe hepatotoxicity post HAART initiation	
Characteristics	**N**	**Overall** *n ,%*	**No** *n ,%*	**Yes** *n ,%*
Age in years	9764	37.4(8.7)*	37.4(8.7)*	35.4(7.0)*
Age Category (in years)	9764			
< 25 years		467(4.8)	461(4.8)	6(6.6)
25-34 years		3800(38.9)	3765(38.9)	35(38.9)
35-44 years		3689(37.8)	3648(37.7)	41(45.6)
>45 years		1808(18.5)	1800(18.6)	8(8.9)
Gender	9764			
Female		6113(62.6)	6061(62.6)	52(57.8)
Male		3651(37.4)	3613(37.4)	38(42.2)
Smoking Status	9764			
No		8730(89.4)	8652(89.4)	78(86.7)
Yes		1034(10.6)	1022(10.6)	12(13.3)
Alcohol intake Status	9764			
Yes		8669(88.8)	8588(88.8)	81(90.0)
No		1095(11.2)	1086(11.2)	9(10.0)
BMI Category (in kg/m^2)	8510			

Normal (18.5 - 25)		4886(57.4)	4841(57.4)	45(56.3)
Underweight (<18.5)		1819(21.4)	1801(21.4)	17(21.2)
Overweight (>25)		1805(21.2)	1788(21.2)	18(22.5)
Baseline Hemoglobin (in g/dL)	9764	11.4(2.2)*	11.4(2.2)*	11.7(2.4)*
Baseline Hemoglobin Category (in g/dL)	9764			
≥8.5		8875(90.9)	8793(90.9)	82(91.1)
<8.5		889(9.1)	881(9.1)	8(8.9)
Baseline CD4 count (in cells/mm^3)	9204	80(29-149) †	81(29-149) †	57(22-126) †
CD4 count Category (in cells/mm^3)	9204			
<50		3385(36.8)	3349(36.7)	36(42.3)
50-100		1917(20.8)	1897(20.8)	20(23.5)
100-200		3133(34.0)	3110(34.1)	23(27.1)
>200		769(8.4)	763(8.4)	6(7.1)
Baseline ALT (in IU/L)	9764	23(16-34) †	23(16-34) †	30(20-41) †
ALT baseline Category (in IU/L)	9764			
<40		7989(81.8)	7222(81.9)	67(74.4)
>40		1775(18.2)	1752(18.1)	23(25.6)
History of Tuberculosis	9758			
No		7849(80.4)	7783(80.5)	66(74.2)
Yes		1909(19.6)	1886(19.5)	23(25.8)
HAART regimen	9764			
Efavirenz-based		8962(91.8)	8887(91.9)	75(83.3)
Nevirapine-based		802(8.2)	787(8.1)	15(16.7)
HIV Stage	7830			
1		3142(40.1)	3109(40.1)	33(42.3)
2		1243(15.9)	1234(15.9)	9(11.5)
3		2570(32.8)	2548(32.9)	22(28.2)
4		875(11.2)	861(11.1)	14(18.0)

*Baseline Haemoglobin and Age at initiation was described using means and standard deviations as there were fairly normally distributed (see appendix D)

†medians and interquartile range used as the data was not normally distributed (see appendix E)

Characteristics of the overall cohort

The average age of the overall cohort was 37.4 years *(std 8.4 years).* The majority of the patients were aged between 25 and 44 years of age. The number of female

patients was about double that of their males. About 10% of the overall cohort smoked while a similar proportion of participants reported taking alcohol.
20% of the individuals were considered underweight, while another 20% was considered overweight.
20% of the overall cohort had a diagnosis of tuberculosis at the time of initiating HAART. The majority of the participants were initiated on an efavirenz-based regimen, a regimen recommended by the Department of Health (DoH) guidelines for initiating antiretroviral therapy in treating naïve patients [46]. 8.2% of the overall cohort was initiated on a nevirapine-based regimen. The median baseline ALT level of the overall cohort was 28 I.U/L (IQR 16-34); with 82 % of individuals with ALT < 40 I.U/L. 9.1% of the cohort had a baseline haemoglobin < 8.5g/dL. The median baseline CD4 count at initiation of HAART for this cohort was 80 cells/ mm^3 (IQR 29-149) which is much lower than the DoH CD4 cut off for initiating HAART of 200 cells/ mm^3 [46].

Characteristics by severe hepatotoxicity post HAART initiation

Individuals who developed severe hepatotoxicity had a mean age of 35.4 years *(std 7.0 years),* two years younger than individuals who did not develop severe hepatotoxicity who had a mean age of 37.4 *(std 8.7 years).*The mean baseline haemoglobin level of individuals who developed severe hepatotoxicity was similar to the mean baseline haemoglobin level for individuals without severe hepatotoxicity*.* There were also no obvious differences in the Body Mass Index of individuals who developed severe hepatotoxicity and those ones who did not.
Individuals who developed severe hepatotoxicity had a lower median baseline CD4 count (57 cells/mm^3; IQR 22-126) compared to individuals who did not have severe

hepatotoxicity (81 cells/mm^3; IQR 29-149). The median baseline ALT level was higher in clients with severe hepatotoxicity (30 I.U/L; IQR 20-41) compared to those individuals without the outcome of interest (23 I.U/L; IQR 16-34).

About a fifth (18.0%) of the individuals who developed severe hepatotoxicity had stage 4 HIV infection while 11% of individuals who did not have severe hepatotoxicity had stage 4 HIV infection.

Characteristics of excluded individuals on the basis of missing baseline ALT results

Below (Table 2), is a comparison of the baseline characteristics of the overall cohort and the individuals excluded from the study on the basis of missing baseline ALT results.

Table 2*:* Comparison of baseline characteristics of the overall cohort and characteristics of the excluded individuals due to missing ALT results

Characteristic	**N**	**Study participants** *n ,%*	**N**	**Excluded individuals with missing baseline ALT** *n ,%*
Age (in years)	9764	37.4(8.7)*	1555	37.1(8.5)*
Age Category (in years)	9764		1555	
< 25 years		467(4.8)		82(5.3)
25-34 years		3800(38.9)		635(40.8)
35-44 years		3689(37.8)		541(34.8)
>45 years		1808(18.5)		297(19.1)
Gender	9764		1555	
Female		6113(62.6)		1012(65.1)
Male		3651(37.4)		543(34.9)
Smoking Status	9764		1555	
No		8730(89.4)		1433(92.2)
Yes		1034(10.6)		122(7.8)

Alcohol intake Status	9764		1555	
Yes		8669(88.8)		1405(90.4)
No		1095(11.2)		150(9.6)
BMI Category (in kg/m^2)	8510		557	
Normal (18.5 - 25)		4886(57.4)		331(59.4)
Underweight (<18.5)		1819(21.4)		115(20.7)
Overweight (>25)		1805(21.2)		111(19.9)
Baseline Hemoglobin (in g/dL)	9764	11.4(2.2)*	1555	10.8(2.5)*
Baseline Hemoglobin Category	9764		1555	
(in g/dL) ≥8.5		8875(90.9)		1497(96.3)
<8.5		889(9.1)		58(3.7)
Baseline CD4 count (in cells/mm^3)	9204	80(29-149) †	499	84(34 - 163) †
CD4 count Category (in cells/mm^3)	9204		499	
<50		3385(36.8)		169(33.9)
50-100		1917(20.8)		111(22.2)
100-200		3133(34.0)		152(30.5)
>200		769(8.4)		67(13.4)
History of Tuberculosis	9758		1555	
No		7849(80.4)		1322(85.0)
Yes		1909(19.6)		233(15.0)
HAART regimen	9764		1555	
Efavirenz-based		8962(91.8)		1263(81.2)
Nevirapine-based		802(8.2)		292(18.8)
HIV Stage	7830		548	
1		3142(40.1)		219(40.0)
2		1243(15.9)		80(14.6)
3		2570(32.8)		178(32.5)
4		875(11.2)		71(12.9)

*Baseline Haemoglobin and Age at initiation was described using means and standard deviations as there were fairly normally distributed (see appendix D)

†medians and interquartile range used as the data was not normally distributed (see appendix E)

The characteristics of the 1555 individuals who were excluded from the study sample looked very similar to those of the overall cohort as can be seen from Table 2 above. Besides differences in HAART regimen and Haemoglobin categories, the excluded individuals looked fairly similar to the overall cohort. The excluded group had more

than double the proportion of individuals on a nevirapine-based regimen compared to the study participants. 9.1% of individuals in the study sample were anaemic compared to only 3.1% in the excluded group.

Incidence of severe hepatotoxicity

Overall incidence rate of severe hepatotoxicity

Out of 9764 participants followed up for a total of 8424 person-years (p-yrs), with a median follow-up time of 1 year, 90 cases of severe hepatotoxicity were observed, corresponding to an overall incidence rate of 10.7 (95% CI: 8.7 – 13.1) cases per 1000 p-yrs of follow-up.

Below (Figure 2), is a Kaplan-Meier plot showing time to severe hepatotoxicity in the first year of initiating HAART.

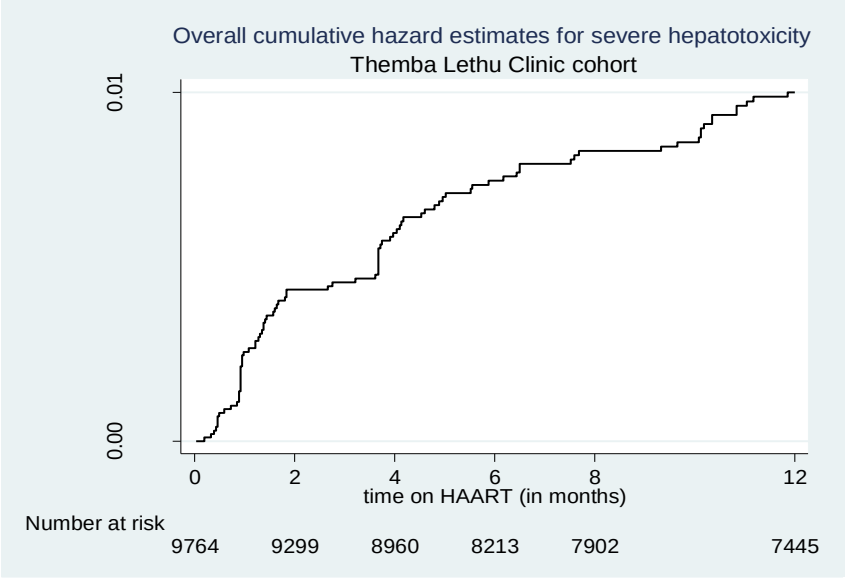


Figure 2*:* Kaplan-Meier plot showing cumulative hazard estimates for severe hepatotoxicity post-HAART

The above Kaplan-Meier plot indicates the overall risk of developing severe hepatotoxicity among the study participants. The cumulative hazard estimates of the cohort, including a risk table showing the numbers at risk for selected follow-up times are also shown.

Period incidence rates of severe hepatotoxicity

The table below (Table 3) shows the overall period incidence rates for severe hepatotoxicity at specified time periods after initiation of HAART.

Table 3 depicts that the greatest risk of developing severe hepatotoxicity occur in the first two months post-HAART initiation with an incidence rate of 26.4 per 1000 p-yrs of follow-up time. The incidence rate decreases with time after initiation of HAART to 5.1 per 1000 p-yrs of follow-up between 6 to 12 months

Table 3: Overall period incidence rates for severe hepatotoxicity at specific time periods post-HAART initiation

Time after HAART initiation	**All cases of severe hepatotoxicity** n (%)	**Period incidence (per 1000 p-yr)** (CI)
0 - 2 months	42 (46.7)	26.4 (19.5-35.7)
2 – 4 months	15 (16.7)	9.9 (5.9-16.3)
4 – 6 months	13 (14.4)	9.2 (5.3-15.9)
6 – 12 months	20 (22.2)	5.1 (3.3-8.0)

Crude estimates of risk factors for severe hepatotoxicity

1. Initiating HAART regimen

Incidence rates of severe hepatotoxicity by HAART regimen

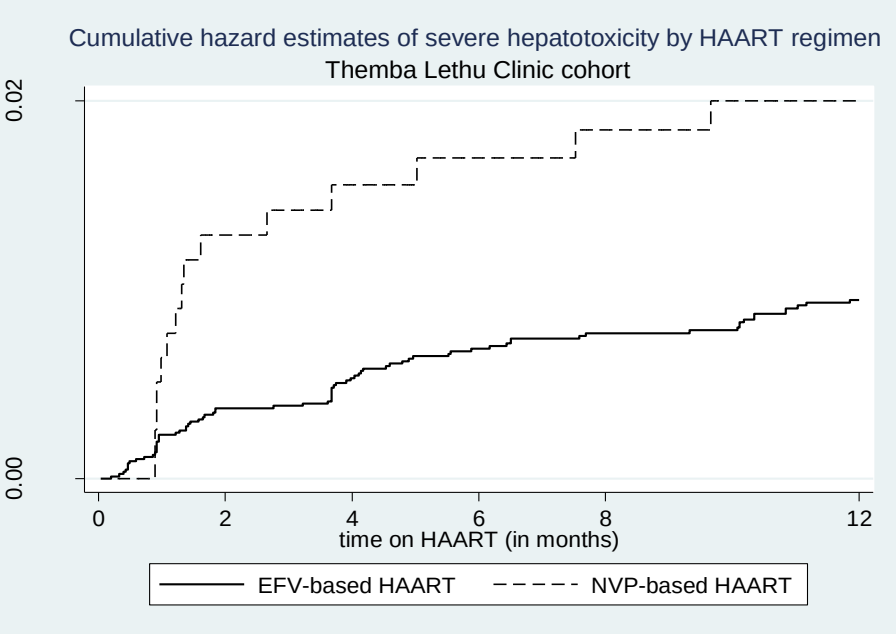
Of the 802 individuals on a nevirapine-based regimen with a total follow-up time of 712 p-yrs, 15 cases developed severe hepatotoxicity. This translates to an incidence rate of 21.1(95% CI: 12.7 – 34.9) cases per 1000 p-yrs of follow-up.

8962 individuals on an efavirenz-based regimen were followed up for a total of 7708 p-yrs. 75 of these participants developed severe hepatotoxicity corresponding to an incidence rate of 9.7 (95% CI: 7.8 – 12.1) cases per 1000 p-yrs of follow-up, which is less than half the incidence rate of a nevirapine-based regimen.

Cumulative hazard estimates for severe hepatotoxicty by HAART regimen

Of the 8962 individuals on an efavirenz-based regimen, 75 (0.8%) developed severe hepatotoxicity compared with 15 (1.9%) of the 802 individuals on nevirapine. Figure 3 below shows the cumulative hazard estimates of severe hepatotoxicity by HAART regimen and a risk table at selected time periods post HAART initiation.

From the Kaplan-Meier plot below (Figure 3), patients on a nevirapine-based regimen have greater hazard of developing hepatotoxicity after initiating HAART compared to their counterparts on an efavirenz-based regimen. The log rank test for equality of hazard was ($\chi^2 = 8.04$, $p = 0.0046$).



Time	0	2	4	6	8	12
n at risk						
NVP	8962	8525	8206	7516	7227	6804
EFV	802	774	754	697	675	641

Figure 3: Kaplan-Meier plot showing cumulative hazard estimates of hepatotoxicity by HAART regimen

2. Baseline laboratory results

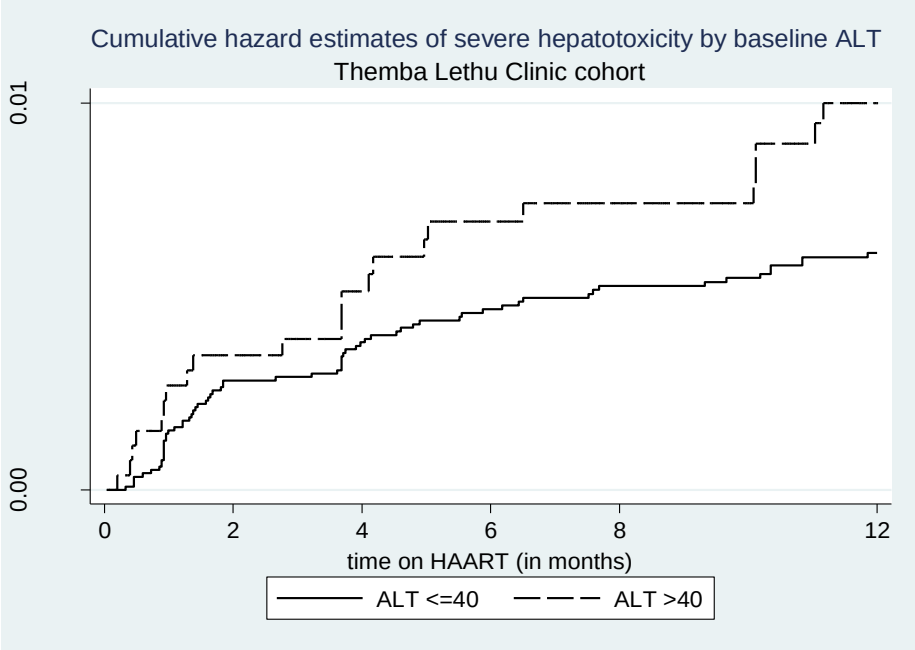
2.1. Baseline ALT category

Incidence rates of severe hepatotoxicity by baseline ALT category

Out of 7989 individuals with a baseline ALT result less than 40 I.U/L with 6955 p-yrs of total follow-up time, 67 cases of severe hepatotoxicity occurred corresponding to an incidence rate of 9.6 (95% CI: 7.6 – 12.2) cases per 1000 p-yrs of follow-up. However, individuals with an ALT result greater than 40 I.U/L at baseline had an incidence rate of 15.7 (95% CI: 10.4 – 23.5) cases per 1000 p-yrs of follow up (23 out of 1775 cases with a follow-up time of 1465 p-yrs) which is double the rate in individuals with an ALT result less than 40 I.U/L.

Cumulative hazard estimates of severe hepatotoxicity by baseline ALT category

Of the 7989 individuals with a baseline ALT less than or equal to 40 I.U/L, 67 (0.8%) developed severe hepatotoxicity compared with 23 (1.3%) of the 1775 individuals with a baseline ALT greater than 40 I.U/L. Figure 4 below shows the cumulative hazard estimates of severe hepatotoxicity by ALT category and a risk table at selected time periods post HAART initiation.



Time	0	2	4	6	8	12
n at risk						
ALT<40	7989	7643	7377	6800	6557	6188
ALT>=40	1775	1656	1583	1413	1345	1257

Figure 4: Kaplan-Meier plot showing cumulative hazard estimates of severe hepatotoxicity by baseline ALT levels

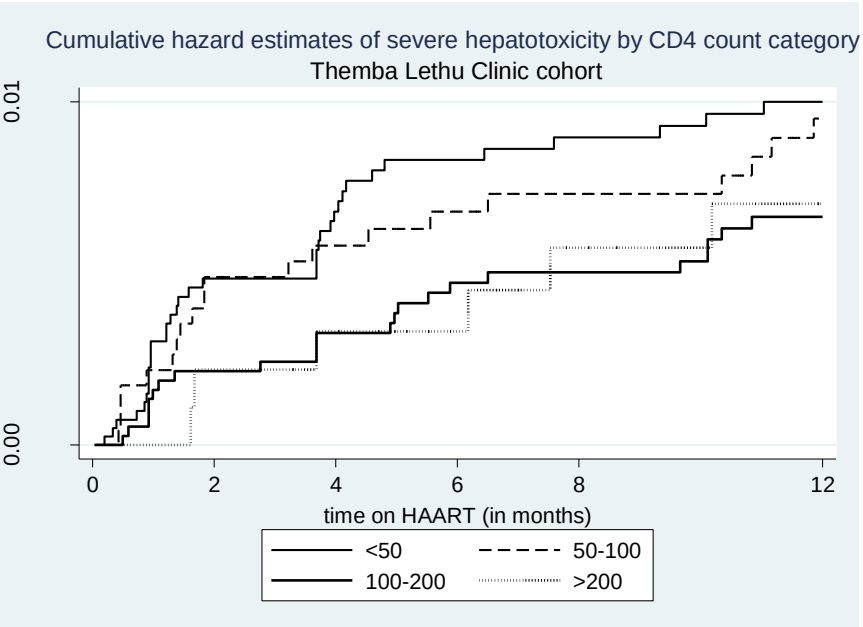
Individuals who had a baseline ALT result of 40 I.U/L or more had increased risk of developing hepatotoxicity compared to those with an ALT result less than 40 I.U/L.

The log-rank test for equality of hazard functions showed that there is a statistically significant difference in hazards for the two groups ($\chi^2 = 3.90$, $p = 0.0482$).

2.2. Baseline CD4 count category

Cumulative hazard estimates for severe hepatotoxicity by CD4 count category

Of the 3385 individuals with a baseline CD4 count less than 50, 36 (1.1%) developed severe hepatotoxicity compared with 20 (1.0%) of the 1917 individuals with a CD4 count between 50 and 100; 23 (0.7%) of the 3133 individuals with a CD4 count between 100; and 200 and 6 (0.8%) of the 769 individuals with a CD4 count greater than 200. Figure 5 below shows the cumulative hazard estimates of severe hepatotoxicity by CD4 count category and a risk table at selected time periods post HAART initiation.



Time	0	2	4	6	8	12
n at risk						
< 50	3385	3127	2938	2606	2498	2338
50 – 100	1917	1834	1779	1648	1584	1500
100 – 200	3133	3040	2969	2783	2683	2531
> 200	769	753	741	696	671	632

Figure 5: Kaplan-Meier plot showing cumulative hazard estimates of hepatotoxicity by CD4 count category

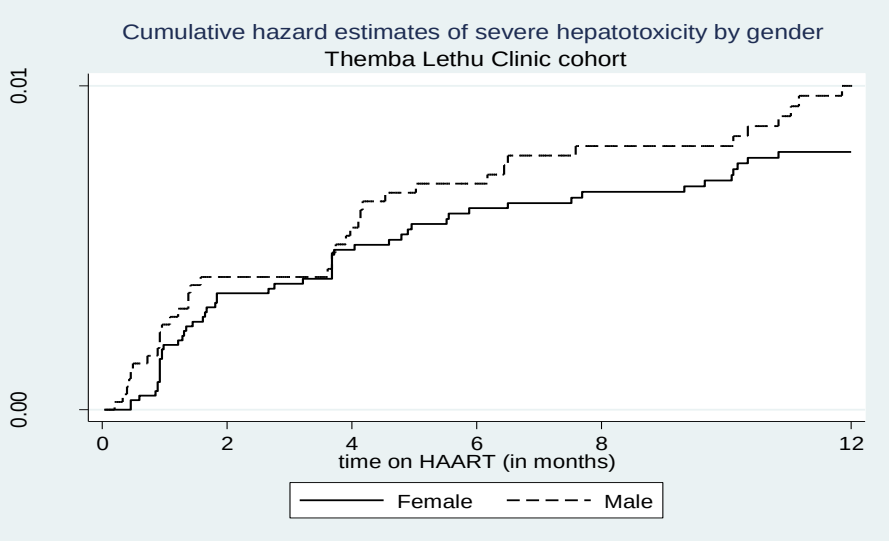
The above Kaplan Meier plot (Figure 6) shows that all the CD4 count categories have similar risk of developing severe hepatotoxicity post-HAART initiation. The log-rank test for equality of hazard functions between CD4 count categories showed that there is no statistically significant difference in hazards for the different categories at α=5% (Log rank test: $\chi^2 = 3.34$, $p = 0.3426$).

3. Demographic features

3.1. Gender

Cumulative hazard estimates for severe hepatotoxicity by gender

Of the 6113 female participants, 52 (0.9%) developed severe hepatotoxicity compared with 38 (1.0%) of the 3651 males. Figure 4 below shows the cumulative hazard estimates of severe hepatotoxicity by gender and a risk table at selected time periods post HAART initiation.



Time	0	2	4	6	8	12
n at risk						
Female	6113	5847	5651	5200	5015	4740
Male	3651	3452	3309	3013	2887	2705

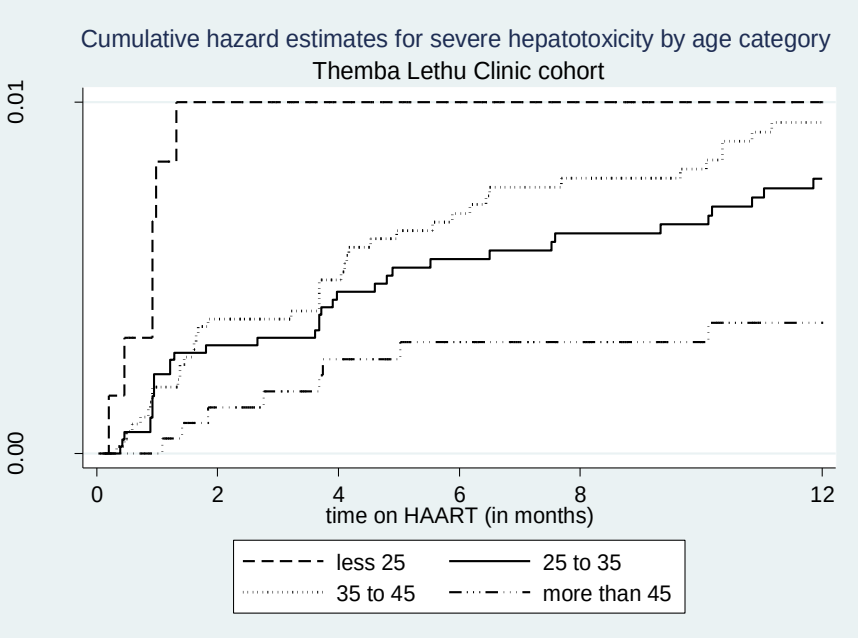
Figure 6: Kaplan-Meier plot showing cumulative hazard estimates of severe hepatotoxicity by gender

From the above Kaplan Meier plot (Figure 5), both genders have similar risk of developing severe hepatotoxicity post HAART initiation. The log-rank test for equality of hazard functions between males and females showed that there is no difference in hazards for the two groups at α=5% (Log rank test: $\chi^2 = 1.07$, p = 0.3001).

3.2. **Age Category**

Cumulative hazard estimates for severe hepatotoxicty by age category

Of the 467 individuals aged less than 25 years, 6 (1.3%) developed severe hepatotoxicity compared with 35 (0.9%) of the 3800 individuals aged between 25 and 34; 41 (1.1%) of the 3689 individuals aged between 35 and 44; and 8 (0.4%) of the 1808 individuals older than 45. Figure 6 below shows the cumulative hazard estimates of severe hepatotoxicity by age category and a risk table at selected time periods post HAART initiation.



Time	0	2	4	6	8	12
n at risk						
< 25	467	449	433	389	370	353
25 – 35	3800	3618	3483	3197	3080	2901
35 – 45	3689	3517	3390	3109	3005	2831
> 45	1808	1715	1654	1518	1447	1360

Figure 7*:* Kaplan-Meier plot showing cumulative hazard estimates of hepatotoxicity by age category

The Kaplan Meier plot above (Figure 6) shows that all the age categories have similar risk of developing severe hepatotoxicity post-HAART initiation. However, the estimates are imprecise. The log-rank test for equality of hazard functions between the age categories showed that there is no statistically significant difference in hazards for the age categories at α=5% (Log rank test: $\chi^2 = 6.54$, p = 0.0881).

Estimating adjusted risk factors for severe hepatotoxicity

Risk factors for severe hepatotoxicity after initiation of HAART were estimated using Cox proportional hazard models. The hazard ratios for the univariate and multivariate models are presented in Table 4 below.

Univariate analysis

In the unadjusted Cox proportional hazard regression model, age at initiation and HAART regimen were significantly associated with severe hepatotoxicity as depicted in Table 4 below.

For every one year increase in age of an individual, there was a 3% reduction in the hazard ratio for developing severe hepatotoxicity. Individuals started on a nevirapine-based regimen had more than double the hazard of developing severe hepatotoxicity compared to their counterparts who were started on an efavirenz-based regimen (HR = 2.19; 95%CI = 1.26 – 3.81; p = 0.006). The hazard for developing severe

hepatotoxicity was 60% higher for individuals with a baseline ALT > 40 I.U/L when compared to individuals with a baseline ALT < 40 I.U/L. This association was imprecise at α = 5% (HR = 1.60; 95%CI = 1.00 – 2.48; p = 0.05).

Multivariate analysis

Age, gender and CD4 count category were chosen up front *(a priori)* to be included in the final multivariate model on the basis of biological plausibility. The initial model had the above factors included and HAART regimen (the most significant factor in unadjusted analysis). The change in estimate method was used to arrive on the final model. Using this method, possible risk factors were added to the initial model one variable at a time. The hazard ratio obtained at each step was compared with the one generated from the previous step. Only those factors which changed the estimates by more than 10% were included in the final model presented in Table 4 below.

In the adjusted model, HAART regimen remained significantly associated with development of severe hepatotoxicity following HAART initiation at 5% significant level. The estimates for baseline ALT category suggested an increased risk for severe hepatotoxicity, though imprecise.

Table 4*:* Factors associated with severe hepatotoxicity after initiating HAART

Characteristics	**Univariate Analysis** HR (95% CI)	p-value	**Multivariate Analysis** HR (95% CI)	p-value
Age (in years)	0.97(0.95 – 0.99)	0.032	0.98 (0.95 – 1.00)	0.078
Age Category (in years)				
< 25 years	1			

25-34 years	0.71 (0.30 – 1.70)	0.447		
35-44 years	0.86 (0.37 – 2.03)	0.731		
>45 years	0.34 (0.12 – 0.99)	0.049		
Gender				
Female	1		1	
Male	1.25 (0.82 – 1.89)	0.301	1.30 (0.84 – 2.03)	0.243
Smoking Status				
No	1			
Yes	1.28 (0.69 – 2.34)	0.433		
Alcohol intake Status				
No	1			
Yes	0.86 (0.43 – 1.71)	0.659		
Baseline Hemoglobin (in g/dL)	0.05 (0.95 – 1.15)	0.345		
Baseline Hemoglobin Category (in g/dL)				
≥8.5	1			
<8.5	1.07 (0.52 – 2.21)	0.857		
CD4 count Category (in cells/mm^3)				
<50	1		1	
50-100	0.93 (0.54 – 1.60)	0.784	0.99 (0.57 – 1.71)	0.962
100-200	0.64 (0.38 – 1.08)	0.094	0.68 (0.40 – 1.16)	0.161
>200	0.67 (0.28 – 1.60)	0.368	0.70 (0.29 – 1.67)	0.418
ALT baseline Category (IU/L)				
<40	1		1	
>40	1.60 (1.00 – 2.58)	0.050	1.62 (1.00 – 2.65)	0.052
History of Tuberculosis				
No	1			
Yes	1.45 (0.90 – 2.33)	0.126		
HAART regimen				
Efavirenz-based	1		1	
Nevirapine-based	2.19 (1.26 – 3.81)	0.006	2.07 (1.13 – 3.79)	0.019
HIV Stage				
1	1			
2	0.70 (0.33 – 1.46)	0.338		
3	0.84 (0.49 – 1.44)	0.530		
4	1.68 (0.90 – 3.13)	0.106		

The hazard of severe hepatotoxicity within the first year of initiating HAART was 2.07 times higher in individuals on a nevirapine-based regimen compared to those on an efavirenz-based regimen after adjusting for baseline ALT, CD4 count, age and gender (HR = 2.07; 95%CI = 1.13 – 3.79; p = 0.019).

Patients with a baseline ALT > 40 I.U/L had a 62% increased hazard of severe hepatotoxicity compared to their counterparts with ALT < 40 I.U/L after adjusting for HAART regimen, age, CD4 count category and gender. However, the estimates of this association was imprecise (HR = 1.62; 95%CI = 1.00 – 2.65; p = 0.052)

Male patients had a 30% increased hazard for severe hepatotoxicity compared to females while adjusting for HAART regimen, baseline ALT, age and CD4 count. This association was however not statistically significant (HR = 1.30; 95%CI = 0.84 – 2.03; p = 0.243). Individuals with CD4 counts between 50 to 100, 100 to 200 and above 200 had a 1%, 32% and 30% reduction (respectively) in hazard for severe hepatotoxicity compared to those individuals with a CD4 count less than 50 at the time of initiating HAART while controlling for HAART, age, ALT and gender. These associations were however not statistically significant at the 5% significance level.

HAART regimen, gender and baseline ALT did not violate the proportional hazard assumption *(appendices F, G & H)*. However, the stphplot for CD4 count suggests minor violation of the proportional hazard assumption as is shown in *Appendix F*. Overall, the whole model did not violate the assumption of proportional hazards (χ^2 = 6.40, p = 0.4935) *(appendix H).*

Interaction terms were tested for but no significant interactions were detected.

Model adequacy was tested for by calculating and plotting Martingale residuals against survival time. *Appendix 1* suggests that the Cox proportional hazard model fits the data poorly.

Sensitivity analysis

To investigate the influence of excluding patients with missing baseline ALT results from the main analysis, a sensitivity analysis was performed. This was done to see whether the exclusion could have biased the hazard ratio estimates presented above.

Firstly, we fit a model including all the individuals with missing baseline ALT values assuming that these individuals all had an ALT value of less than 40 I.U/L. The estimates for this scenario were then determined.

We then fit a second model, this time including all the individuals with missing baseline ALT values assuming their baseline ALT value was greater than 40I.U/L. The estimates for such a situation were also determined.

The estimates for these two scenarios were then compared to the estimates of the main analysis as is depicted in Table 5.

Table 5: Factors associated with severe hepatotoxicity: main analysis *(adjusted)* and sensitivity analyses *(adjusted)*

Characteristic	**Main analysis** *(adjusted)* HR (95% CI) p-value	**Missing ALT included as ALT < 40** *(adjusted)* HR (95% CI) p-value	**Missing ALT included as ALT > 40** *(adjusted)* HR (95% CI) p-value
Age (in years)	0.98 (0.95– 1.00)0.078	0.97 (0.95– 0.99)0.043	0.97 (0.95- 0.99)0.042
Gender			
Female	1	1	1

Male	1.30 (0.82– 2.03)0.243	1.37 (0.89- 2.10)0.151	1.37 (0.90- 2.11)0.145
HAART regimen			
Efavirenz-based	1	1	1
Nevirapine-based	2.07 (1.13– 3.79)0.019	1.98 (1.10- 3.56)0.022	1.96 (1.09- 3.52)0.024
ALT baseline (IU/L)			
Less than 40	1	1	1
Greater than 40	1.62 (1.00– 2.65)0.052	1.59 (0.98- 2.58)0.059	1.61 (1.03- 2.51)0.037
CD4 count (cells/mm^3)			
Less than 50	1	1	1
50 to 100	0.99 (0.57– 1.71)0.962	0.95 (0.55–1.65) 0.868	0.95 (0.55–1.64) 0.855
100 to 200	0.68 (0.40– 1.16)0.161	0.75 (0.45–1.25) 0.276	0.75 (0.45–1.25) 0.276
Greater than 200	0.70 (0.29– 1.67)0.418	0.88 (0.41–1.91) 0.749	0.87 (0.40–1.88) 0.720

The estimates obtained following conducting sensitivity analyses *(Table 5)* shows that although there are minor deviations from the main analysis results in the two scenarios, the confidence intervals of these estimates look very similar. It is thus unlikely that excluding records with missing ALT results has biased the estimates in the main analysis significantly.

Correlation between recorded clinical diagnosis and laboratory diagnosis of severe hepatotoxicity

Correlation between a recorded diagnosis of hepatotoxicity by attending physicians and laboratory diagnosis (as used in the main analysis presented above) was calculated using values given in the 2 x 2 table in *Table 6* below. A total of 110 clinical diagnoses of hepatotoxicity were recorded compared to 90 laboratory diagnoses. Laboratory diagnosis was used as the gold standard for diagnosis of severe hepatotoxicity. Clinicians only diagnosed and recorded 19 out of the 90 individuals who had biochemically confirmed severe hepatotoxicity. This corresponds to a sensitivity of 21.1%. Of the 9674 individuals without a laboratory diagnosis of

severe hepatotoxicity, the attending clinician diagnosis agreed with 9583 of these, corresponding to a specificity of 99.1%

Table 6*:* 2 x 2 table showing number of patients diagnosed clinically and biochemically of severe hepatotoxicity at Themba Lethu clinic

		Laboratory diagnosis of severe hepatotoxicity		
		Yes	No	**Total**
Clinical diagnosis of hepatotoxicity	Yes	19	91	110
	No	71	9583	9654
	Total	90	9674	9764

The amount of agreement above chance between Laboratory diagnosis and recorded clinical diagnosis of severe hepatotoxicity, (kappa statistic- κ) was 18.2%. This means that clinicians correctly diagnosed and recorded whether an individual had severe hepatotoxicity or not in only 18.2% of the study participants.

CHAPTER FOUR

DISCUSSION

Summary

The study's main objectives were to determine the incidence and risk factors for severe hepatotoxicity following initiation of HAART in a South African cohort. The overall incidence rate of severe hepatotoxicity of 10.7 per 1000 p-yrs of follow-up time was substantially lower than incidence rates reported in a South African mine [26] and in Italy [30]. HAART regimen was consistently the strongest risk factor associated with the development of severe hepatotoxicity after adjusting for age, gender, baseline ALT and CD4 count in a multivariate Cox proportional hazard regression model. Though imprecise, the estimate for baseline ALT category suggested an increased risk of severe hepatotoxicity in individuals with a baseline ALT > 40 I.U/L

Baseline characteristics

The baseline characteristics of the cohort differed from the populations studied in previous studies in several aspects.

Participants in an Italian cohort that had a high incidence rate of severe hepatotoxicity were all co-infected with HCV [30] whereas participants in our study did not have their baseline HCV status evaluated, though it is reportedly low in sub-Saharan Africa. A study conducted in Malawi reported an HCV prevalence of only 4.5% [31]. The average CD4 count of this cohort was 80 cells/mm^3, a value which is

lower than an average CD4 count of 103 cells/mm^3 observed in a fairly large and representative cohort of 45 000 South African adults at baseline [52].

There is substantial evidence that suggest that women disproportionately access antiretroviral services when compared to men in sub-Saharan Africa, even when the higher HIV infection prevalence in females is accounted for [53]. The gender distribution of the study sample reflects this pattern. However, studies on cohorts in Europe [30], Asia [27] and one other in South Africa [26] had greater proportions of male patients compared to this cohort. This is possibly due to the fact that HIV/AIDS in other parts of the world is driven mainly by men having sex with men or intravenous drug users, whereas the epidemic is mainly driven by heterosexual relationships in sub-Saharan Africa. The study on a South African cohort was done in a mine setting where the workforce is predominantly male.

The proportion of individuals on an efavirenz-based regimen in this cohort was greater than Asian cohorts [27-28] that largely consisted of individuals on a nevirapine-based regimen. This difference is largely explained by the fact that efavirenz is preferred over nevirapine in first line HAART regimens in areas of high tuberculosis prevalence such as sub-Saharan Africa. This is evidenced by the high proportion of individuals with a diagnosis of tuberculosis at the time of commencing HAART.

Efavirenz-based regimens are preferred over nevirapine-based ones because rifampicin (one of the anti-tuberculous drugs) is a powerful enzyme inducer which results in significant reductions in nevirapine blood concentrations in patients on both treatments [47]. Furthermore, co-administration of nevirapine and anti-tuberculous drugs has been traditionally viewed as highly hepatotoxic. Of the 1909 individuals

who had tuberculosis at the time of initiating HAART, only 34 of them were started on a nevirapine-based regimen. No cases of severe hepatotoxicity were observed in individuals who were started on a nevirapine-based regimen while they had a diagnosis of tuberculosis. We were therefore unable to assess for effect modification between HAART regimen and tuberculosis infection status.

Incidence of severe hepatotoxicity

The cohort had an overall incidence rate of 10.7/1000 p-yrs which is much lower than incidence rates reported previously in other studies. For example, one Italian cohort that included individuals with HCV/HIV co-infection had an overall incidence rate of 177.1/1000 p-yrs. This large difference in incidence could be explained by the fact that HCV infection leads to significant underlying liver damage in an individual thereby increasing the susceptibility to potential hepatotoxic drugs. Furthermore, HCV/HIV co-infection has been demonstrated to result in accumulation of functional HIV-specific T-cells in the liver thereby resulting in accelerated progression of liver disease [36]. Even though HCV infection status is not measured at baseline in this cohort, the low rates of HIV/HCV co-infection in southern Africa [31] could explain the low incidence rates of severe hepatotoxicity in this cohort.

A South African cohort in a mining environment had an incidence rate of severe hepatotoxicity of 77.0 events per 1000 p-yrs of follow-up time within a year of initiating HAART [26]. The individuals included in this cohort differed from our cohort in that more than half of the patients were on anti-tuberculous treatment at initiation of HAART compared to only a fifth in our study cohort. Three of the four drugs used

as first-line agents in tuberculosis treatment (Isoniazid, rifampicin and pyrazinamide) are known hepatotoxins. Furthermore, tuberculosis may result in immune reconstitution inflammatory syndrome (IRIS) if HAART is started at the same time with tuberculosis drugs. Usually, IRIS leads to transaminase elevation due to immune surveillance of mycobacterial antigens in the liver. The lower incidence rate of severe hepatotoxicity in our cohort compared to the South African mining cohort could therefore be attributed to a small proportion of individuals on anti-tuberculous drugs at initiation of HAART. Previous studies have also demonstrated increased incidence of hepatotoxicity in patients on both HAART and anti-tuberculous treatment [48-49].

Our findings showed that the first two months of initiating HAART had the highest period incidence rate of severe hepatotoxicity. Thereafter, the incidence rate gradually decreases. This is in keeping with the findings of previous studies [22, 50-51]. The predominance of cases of severe hepatotoxicity early during treatment suggests that HAART-induced hepatotoxicity is less likely to be problematic with increasing treatment duration.

Risk factors for severe hepatotoxicity

Univariate analysis showed that only age and HAART regimen were significantly associated with the development of severe hepatotoxicity following initiation of HAART. The estimates for baseline ALT also suggested an increased risk of severe hepatotoxicity, though imprecise. However, after adjusting for potential confounders in a multivariate model, HAART regimen was the only factor independently associated with the development of severe hepatotoxicity.

The increased risk of severe hepatotoxicity in individuals on a nevirapine-based regimen compared to those on an efavirenz-based regimen (2.07 times higher) follows similar trends observed in previous studies [14-15, 22]. It is for this reason that a “black-box” warning has been issued for nevirapine-based HAART [8].

Correlation between recorded clinical diagnosis and laboratory diagnosis

The correlation between recorded clinical diagnosis and laboratory diagnosis of severe hepatotoxicity during follow-up visits was only 18.2%. This proportion is very low considering the fact that hepatotoxicity can lead to treatment interruption, clinical hepatitis and even death.

Several factors may account for the low correlation between recorded clinical diagnosis and laboratory diagnosis of severe hepatotoxicity. Firstly, the high number of patients may put a lot of pressure on doctors to an extent that they are less thorough during patient examination and review of results. Clinical outpatient settings may also not have information from inpatient diagnosis recorded thereby missing these patients. The definition for severe hepatotoxicty used by the clinicians could have been different from the study definitions thereby resulting in the low correlation. Clinicians were not the only people entering data into Therapy Edge-HIV ^{TM}. The use of lay data capturers to enter data into Therapy Edge-HIV ^{TM} in previous years may have also contributed to the low correlation since they are non-medical staff. Furthermore, doctors might not actively look for the diagnosis of hepatotoxicity because of limited knowledge about the possible consequences of the clinical condition.

Strengths and Limitations

While the study reflects what happens in real clinical settings, the results should be interpreted with some caution considering a number of limitations.

Loss to follow-up is a common limitation in observational cohort studies and may introduce bias to estimates if the individuals lost to follow-up had a different pattern of exposure variables and severe hepatotoxicity from those retained in the cohort. The results of this study should therefore be interpreted with some caution considering the attrition rate of 20.3%

In reality, all HAART drugs have the potential of causing severe hepatotoxicity [8-9] but the attribution of severe hepatotoxicity to a single agent (nevirapine-based or efavirenz-based regimens) can be arbitrary and not reflect the real contribution of each drug to liver toxicity. However, the NNRTI class of antiretroviral drugs have been implicated in most cases of liver toxicity [8-9] and hence the categorisation. The above results should therefore be interpreted while aware of the fact that all HAART drugs have a potential of causing severe hepatotoxicity.

It is important to recognise possible limitations of this study in terms of HAART regimen comparisons. Different frequencies in the measurement of liver function, which resulted in more frequent ALT monitoring in the nevirapine-based cohort, may have led to an increased detection of severe hepatotoxicity in that group compared to the efavirenz-based cohort. However, the observed association is consistent with findings from studies done elsewhere [14, 22, 28]. This finding suggests that careful management of patients on a nevirapine-based regimen, with a strict patient follow-up should be done.

Smoking status, alcohol intake status, baseline BMI, age at initiation, baseline CD4 count, history of tuberculosis at initiation and gender did not appear to have any

significant effect on development of severe hepatotoxicity following initiation of HAART. However, variables which are not reliably measured in individuals, especially alcohol intake, could have resulted in residual confounding.

Unlike in experimental studies where treatment arms are randomly assigned to individuals, in observational studies clinicians may assign an individual with a high risk of developing hepatotoxicity a regimen which is assumed to have better liver tolerability. Regardless of this shortfall, the study results reflect the experience of a large cohort in a real clinical setting compared to randomised controlled trials.

HBV and HCV infection status of individuals are not routinely measured at the start of HAART at Themba Lethu clinic. While HIV/HBV co-infection is more common in some parts of sub-Saharan Africa than in resource-rich settings [20, 32], HIV/HCV co-infection has been reportedly less common [31]. These factors have been cited several times in previous studies as predictors of severe hepatotoxicity following HAART initiation [12-13, 26, 28, 33-35]. Absence of these variables might have led to exaggeration or lack of association between a possible predictor and severe hepatotoxicity as hepatitis infection status would not be adjusted for during analysis. These results should therefore be interpreted while cognisant of this fact.
Inclusion of these and other possible predictors of hepatotoxicity in the analysis would help improve model fitness.
Interpretation of the study results should also be made while aware of the fact that there may have been residual confounding. Variables such as smoking and alcohol use can cause limitations in the ability to control for confounding due to the imprecise

nature of their measurement and may result in residual confounding. However, no studies in our setting have shown that this is likely to be a problem.

Individuals excluded from the study on the basis of absent baseline ALT results could have biased our estimates if they had a different pattern of exposure variables and severe hepatotoxicity from those retained in the cohort. The differences in HAART regimen and baseline haemoglobin in patients excluded from the study on basis of missing baseline ALT results and the study sample warrants a cautious interpretation of the results. As Nevirapine is confirmed as a risk factor for hepatotoxicity, excluding these patients may have led to the underestimation of the estimate of severe hepatotoxicity

Secondary data analysis of a prospective cohort study is highly dependent on good records and therefore if the database has missing data or some inaccuracies in patients' information, misclassification of exposure variables can occur. This might therefore lead to bias in estimates. Therapy Edge-HIV ^{TM} uses an electronic data capturing system which minimises errors during data entry. All data used in this study was obtained from variables already captured on this electronic database.

Generalizability

This study presents data from a single urban government antiretroviral clinic. While the cohort is large, possible differences in characteristics of individuals in this cohort and other antiretroviral roll-out clinics in South Africa may limit the generalizability of our study findings beyond the study population. Themba Lethu clinic mainly caters for the urban populace that may have socio-demographic, clinical and treatment factors which may be different from their rural counterparts and those accessing care in the private sector.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

The precision of the above estimates is, to some extent, compromised by a number of limitations. The poor retention of patients in this cohort increases the uncertainty of the calculated risks of severe hepatotoxicity. In order to improve follow-up of clients in the cohort, a strong system of obtaining data on patients who are transferred to other health facilities should be considered.

The correlation of clinical and laboratory diagnosis of severe hepatotoxicty was low, considering the possible consequences of severe hepatotoxicity. It is therefore highly recommended that the factors that may impair the clinicians' ability to correctly diagnose severe hepatotoxicity and data entry need to be addressed. These include the review of the doctor-patient ratio and conducting refresher courses aimed at equipping doctors with knowledge to help in the diagnosis.

The high incidence rate of severe hepatotoxicity in the first two months of initiating HAART necessitates more frequent and careful monitoring of blood ALT levels early during therapy. This will identify the majority of the cases severe hepatotoxicity and allow appropriate interventions to be instituted.

Poor model fitness in analysis suggests that there may be important predictors of severe hepatotoxicity which were not included in the model. One such factor may have been HBV infection status. It is therefore highly recommended that further

studies that measure important possible predictors of severe hepatotoxicty like HBV infection status be conducted.

In order to minimise possible effects of detection bias, studies which will investigate factors for hepatotoxicity when ALT levels are measured at similar intervals for both the nevirapine-based and efavirenz-based groups need to be undertaken.

Further studies which include participants from different South African settings are highly recommended in order to obtain results which can be generalised.

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Appendix A

Signed Standard Operation Procedure (SOP) of the Clinical HIV Research Unit (CHRU)

Clinical HIV Research Unit, Department of Medicine

Standard Operating Procedure: Process to be followed for granting access to data for research on the Themba Lethu Clinical Cohort and other Cohorts stored on the TherapyEdge database

Requests from individuals wanting to make use of data from the Themba Lethu Clinical Cohort and other Cohorts stored on the TherapyEdge database for research purposes must follow the following procedure and sign this document:

1. A written request to use the data set must be sent to the Regulatory Manager of the Clinical HIV Research Unit, Department of Medicine, Helen Joseph Hospital (Marleen Naidoo; manaidoo@witshealth.co.za).
   - 1.1. The request should include:
     - 1.1.1. Names of individuals who will be using the data set and their affiliations.
     - 1.1.2. Information on whether the research is for degree purposes and details of the institution that will grant the degree.
     - 1.1.3. A proposal detailing the objectives of using the data, planned analysis, planned public distribution of results and date of finalization.
   - 1.2. The Regulatory Manager will communicate the request to Dr Ian Sanne, researchers in the Clinical HIV Research Unit and the Director(s) of the relevant site(s).
     - 1.2.1. Where the analysis involves the Themba Lethu Clinical Cohort a copy of the proposal will be submitted to the Head of the Department of Medicine at Helen Joseph Hospital.

2. A consensus decision of the Clinical HIV Research Unit and the relevant site director on whether to grant permission for data use will be required in all cases.
   - 2.1. Where the analysis involves the Themba Lethu Clinical Cohort the Regulatory Manager of the CHRU will ensure that the relevant documentation required by the Helen Joseph Hospital is completed and submitted to the office of the CEO of Helen Joseph Hospital for approval.
   - 2.2. Once permission has been agreed by the above groups, a decision will be communicated to the applicant by the Regulatory Manager of the CHRU.

3. By signing this SOP the applicant agrees to the following statements of assurance:
   3.1. All references to the data either in public verbal presentation or in print must credit the Clinical HIV Research Unit, Right to Care, the Department of Medicine at Helen Joseph Hospital (for the Themba Lethu Cohort only) and the specific cohort as the source.
   3.2. Should the applicant wish to use the data for analysis beyond the originally submitted proposal, a further written request will be required and the procedures outlined above followed.
   3.3. A final draft of the results of the research will be submitted to the CHRU for information and comment preferably before it is submitted for publication or public presentation.
   3.4. The authorship of the paper will be decided using internationally recognized criteria, and must recognize the CHRU and Department of Medicine researchers appropriately, as well as any international researchers involved in the data analysis.
   3.5. The applicant will also be required to submit a copy of the final product resulting from use of the data set to the Regulatory Manager of the CHRU.
   3.6. The data set will not be shared, copied or provided to anyone other than the person/s outlined in the proposal

4. The Human Research Ethics Committee (Medical) of the University of the Witwatersrand has approved the use of the electronic medical records stored on TherapyEdge subject to conditions (particularly with regard to the identity of participants) outlined in protocol (M060626).
   4.1. The applicant must comply with the conditions laid out in this protocol.
   4.2. It will be the responsibility of the applicant to ensure that approval to do their study is covered by the protocol.
   4.3. The applicant will be responsible for obtaining approval from any other authorities or Internal Review Boards as may be necessary.

5. If possible, the applicant should make an oral presentation to the Clinical HIV Research Unit at the start of the investigation and again once it has been concluded. This will be part of the regular academic programme of the Clinical HIV Research Unit.

6. A file will be maintained in the offices of the Clinical HIV Research Unit for all correspondence in this regard. In particular:
   6.1. Correspondence documenting the approval process as out lined in Point 2 above
   6.2. The signed agreement of the applicant (this SOP).
   6.3. A copy of the final product resulting from use of the data.

I have read and accept these conditions.

Applicant: DR MUNAMATO MIRIRA

Date: 15 OCTOBER 2010

Appendix B

Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Munamato Mirira

CLEARANCE CERTIFICATE	**M10936**
PROJECT	Incidence of and Risk Factors for Hepatotoxicity following Antiretroviral Inflation in Patients Attending Themba Lethu Clinic, Johannesburg
INVESTIGATORS	Dr Munamato Mirira.
DEPARTMENT	School of Public Health
DATE CONSIDERED	01/10/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 01/10/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Mhari Maskew

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix C

Approval letter from the Chief Executive Officer of Helen Joseph Hospital

Clinical HIV Research Unit, Department of Medicine

Helen Joseph Hospital, Themba Lethu Clinic, Perth Road, Westdene, Johannesburg 2092, South Africa
Postnet Suite 176, Private Bag X2600, Houghton 2041, South Africa • Tel: +27 11 276-8800 • Fax: +27 11 482-2130

05 November 2010

Dr NL Hlongwane
Senior Superintendent
Helen Joseph Hospital
Perth Road
Westdene

By Hand

Dear Dr Hlongwane

RE: Dr Munamato Mirira: ETHICS REF NO: M10936

"Incidence Of And Risk Factors For Hepatotoxicity Following Antiretroviral Initiation In Patients Attending Themba Lethu Clinic, Johannesburg"

This letter serves to confirm that Dr M Mirira is a employee at Department of Epidemiology and Biostatistics, School of Public Health and wishes to conducted research study here at Helen Joseph Hospital.

The research has already been approved by the Human Research Ethics Committee (University of the Witwatersrand) under protocol M10936.

As the proposed study is purely research and will not impact on the hospital in anyway.

Yours sincerely,

Mrs Marlene Naidoo
Regulatory Manager
Clinical HIV Research Unit
Themba Lethu Clinic
Helen Joseph Hospital
Perth Road, Westdene, Johannesburg
Tel: +27 11 276 8809

Gauteng Department of Health

Helen Joseph Hospital

PERMISSION FOR RESEARCH

DATE: 29 October 2010

NAME OF RESEARCH WORKER: Dr Munamato Mirira

CONTACT DETAILS OF RESEARCHER (INCLUDE ALTERNATE RESEARHER):
Department of Epidemiology and Biostatistics
School of Public Health
Tel: 072 069 7016

TITLE OF RESEARCH PROJECT Incidence Of And Risk Factors For Hepatotoxicity Following Antiretroviral Initiation In Patients Attending Themba Lethu Clinic, Johannesburg

OBJECTIVES OF STUDY (Briefly or include a protocol):

- To describe the characteristics of patients initiated on antiretroviral therapy
- To describe the prevalence of hepatotoxicity among patients at initiation of antiretroviral therapy
- To determine the incidence of hepatotoxicity within 12 months of initiating antiretroviral therapy
- To investigate factors associated with incident hepatotoxicity in patients initiated on antiretroviral therapy

METHODOLOGY (Briefly or include a protocol):
The study is a secondary analysis of prospective cohort data among patients initiating HAART at Themba Lethu Clinic between 1 April 2004 and 30 June 2009.

CONFIDENTIALITY OF PATIENTS MAINTAINED: Yes

COSTS TO THE HOSPITAL: NIL

APPROVAL OF HEAD OF DEPARTMENT:

APPROVAL OF CRHS OF WITS UNIVERSITY: Yes

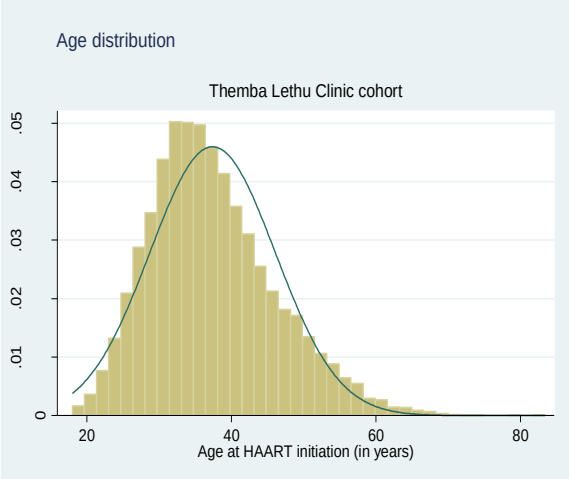
SUPERINTENDENT PERMISSION:

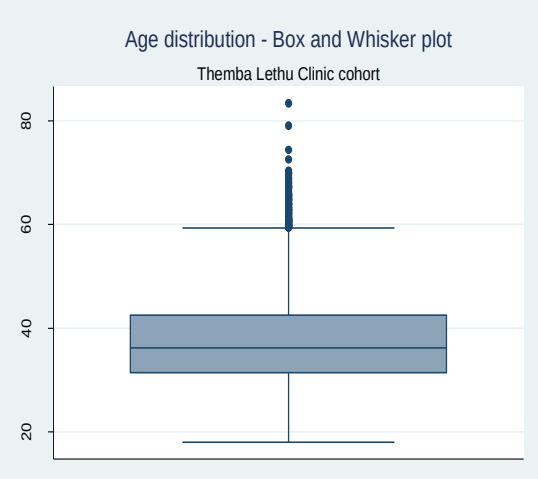
Signature: J.M. Kirby Date: 24/11/2010

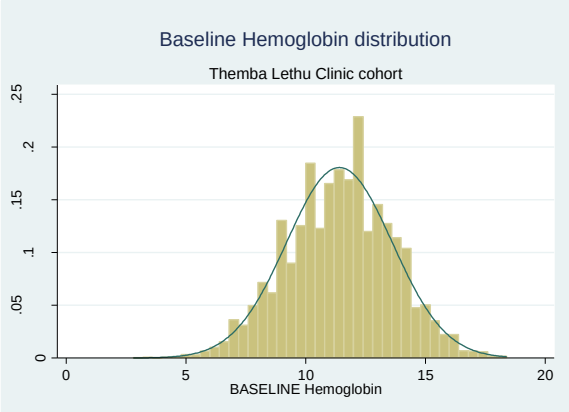
Subject to any restrictions: no.

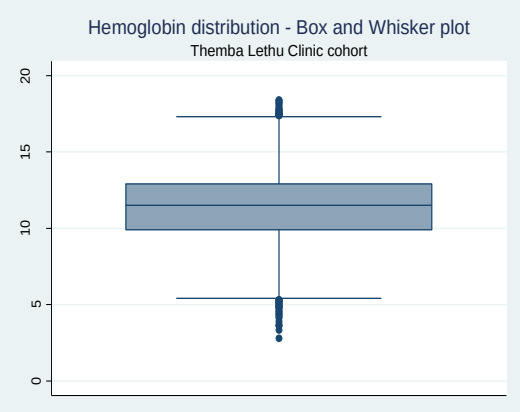
Appendix D

Age and haemoglobin distribution





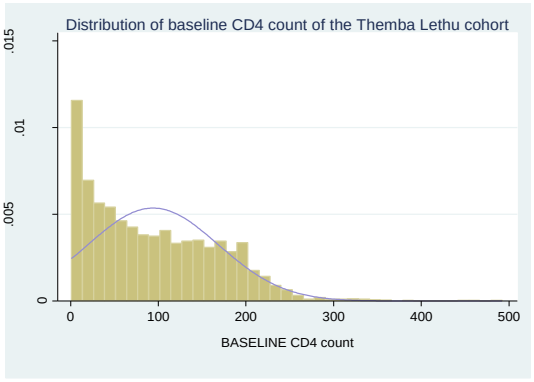


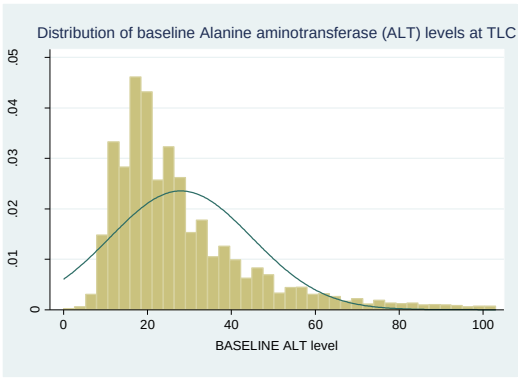


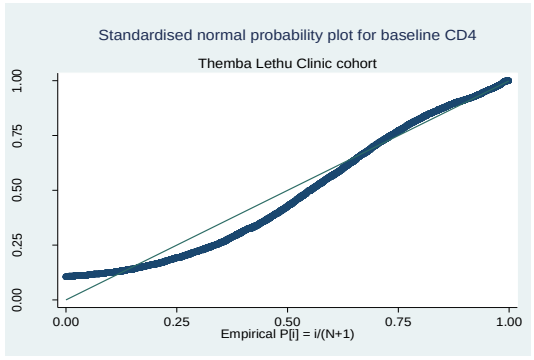
The histograms and Box and Whisker plots above suggest normal distribution of Age at initiation and baseline Haemoglobin and therefore it is appropriate to use means and standard deviations to describe these characteristics

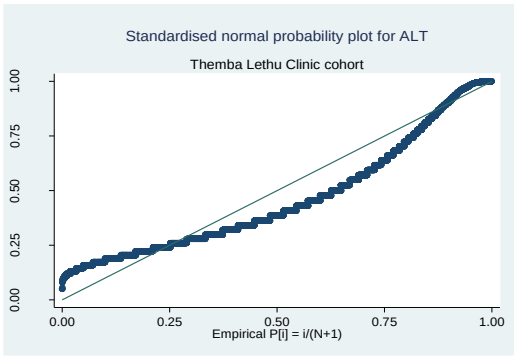
Appendix E

Baseline CD4 count and ALT distribution





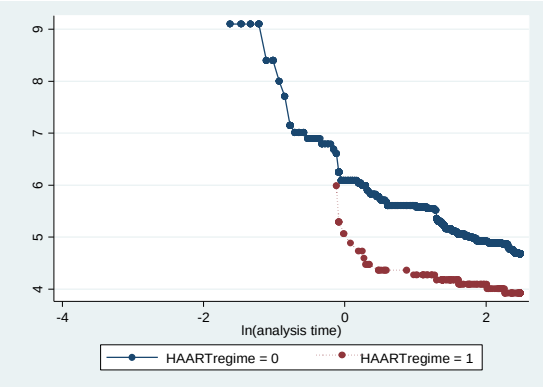


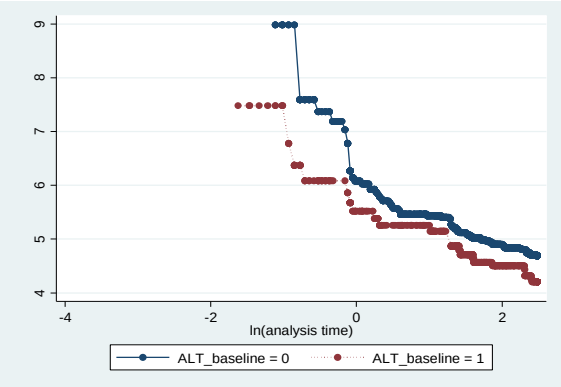


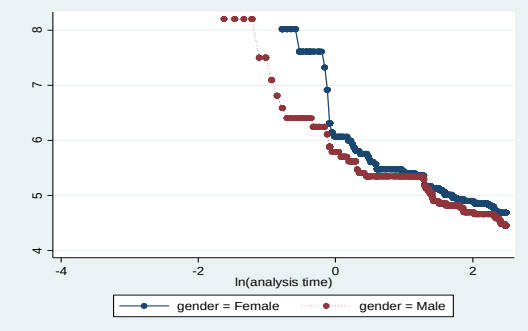
The histograms (with superimposition of the normal curve) and the probability plots do not suggest normal distribution of baseline CD4 count and baseline ALT levels and therefore it is appropriate to use interquartile ranges to describe these characteristics

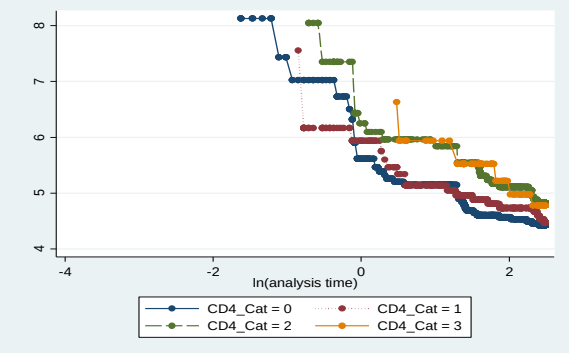
Appendix F

Stphplot for baseline ALT, gender and HAART regimen





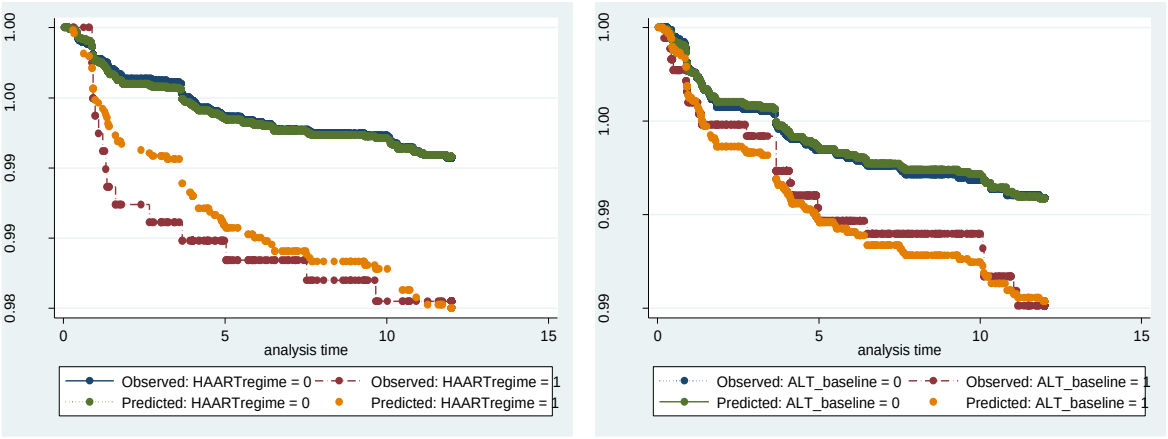




Stphplot: The plots of ALT, gender and HAART regimen against log of follow-up time shown above suggests that the proportional hazard assumption is not violated due to the parallel nature of the plots. However, the CD4 count plot suggests some minor violation of this assumption

Appendix G

K-M plots plotted against predicted values



Stcoxkm*:* K-M plots plotted against Cox predicted values. The proportional hazard assumption is unlikely to have been violated as the observed values are very close to the predicted

Appendix H

Global Spthtest

The spthtest

Test of proportional-hazards assumption

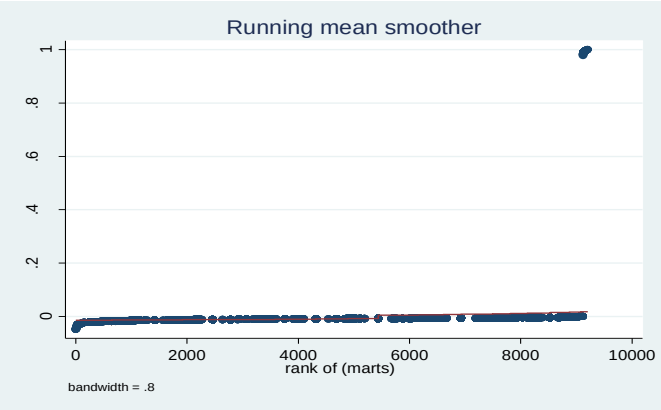
Time: Time

	rho	chi2	df	Prob>chi2
_Igender_2	0.05459	0.25	1	0.6205
_ICD4_Cat_1	0.10398	0.89	1	0.3451
_ICD4_Cat_2	0.18926	2.88	1	0.0899
_ICD4_Cat_3	0.16645	2.28	1	0.1313
age_at_ini~n	-0.03336	0.08	1	0.7789
_IHAARTreg~1	-0.15444	1.94	1	0.1634
ALT_baseline	0.09735	0.79	1	0.3727
global test		6.40	7	0.4935

Spthtest: globally, the assumption of proportional hazards was met as p>0.005.

Appendix: I

Martingale residuals plotted against survival time



Model adequacy was tested by calculating and plotting Martingale residuals against survival time. The plot suggests that the Cox model fits the data poorly