# Late mortality, loss to follow-up, and associated factors in adults on long term antiretroviral therapy in Khayelitsha

by

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*To Magali, Maéline, and Naomi.*

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# TABLE OF CONTENTS





University of Cape Town

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# TABLE OF FIGURES AND TABLES

## FIGURES



## TABLES



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# LIST OF ABBREVIATIONS

| | |
|---|---|
| 3TC | Lamivudine |
| AIC | Aikaike's information criterion |
| AIDS | Acquired immune deficiency syndrome |
| ALT | Alanine transaminase |
| ART | Antiretroviral Therapy |
| ARV | Antiretroviral |
| AZT | Zidovudine |
| CI | Confidence interval |
| D4T | Stavudine |
| DDI | Didanosine |
| EFV | Efavirenz |
| EPTB | Extra-pulmonary tuberculosis |
| HIV | Human immunodeficiency virus |
| HR | Hazard ratio |
| IQR | Interquartile range |
| LPV/r | Lopinavir/ritonavir |
| LTFU | Lost to follow-up |
| MSF | Médecins sans Frontières |
| NVP | Nevirapine |
| PMTCT | Prevention of Mother to Child Transmission |
| PTB | Pulmonary tuberculosis |
| py. | Person-years |
| TB | Tuberculosis |
| TDF | Tenofovir |
| VCT | Voluntary counselling and testing |

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# ABSTRACT

OBJECTIVES: To estimate baseline characteristics, survival, and factors associated with mortality and losses to follow-up in patients on antiretroviral therapy (ART), and compare the periods before (*early*) and after (*late*) 3 months on treatment.

DESIGN: Prospective cohort study.

SETTING: Community-based, public sector, ART programme in the South African township of Khayelitsha. Adult HIV prevalence, tuberculosis (TB) case finding rate, and poverty are very high.

SUBJECTS, PARTICIPANTS: 3,595 patients started on ART between 12 March 2001 and 31 December 2005, and followed-up until 31 December 2006. Age below 14 years and previous exposure to more than one month of ART were exclusion criteria.

MAIN OUTCOME MEASURES: Time to death and time to loss to follow-up. All-cause mortality was ascertained through notification of the clinic or active defaulter tracing. Patients were defined as lost to follow-up when their most recent visit to the clinic occurred before 1 July 2006, and they were not known to have died or been transferred to another facility.

METHODS: Kaplan-Meier product-limit methods were used to estimate survival and cumulative probability of loss to follow-up. Cox proportional hazards regression was used to identify associated factors.

RESULTS: Mortality decreased rapidly during the first year on ART and remained constant thereafter. The early and late death rates were 23.9 and 3.4/100 person-years respectively. The rate of loss to follow-up decreased gradually over time, from 9.4/100 person-years during the early period to 3.0 during the late period. The cumulative probability of being in care at 5 years was 72% (95% CI 68-76%). Baseline factors associated with late mortality were: male gender, age, $CD4^+$ T-lymphocyte count, weight, Kaposi sarcoma, oesophageal candidiasis, chronic diarrhoea, and wasting syndrome. Tuberculosis at baseline was protective (HR 0.58, 95% CI 0.40-0.84). Incident opportunistic infections on ART were strongly associated with death. 42% of deaths had tuberculosis at the time of their last visit. Efavirenz in the starting regimen and low weight were associated with early loss to follow-up. A diagnosis of AIDS was protective. Younger age, lower weight, and previous exposure to prevention of mother to child transmission were associated with late losses to follow-up, whilst previous tuberculosis was protective.

CONCLUSIONS: Mortality and loss to follow-up were highest during the first months on ART. Enhanced clinical monitoring and adherence support during this period could potentially improve outcomes. Increased access to ART is necessary to achieve initiation at an earlier stage. Important differences emerge when considering factors associated with mortality and loss to follow-up beyond the early months on ART in this setting. Focused adherence support for youth and young mothers could potentially decrease losses to follow-up. Further research is needed into the protective effect of tuberculosis on mortality beyond the very early period on ART.

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# 1. INTRODUCTION AND LITERATURE REVIEW

The human immunodeficiency virus (HIV) has become the major cause of mortality in South Africa (Dorrington, Bourne et al. 2001), being responsible for 39% of the premature mortality burden in 2000 (representing 4,665,410 years of life lost)(Bradshaw, Groenewald et al. 2003). Worldwide, an estimated 33.2 million people were living with HIV in 2007; an estimated 2.5 million became newly infected and an estimated 2.1 million lost their lives to AIDS (UNAIDS and WHO 2007). In high income countries, the introduction of combination antiretroviral therapy (ART) in 1996 was associated with a sharp decline in mortality (Crum, Riffenburgh et al. 2006) and an increase in life expectancy of people living with HIV (Fang, Chang et al. 2007; Lohse, Hansen et al. 2007). In poorer countries - especially Sub-Saharan Africa, where the majority of HIV-infected people live - ART was introduced later, and at a slower pace (UNAIDS 2006). Data on mortality on ART in low income countries are limited.

Khayelitsha is a township at the periphery of Cape Town, South Africa, with an estimated population of nearly half a million (Maverick 2006). Socio-economic conditions are poor, with high rates of unemployment and poverty, a majority of informal housing, and a high mobility of the population (i.e. frequent changes of residence, both in Khayelitsha and between the Eastern and Western Cape Provinces). The antenatal HIV prevalence rose from 15% in 1999 to 33% in 2005 (Department of Health Western Cape 2006). The tuberculosis (TB) case finding rate in 2005 was 1,600/100,000 (Azevedo 2007). In the last prevalence survey of drug resistant TB in the Western Cape Province, multi-drug resistant TB (MDR TB) was detected in 0.9 % of the isolates recovered from patients with newly diagnosed TB and 3.9% of isolates recovered from patients with previously treated TB.

In February 2000, Médecins Sans Frontières (MSF), the Western Cape Department of Health, and the University of Cape Town opened three dedicated clinics for the treatment of HIV. With the introduction of ART in May 2001, this became one of the oldest public sector ART programmes in Africa (Médecins Sans Frontières South Africa, Department of Public Health at the University of Cape Town et al. 2003; Coetzee, Hilderbrand et al. 2004). While smaller numbers of patients were started on ART between 2001 and 2003, enrolment onto ART was scaled up massively with the integration of the Khayelitsha programme into the national rollout during the first

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quarter of 2004. By early 2006 it was estimated that half of those in need of ART were accessing it in Khayelitsha, and the services have subsequently struggled to keep pace with demand due to the increasing patient load. This has been associated with greater proportions of patients lost to care after 2004 (Van Cutsem, Hilderbrand et al. 2007). As acceleration of enrolment allowed for a greater proportion of those in need for ART to access it, this was associated by an increase in baseline CD4+ T-lymphocyte count (a marker of disease progression) resulting in an overall decline of mortality on ART in Khayelitsha over time (Van Cutsem, Hilderbrand et al. 2007).

## 1.1. LATE MORTALITY

ART programmes in low income countries have been typically characterized by a very high mortality during the first 3 months of therapy, followed by a subsequent decline in the following years (Lawn, Myer et al. 2005; Braitstein, Brinkhof et al. 2006; Etard, Ndiaye et al. 2006; Lawn, Myer et al. 2006; Zachariah, Fitzgerald et al. 2006; Parkes, Levin et al. 2007). Mortality can thus be divided into early and late mortality. Overall mortality on ART remains higher in low income countries as compared to high income countries (Braitstein, Brinkhof et al. 2006).

In high income countries, the proportion of deaths associated with infectious diseases has declined sharply, in parallel with an increase of deaths associated with heart and liver disease (Crum, Riffenburgh et al. 2006). Although extensive literature exists on the factors associated with death and specific mortality in people on ART in high income countries, little has been published on this topic in Africa.

In addition, due to the relative novelty of ART on this continent, the existing literature focuses mainly on the description of mortality during the first one or two years on treatment (Ivers, Kendrick et al. 2005; Lawn, Myer et al. 2005; Braitstein, Brinkhof et al. 2006; Etard, Ndiaye et al. 2006; Lawn, Myer et al. 2006; Zachariah, Fitzgerald et al. 2006; Moh, Danel et al. 2007; Parkes, Levin et al. 2007). Baseline factors found in previous studies to be associated with mortality on ART in resource poor countries include WHO stage 3 or 4, high viral load, past history of tuberculosis, low body mass index (BMI), low haemoglobin, and low CD4+ T-lymphocyte count. Associated factors

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during follow-up on ART were low CD4+ T-lymphocyte count and a detectable viral load.

Little is known on the outcomes in people who have been on ART for longer periods of time. Since Khayelitsha is one of the oldest ART programmes in Africa, it provides a unique opportunity to describe mortality in this population.

## 1.2. LOSSES TO FOLLOW-UP

Patients lost to follow-up constitute a major problem faced by ART programmes in low income countries. Firstly, because the majority of patients lost to follow-up interrupt their treatment, resulting in a decrease of their CD4+ T-lymphocyte count and the consequent increase in related morbidity and mortality. Secondly, treatment interruption is one of the main risk factors for the emergence of drug resistance and failure of subsequent ART in patients who return into care after a period of defaulting (Oyugi, Byakika-Tusiime et al. 2007).

Loss to follow-up also poses problems in the analysis of programme outcomes. To estimate cumulative incidence using survival analysis one needs to assume that patients lost to follow-up have the same probability of having the outcome as patients who remain in the study (Szklo and Nieto 2004). If patients lost to follow-up are more likely to have died, there is informative censoring (Kirkwood and Sterne 2003). This would lead to a biased overestimation of survival. The Antiretroviral Treatment in Lower Income Countries (ART-LINC) cohort collaboration has shown that in programmes with passive follow-up, reported mortality is lower than in programmes with active follow-up, suggesting that a large proportion of patients lost to follow-up have died (Braitstein, Brinkhof et al. 2006). Whilst during the first years of the programme in Khayelitsha patients lost to follow-up were actively traced, this system has not coped with the increase in enrolment which occurred after 2004 (Van Cutsem, Hilderbrand et al. 2007).

In addition, while major efforts have been made to scale up access to ART in Africa, programme outcomes are being scrutinized by scientists, funders, and policymakers. Patient retention and the extent of losses to follow-up have been the focus of much

University of Cape Town

debate recently (Muller, Boulle et al. 2007; Smart 2007), with some authors painting a very sombre picture (Rosen, Fox et al. 2007; Wakabi 2008). A recent systematic review of ART programmes in Africa stated that retention in care at 2 years is about 60%, with loss to follow up as the major cause of attrition, followed by death (Rosen, Fox et al. 2007). While these results are difficult to interpret due to varying definitions for loss to follow-up (Muller, Boulle et al. 2007) and the heterogeneity of the programmes analyzed, it highlights the need for better understanding of losses to follow-up and description of programmes with good patient retention.

A large study looking at factors associated with non-adherence in patients on ART in France found moderate and poor adherence to be independently associated with younger age, perceived treatment side effects, dosing frequency different from twice daily, a protease inhibitor based regimen, depression, and lack of support from the main partner (Carrieri, Leport et al. 2006). While a number of articles have been published on quantitative as well as qualitative determinants of adherence to ART (Vervoort, Borleffs et al. 2007) in rich countries, very little evidence exists on adherence in resource poor settings.

A recent retrospective study of loss to follow-up among patients attending an urban ARV clinic in Johannesburg, South Africa, described 16.4% of loss to care over a one year period. Almost half of the patients lost (48%) had died. Age, gender, and ethnicity were not found to be predictive of loss to follow-up. Common reasons for non-death losses were relocation or transfer to another clinic, and hospitalization or non-fatal illnesses (Dalal, MacPhail et al. 2008).

A retrospective study of a large-scale ARV programme in western Kenya found a death rate of 5.4% and a loss to follow-up rate of 24.5% at 39 months. The authors suggest that a high proportion of loss to follow-up might be attributable to death, seen that a passive reporting system was used. Male gender was found to be predictive of loss to follow-up (Wools-Kaloustian, Kimaiyo et al. 2006).

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## 1.3.PREVIOUS STUDIES IN SOUTH AFRICA

In 2004, the first report on the ART programme in Khayelitsha described outcomes in 287 ART-naïve adults followed for a median duration of 13.9 months (Coetzee, Hilderbrand et al. 2004). The survival probability at 12 and 24 months on treatment was 86.3% (95% CI 81.7-89.8%); all deaths occurred before 12 months on ART (71% before 3 months), and 4 patients were lost to follow-up. Lower initial $CD4^+$ T-lymphocyte count and a diagnosis of Kaposi sarcoma were associated with death in multivariate analysis. A mortality review identified 95% (36/38) of the deaths to be HIV-related; 52% (20/38) were attributed to advanced HIV disease, 8% (3/38) to tuberculosis, 8% (3/38) to Kaposi sarcoma, and 16% (6/38) to disease progression due to treatment interruption or poor adherence. The study was limited by the short duration of follow-up and the small number of people at risk beyond one year on treatment.

In 2006, a cohort study in a similar setting in Cape Town reported outcomes of 1235 patients referred for ART, of whom 927 (75%) were started on ART over 3 years (Lawn, Myer et al. 2006). Deaths that occurred before initiation of ART accounted for 46% (56/121) of total mortality; deaths during the first 4 months on ART for 40% (49/121), and deaths that occurred after 4 months for 13% (16/121). The mortality rate before initiation of ART was very high at 33.3/100 person-years (95% CI 25.5-43.0); this decreased to 19.1/100 person-years (95% CI 14.4-25.2) during the first 4 months on ART, and was only 2.9/100 person-years (95% CI 1.8-4.8) after this period. Baseline characteristics of pre-treatment deaths and deaths that occurred during the first 4 months on ART were similar. Multivariate analysis identified decreasing baseline $CD4^+$ T-lymphocyte count and WHO stage 3 or 4 as factors associated with death. Deaths on ART before 4 months on treatment were associated with advanced WHO stage, lower baseline $CD4^+$ T-lymphocyte count, and male sex. Deaths occurring after 4 months on treatment were only associated with the increase in $CD4^+$ T lymphocyte at 4 months. The analysis of associations with late mortality was limited by small numbers of deaths occurring after 4 months on ART. In an earlier publication on the same cohort, likely causes of death on ART were cryptococcal meningitis (16%), tuberculosis (13%), wasting syndrome (13%), Kaposi sarcoma (11%), and chronic respiratory disease (11%) (Lawn, Myer et al. 2005). The authors attributed the fact that most cryptococcal-related mortality occurred during the first few weeks on ART to

University of Cape Town

immune reconstitution syndrome. However, this finding remains isolated in the literature.

More recently, Fairall and colleagues described the outcomes of the public sector antiretroviral program in the Free State, South Africa (Fairall, Bachmann et al. 2008). They followed 14,267 patients, of whom 3,619 received ART for up to 19 months (median 6 months; interquartile range 3-9 months). Fifty-three percent (2,430/4,570) of patients followed up for at least one year were known to have died. Eighty-seven (2,105/2,430) of these never accessed ART. Cotrimoxazole prophylaxis, ART, and tuberculosis at baseline were associated with lower mortality. Older age, lower $CD4^+$ T-lymphocyte count, and decreased (baseline-adjusted) weight were associated with higher mortality.

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## 1.4. METHODOLOGICAL ISSUES

All the studies describing mortality on ART, losses to follow-up, and associated factors identified during the literature review were cohort studies, either prospective or retrospective. This is the best study design to examine survival and associated factors in large ART programmes. Randomized controlled trials are excluded for obvious ethical reasons since the efficiency of ART is not disputed, and case-control studies do not estimate rates. Nested case-control or case-cohort studies would be alternative but inferior designs as these would entail a higher risk of selection bias.

The statistical methods most often used were Kaplan-Meier product-limit methods and Cox proportional hazards regression analysis. More complex methods encountered were random-effects Weibull regression models of several datasets with multiple imputations of missing data (Braitstein, Brinkhof et al. 2006), marginal structural regression models adjusted for baseline and time-varying covariates (Fairall, Bachmann et al. 2008), and a combination of probit generalized estimating equations models with an adaptation of the 2-stage Heckman approach to correct for the bias introduced by missing data (Carrieri, Leport et al. 2006).

The definition of lost to follow-up varied greatly between studies. Various definitions encountered include: "patients who had not attended services for 3 months or more

beyond their last scheduled appointment and who could not be traced" (Coetzee, Hilderbrand et al. 2004), "patients receiving ART who were 14 weeks late for a scheduled clinic or pharmacy visit and who were neither transfers-out nor relocations" (Lawn, Myer et al. 2006), "if the last visit was recorded during the first year after starting HAART and the patient had at least 1 year of additional potential follow-up until the closing date of the database" (Braitstein, Brinkhof et al. 2006). In summary, definitions of lost to follow-up ranged between being 3 months and 1 year late for scheduled consultation or medication pick-up (Rosen, Fox et al. 2007).

As programmes mature, investigators are increasingly finding it difficult to describe a single set of associations with outcomes over the full duration of follow-up due to the hazards not remaining proportional over time. This is anticipated as baseline factors are more likely to be strongly associated with early mortality than after a significant immune recovery has occurred. For this reason a number of authors have elected to explore associations with outcomes separately, before and after a defined duration of follow-up.

Definitions of *early* mortality ranged from one to four months on ART. In this study, *early* mortality and losses to follow-up were defined as occurring during the first three months on ART, whilst *late* was defined as occurring at or after three months on ART. The choice of this cut-off was based on the existing literature (Lawn, Myer et al. 2006; Moore, Yiannoutsos et al. 2007) rather than on the actual shape of the hazard function in this cohort.

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# 2. RATIONALE

HIV/AIDS kills 2.5 million people every year worldwide and has become the first cause of death in South Africa (Dorrington, Bourne et al. 2001; UNAIDS and WHO 2007). Changing patterns of mortality after the introduction of antiretroviral therapy are well described in high income countries. However, in Africa, ART was introduced later and at a slower pace than in Europe and North America. Whilst there is some data on early mortality and losses to follow-up, description of outcomes and associations with outcomes in patients who have been on ART for longer periods is lacking. Additional data on outcomes of people on long term ART and associated factors is needed to better

understand the factors associated with mortality and losses to follow-up, to inform policy, and as a measure of programme efficiency.

## 3. AIM

To describe mortality, losses to follow-up, associated factors and changes with duration of follow-up in people started on ART between May 2001 and January 2006.

## 4. OBJECTIVES

In ARV-naïve adults who initiated ART in Khayelitsha:

1. To describe baseline characteristics
2. To describe *early* and *late* survival and associated factors
3. To describe *early* and *late* losses to follow-up and associated factors

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# 5. METHODS

## 5.1. STUDY SETTING AND BACKGROUND

Antiretroviral therapy was started in Khayelitsha in May 2001 by the international humanitarian organization Médecins sans Frontières in collaboration with the Provincial Government of the Western Cape and the University of Cape Town in three community health centres. Before this date, care for people living with HIV in South Africa's public sector was limited to treatment and prophylaxis of opportunistic infections. Diagnosis of HIV was perceived as a death sentence and very few people were accessing voluntary counselling and testing services as stigma around the disease was high.

Until the end of 2003, the South African government was opposed to the provision of antiretroviral therapy within the public sector. As a consequence of this, the ART programme in Khayelitsha was initiated as a study and entirely staffed and funded by MSF during the first years. Generic antiretroviral drugs were imported from the national ART programme in Brazil. Detailed descriptions of the first years of the programme have been published elsewhere (Médecins Sans Frontières and University of Cape Town Department of Public Health 2002; Médecins Sans Frontières South Africa, Department of Public Health at the University of Cape Town et al. 2003; Coetzee, Boulle et al. 2004; Coetzee, Hilderbrand et al. 2004).

Treatment protocols were adapted several times before the introduction of the South African national treatment guideline in 2004 (South African National Department of Health 2004). This resulted in variation of the drugs being part of the first line regimen depending on the year of initiation.

The profile of the population attending the clinics also changed over time. Initially antiretroviral therapy was novel and controversial in South Africa, and patients presenting to the programme were either very ill, well informed, or both. After inception of the national antiretroviral plan in 2004, enrolment on treatment increased massively. As ART became more accessible, stigma decreased. This resulted in increased numbers of people accessing voluntary counselling and testing (VCT) services, followed by increased and earlier presentation to the ART programme. From



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below 1000 per year pre-2001, the number of people attending VCT increased from 16,000 in 2003 to nearly 27,000 in 2005, among whom 8,676 (33%) were HIV-infected (Van Cutsem, Hilderbrand et al. 2007).

From small-scale and nurse-based in 2001 to 2003 – each clinic was run by one doctor, two nurses, and two adherence counsellors – the programme evolved to large volume treatment sites from 2004 onwards. At the end of 2007, more than 7000 patients were on ART in Khayelitsha, with more than 2000 patients per clinic. The staffing increased to three to four doctors, three to four nurses, six adherence counsellors, and two to three clerical staff per clinic. This evolution has been associated with longer waiting times, depersonalisation of care, managerial complications, and increased and earlier loss to follow-up from the programme (Van Cutsem, Hilderbrand et al. 2007).

Treatment of tuberculosis was provided by the national TB control programme in the three sites. In the largest of the three treatment sites, the TB and HIV programmes were integrated in 2003.

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## 5.2. TREATMENT REGIMENS AND CLINICAL PROTOCOLS

From the end of 2003 onwards, the Western Cape provincial protocol (Provincial Administration of the Western Cape 2004) and the South African National guidelines (South African National Department of Health 2004) were followed. These recommend a first line regimen consisting of stavudine and lamivudine as the nucleoside reverse transcriptase (NRTI) backbone, and nevirapine as the non-nucleoside reverse transcriptase (NNRTI) backbone. For patients with tuberculosis (TB) nevirapine was replaced by efavirenz. From 2001 to 2003, zidovudine was used as the NRTI backbone, instead of stavudine. Efavirenz was the preferred NNRTI in 2002 and 2003, whilst a nevirapine-based regimen was initiated in the majority of patients starting in 2001, 2004, and 2005 (Boulle, Van Cutsem et al. 2006). Prevention of mother-to-child transmission was initiated in 1999 by the Provincial Government of the Western Cape. The initial standard regimen for the mother consisted of oral zidovudine from 34 weeks of gestation until delivery, and nothing for the baby. In 2004, a single dose of nevirapine during childbirth was added to this regimen, as well as a dose of nevirapine and 7 days prophylaxis with zidovudine to the baby.

Criteria for initiation of ART in South Africa are a $CD4^+$ T-lymphocyte count below 200 cells/μl or WHO stage IV disease. All patients attend at least three adherence counselling sessions before being started on ART. After initiation, routine clinic visits are scheduled at two weeks, and then monthly. Routine laboratory investigations initially included at baseline were a viral load, $CD4^+$ T-lymphocyte count, haemoglobin, platelet count, alanine transaminase (ALT), and leukocyte differential count; haemoglobin and leukocyte differential count was measured at two weeks, 1 month, 2 months, 3 months, and then six monthly in patients on zidovudine; ALT was measured at the same intervals in patients on nevirapine. $CD4^+$ T-lymphocyte count and viral loads were done six monthly after initiation. Adverse events were graded using the AIDS Clinical Trial Toxicity (ACTG) scale (Centers for Disease Control and Prevention 1992).

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## 5.3.STUDY DESIGN

This is a prospective cohort study. Khayelitsha is a sentinel site for monitoring and evaluation of the ARV programme in the Western Cape. Enhanced routine surveillance and data collection has been maintained since inception of the programme with a view to describe a range of clinical outcomes and their associations.

## 5.4.POPULATION AND PERIOD OF OBSERVATION

The study population consists of adults (14 years and older) started on ART in Khayelitsha between 12 March 2001 and 30 December 2005. Individuals who had been on ART for more than one month prior to initiation of treatment, and children younger than 14 years at initiation were excluded from the analysis. Pregnant women who had received ARV drugs as part of prevention of mother to child transmission (PMTCT) were not excluded. All patients were included; hence there was no sampling within the study population.

Patients were observed from ART initiation until 30 December 2006. Right censoring occurred when a patient died (with loss to follow-up as the outcome), was lost to

follow-up (with mortality as the outcome), or transferred to another site. Observations were censored at the date of death or transfer to another clinic; for losses to follow-up the date of the last visit at the clinic was used.

## 5.5.OUTCOME MEASURES

The primary outcome measures were time to death and time to loss to follow-up. Outcome was recorded as a categorical variable in the database (HIV-related death, non-HIV related death, death without known cause, lost to follow-up, and transferred out) and as separate binary variables for all deaths, HIV-related deaths, and lost to follow-up.

Dates of the outcomes, initiation on ART, and every clinical visit were recorded as continuous variables. Time was measured from the date on which ART was initiated until the date of death, transfer out, or most recent visit at the clinic before 1 January 2007.

Death was ascertained through notification of the clinic by family or friends, home visits to patients lost to follow-up by a nurse and an adherence counsellor, telephonic consultation of hospital records, and consultation with patients in the support groups.

For the purpose of this analysis, patients were defined as lost to follow-up when their most recent clinic visit occurred before 1 July 2006, and they were not known to have died or been transferred out. The definition of loss to follow-up as at least six months without a clinic visit was chosen to reflect a compromise measure between the three months without a visit operational definition used by the clinics in the Western Cape to identify recent defaulters (Coetzee, Hilderbrand et al. 2004) and the one year definition used more widely in publications describing outcomes of ARV programmes (Braitstein, Brinkhof et al. 2006).

Procedures in place for tracing patients who missed their scheduled appointment for more than one week consisted of two telephone calls by the clinic counsellors, followed by a home visit by a nurse and a counsellor if the calls were unsuccessful. The number of counsellors increased from two per clinic in 2001 to six per clinic in 2006.

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## 5.6. EXPOSURE VARIABLES

Demographic data, patient history, and referral information were recorded at first presentation of patients to the clinic. Since data were collected as part of routine surveillance within a busy public sector clinic, it was not possible to collect data on socio-economic factors such as income, education, housing, or immigration from outside the Western Cape. Clinical information and results of laboratory investigations were recorded at the date of the clinic visit. When laboratory investigations occurred without a clinical visit, the results were allocated to the closest visit. Gender, previous treatment for tuberculosis, pregnancy, the presence of an AIDS-defining illness, treatment interruption, and being on second line regimen were recorded as a binary variables; clinic, source of referral, WHO clinical stage, initial and current nucleoside reverse transcriptase inhibitors (NRTI) or other antiretroviral drug, the presence of individual opportunistic infections, and adverse event were entered as categorical variables; date of birth, age (years), date of diagnosis of HIV, $CD4^+$ T-lymphocyte count (cells/μl), viral load (copies/μl), weight (kg), and dates of clinical consultation were recorded as continuous variables.

The variable 'AIDS' was equivalent to WHO stage 4 disease. It was kept as a crude measure of disease severity; WHO stage added some gradation to this measure, whilst specific opportunistic infections were the most detailed measure. Since not all opportunistic infections were recorded, WHO stage did reflect some additional information on disease severity.

Opportunistic infections and adverse events were recorded as new, ongoing, or recurrent. Specific opportunistic infections that were captured electronically were: pulmonary and extra-pulmonary tuberculosis, invasive cervical cancer, oral candidiasis, oesophageal candidiasis, cardiomyopathy, coccidioidomycosis, cryptococcal meningitis, cryptosporidiosis, cytomegalovirus retinitis, chronic diarrhoea, herpes simplex infection, herpes zoster infection, HIV encephalopathy, human papillomavirus infection, isosporiasis, Kaposi sarcoma, non-Hodgkin lymphoma, and wasting syndrome. Chronic diarrhoea and wasting syndrome were defined according to the revised CDC case definitions for AIDS (Centers for Disease Control and Prevention 1987) as respectively 'diarrhoea lasting for more than one month', and 'involuntary weight loss of 10% of baseline body weight plus either chronic diarrhoea (two loose stools per day for more than 30 days) or chronic weakness and documented

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fever (for 30 days or more, intermittent or constant) in the absence of a concurrent illness or condition other than HIV infection that would explain the findings'. Adverse events were graded according to ACTG (Centers for Disease Control and Prevention 1992).

## 5.7.SAMPLE SIZE & POWER CALCULATION

3,595 patients were started on ART during the observation period, and were followed up during a total of 6,563 person-years. On 31 December 2006, 375 (10%) patients were confirmed to have died, 177 (5%) had been transferred out, and 254 (7%) were lost to follow-up (i.e. had not attended the clinics for at least six months).

Using the method of Hsieh and Lavori for sample size calculations for the Cox proportional hazards regression model with non-binary variables (Hsieh and Lavori 2000) and Schoenfeld's method for binary variables (Schoenfeld 1983), it was determined that with a sample size of 3,595, for a significance level of 0.05, a power of 0.8, a probability of failure of 0.1, and a standard deviation of the covariate of 0.5, the minimal detectable value by a two-sided Wald test was a hazard ratio of 0.74 (StataCorp 2007) . For a probability of failure of 0.02, however, the minimal detectable value for the hazard ratio would have been 0.52. Probabilities of failure were informed by published results of earlier periods of the Khayelitsha cohort, where mortality and losses to follow-up at three months were respectively 0.1 and 0.01 in 2002 and from 2004 onwards there was a trend towards increased loss to follow-up and decreased mortality (Coetzee, Hilderbrand et al. 2004; Van Cutsem, Hilderbrand et al. 2007).

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## 5.8.MEASUREMENTS

Demographic data were entered by clerks directly into an electronic database at patients' first visit to the clinic. During following consultations variables were prospectively captured on a paper-based monitoring sheet by the attending clinicians and subsequently entered into the electronic database by a data capturer.

There were no standardized case definitions of opportunistic infections; diagnosis was based on clinical judgement of the attending clinician. For tuberculosis, standard definitions as defined by the South African national guidelines were used (The South African Tuberculosis Control Programme 2000). Pulmonary TB refers to disease involving the lung parenchyma. Disease confined to other areas (including the pleura and hilar lymphadenopathy) constitute extra-pulmonary TB. A patient with both pulmonary and extra-pulmonary TB constitutes a case of pulmonary TB.

Available means for the diagnosis of tuberculosis included smear microscopy, culture, chest radiography, abdominal ultrasound, and computed tomography scanning for cerebral lesions. A clinical algorithm was used for the early diagnosis of smear negative pulmonary tuberculosis. This included monitoring weight, clinical symptoms, C-reactive protein, haemoglobin, and Karnofsky score.

Laboratory analyses were performed by the National Health Laboratory System. CD4+ T-lymphocyte counts were performed by flow cytometry and HIV RNA PCR viral load by nucleic acid sequence-based amplification procedure (NASBA) Nuclisens HIV-1 QT assay (BioMerieux, Boxtel, Netherlands). Smear microscopy for tuberculosis was performed on concentrated auramine stain examined under x500 magnification, using a fluorescent microscope, and graded according to IUATLD guidelines. Culture of mycobacteria was performed using the BACTEC MGIT 960 system (BD Diagnostic Systems, Sparks, MD, USA), and Mycobacterium Tuberculosis complex was identified using Ziehl-Nielsen staining and p-nitrobenzoic acid testing.

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## 5.9. RELIABILITY

Two quality assessors regularly examined all data in the electronic database for discrepancies, doubtful or unlikely information, and missing data, and verified electronic data against paper-based data. Data capturers and admin-clerks underwent regular training and attended weekly meetings. A quality control manager was responsible for ongoing folder reviews and communication with clinicians and data capturing staff concerning the quality of data entering (paper and electronic). A number of validity checks were introduced in the design of the database to prevent frequently occurring mistakes in data entering and capturing.

## 5.10. DATA MANAGEMENT

Data were prospectively captured on site into the electronic database by trained data capturers. Quality assessors and senior data management staff regularly cleaned and synchronized the data of each clinic into one central database.

## 5.11. STATISTICAL ANALYSIS

Data were analysed using Stata™ statistical software: release 10.0 (StataCorp. 2007. College Station, TX: Stata Corporation). Data exports for analysis from the electronic database to Stata™ were anonymous.

The primary response variables were of the *time-to-event type*: time (years) from initiation of ART until death or loss to follow-up. One year was defined as 365.25 days. Analyses were repeated for each of the two response variables in patients who had been on ART for less than three months and in patients who had been on ART for three months or more. These two periods are referred to respectively as *early* and *late* mortality and loss to follow-up in this study.

### EXPLORATORY ANALYSIS

Data exploratory analysis was performed as follows (Kirkwood and Sterne 2003). Distributions and summary statistics of individual variables were examined by using histograms and the Shapiro-Wilks test to test for normality of continuous variables, summary statistics (mean, standard deviation, median, inter-quartile range, and range), frequency tables for categorical variables, and median survival time for time-to-event variables.

Possible associations between the response and covariate variables were examined by using scatter plots, box plots by different categories, and contingency tables. Spearman's rank test was used to test for correlations between non-normally distributed variables. The Wilcoxon rank-sum (Mann-Whitney) test was used to test for differences between medians; Pearson's chi-squared test and Fisher's exact test were used to test for differences between proportions. A Kruskal-Wallis test and

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Cuzick's non-parametric test (Cuzick 1984) was used to test for trend across ordered categories.

The response variable being of the time-to-event type, survival analysis was chosen as the appropriate method of analysis. Summary statistics (time at risk, incidence rate, number of subjects, and 25th, 50th, and 75th percentile of survival time) were calculated for the two response variables. Estimates of the hazard function were plotted using weighted kernel-density estimates based on an Epanechinikov function (StataCorp 2003). Estimates of the Kaplan-Meier survival and failure function, as well as the Nelson-Aalen cumulative hazard function were plotted for *early* and *late* mortality and loss to follow-up. Adjusted and unadjusted stratified curves were produced for the categorical variables to look for potential associations. A logrank test was used to test for equality of survivor functions across binary categories. A stratified logrank test was used when adjustment for potential confounding was necessary. When three or more ordered categories were compared, a test for trend was used.

Conditions present at the last visit before death for early and late deaths were calculated as proportions of the total number of deaths. Differences between medians and proportions were assessed as described above.

## DATA CLEANING

Range checks were conducted and outliers with biologically impossible values were replaced by missing data (e.g. weight above 150 kg or below 15 kg). Outliers with biologically possible data were left unchanged. Consistency checks were conducted and inconsistent data were replaced where possible (e.g. gender and pregnancy were cross-tabulated and pregnancy was changed to 'absent' in men).

## DATA REDUCTION

Irrelevant variables and observations occurring before or after the period of observation were dropped. The continuous variables age, weight, and $CD4^+$ T-lymphocyte count were regrouped into categories. WHO stages 1 & 2 were grouped together as these are clinically very similar and represented a small proportion of the observations. Origin of referral was regrouped from 26 into 5 categories, with individual hospices and hospitals being regrouped in one 'Hospital' category, individual

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primary care clinics into a 'Primary Health Clinics' category; referrals from the prevention of mother-to-child transmission programme (PMTCT) and TB clinics remained categories on their own, and a new category 'Other' was created, regrouping the categories private clinician, home-based care, non-governmental organization (NGO), none, outside of Khayelitsha, lay counsellor, Treatment Action Campaign (TAC), Youth Clinic Khayelitsha, and other. The years of initiation of ART 2001 and 2002 were regrouped into one category for some of the analyses as only a small number of patients were seen during those years. For the analysis of loss to follow-up, 2003 was added to this category since there was no early loss to follow-up during the first two years. Other data manipulations included renaming of variables, creation of new time variables, creation of new variables for opportunistic infections present at baseline, weight changes, $CD4^+$ T-lymphocyte changes, viral load changes over time, and viral non-suppression.

## UNIVARIATE ANALYSIS

Univariate Cox proportional hazards regression analysis was used to investigate the effect of individual covariates on the relative hazard of death and lost to follow-up in patients on ART for less than three months and in patients on ART for at least three months. A Wald test for linear hypotheses after estimation was used for ordinal variables (StataCorp 2007).

Separate tables were constructed for baseline characteristics and time-varying covariates as their clinical significance is different, as well as the way they impact on the relative hazard. Baseline characteristics are present before initiation on ART and are thus not influenced by treatment. Their impact on the relative hazard is constant. Time-varying covariates represent events that occur on ART, and whose influence on the hazard is changing with time. While proportional hazards regression with baseline covariates explore how characteristics present at initiation of treatment are associated with death or loss to follow-up, regression with time-varying covariates explores associations with events that occur on ART. For clinical purposes, analysis of baseline covariates helps to identify categories of patients who are at higher risk of death or loss to follow-up so that they can be given enhanced attention. Analysis of time-varying covariates aims at identifying variables that should be monitored in patients on treatment.

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For baseline characteristics, models were fitted only for opportunistic infections present in 1% or more of all patients. Since not all WHO stage defining opportunistic infections were included, WHO stage still captures some information not reflected by the individual opportunistic infections.

For time-varying - on ART - covariates, models were fitted for all opportunistic infections, but only shown if enough observations were present to perform the regression. Weight gain and CD4 gain were defined as the difference between the value on the current visit and the baseline value. Different categories of $CD4^+$ T-lymphocyte counts were created to reflect the larger numbers of increased values on ART. Failure to suppress viral load was defined as having a viral load above 400 copies/μl at any time point after initiation.

## MODEL BUILDING

The strategy for selection of variables to be included in the multivariate Cox proportional hazards regression models was based on purposeful selection (Hosmer and Lemeshow 1999). All variables with p-values below 0.25 in the univariate analyses were included in an initial model. Known confounders (gender, age, baseline $CD4^+$ T-lymphocyte count), and variables judged to be clinically important (tuberculosis at initiation, previous exposure to PMTCT, efavirenz and stavudine) were always included in the models, regardless of their univariate level of significance.

For the analysis of mortality, it was chosen to include the most frequent opportunistic infections (present in > 1% of total patients) rather than WHO stage, as this yielded more detailed information and reflected the data better. Rarely recorded opportunistic infections were not included as they didn't contribute to the model due to the small number of observations. For the analysis of loss to follow-up, AIDS at baseline was chosen as a marker of disease severity, since individual opportunistic infections were weakly or not associated with loss to follow-up and didn't contribute to the model.

Then, covariates with p-values from the Wald tests of the individual hazard ratios above 0.25 were removed from the initial model one by one. After removal of each covariate, it was assessed whether there were important changes in the coefficients of the remaining covariates. A change in the direction of the association, or a difference of 20% or more was defined as important. After removal of all covariates with a p-value

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above 0.25, variables excluded from the initial model were introduced in the reduced model to check for confounding or statistical significance. Covariates with p-values below 0.25 were added to the model.

All models were then compared using Aikaike's information criterion (AIC). The model with the lowest AIC, and that contained all the clinically important variables, was chosen as the final model. Results of the manual model building were compared with results from stepwise procedures to check whether important covariates had been omitted.

## MULTIVARIATE MODELS

Four multivariate Cox proportional hazards regression models were constructed, looking at baseline factors associated with early mortality, late mortality, early loss to follow-up and late loss to follow-up. The model for early mortality was built using stratified Cox regression, to accommodate for the non proportional hazard of gender during this period, while still controlling for confounding by gender.

An attempt to construct multivariate models with time-varying covariates only was made; however, this was unsuccessful due to the insufficient number of observations leading to model instability due to small cells. Time-varying covariates were thus subsequently added to baseline characteristics in the four initial multivariate models following the same model building strategy.

Covariates on the causal pathway between baseline variables and the outcome were not added to the model. For example, baseline oesophageal candidiasis can lead to weight loss, which is associated with mortality. Oesophageal candidiasis and weight gain were thus not introduced in the same model.

## MODEL CHECKING

The following diagnostics were performed for all models. First, a link test for single equation models was performed to assess model specification (Cleves, Gould et al. 2002; StataCorp 2003; StataCorp 2007). The link test considers the specification of the dependent variable and the explanatory variables that are properly specified, under the assumption that the model contains the right explanatory variables (Cleves, Gould et al.

University of Cape Town

2002).

Then the proportional hazards assumption was checked by testing, for individual covariates and globally, the null hypothesis of zero slope in a generalized regression of the scaled Schoenfeld residuals on the functions of time (Cleves, Gould et al. 2002; StataCorp 2003). Graphical assessment of the proportional hazards assumption was performed through the examination of "log-log" plots for each category of the nominal or ordinal covariates. "Log-log" plots are plotting -ln(-ln(survival)) curves for each category of covariates against ln(analysis time) (StataCorp 2003). Unadjusted log-log plots were compared with plots adjusted for the other covariates in the model.

A proportional hazards model stratified by gender was fitted for mortality in patients who were on ART for less than three months to accommodate non-proportional hazards of gender during that period (Hosmer and Lemeshow 1999). The test of non-zero slope assumes homogeneity of variance across risk sets. This assumption may not hold in stratified Cox models (Cleves, Gould et al. 2002). Therefore, the test for proportional hazards assumption was checked separately for each stratum.

Overall model fit was assessed by plotting the cumulative hazard against the cumulative Cox-Snell residuals. If the Cox regression model fits the data, the Cox-Snell residuals have a standard censored exponential distribution with a hazard ratio of 1 and the plot of the cumulative hazard versus the Cox-Snell residuals should be a straight line with slope 1 (Cleves, Gould et al. 2002; Little 2007).

Cumulative martingale residuals and deviance residuals were generated and plotted versus the linear predictor to assess model fit and identify outliers and potentially influential observations.

University of Cape Town

# 6. ETHICAL CONSIDERATIONS

Ethical clearance has been obtained from the University of Cape Town Health Sciences Faculty Research Ethics Committee for *enhanced routine surveillance of an HIV clinic population in Khayelitsha (REC REF 395/2005)*. The data used for this study are part of routine monitoring of the ART programme in the Western Cape and no additional data items were collected solely for research purposes. All data was dealt with confidentially and anonymity of patients was respected by the researchers. The database containing names is password-protected and the exported data used for analysis was anonymous. Patients did not obtain immediate benefit from this study. However, a better understanding of mortality and loss to follow-up is needed to improve the programme functioning and future outcomes. In this sense, this study might result in indirect benefits to participants who are still alive and in care. In terms of justice, patients are part of the population who stand to benefit from the research. Study results will be disseminated to local staff and provincial policymakers, especially the ARV directorate of the Provincial Government of the Western Cape.

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# 7. RESULTS

## 7.1.COHORT DESCRIPTION

### COHORT CHARACTERISTICS

Characteristics of the whole cohort as well as from deaths, losses to follow-up and patients alive and in care are shown in Table 1. The majority of patients were women between 25 and 34 years, with a median weight of 58 kg (IQR 51-67 kg) and a median baseline $CD4^+$ T-lymphocyte count of 89 cells/μl (IQR 37-148 cells/μl). Almost one third were on treatment for tuberculosis when they started on ART, whilst 45% had been previously treated for tuberculosis, and 40% had WHO stage 4 disease. Sixty-nine percent were referred from primary health care services, primarily community health centres (42%), but also TB clinics (18%) and the prevention of mother to child programme (9%). Amongst women, 6% were pregnant when they started ART, and 14% had been exposed to nevirapine and/or zidovudine to prevent mother to child transmission during pregnancy. Seventy-nine percent of patients were started on stavudine, and 50% on nevirapine, reflecting the standard first line regimen in South Africa. The large proportion of patients started on efavirenz reflected both the high prevalence of tuberculosis, and the fact that efavirenz was the preferred non-nucleoside reverse transcriptase inhibitor in the programme in 2002 and 2003. Enrolment increased exponentially, with 19% of the patients started from 2001 to 2003, and 50% of all patients in 2005. The median time between the date on which the baseline CD4 count was taken and initiation on treatment was 50 days (IQR 9-97).

Baseline characteristics are broken down by outcome group in table 1. Differences between patients who died or were lost to follow-up and patients alive and in care are not discussed in this section, as they are discussed in detail when the univariate analyses are presented below.

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TABLE 1 COHORT CHARACTERISTICS

| | All | | Alive in care | | Dead | | Lost to follow-up | |
|---|---|---|---|---|---|---|---|---|
| **Number** | 3595 | | 2789 | 78% | 375 | 10% | 254 | 7% |
| **Baseline weight, kg (median, IQR)** | 58.3 | 51.4-67 | 59.3 | 52.5-68 | 53 | 45.5-61 | 56.7 | 50-63 |
| **Baseline viral load, log copies/µl (median, IQR)** | 5.1 | 4.6-5.6 | 5.1 | 4.5-5.6 | 5.2 | 4.8-5.7 | 5.2 | 4.7-5.6 |
| **Women** | 2551 | 71% | 1988 | 71% | 249 | 66% | 176 | 69% |
| **Age categories (years)** | | | | | | | | |
| **14 - 24** | 384 | 11% | 285 | 10% | 40 | 11% | 32 | 13% |
| **25 - 34** | 1846 | 51% | 1443 | 52% | 174 | 46% | 141 | 56% |
| **35 - 44** | 1024 | 28% | 800 | 29% | 114 | 30% | 66 | 26% |
| **45 - 54** | 290 | 8% | 223 | 8% | 40 | 11% | 12 | 5% |
| **>= 55** | 51 | 1% | 38 | 1% | 7 | 2% | 3 | 1% |
| **Median, IQR** | 32 | 28-38 | 32 | 28-38 | 33 | 29-39 | 30 | 27-37 |
| **Baseline CD4 categories (cells/ µL)** | | | | | | | | |
| **< 25** | 637 | 18% | 416 | 15% | 146 | 39% | 36 | 15% |
| **25 - 49** | 479 | 14% | 349 | 13% | 72 | 19% | 31 | 13% |
| **50 - 99** | 790 | 23% | 632 | 23% | 61 | 16% | 56 | 24% |
| **100 - 199** | 1388 | 40% | 1145 | 43% | 79 | 21% | 97 | 41% |
| **≥ 200** | 187 | 5% | 149 | 6% | 16 | 4% | 16 | 7% |
| **Median, IQR** | 89 | 37-148 | 95 | 44-153 | 39 | 13-100 | 93.5 | 43.5-148 |
| **AIDS at initiation** | 1443 | 40% | 1035 | 37% | 246 | 66% | 87 | 34% |
| **WHO Stage in initiation** | | | | | | | | |
| **1 & 2** | 548 | 12% | 278 | 10% | 5 | 1% | 29 | 11% |
| **3** | 1702 | 47% | 1307 | 47% | 85 | 23% | 119 | 47% |
| **4** | 1443 | 40% | 1204 | 43% | 285 | 76% | 106 | 42% |
| **Opportunistic infections at initiation** | | | | | | | | |
| **Tuberculosis (all)** | 1131 | 31% | 858 | 31% | 130 | 35% | 84 | 33% |
| **Pulmonary TB (PTB)** | 786 | 22% | 596 | 21% | 90 | 24% | 59 | 23% |
| **Extra-Pulmonary TB (EPTB)** | 392 | 11% | 296 | 11% | 4 | 13% | 27 | 11% |
| **Kaposi Sarcoma** | 103 | 3% | 52 | 2% | 41 | 11% | 7 | 3% |
| **Oro-pharyngeal candidiasis** | 90 | 3% | 1 | 0% | 18 | 5% | 1 | 0% |
| **Cryptococcal meningitis** | 73 | 2% | 46 | 2% | 18 | 5% | 2 | 1% |
| **Chronic diarrhoea** | 53 | 1% | 28 | 1% | 20 | 5% | 2 | 1% |
| **PTB + EPTB** | 47 | 1% | 34 | 1% | 9 | 2% | 2 | 1% |
| **Oesophageal candidiasis** | 38 | 1% | 24 | 1% | 12 | 3% | 1 | 0% |
| **Wasting Syndrome** | 23 | 1% | 5 | 0% | 16 | 4% | 1 | 0% |
| **HIV encephalopathy** | 17 | 0% | 12 | 0% | 4 | 1% | 1 | 0% |
| **Cryptosporidiosis** | 11 | 0% | 8 | 0% | 2 | 1% | 0 | 0% |
| **Toxoplasmosis** | 10 | 0% | 8 | 0% | 2 | 1% | 0 | 0% |
| **Cytomegalovirus** | 10 | 0% | 5 | 0% | 4 | 1% | 0 | 0% |
| **Pneumocystis Jiroveci pneumonia** | 8 | 0% | 6 | 0% | 1 | 0% | 0 | 0% |
| **Herpes Zoster Virus** | 7 | 0% | 7 | 0% | 0 | 0% | 0 | 0% |
| **Initial ARV Regimen** | | | | | | | | |
| **AZT/3TC/NVP** | 303 | 8% | 238 | 9% | 23 | 6% | 22 | 9% |
| **AZT/3TC/EFV** | 464 | 13% | 327 | 12% | 86 | 23% | 25 | 10% |
| **D4T/3TC/NVP** | 1500 | 42% | 1221 | 44% | 115 | 31% | 94 | 37% |
| **D4T/3TC/EFV** | 1317 | 37% | 997 | 36% | 147 | 40% | 112 | 44% |
| **Origin of referral** | | | | | | | | |
| **Hospital** | 388 | 11% | 286 | 10% | 53 | 14% | 30 | 12% |
| **MTCT** | 323 | 9% | 264 | 9% | 17 | 5% | 25 | 10% |
| **Other** | 718 | 20% | 510 | 18% | 97 | 26% | 56 | 22% |
| **Primary Health Clinics** | 1522 | 42% | 1223 | 44% | 135 | 36% | 102 | 40% |
| **TB Clinics** | 644 | 18% | 506 | 18% | 73 | 19% | 41 | 16% |
| **Prior TB** | 1630 | 45% | 1253 | 45% | 217 | 58% | 92 | 37% |
| **Exposure to PMTCT** | 366 | 10% | 306 | 11% | 27 | 7% | 17 | 7% |
| **Pregnancy at initiation** | 151 | 4% | 125 | 4% | 5 | 1% | 14 | 6% |
| **Months of follow-up (median, IQR)** | 27 | 17-43 | 30 | 21-47 | 10 | 6-23 | 14 | 6-29 |
| **Months on ART (median, IQR)** | 19 | 13-29 | 22 | 16-31 | 3 | 1-9 | 8 | 2-17 |
| **Days between baseline CD4 and start of ART (median, IQR)** | 50 | 8-97 | 53 | 8-99 | 33 | 7-84 | 48 | 11-89 |
| **On Second Line Regimen** | 135 | 4% | 123 | 4% | 5 | 1% | 6 | 2% |
| **Ever interrupted treatment** | 244 | 7% | 199 | 7% | 6 | 2% | 22 | 9% |
| **Year of initiation on ART** | | | | | | | | |
| **2001** | 82 | 2% | 57 | 2% | 18 | 5% | 3 | 1% |
| **2002** | 207 | 6% | 140 | 5% | 44 | 12% | 9 | 4% |
| **2003** | 394 | 11% | 283 | 10% | 68 | 18% | 22 | 9% |
| **2004** | 1102 | 31% | 834 | 30% | 122 | 33% | 83 | 33% |
| **2005** | 1810 | 50% | 1475 | 53% | 123 | 33% | 137 | 54% |

Outcomes (alive in care, dead, lost to follow-up) are for the entire study period (i.e. from May 2001 to 31 December 2006). IQR=inter-quartile range, ARV=antiretroviral, AZT=zidovudine, 3TC=lamivudine, NVP=nevirapine, EFV=efavirenz, D4T=stavudine, MTCT=Mother to child transmission programme, TB=tuberculosis, PMTCT= prevention of mother to child transmission.

Missing data : weight: 235 (6.5%); viral load: 570 (15.9%); gender: 0; age: 0; cd4: 114 (3.2%); aids: 0; WHO stage: 2 (0.06%); opportunistic infections: 0; ARV regimen: 0 (11 patients were on non-standard regimens); Origin of referral: 9 (0.3%); Prior TB: 0; PMTCT: 13 (0.4%); all other variables: 0.

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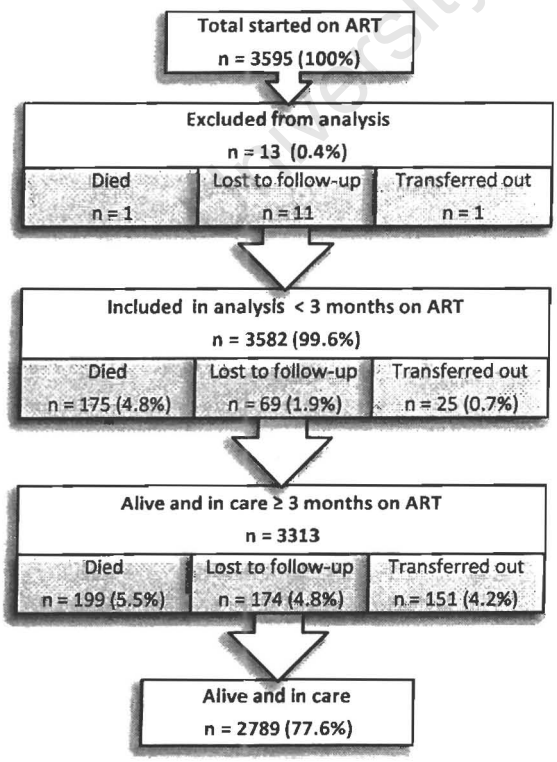
3,595 patients were started on ART between March 2001 and December 2005, of which 3,582 were included in the analysis (figure 1), contributing to 6,553 person-years of observation (731 person-years before 3 months and 5,822 person-years from 3 months onwards). 13 patients were excluded because the outcome occurred on the day of initiation on ART. In total, 617 (17.2%) patients were lost to care (i.e. died or were lost to follow- up): 244 (6.8%) during the first three months, and 373 (10.4%) after at least three months on ART. The total number of consultations during the study period was 91,137. Median time of follow-up on ART was 19 months (IQR 13-29) for all patients, 20 months (IQR 15-30) for patients who had been at least 3 months on ART, and 1 month (IQR 0.6-1.9) for patients who were lost to care before 3 months. The median survival time was 3.4 months (IQR 1.0-9.3) for patients who died and 7.8 months (IQR 2.0-17.3) for patients who were lost to follow-up. Smoothed hazard estimates (figure 2) showed a high initial risk of death, followed by a rapid decline during the first year. The risk of death remained almost constant after one year. The risk of loss to follow-up was highest immediately after ART initiation, though less than half the risk of death, and thereafter declined gradually to reach plateau after three years.

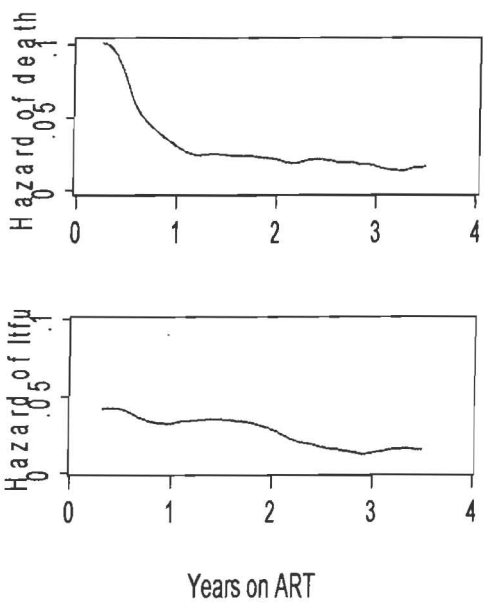
University of Cape Town

FIGURE 1 FLOW DIAGRAM SHOWING OUTCOME EVENTS DURING THE PERIOD OF OBSERVATION.




ART: antiretroviral therapy. Percentages are of the total number started on ART

FIGURE 2 SMOOTHED HAZARD ESTIMATES OVER TIME



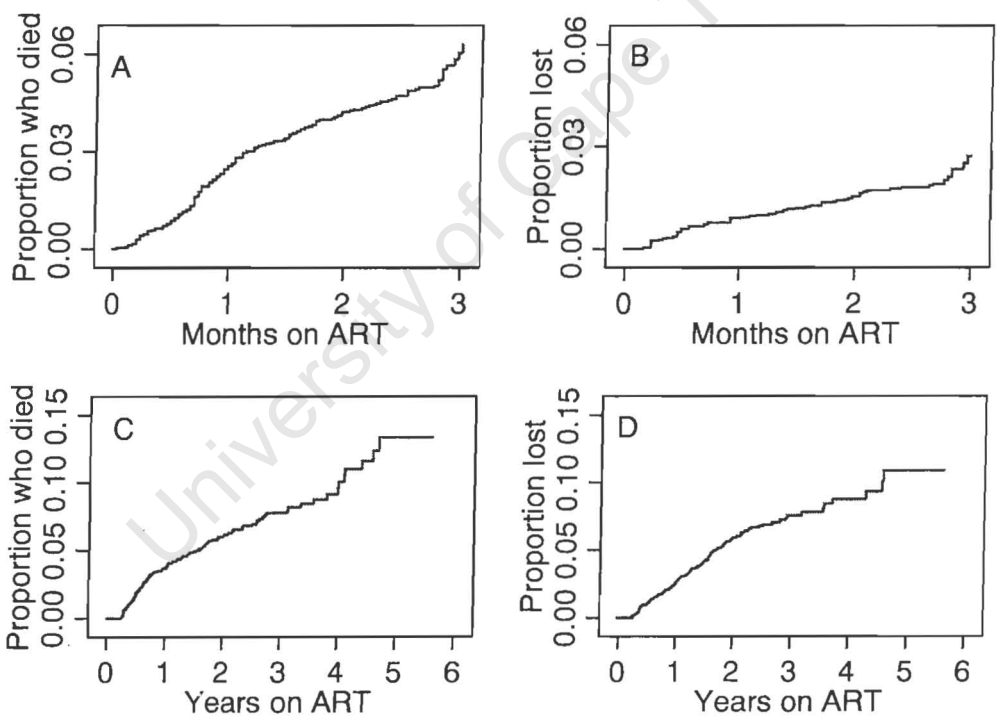

Ltfu: lost to follow-up. ART: antiretroviral therapy

The cumulative probability of dying was highest during the first month (2.3%), accounting for 23% of all deaths (85/374), and remained high during the first year, after which it stabilised at a lower level (between 1 and 4% per year) (figure 3 and table 2). 46.7% (175/374) of all deaths occurred during the first three months.

The cumulative probability of being lost to follow-up remained stable at 4% per year during the first two years, and was halved in the following years. Whilst the probability of death remained higher than that of loss to follow-up over the entire period, the gap was markedly reduced after two years on ART.

The cumulative probability of being lost to care at 5 years was 28% (95% CI 24-32%). The probabilities of being lost to follow-up at 1, 2, and 5 years were respectively 4% (95% CI 4-5%), 8% (95% CI 7-9%), and 13% (95% CI 10-16%).

FIGURE 1 KAPLAN-MEIER FAILURE CURVES OF MORTALITY AND LOSSES TO FOLLOW-UP




Kaplan-Meier failure curves for A. Mortality during the first 3 months B. Losses to follow-up during the first 3 months C. Mortality from 3 months to the end of the study period D. Losses to follow-up from 3 months to the end of the study period. Time to failure was calculated from initiation on ART to death/loss to follow-up or censoring. ART: antiretroviral therapy. Lost: lost to follow-up. Early mortality was twice as high as early losses to follow-up (respectively 6% versus 3% at 3 months), whilst late mortality reached similar levels at 5 years compared to late losses to follow-up. The curve for mortality flattens after one year, whilst the curve for losses to follow-up keeps the same slope until 3 years on ART.

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TABLE 2 CUMULATIVE ESTIMATES OF MORTALITY AND LOSS TO FOLLOW-UP

| Time on ART | 0 | 3 months | 6 months | 1 year | 2 years | 3 years | 4 years | 5 years |
|---|---|---|---|---|---|---|---|---|
| **Deaths** | 0 | 175 | 59 | 60 | 51 | 17 | 7 | 5 |
| **Percentage** | 0 | 5% | 7% | 8% | 11% | 12% | 14% | 18% |
| **95% CI** | | (4-6%) | (6-8%) | (8-9%) | (10-12%) | (11-14%) | (12-17%) | (14-22%) |
| **Lost to follow-up** | 0 | 69 | 32 | 48 | 71 | 16 | 4 | 3 |
| **Percentage** | | 2% | 3% | 4% | 8% | 9% | 11% | 13% |
| **95% CI** | | (2-3%) | (2-4%) | (4-5%) | (7-9%) | (8-11%) | (9-12%) | (10-16%) |
| **Number in care** | 3582 | 3316 | 3194 | 2954 | 1346 | 492 | 202 | 55 |
| **Percentage surviving** | 100% | 93% | 91% | 88% | 83% | 80% | 77% | 72% |
| **95% CI** | | (92-94%) | (90-92%) | (86-89%) | (81-84%) | (78-81%) | (74-79%) | (68-76%) |

CI: confidence interval. ART: antiretroviral. Proportions are estimated Kaplan-Meier failure functions.

## TRENDS OVER TIME

The overall death rate was 5.7/100 person-years; it was 23.9/100 person-years during the first three months, and fell to 3.4/100 person-years for late mortality. From three months to one year it was 5.2/100 person-years, and after one year it was stable at 2/100 person-years.

The incidence rate of loss to follow-up was 3.7/100 person-years overall: 9.4 during the first three months, and 3.0 afterwards. The incidence rate of loss to care was 33.3/100 patient-years during the first three months, 6.4 after that, and 9.4 overall.

Early mortality rates decreased between 2001 and 2005, whilst late mortality remained stable (table 3).

TABLE 3 EVOLUTION OF RATES OVER YEARS OF INITIATION

| | Rate of ltfu | | Mortality rate | | Rate of loss to care | |
|---|---|---|---|---|---|---|
| | early | late | early | late | early | late |
| **2001** | 0.0 | 0.9 | 40.5 | 3.3 | 40.5 | 4.3 |
| **2002** | 0.0 | 1.3 | 47.0 | 3.5 | 47.0 | 4.8 |
| **2003** | 5.1 | 1.8 | 39.2 | 3.8 | 44.3 | 5.6 |
| **2004** | 4.0 | 3.6 | 24.7 | 3.3 | 28.7 | 6.9 |
| **2005** | 15.3 | 4.0 | 16.7 | 3.3 | 32.0 | 7.3 |

Rows are years of initiation on ART. Rates are in person-years of observation. ART: antiretroviral therapy. Ltfu: lost to follow-up

Early as well as late loss to follow-up increased over time (figure 4). For example, probability of loss to follow-up at 3 months was 0% in 2001/2002, 1% in 2003/2004, and 3% in 2005. Probability of loss to follow-up at one year was 0%, 0%, 1.5%, 3%, and 6.5% respectively in 2001, 2002, 2003, 2004, and 2005. Early loss to care decreased over the years and late loss to care increased. As a result, overall loss to care

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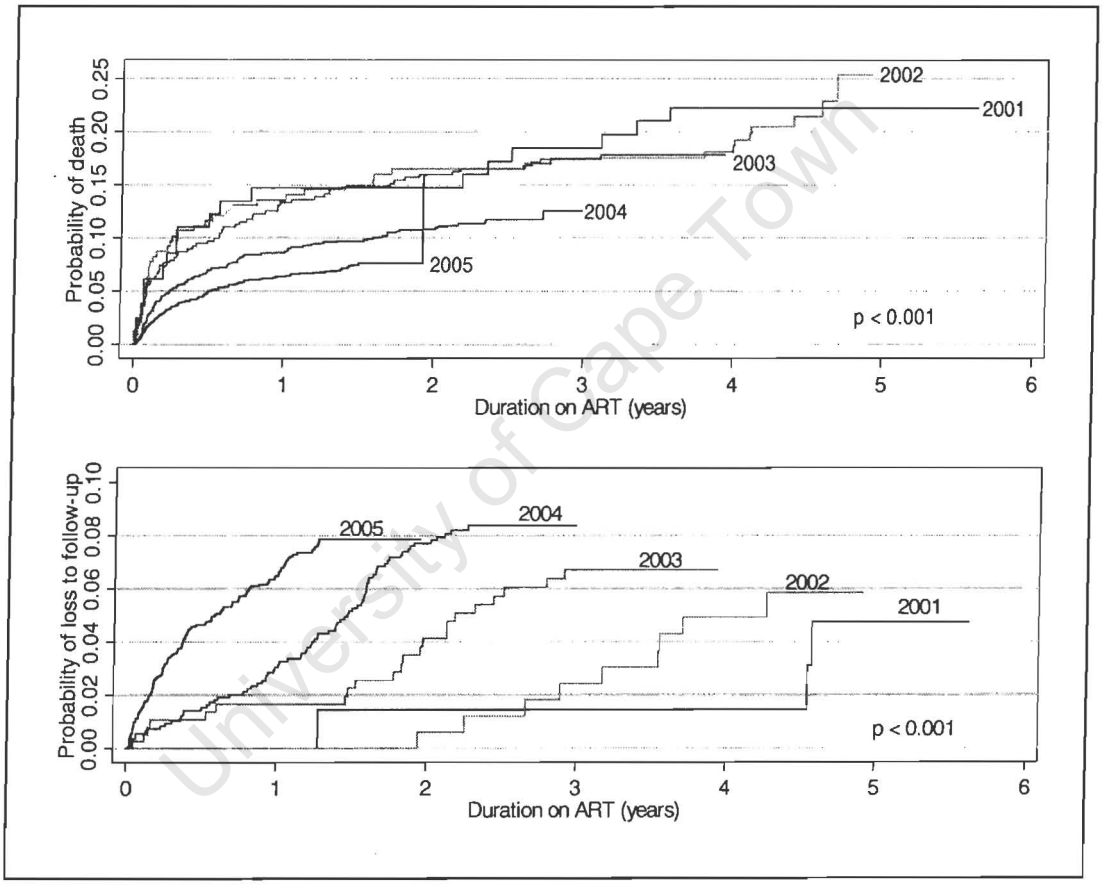
remained stable. A test for trend showed no evidence against the null hypothesis of equal survivor functions for remaining in care (p = 0.504) (figure 5).

Decrease of mortality was associated with a concomitant increase in median $CD4^+$ T-lymphocyte count at initiation on ART, which was in 2001, 2002, 2003, 2004, 2005, respectively 48 (IQR 13-88), 48 (IQR 18-115), 79 (IQR 28-129), 91 (IQR 42-147), and 109 (IQR 51-164).

FIGURE 2 KAPLAN-MEIER FAILURE CURVES BY YEAR OF INITIATION




Kaplan-Meier failure curves for death (upper graph) and loss to follow-up (lower graph). Time to failure was calculated from initiation on ART to death/loss to follow-up or censoring. Curves are stratified by year of initiation on ART. The plots include 3582 participants with respectively 82, 207, 394, 1098, and 1801 started in 2001, 2002, 2003, 2004, and 2005. ART: antiretroviral therapy. P-values are for tests for trend of equal survivor functions. The cumulative probability of death remained similar between 2001 and 2003, and decreased in 2004 and 2005. The cumulative probability of loss to follow-up increased and occurred earlier after initiation of ART with every calendar year.

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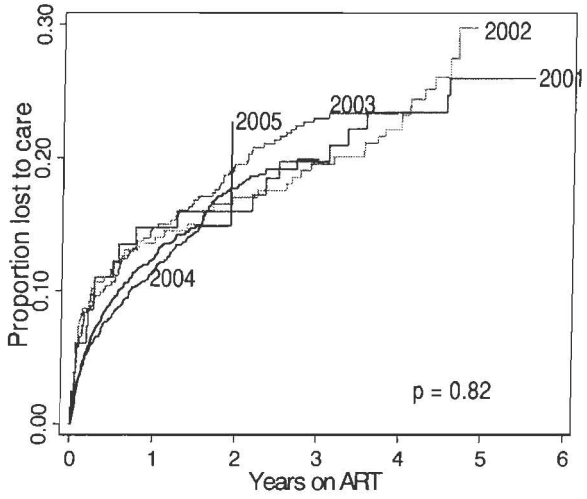
FIGURE 3 KAPLAN-MEIER FAILURE CURVES FOR LOSS TO CARE

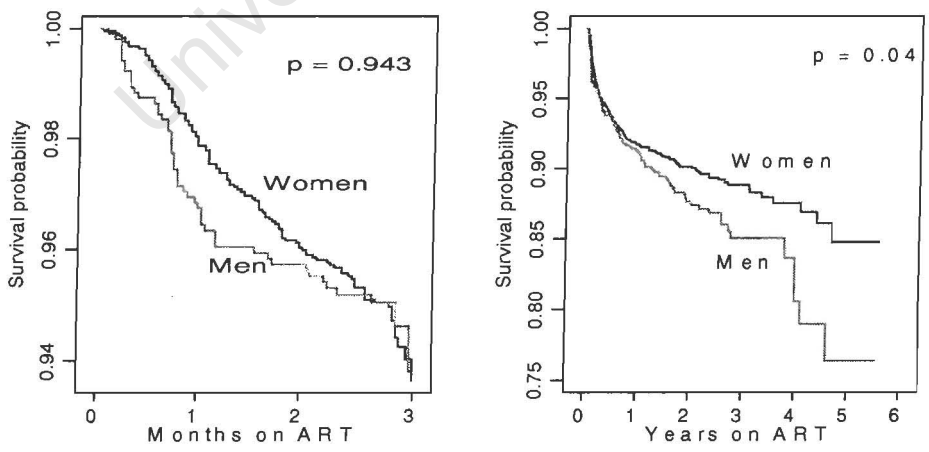



ART: antiretroviral therapy. P–value is for logrank test. Test for trend: p = 0.504
There was no difference in the cumulative probability of loss to care between years of initiation.

## EXPLORATORY ANALYSIS WITH PRODUCT-LIMIT METHODS

With unadjusted Kaplan-Meier analysis, the cumulative probability of late death was higher for men than for women after the first year on ART (figure 6). Although a similar trend was visible for early mortality, there was only weak evidence against the null hypothesis of equal survivor functions.

FIGURE 4 KAPLAN-MEIER SURVIVAL CURVES BY GENDER FOR EARLY AND TOTAL MORTALITY




ART: antiretroviral therapy. P-values are from logrank tests for equality of survivor functions. There was strong evidence against the null hypothesis of equal survival functions between men and women for late, but not for early mortality.

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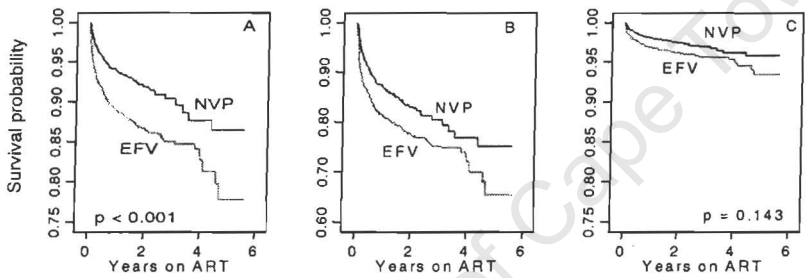
Probability of death appeared higher in patients who were started on efavirenz as compared to patients who were started on nevirapine (figure 7). This difference was reduced but still present after adjustment for gender, CD4 count, and tuberculosis. There was weak evidence against the null hypothesis of equal survivor functions after adjusting for severity of disease by performing the analysis in patients with WHO stage 1 or stage 2 disease. However, this might have been due to small numbers (only 10% of all patients started were in WHO stage 1 and 2). Stratified log-rank tests by tuberculosis, gender, AIDS, or severity of disease (approximated by grouping WHO stages 1 & 2 together as opposed to WHO stages 3 & 4) all provided strong evidence against the null hypothesis of equal survivor functions ($p < 0.001$ for tuberculosis, gender, and AIDS, and $p = 0.001$ for severity of disease).

FIGURE 5 CUMULATIVE PROBABILITY OF DEATH ON EFAVIRENZ VERSUS NEVIRAPINE




Kaplan-Meier survival estimates plotted versus time on ART. A: patients started on efavirenz (EFV) vs. nevirapine (NVP), unadjusted. B: adjusted for zero values of baseline CD4 count, female gender, and tuberculosis. C: estimates stratified by EFV vs. NVP, only patients in WHO stage 1&2 included. P-values are from log-rank tests. NNRTI: non-nucleoside reverse transcriptase inhibitor.

The estimated cumulative probability of death was higher in patients with low $CD4^+$ T-cell counts (figure 3). This difference remained important for early and late mortality. There was a trend towards increased probability of late mortality in older age groups (figure 8). This trend was not present during the first year on ART.

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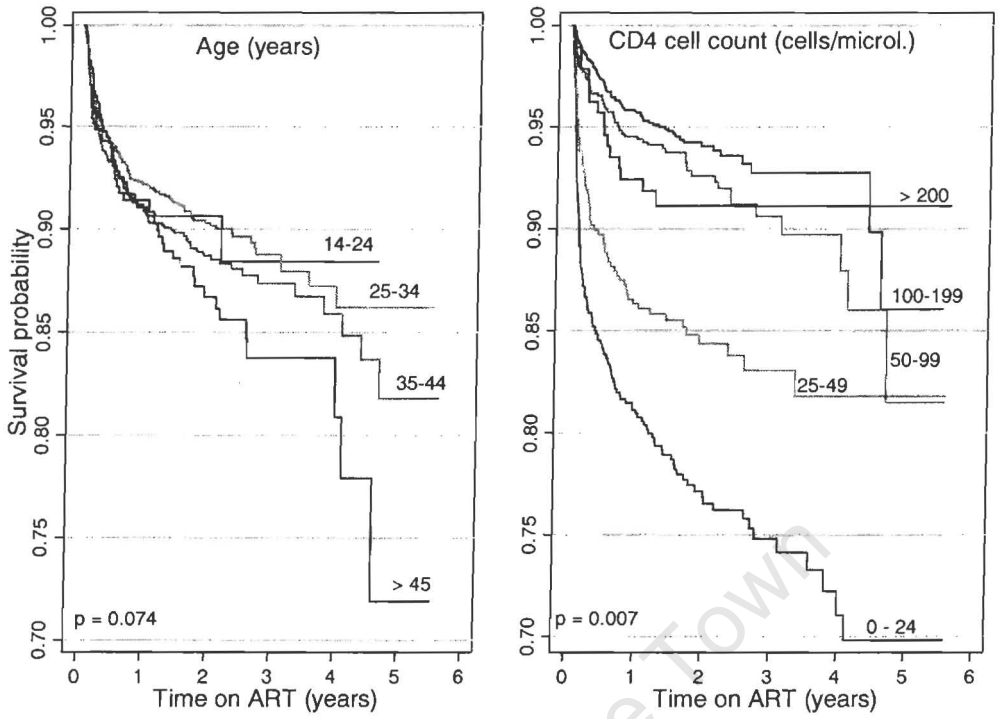
FIGURE 6 CUMULATIVE PROBABILITY OF DEATH BY BASELINE CD4 AND AGE CATEGORIES




Kaplan-Meier survival estimates by CD4⁺ T-cell lymphocyte count and age at initiation of ART. P-values are from tests for trend of survivor functions. Patients with CD4 counts below 50 cells/μl had a markedly increased probability of dying.

Unadjusted estimates showed an increased probability of death in patients with tuberculosis at initiation of ART (figure 9). The increase in risk tended to decrease over time. When adjusted for zero values of CD4⁺ T-lymphocyte count at initiation, female gender, and the absence of AIDS at initiation, this difference disappeared during the early period, and patients with tuberculosis seemed to have a decreased risk of death after 1 year on ART.

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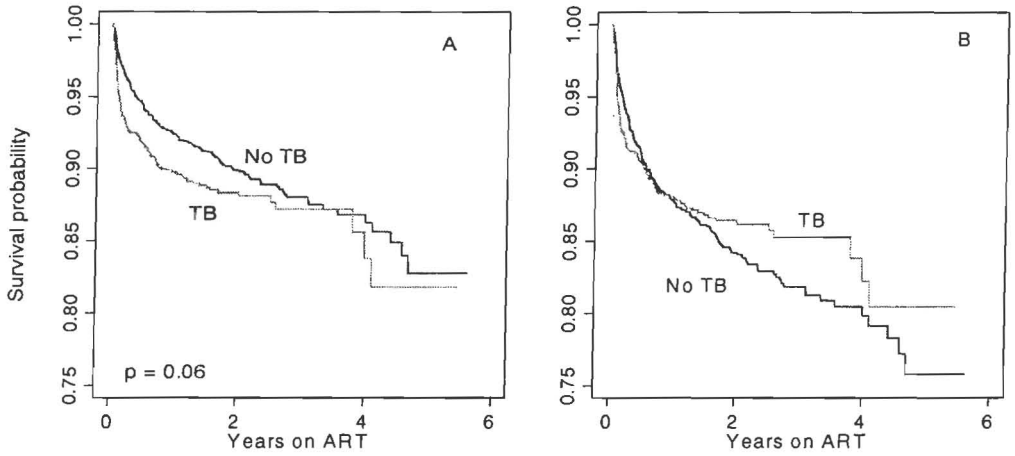
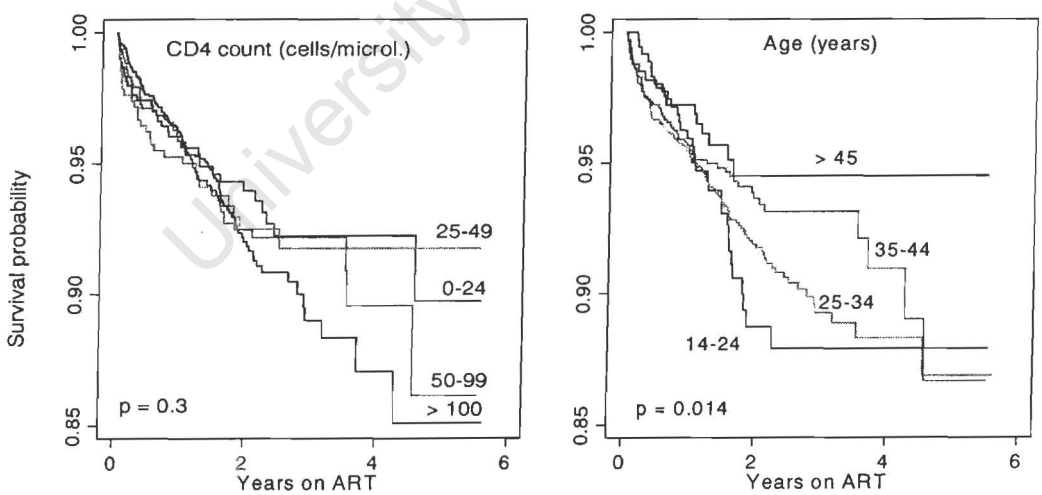
FIGURE 7 CUMULATIVE PROBABILITY OF DEATH IN PATIENTS WITH AND WITHOUT TUBERCULOSIS AT INITIATION OF ART




Kaplan-Meier survival estimates for patients with and without tuberculosis (TB). Unadjusted in A. Adjusted for zero values of baseline $CD4^+$ T-lymphocyte cell count, female gender, and the absence of AIDS diagnosis in B. p-value is from log-rank test.

As opposed to mortality, the cumulative probability of loss to follow-up increased with decreasing age, the age group between 14 and 24 years being the most at risk (figure 10). There was no difference between age groups during the first year on ART.

FIGURE 8 LOSSES TO FOLLOW-UP ACCORDING TO AGE AND CD4 COUNT AT INITIATION ON ART




Kaplan-Meier survival estimates. P-values are from tests for trend of survivor functions.

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## 7.2. MORTALITY

### UNIVARIATE ANALYSIS WITH BASELINE COVARIATES

Results of univariate Cox proportional hazards regression on covariates present at initiation of ART are described in table 4. Male gender and older age were associated with the hazard of death in late mortality, whilst there was no evidence of associations for early mortality. Among patients who had been on ART for at least three months, men had a 48% increased hazard of death as compared to women, and patients older than 45 years had twice the hazard of patients between 25 and 34 years.

Low baseline weight and $CD4^+$ T-lymphocyte count, a diagnosis of AIDS, and WHO stage 3 or 4 were associated with an increased hazard of death for the early and late periods. However, this effect was stronger for early mortality (table 4). Baseline viral load was associated with an increased hazard of death for *late* (HR 1.24, 95% CI 1.01-1.52), but not *early* mortality (HR 1.18, 95% CI 0.94-1.47).

Tuberculosis and oral candidiasis were associated with *early*, but not *late* mortality. A history of previous treatment for tuberculosis was associated with an increased hazard of death during the early and the late periods. Kaposi sarcoma, cryptococcal meningitis, chronic diarrhoea, oesophageal candidiasis, and wasting syndrome were associated with an increased hazard of death. Whilst the effect of Kaposi sarcoma and wasting syndrome was smaller for late mortality, the opposed was true for oesophageal candidiasis, which had a higher hazard ratio for late compared to early mortality.

The presence of efavirenz within the initial ART regimen was associated with an increased hazard of death. This effect was stronger during the first three months, but remained after this period. Stavudine was not associated with mortality.

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TABLE 2 ASSOCIATIONS OF BASELINE COVARIATES WITH THE HAZARD OF DEATH

| | | < 3 months on ART | | | ≥ 3 months on ART | | |
|---|---|---|---|---|---|---|---|
| | | HR | (95% CI) | p | HR | (95% CI) | P |
| **Male gender** | | 1.01 | (0.73 - 1.40) | 0.943 | 1.48 | (1.11 - 1.98) | 0.007 |
| **Baseline Age (years)** | | | | | | | |
| | **14 – 24** | 1.11 | (0.68 - 1.81) | 0.772 | 1.12 | (0.69 - 1.82) | 0.011 |
| | **25 – 34** | 1.00 | | | 1.00 | | |
| | **35 – 44** | 1.17 | (0.83 - 1.64) | | 1.19 | (0.85 - 1.66) | |
| | **> 45** | 0.93 | (0.54 - 1.60) | | 1.99 | (1.33 - 2.98) | |
| **Baseline Weight (kg)** | | 0.92 | (0.90 - 0.93) | <0.001 | 0.98 | (0.97 - 0.99) | 0.002 |
| **Baseline CD4 cell count (cells/μl)** | | | | | | | |
| | **1 - 24** | 7.98 | (5.07 - 12.57) | <0.001 | 2.51 | (1.74 - 3.62) | <0.001 |
| | **26 - 49** | 4.75 | (2.85 - 7.91) | | 1.78 | (1.16 - 2.73) | |
| | **51 - 99** | 1.68 | (0.95 - 2.98) | | 1.14 | (0.76 - 1.73) | |
| | **101 - 199** | 1.00 | | | 1.00 | | |
| | **200** | 2.15 | (0.92 - 4.98) | | 1.16 | (0.57 - 2.34) | |
| **Baseline Viral load (log)** | | 1.18 | (0.94 - 1.47) | 0.163 | 1.24 | (1.01 - 1.52) | 0.041 |
| **AIDS** | | 4.25 | (3.03 - 5.95) | <0.001 | 2.10 | (1.58 - 2.78) | <0.001 |
| **WHO Stage at initiation** | | | | | | | |
| | **1&2** | 1.00 | | <0.001 | 1.00 | | <0.001 |
| | **3** | 2.74 | (0.98 - 7.65) | | 2.47 | (1.14 - 5.37) | |
| | **4** | 10.12 | (3.74 - 27.37) | | 4.61 | (2.15 - 9.91) | |
| **Opportunistic infections at initiation** | | | | | | | |
| | **Tuberculosis (all)** | 1.90 | (1.41 - 2.55) | <0.001 | 0.77 | (0.56 - 1.07) | 0.116 |
| | **Pulmonary TB** | 1.71 | (1.24 - 2.35) | 0.001 | 0.76 | (0.52 - 1.11) | 0.152 |
| | **Extra-Pulmonary TB** | 1.54 | (1.03 - 2.31) | 0.035 | 1.08 | (0.69 - 1.70) | 0.743 |
| | **Oro-pharyngeal candidiasis** | 2.38 | (1.25 - 4.50) | 0.008 | 1.27 | (0.60 - 2.70) | 0.539 |
| | **Kaposi Sarcoma** | 6.26 | (4.10 - 9.57) | <0.001 | 4.24 | (2.54 - 7.07) | <0.001 |
| | **Cryptococcal meningitis** | 2.24 | (1.10 - 4.56) | 0.025 | 2.46 | (1.30 - 4.66) | 0.006 |
| | **Chronic diarrhoea** | 5.58 | (3.10 - 10.03) | <0.001 | 3.52 | (1.74 - 7.15) | <0.001 |
| | **Oesophageal candidiasis** | 2.92 | (1.20 - 7.12) | 0.018 | 3.56 | (1.67 - 7.56) | 0.001 |
| | **Wasting Syndrome** | 18.04 | (10.23 - 31.81) | <0.001 | 5.34 | (1.70 -16.71) | 0.004 |
| **Initial ARV regimen containing** | | | | | | | |
| | **Efavirenz** | 2.33 | (1.70 - 3.21) | <0.001 | 1.35 | (1.02 - 1.79) | 0.037 |
| | **Stavudine** | 0.78 | (0.56 - 1.10) | 0.154 | 0.79 | (0.57 - 1.09) | 0.146 |
| **Origin of referral** | | | | | | | |
| | **Primary Health Clinics** | 1.00 | | | 1.00 | | |
| | **Hospital** | 2.42 | (1.55 - 3.79) | <0.001 | 1.01 | (0.63 - 1.61) | 0.965 |
| | **PMTCT[a] Programme** | 0.44 | (0.18 - 1.10) | 0.078 | 0.63 | (0.35 - 1.16) | 0.142 |
| | **Other[b]** | 1.96 | (1.32 - 2.92) | 0.001 | 1.24 | (0.87 - 1.76) | 0.233 |
| | **TB Clinics** | 1.91 | (1.28 - 2.87) | 0.002 | 0.84 | (0.56 - 1.27) | 0.415 |
| **Previous TB treatment** | | 1.95 | (1.44 - 2.65) | <0.001 | 1.35 | (1.02 - 1.79) | 0.034 |
| **Previous exposure to PMTCT[a]** | | 2.16 | (1.10 - 4.23) | 0.024 | 1.21 | (0.75 - 1.97) | 0.440 |
| **Pregnancy** | | 0.24 | (0.06 - 0.98) | 0.047 | 0.42 | (0.14 - 1.33) | 0.140 |
| **Time between baseline CD4 and start of ART (months)** | | 0.93 | (0.87 – 0.99) | 0.051 | 0.95 | (0.89 - 1.01) | 0.111 |
| **Year of initiation on ART** | | | | | | | |
| | **2001** | 2.43 | (1.11 - 5.32) | <0.001 | 1.70 | (0.84 - 3.42) | 0.091 |
| | **2002** | 2.81 | (1.69 - 4.65) | | 1.65 | (0.98 - 2.77) | |
| | **2003** | 2.33 | (1.51 - 3.59) | | 1.76 | (1.15 - 2.69) | |
| | **2004** | 1.48 | (1.03 - 2.13) | | 1.29 | (0.90 - 1.85) | |
| | **2005** | 1.00 | | | 1.00 | | |

HR: Hazard ratio; CI: confidence interval; Results from univariate Cox proportional hazards models. p-values are for Wald tests; [a] prevention of mother-to-child transmission with either zidovudine, nevirapine or both; [b] other refers to home based care, lay counsellor, non-governmental organization, private clinician, youth clinic, unknown or none.

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Patients referred from primary health care clinics had a decreased hazard of death compared to referrals from hospitals, TB clinics, or other health services. Women referred from the prevention of mother to child transmission (PMTCT) programme had a lower hazard of death, as did women who were pregnant at initiation on ART. As prevention of mother to child was given in the maternity ward, a higher proportion of pregnant women were referred by the PMTCT programme (64%) than from other programmes ($\chi^2 = 592$, $p < 0.001$). Women with previous exposure to PMTCT had an increased hazard of death during the first three months, and were more likely to be pregnant ($\chi^2 = 7.2$, $p = 0.007$). Bivariate analysis adjusting for baseline CD4$^+$ T-lymphocyte count cancelled the association between exposure to PMTCT and the hazard of death (HR 1.61, 95% CI 0.82-3.17, $p = 0.163$). 25 (17%) of 151 women who were pregnant at initiation were recorded as having had previous exposure to PMTCT, suggesting underreporting of PMTCT.

A shorter waiting time before starting ART was associated with an increased hazard of death. This is probably a reflection of fast-tracking on treatment of patients with low CD4$^+$ T-cell counts and/or WHO stage 4 disease, especially Kaposi sarcoma.

As shown in the exploratory analysis, the mortality rate was higher during the first years of the programme and decreased after 2003. This was more marked for early than for late mortality.

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## UNIVARIATE ANALYSIS WITH TIME-VARYING COVARIATES ON ART

Weight was recorded for 67% of all consultations (60,836/91,137) and ranged from 25 kg to 147 kg. Median weight gain was 4 kg at 6 months (IQR 0.5-9) and 6 kg at one year (IQR 1-12). Weight at every visit more than baseline weight was negatively associated with the hazard of death, and this for early as well as late mortality (table 5). The hazard of death increased sharply when the weight dropped below 50 kg. For one kg of weight gain the hazard of death decreased by 13% and 10% respectively for early and late mortality.

Associations with CD4$^+$ T-lymphocyte count and viral load could not be measured before three months on ART, since these investigations are routinely done at six

months, and the number of observations before three months was too small. $CD4^+$ T-lymphocyte counts below 50 cells/μl were strongly associated with late mortality. Gain in $CD4^+$ T-lymphocyte count was protective. Failure to suppress viral load below 400 copies/μl at any time point resulted in a three-fold increase in the hazard of death (HR 3.0, 95% CI 2.17-4.16).

All occurrences of opportunistic infections while on ART were strongly associated with an increased hazard of death. This was true for early as well as late mortality, except for wasting syndrome which didn't occur after three months on ART. Wasting syndrome, chronic diarrhoea, and oesophageal candidiasis all had hazard ratios above 10.

TABLE 3 ASSOCIATIONS OF ON-TREATMENT COVARIATES WITH THE HAZARD OF DEATH

| | < 3 months | | ≥3 months | |
|---|---|---|---|---|
| | HR (95% CI) | p | HR (95% CI) | p |
| **Weight categories (kg)** | | | | |
| 20 - 29 | 106.87 (21.46 - 532.22) | <0.001 | 0.00 (. - .) | <0.001 |
| 30 - 39 | 31.82 (11.90 - 85.06) | | 90.48 (38.82 - 210.87) | |
| 40 - 49 | 8.16 (3.32 - 20.07) | | 11.32 (5.32 - 24.08) | |
| 50 - 59 | 3.10 (1.25 - 7.68) | | 2.63 (1.22 - 5.66) | |
| 60 - 69 | 1.00 | | 1.00 | |
| 70 - 79 | 1.47 (0.42 - 5.22) | | 1.51 (0.63 - 3.63) | |
| ≥ 80 | 1.19 (0.24 - 5.91) | | 1.82 (0.74 - 4.48) | |
| **Weight gain (kg)** | 0.87 (0.83 - 0.92) | <0.001 | 0.90 (0.88 - 0.92) | <0.001 |
| **CD4 count (10 cells/μl)** | | | 0.94 (0.90 - 0.98) | 0.002 |
| **CD4 count categories (cells/μl)** | | | | |
| 0 - 24 | | | 22.66 (4.94 - 103.96) | 0.002 |
| 25 - 49 | | | 6.62 (0.73 - 59.94) | |
| 50 - 99 | | | 2.45 (0.27 - 22.07) | |
| 100 - 199 | | | 3.10 (0.87 - 11.04) | |
| 200 - 349 | | | 1.00 | |
| 350 - 499 | | | 0.45 (0.05 - 4.01) | |
| ≥ 500 | | | 1.48 (0.27 - 8.28) | |
| **Gain in CD4 count (10 cells/μl)** | | | 0.95 (0.91 - 0.99) | 0.008 |
| **Viral load (log copies/μl)** | | | 1.75 (1.23 - 2.48) | 0.002 |
| **Failure to suppress viral load** | | | 3.00 (2.17 - 4.16) | <0.001 |
| **Opportunistic infections** | | | | |
| Tuberculosis (all) | 2.46 (1.83 - 3.32) | <0.001 | 5.03 (3.72 - 6.80) | <0.001 |
| Wasting Syndrome | 32.85 (17.25 - 62.56) | <0.001 | 0.00 (0.00 - .) | 1.000 |
| Oesophageal candidiasis | 11.67 (5.46 - 24.94) | <0.001 | 20.21 (8.28 - 49.34) | <0.001 |
| Chronic diarrhoea | 11.89 (7.37 - 19.19) | <0.001 | 15.17 (8.22 - 27.99) | <0.001 |
| Oro-pharyngeal candidiasis | 6.47 (3.41 - 12.28) | <0.001 | 13.62 (6.69 - 27.75) | <0.001 |
| HIV encephalopathy | 8.54 (3.17 - 23.05) | <0.001 | 8.69 (2.77 - 27.28) | <0.001 |
| Kaposi Sarcoma | 6.26 (4.10 - 9.57) | <0.001 | 4.24 (2.54 - 7.07) | <0.001 |
| Cytomegalovirus | 5.61 (1.79 - 17.56) | 0.003 | 2.85 (0.40 - 20.39) | 0.296 |
| Toxoplasmosis | 4.09 (1.01 - 16.50) | 0.048 | 4.40 (1.09 - 17.74) | 0.037 |
| Cryptococcal meningitis | 2.19 (1.12 - 4.28) | 0.022 | 3.18 (1.88 - 5.39) | <0.001 |
| Pulmonary TB | 2.07 (1.51 - 2.84) | <0.001 | 3.88 (2.74 - 5.50) | <0.001 |
| Extra-Pulmonary TB | 1.99 (1.36 - 2.90) | <0.001 | 4.14 (2.79 - 6.15) | <0.001 |

HR: Hazard ratio. CI: confidence interval. Failure to suppress viral load is defined as any viral load above 400 copies/μl. TB: tuberculosis.

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## MULTIVARIATE ANALYSIS WITH BASELINE COVARIATES

For early mortality, the multivariate model was stratified by gender to accommodate the non-proportional hazard of gender during this time-period (figure 6) and still be able to adjust for potential confounding. When included in the model, there was little evidence for an association between gender and the hazard of early mortality (HR 0.80, 95% CI 0.55-1.17).

Early and late mortality were associated with low baseline weight, low baseline $CD4^+$ T-lymphocyte count, and Kaposi sarcoma (table 6). The associations were stronger for early compared to late mortality. Associations with chronic diarrhoea and wasting syndrome were similar between early and late mortality.

In the final model, early mortality was positively associated with efavirenz, stavudine, previous tuberculosis, and year of initiation. Stavudine was strongly correlated with year of initiation ($\chi^2 = 5.8*10^4$, $p < 0.001$), as the standard regimen was changed from zidovudine to stavudine during 2003.

Late mortality was associated with male gender, increasing age, and oesophageal candidiasis. Tuberculosis at baseline was protective (HR 0.58, 95% CI 0.40-0.84). As previous tuberculosis did not contribute to the model and was not associated with late mortality it wasn't included in the final model.

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TABLE 4 MULTIVARIATE REGRESSION MODELS OF MORTALITY ON ART

| | | < 3 months on ART[a] | | | ≥ 3 months on ART | | |
|---|---|---|---|---|---|---|---|
| | | HR | 95% CI | p | HR | 95% CI | p |
| **Male sex** | | | | | **1.40** | **(1.02 - 1.93)** | **0.037** |
| **Baseline age (years)** | **14 - 24** | 0.95 | (0.56 - 1.62) | 0.197 | **1.29** | **(0.79 - 2.12)** | **0.054** |
| | **25 - 34** | 1.00 | | | **1.00** | | |
| | **35 - 44** | 1.46 | (1.01 - 2.10) | | **1.14** | **(0.80 - 1.61)** | |
| | **> 45** | 1.07 | (0.57 - 2.00) | | **1.81** | **(1.18 - 2.78)** | |
| **Baseline weight (per 10 kg)** | | **0.57** | **(0.47 - 0.66)** | **<0.001** | **0.87** | **(0.76 - 0.99)** | **0.029** |
| **Baseline CD4 cell count (cells/µl)** | **0 - 24** | **3.72** | **(2.25 - 6.16)** | **<0.001** | **1.85** | **(1.23 - 2.77)** | **0.008** |
| | **25 - 49** | **2.42** | **(1.39 - 4.20)** | | **1.66** | **(1.06 - 2.60)** | |
| | **50 - 99** | **1.13** | **(0.61 - 2.08)** | | **1.05** | **(0.68 - 1.60)** | |
| | **100 - 199** | **1.00** | | | **1.00** | | |
| | **> 200** | **1.52** | **(0.64 - 3.63)** | | **0.83** | **(0.39 - 1.78)** | |
| **Tuberculosis** | | 1.16 | (0.80 - 1.69) | 0.421 | **0.58** | **(0.40 - 0.84)** | **0.004** |
| **Oral candidiasis** | | 1.60 | (0.79 - 3.23) | 0.188 | 0.97 | (0.44 - 2.15) | 0.949 |
| **Kaposi sarcoma** | | **5.46** | **(3.48 - 8.55)** | **<0.001** | **4.55** | **(2.69 - 7.71)** | **< 0.001** |
| **Cryptococcal meningitis** | | 0.79 | (0.36 - 1.75) | 0.560 | 1.72 | (0.86 - 3.44) | 0.124 |
| **Oesophageal candidiasis** | | 1.00 | (0.39 - 2.54) | 0.994 | **2.93** | **(1.30 - 6.59)** | **0.009** |
| **Chronic diarrhea** | | **2.12** | **(1.08 - 4.15)** | **0.029** | **2.42** | **(1.16 - 5.03)** | **0.018** |
| **Wasting syndrome** | | **5.75** | **(3.01 - 10.97)** | **<0.001** | **5.17** | **(1.60 - 16.69)** | **0.006** |
| **Efavirenz** | | **1.50** | **(1.00 - 2.24)** | **0.048** | 1.23 | (0.86 - 1.77) | 0.254 |
| **Stavudine** | | **2.01** | **(1.02 - 3.97)** | **0.045** | 0.90 | (0.50 - 1.65) | 0.743 |
| **Year of initiation** | **2001/2002** | **3.35** | **(1.48 - 7.58)** | **0.034** | 1.06 | (0.50 - 2.26) | 0.587 |
| | **2003** | **1.96** | **(1.07 - 3.58)** | | 1.31 | (0.70 - 2.45) | |
| | **2004** | **1.24** | **(0.83 - 1.85)** | | 1.23 | (0.84 - 1.79) | |
| | **2005** | **1.00** | | | 1.00 | | |
| **Exposure to PMTCT** | | 1.35 | (0.67 - 2.72) | 0.396 | 0.80 | (0.48 - 1.33) | 0.390 |
| **Previous tuberculosis** | | **1.57** | **(1.12 - 2.19)** | **0.008** | | | |

[a] Model stratified by gender. Results are from two multivariate Cox proportional hazards regression models: one with early, and one with late mortality as the outcome. All covariates were present at baseline. ART: antiretroviral therapy. PMTCT: prevention of mother to child transmission.

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## ALTERNATIVE MULTIVARIATE MODELS

Replacement of baseline opportunistic infections by episodes on ART in the model for early mortality shown in table 6 did not result in large differences, except for increasing the hazard ratios for the diseases already associated in the baseline model, and reversing the association of efavirenz with the hazard of death (HR 0.78, 95% CI 0.44-1.38).

Viral load at baseline wasn't included in the final model because it was missing in 15.9% (N=570) of the patients due to a protocol change in 2005 which abandoned baseline viral load testing. However, when added to the model with baseline

covariates, baseline viral load was not associated with early mortality (HR 0.96, 95% CI 0.75-1.23, p = 0.733).

When opportunistic infections occurring on ART, instead of at baseline, were included into the model for late mortality, all were strongly associated with the hazard of death: tuberculosis (HR 4.06, 95% CI 2.96-5.57), oral candidiasis (HR 7.83, 95% CI 2.96-5.57), Kaposi sarcoma (HR 3.72, 95% CI 3.69-16.66), cryptococcal meningitis (HR 2.29, 95% CI 1.31-4.02), oesophageal candidiasis (HR 6.46, 95% CI 2.31-18.06), and chronic diarrhoea (HR 10.58, 95% CI 5.43-20.60). Inclusion of these time-varying covariates did not notably modify the hazard ratios for the other covariates.

When included in the final model, there was weak evidence of an association between baseline viral load and late mortality (HR 1.21, 95% CI 0.97-1.52, p = 0.098). Failure to suppress the viral load was positively associated with late mortality when added to the final model (HR 2.85, 95% CI 2.00-4.06). Weight, weight gain, and CD4+ T-lymphocyte response were negatively associated with late mortality when included one by one in the model. However, when multiple time-varying covariates were added together in the final model, this resulted in model instability and very large confidence intervals due to small cells.

When disease severity was adjusted for by including WHO stage or AIDS rather than individual opportunistic infections, hazard ratios for the other covariates, including efavirenz, remained almost unchanged. The hazard ratio for AIDS was 2.0 (IQR 1.38-2.91) for early and 1.6 (IQR 1.17-2.18) for late mortality.

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## A CLOSER LOOK AT CLINICAL PARAMETERS CLOSE TO THE TIME OF DEATH

Conditions present on the last clinic visit before death are described in table 7. The data provided strong evidence against the null hypotheses of equal proportions between early and late mortality for WHO stage, last CD4+ T-lymphocyte count and viral load before death, failure to suppress viral load below 400 copies/µl, pulmonary tuberculosis, and wasting syndrome, as measured by Wilcoxon rank sum test and Pearson's chi-square test or Fisher's exact test (p-values respectively 0.006, < 0.001,

0.01, <0.001, 0.013, and <0.001). Being on second line was different at the 10% level of significance (p = 0.063).

Patients who died during the first three months on ART were more likely to have WHO stage 4 disease, a markedly lower $CD4^+$ T-lymphocyte count, a higher viral load, and were more frequently diagnosed with wasting syndrome and tuberculosis (table 7). All these measures indicate more advanced disease among early deaths compared to late deaths. Weight at the last visit was not different between early and late deaths. Strikingly, almost half of early deaths, and more than a third of late deaths were on treatment for tuberculosis. Among the 158 patients who had tuberculosis, respectively for early and late mortality 9 and 3 patients also had diarrhoea, and 10 and 9 Kaposi sarcoma. Sixty-four percent (112/176) of early deaths and 43% (86/199) of late deaths had tuberculosis, chronic diarrhoea, or Kaposi sarcoma. Seventy-one percent (125/176) of early deaths and 52% (103/199) of late deaths had an opportunistic infection recorded during their last visit. A quarter of the late deaths had virological failure.

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TABLE 5 CONDITIONS PRESENT ON THE LAST VISIT BEFORE DEATH

| | All | | Early | | Late | |
|---|---|---|---|---|---|---|
| | N | | N | | N | |
| **N** | 375 | | 176 | | 199 | |
| **WHO stage** | | | | | | |
| **1 & 2** | 5 | 1% | 3 | 2% | 2 | 1% |
| **3** | 85 | 23% | 34 | 19% | 51 | 26% |
| **4** | 285 | 76% | 139 | 79% | 146 | 73% |
| **Median CD4 count (cells/μl)** | 134 | (15 - 266) | 14 | (7 - 107) | 147 | (86 - 270) |
| **Median viral load (log copies/μl)** | 3.2 | (2.1 - 6.2) | 5.5 | (4.7 - 6.1) | 2.1 | (2.1 - 4.0) |
| **Virological failure** | 58 | 15% | 8 | 5% | 50 | 25% |
| **On second line** | 5 | 1% | 0 | 0% | 5 | 3% |
| **Median weight gain (kg)** | 1 | (-2 - 5.1) | 0.5 | (-0.5 - 3.6) | 5.5 | (0.7 - 11.5) |
| **Opportunistic infections** | | | | | | |
| **Tuberculosis** | 158 | 42% | 87 | 49% | 71 | 36% |
| **Pulmonary tuberculosis** | 101 | 27% | 58 | 33% | 43 | 22% |
| **Extra-pulmonary tuberculosis** | 63 | 17% | 33 | 19% | 30 | 15% |
| **Kaposi sarcoma** | 41 | 11% | 25 | 14% | 16 | 8% |
| **Diarrhea** | 30 | 8% | 19 | 11% | 32 | 16% |
| **Cryptococcosis** | 24 | 6% | 9 | 5% | 11 | 6% |
| **Oral candida** | 18 | 5% | 10 | 6% | 8 | 4% |
| **Oesophageal candida** | 12 | 3% | 7 | 4% | 5 | 3% |
| **Wasting syndrome** | 10 | 3% | 10 | 6% | 0 | 0% |
| **Herpes simplex** | 8 | 2% | 5 | 3% | 3 | 2% |
| **Encephalopathy** | 7 | 2% | 4 | 2% | 3 | 2% |
| **Cytomegalovirus** | 4 | 1% | 3 | 2% | 1 | 1% |
| **Toxoplasmosis** | 4 | 1% | 2 | 1% | 2 | 1% |
| **Pneumocystis jerovecii pneumonia** | 3 | 1% | 2 | 1% | 1 | 1% |
| **Cervical cancer** | 3 | 1% | 2 | 1% | 1 | 1% |
| **Cardiomyopathy** | 1 | 0% | 0 | 0% | 1 | 1% |
| **Cryptosporidiosis** | 1 | 0% | 1 | 1% | 0 | 0% |
| **Non Hodgkin lymphoma** | 1 | 0% | 0 | 0% | 1 | 1% |
| **Grade 3 or 4 adverse drug reactions** | | | | | | |
| **Transaminitis** | 1 | 0% | 1 | 1% | 0 | 0% |
| **Anaemia** | 5 | 1% | 2 | 1% | 3 | 2% |

Values between brackets are inter-quartile ranges.

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## 7.3. LOSS TO FOLLOW-UP

### UNIVARIATE ANALYSIS WITH BASELINE COVARIATES

An increased hazard of early loss to follow-up was associated with lower baseline weight, a higher baseline viral load, efavirenz in the initial regimen, and increasing year of initiation (table 8). There was weak evidence that a diagnosis of AIDS was associated with increased early loss to follow-up (HR 0.62, 95% CI 0.37-1.40). Age was not associated with early loss to follow-up.

Late loss to follow-up was associated with younger age, lower baseline weight, stavudine in the initial ART regimen, and year of initiation. There was weak evidence that PMTCT was associated with an increased risk of late loss to follow-up (HR 1.64, 95% CI 0.91-2.95). A history of previous treatment for tuberculosis was protective.

Baseline weight was the only variable (besides year of initiation) to be associated with both early and late loss to follow-up. The association was markedly stronger for early than for late loss to follow-up.

Interestingly, the direction of the trend between $CD4^+$ T-lymphocyte count and loss to follow-up was reversed between early and late mortality. Patients with higher baseline $CD4^+$ T-cell lymphocyte counts had a trend towards a lower risk of early, but a higher risk of late loss to follow-up.

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TABLE 6 ASSOCIATIONS OF BASELINE COVARIATES WITH THE HAZARD OF LOSS TO FOLLOW-UP

| | | < 3 months on ART | | | ≥ 3 months on ART | | |
|---|---|---|---|---|---|---|---|
| | | HR | 95%CI | p | HR | 95%CI | p |
| **Male sex** | | 1.40 | 0.86 - 2.29 | 0.177 | 1.04 | 0.75 - 1.44 | 0.815 |
| **Baseline Age categories (years)** | | | | | | | |
| | **14 - 24** | 0.35 | 0.11 - 1.14 | 0.305 | **1.44** | **0.95 - 2.20** | **0.016** |
| | **25 - 34** | 1.00 | | | **1.00** | | |
| | **35 - 44** | 0.95 | 0.56 - 1.61 | | **0.73** | **0.50 - 1.06** | |
| | **≥ 45** | 0.66 | 0.26 - 1.66 | | **0.57** | **0.30 - 1.10** | |
| **Baseline Weight (per 10 kg)** | | **0.58** | **0.45 - 0.73** | **<0.001** | **0.87** | **0.76 - 0.99** | **0.039** |
| **Baseline CD4 cell count** | | | | | | | |
| | **1 - 24** | 1.60 | 0.82 - 3.10 | 0.343 | 0.60 | 0.37 - 0.98 | 0.130 |
| | **25 - 49** | 1.78 | 0.88 - 3.57 | | 0.67 | 0.40 - 1.11 | |
| | **50 - 99** | 1.30 | 0.68 - 2.48 | | 0.84 | 0.57 - 1.23 | |
| | **≥ 100** | 1.00 | | | 1.00 | | |
| **Baseline Viral load (log copies/μl)** | | **1.50** | **1.02 - 2.20** | **0.038** | 1.14 | 0.91 - 1.42 | 0.253 |
| **AIDS** | | **0.62** | **0.37 - 1.04** | **0.069** | 0.86 | 0.64 - 1.18 | 0.357 |
| **WHO Stage at initiation** | | | | | | | |
| | **1&2** | 1.00 | | 0.182 | 1.00 | | 0.553 |
| | **3** | 0.91 | 0.46 - 1.77 | | 0.88 | 0.55 - 1.40 | |
| | **4** | 0.57 | 0.27 - 1.19 | | 0.78 | 0.48 - 1.26 | |
| **Efavirenz** | | **2.14** | **1.30 - 3.53** | **0.003** | 1.08 | 0.80 - 1.46 | 0.609 |
| **Stavudine** | | 1.65 | 0.85 - 3.23 | 0.141 | **1.78** | **1.18 - 2.69** | **0.006** |
| **Origin of referral** | | | | | | | |
| | **Primary Health Clinics** | 1.00 | | | 1.00 | | |
| | **Hospital** | 0.78 | 0.32 - 1.85 | 0.566 | 1.16 | 0.71 - 1.87 | 0.557 |
| | **MTCT** | 0.99 | 0.44 - 2.23 | 0.971 | 1.09 | 0.64 - 1.86 | 0.751 |
| | **Other** | 0.70 | 0.34 - 1.42 | 0.320 | 1.17 | 0.79 - 1.73 | 0.427 |
| | **TB Clinics** | 0.95 | 0.50 - 1.81 | 0.877 | 0.89 | 0.57 - 1.39 | 0.604 |
| **Prior TB** | | 0.78 | 0.48 - 1.27 | 0.320 | **0.64** | **0.47 - 0.87** | **0.004** |
| **On TB treatment at initiation of ART** | | 1.25 | 0.77 - 2.05 | 0.369 | 1.22 | 0.89 - 1.67 | 0.217 |
| **PMTCT** | | 1.91 | 0.70 - 5.25 | 0.208 | 1.64 | 0.91 - 2.95 | 0.097 |
| **Pregnancy at initiation of ART** | | 1.65 | 0.66 - 4.09 | 0.282 | 1.59 | 0.81 - 3.13 | 0.176 |
| **Time between baseline cd4 and start of ART (months)** | | 0.96 | 0.86 - 1.08 | 0.498 | 1.00 | 0.94 - 1.07 | 0.957 |
| **Year of initiation on ART** | | | | | | | |
| | **2001/2002** | **1.00** | | **< 0.001** | **1.00** | | **0.002** |
| | **2003** | **2.21** | **0.99 - 4.90** | | **1.84** | **0.81 - 4.20** | |
| | **2004** | **3.70** | **1.75 - 7.83** | | **3.49** | **1.62 - 7.51** | |
| | **2005** | **5.75** | **2.72 - 12.16** | | **3.94** | **1.80 - 8.61** | |

Univariate Cox proportional hazards regression models. HR: Hazard ratio. CI: confidence interval. ART: antiretroviral therapy. TB: tuberculosis. MTCT: mother to child transmission. PMTCT: prevention of mother to child transmission.

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## UNIVARIATE ANALYSIS WITH TIME-VARYING COVARIATES ON ART

The only on-treatment variable associated with early loss to follow-up was weight, with every additional kg being associated with a 5% decrease in the hazard (table 9). There was weak evidence of a negative association of the hazard of loss to follow-up with weight gain (HR 0.92, 95% CI 0.85-1.01), and of a positive association with oesophageal candidiasis (HR 4.40, 95% CI 0.61-31.86) and chronic diarrhoea (HR 3.06, 95% CI 0.75-12.54). However, the small number of these events resulted in large confidence intervals.

The hazard of late loss to follow-up was negatively associated with baseline weight, weight loss, and an increase in $CD4^+$ T-lymphocytes of more than 100 cells/μl. It was positively associated with failure to suppress viral load below 400 copies/μl, pulmonary tuberculosis and HIV encephalopathy. Associations with some opportunistic infections could not be estimated due to small numbers of episodes of individual opportunistic infections after three months on ART. Interestingly the largest hazard ratio was for the association with HIV encephalopathy.

TABLE 7 ASSOCIATIONS OF ON-TREATMENT COVARIATES WITH THE HAZARD OF LOSS TO FOLLOW-UP

| | < 3 months | | ≥3 months | |
|---|---|---|---|---|
| | HR (95% CI) | p | HR (95% CI) | p |
| **Weight (kg)** | **0.95 (0.92 - 0.98)** | **0.003** | **0.97 (0.95 - 0.98)** | **<0.001** |
| **Weight gain (kg)** | 0.92 (0.85 - 1.01) | 0.069 | **0.97 (0.95 - 0.99)** | **0.015** |
| **CD4 count categories (cells/μl)** | | | | |
| **0 - 99** | | | 2.00 (0.40 - 9.99) | 0.856 |
| **100 - 199** | | | 1.85 (0.56 - 6.10) | |
| **200 - 349** | | | 1.00 | |
| **350 - 499** | | | 1.36 (0.43 - 4.27) | |
| **≥ 500** | | | 1.30 (0.35 - 4.79) | |
| **Gain in CD4 count > 100 cells/μl** | | | **0.26 (0.11 - 0.61)** | **0.002** |
| **Viral load (log copies/μl)** | | | 1.40 (0.92 - 2.13) | 0.121 |
| **Failure to suppress viral load** | | | **2.40 (1.68 - 3.43)** | **<0.001** |
| **Opportunistic infections** | | | | |
| **Tuberculosis (all)** | 1.36 (0.83 - 2.24) | 0.221 | **2.11 (1.41 - 3.16)** | **<0.001** |
| **Oesophageal candidiasis** | 4.40 (0.61 - 31.86) | 0.142 | | |
| **Chronic diarrhoea** | 3.06 (0.75 - 12.54) | 0.119 | | |
| **Oro-pharyngeal candidiasis** | 1.60 (0.22 - 11.51) | 0.643 | | |
| **HIV encephalopathy** | | | **7.32 (1.81 - 29.56)** | **0.005** |
| **Kaposi Sarcoma** | 1.70 (0.53 - 5.39) | 0.371 | | |
| **Cryptococcal meningitis** | 0.59 (0.08 - 4.23) | 0.597 | 0.23 (0.03 - 1.62) | 0.139 |
| **Pulmonary TB** | 1.09 (0.61 - 1.97) | 0.762 | **2.35 (1.49 - 3.71)** | **<0.001** |
| **Extra-Pulmonary TB** | 1.45 (0.74 - 2.84) | 0.275 | 1.45 (0.74 - 2.85) | 0.281 |

Univariate Cox proportional hazards models. ART: antiretroviral therapy. HR: hazard ratio. CI: confidence interval. TB: tuberculosis.

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## MULTIVARIATE ANALYSIS WITH BASELINE COVARIATES

In a multivariate model, a lower baseline weight, being started on efavirenz, and year of initiation were associated with an increased hazard of early loss to follow-up (table 10). A diagnosis of AIDS was associated with a decreased hazard. There was weak evidence of a strong positive association between previous exposure to PMTCT and the hazard of early loss to follow-up (HR 6.45, 95% CI 0.88-47.26). This was limited by the sample size. Age was not associated with early loss to follow-up on a continuous scale or as a categorical variable.

Late loss to follow-up was positively associated with younger age, lower baseline weight, being started on efavirenz, and a history of exposure to PMTCT. Previous tuberculosis was protective. AIDS and year of initiation were not associated with late loss to follow-up.

TABLE 8 MULTIVARIATE REGRESSION MODELS OF LOSS TO FOLLOW-UP

| | | Early | | | Late | | |
|---|---|---|---|---|---|---|---|
| | | HR | 95% CI | p | HR | 95% CI | p |
| **Sex** | | 1.13 | (0.65 - 1.99) | 0.660 | 1.12 | (0.77 - 1.63) | 0.547 |
| **Age (years)** | **14 - 24** | 0.23 | (0.05 - 0.96) | 0.155 | **1.43** | **(0.90 - 2.27)** | **0.008** |
| | **25 - 34** | 1.00 | | | **1.00** | | |
| | **35 - 44** | 0.78 | (0.44 - 1.41) | | **0.63** | **(0.42 - 0.95)** | |
| | **≥ 45** | 0.57 | (0.22 - 1.47) | | **0.52** | **(0.26 - 1.05)** | |
| **Baseline weight (per 10 kg)** | | **0.52** | **(0.39 - 0.69)** | **<0.001** | **0.84** | **(0.73 - 0.98)** | **0.028** |
| **Baseline CD4 cell count (cells/µl)** | | | | | | | |
| | **0 - 24** | 1.67 | (0.80 - 3.51) | 0.410 | 0.60 | (0.35 - 1.01) | 0.205 |
| | **25 - 49** | 1.73 | (0.82 - 3.65) | | 0.68 | (0.40 - 1.17) | |
| | **50 - 99** | 1.49 | (0.75 - 2.95) | | 0.89 | (0.60 - 1.33) | |
| | **> 100** | 1.00 | | | 1.00 | | |
| **AIDS at initiation** | | **0.49** | **(0.28 - 0.88)** | **0.016** | 0.85 | (0.60 - 1.21) | 0.370 |
| **Efavirenz** | | **2.32** | **(1.23 - 4.35)** | **0.009** | **1.46** | **(1.01 - 2.10)** | **0.044** |
| **Stavudine** | | 1.71 | (0.41 - 7.13) | 0.464 | 1.52 | (0.75 - 3.05) | 0.244 |
| **Year of initiation:** | **2001-2003** | **1.00** | | **0.005** | 1.00 | | 0.205 |
| | **2004** | **1.50** | **(0.31 - 7.40)** | | 1.96 | (0.93 - 4.11) | |
| | **2005** | **4.19** | **(0.98 - 17.92)** | | 1.73 | (0.82 - 3.63) | |
| **Exposure to PMTCT** | | 6.45 | (0.88 - 47.26) | 0.067 | **2.11** | **(1.09 - 4.08)** | **0.027** |
| **Previous tuberculosis** | | | | | **0.63** | **(0.45 - 0.89)** | **0.008** |
| **Tuberculosis at initiation** | | 0.67 | (0.37 - 1.21) | 0.183 | | | |

Multivariate Cox proportional hazards models of early and late loss to follow-up. ART: antiretroviral therapy. HR: hazard ratio. CI: confidence interval. PMTCT: prevention of mother to child transmission.

The independent association of efavirenz with an increased risk of early as well as late loss to follow-up was an unexpected finding, as existing literature suggests equivalent or better adherence with efavirenz compared to nevirapine (Braithwaite, Kozal et al.



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2007). When the model was fitted controlling for every year of initiation instead of grouping 2001 to 2003 together, the evidence for this association was weakened for late loss to follow-up (HR 1.38, 95% CI 0.95-2.01, p = 0.089) without altering the other associations. Model fit as measured by Aikaike's information criterion was slightly better for the model presented in Table 8 compared to this model. The association of late loss to follow-up with efavirenz is thus fragile and should not be interpreted as a true effect. The small number of events before three months on ART during the first three years did not allow separating 2001 to 2003 for the analysis of early loss to follow-up.

## ALTERNATIVE MULTIVARIATE MODELS

When time-varying covariates were introduced into the multivariate model for late loss to follow-up, no major changes occurred in the hazard ratios of baseline covariates, except for widening confidence intervals due to smaller numbers across risk sets. Loss of weight and failure to suppress viral load below 400 copies/μl were independently associated with an increased risk of late loss to follow-up (respectively HR 0.97, 95% CI 0.94-0.99, p = 0.012; HR 2.71, 95% CI 1.65-4.44, p < 0.001). Tuberculosis on ART showed a trend towards increased risk (HR 1.62, 95% CI 0.87-3.04). To avoid problems related to collinearity, age was removed from the model when failure to suppress viral load was introduced

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## 7.4.MODEL CHECKING

Model diagnostics are described for the stratified multivariate Cox proportional hazards model of mortality in patients who were less than three months on ART. Identical diagnostics were performed for the other models in the analysis (data not shown).

The test of non-zero slope of the scaled Schoenfeld residuals on the functions of time in a generalized regression showed a violation of Cox's proportional hazards assumption when gender was kept in the model (global test, $p = 0.119$; test for gender, $p < 0.001$). Further examination of a log-log plot by gender and adjusted for all the other covariates in the model showed curves that were almost parallel for men and women (figure 11). Examination of a Kaplan-Meier survival curves by gender revealed increased mortality among men compared to women during the first two months on ART and a reversal of this trend towards the third month (figure 6). Male gender did not appear to be a significant risk factor for mortality in the multivariate model (HR, 0.80; 95% CI, 0.55 – 1.17; $p = 0.254$). However, its inclusion in the model violated the proportional hazards assumption. Consequently, it was decided to fit a model stratified by gender to control for potential unmeasured confounding whilst accommodating for gender's nonproportional hazards.

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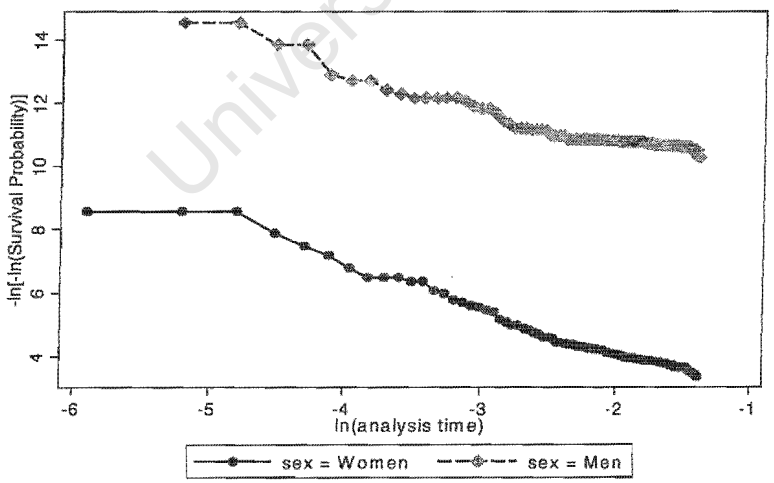
FIGURE 9 'LOG-LOG' PLOT BY GENDER FOR EARLY MORTALITY




Analysis time is in years on ART. Plot is adjusted for age categories, baseline weight, baseline CD4 categories, tuberculosis, oral candidiasis, Kaposi sarcoma, cryptococcal meningitis, oesophageal candidiasis, chronic diarrhoea, wasting syndrome, efavirenz, stavudine, year on initiation on ART, exposure to prevention of mother to child transmission, and previous tuberculosis.

For the stratified model, the p-value for the coefficient on the squared linear predictor from the link test stratified by gender was 0.545, hereby not revealing any specification problems. The test of non-zero slope of scaled Schoenfeld residuals on functions of time didn't show violations of the proportional hazards assumption overall ($\chi2 = 22.12$; $p = 0.453$) or with regard to individual covariates (table 11). The same was true when the proportional hazards assumption was checked separately for each stratum (women: $\chi2 = 22.77$, $p = 0.415$; men: $\chi2 = 27.25$, $p = 0.202$). Log-log plots of each category of nominal or ordinal variable, adjusted for the other covariates in the model, were generated. The parallel curves confirmed that the proportional hazards assumption was not violated.

TABLE 9 TEST OF PROPORTIONAL HAZARDS ASSUMPTION

| | $\chi^2$ | p | | $\chi^2$ | p |
|---|---|---|---|---|---|
| **Age (years)** | | | | | |
| **14 - 24** | 1.67 | 0.196 | **Cryptococcal meningitis** | 0.04 | 0.833 |
| **35 - 44** | 0.78 | 0.377 | **Oesophageal candidiasis** | 0.64 | 0.425 |
| **≥ 45** | 1.88 | 0.170 | **Chronic diarrhea** | 0.09 | 0.769 |
| **Weight** | 0.54 | 0.462 | **Wasting syndrome** | 3.63 | 0.057 |
| **CD4 count (cells/μl)** | | | **Efavirenz** | 0.03 | 0.853 |
| **0 - 24** | 0.03 | 0.854 | **Stavudine** | 0.07 | 0.798 |
| **25 - 49** | 0.3 | 0.584 | **Year of initiation** | | |
| **50 - 99** | 0.4 | 0.529 | **2001/2** | 1.79 | 0.180 |
| **≥ 200** | 0.98 | 0.322 | **2003** | 1.77 | 0.183 |
| **Tuberculosis** | 0.67 | 0.415 | **2004** | 1.23 | 0.267 |
| **Oral candidiasis** | 2.39 | 0.122 | **PMTCT** | 0.36 | 0.546 |
| **Kaposi sarcoma** | 1.18 | 0.278 | **Previous TB** | 1.09 | 0.297 |

Test of non-zero slope of scaled Schoenfeld residuals on functions of time for multivariate Cox proportional hazards model of early mortality, stratified by sex.

A plot of the cumulative hazard versus the cumulative Cox-Snell residuals showed a good overall model fit (figure 12). Plots of martingale residuals and deviance residuals against the linear predictor (figure 13) indicated a good model fit as well and identified some observations as potentially influential or as being outliers. Fitting the model after excluding these variables didn't yield different results. Consequently, no observations were excluded from the analysis.

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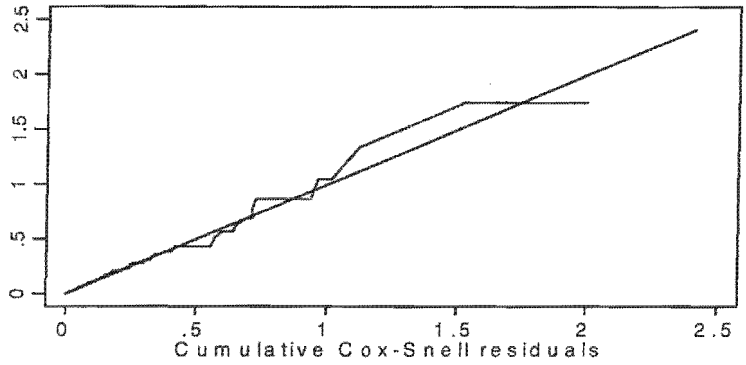
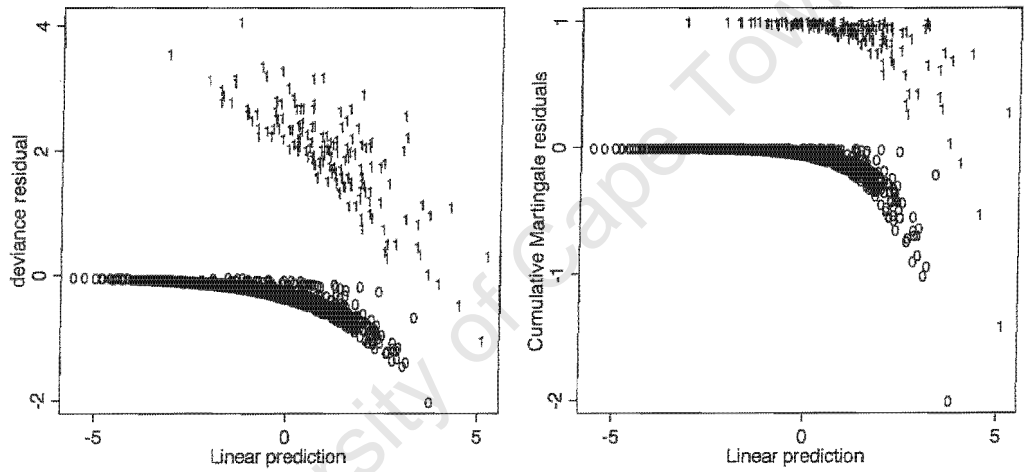
FIGURE 10 PLOT OF THE CUMULATIVE HAZARD VERSUS CUMULATIVE COX-SNELL RESIDUALS




A plot of the cumulative hazard (using the Cox-Snell residuals as the time variable and the data's original censoring variable) versus the Cox-Snell residuals showed a standard exponential distribution with a hazard ratio that is close to 1. This demonstrates a good model fit.

FIGURE 11 PLOTS OF DEVIANCE RESIDUALS AND CUMULATIVE MARTINGALE RESIDUALS AGAINST THE LINEAR PREDICTOR




Plots of the deviance residuals and cumulative Martingale residuals against the linear predictor showed a random scatter, with censored observations (labelled '0' on the graph; '1' corresponds to deaths) clumped together below zero. This suggests a good fit of the model.

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# 8. DISCUSSION

## 8.1. RATES OF MORTALITY AND LOSS TO FOLLOW-UP

This analysis of a prospective cohort of patients on ART included 3,595 patients followed over a period of 5 years and totalling 6,553 patient-years of observation. As described for other cohorts in Africa, mortality was very high during the first year, and decreased sharply afterwards (Braitstein, Brinkhof et al. 2006; Etard, Ndiaye et al. 2006; Fairall, Bachmann et al. 2008). The stable mortality rate of 2.2/100 person-years from one to five years on ART is slightly higher than the rate of 0.9 at 5 years reported by Etard et al. on long term outcomes of ART in Senegal (Etard, Ndiaye et al. 2006), similar to the overall post-1998 mortality rate of 2.6/100 person-years in the EuroSIDA cohort (Mocroft, Ledergerber et al. 2003), but higher than the mortality rates of 1.1 and 0.2 respectively in 2002 and 2003 in a US military cohort described by Crum (Crum, Riffenburgh et al. 2006). The higher mortality on ART in resource poor countries as compared to rich countries is mainly due to very high early mortality linked to late presentation of patients with advanced immune suppression. Late mortality however remains slightly higher in resource poor countries. This appears in this study to be related to the fact that patients who present with advanced disease have a higher risk of early as well as late mortality.

The rate of loss to follow-up increased in parallel with enrolment to reach 15/100 person-years during the first three months in 2005. Nevertheless, overall cumulative estimates of loss to follow-up remained low compared to the 15% at one year reported by ART-LINC (Braitstein, Brinkhof et al. 2006). This was only 4% at 1 year, 8% at 2 years, and 13% at 5 years.

Retention in care was 83% at two years and 72% at 5 years, comparing favourably with the 60% retention at two years in African cohorts published by Rosen et al. (Rosen, Fox et al. 2007). Mortality was the main cause of attrition (60%), which contrasts with other African cohorts in which loss-to-follow up was found to be the main cause (56%) (Rosen, Fox et al. 2007)

Almost half (47%) of the deaths and almost a third (28%) of loss to follow-up occurred during the first three months on ART. These findings highlight the need for enhanced



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clinical monitoring and adherence support during the initial period on ART. In addition, the high proportion of attrition attributed to mortality is an indicator of the good quality of the active tracing system in place.

The early mortality rate decreased from more than 40/100 patient-years in 2001-2002 to less than 17/100 person-years in 2005, as the CD4+ T-lymphocyte count at initiation increased from 48 to 109 cells/μl. The late mortality rate, however, remained stable at around 3/100 patient-years, whilst both the early and the late rates of loss to follow-up increased during this period, respectively from 0 to 15, and from 0.9 to 4/100 patient-years. This resulted in a net decrease in the rate of early loss to care, an increase in the rate of late loss to care, and almost a doubling of the total rate of loss to care, from 6 (95% CI 4-9) in 2001 to 11/100 patient-years (95% CI 10-13) in 2005. There was no difference in cumulative probability of loss to care between years of initiation.

In this programme increased coverage resulted in decreasing mortality associated with the rise of baseline CD4+ T-lymphocyte count. However, the surge from 4,157 ARV consultations in 2001 to 34,825 in 2005, without increasing the number of sites, was associated with earlier and increasing loss to follow-up. This trend has recently been described in many other African cohorts.

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## 8.2. FACTORS ASSOCIATED WITH MORTALITY

In accordance with existing studies on early mortality (Dillingham, Pinkerton et al. 2006; Erikstrup, Kallestrup et al. 2007), baseline markers of advanced HIV disease such as a low CD4+ T-lymphocyte count, WHO stage 3 and 4, presence of AIDS-defining illnesses, and low weight were independently associated with mortality. While this was known for early mortality, the fact that severe disease at baseline was also associated with late mortality – albeit to a lesser extent - is an important finding.

Wasting syndrome and Kaposi sarcoma at initiation on ART were independently associated with a 5-fold increase in the risk of early as well as late mortality. Both conditions had already been reported in separate studies as significant risk factors of mortality (Brodt, Kamps et al. 1998; Moore, Yiannoutsos et al. 2007). Patients with low

weight and chronic diarrhoea should be very closely monitored. Research into optimal diagnosis and treatment of these conditions is a priority.

Low baseline weight, as well as low weight at any time point, was negatively associated with mortality. The hazard of death decreased by 6% for every additional kilogram of baseline weight. Late mortality was weakly associated with baseline weight, but strongly associated with weight at any time point. Previous studies had identified body mass index (BMI) as one of the principal risk factors for early mortality (Zachariah, Fitzgerald et al. 2006). BMI was not calculated in this study as heights had not been routinely measured. Weight gain was associated with a lower risk of death. These findings support the use of weight as an essential parameter to monitor clinical progress on ART, and suggest that weight loss on ART should prompt enhanced clinical attention.

In terms of treatment regimens, efavirenz and stavudine were positively associated with early, but not late mortality in multivariate analyses. The association with stavudine was probably confounded by anaemia. The absence of haemoglobin data in the dataset did not allow this to be tested in multivariate analysis. In patients with haemoglobin below 8 mg/dl stavudine was initiated instead of zidovudine in the years where zidovudine was preferred in the initial regimen. As low haemoglobin has been shown to be positively associated with mortality, it is likely that patients who were started on stavudine had a higher baseline risk of dying when compared to zidovudine. For efavirenz, there is no similar known confounder. However, in a context where more than a third of patients starting ART are on treatment for tuberculosis, and unmasking of undiagnosed tuberculosis after starting ART is common, clinicians would tend to start sicker patients on efavirenz rather than nevirapine. In addition, in patients with liver disease or a history of alcoholism efavirenz would be preferred above nevirapine. Consequently, it is probable that the association of death with efavirenz is attributable to indication bias rather than a true effect. The attenuation of the effect of efavirenz on mortality when episodes of opportunistic infections on ART were added in the multivariate model supports this hypothesis.

A history of tuberculosis was associated with increased early mortality, whilst tuberculosis at initiation was associated with decreased late mortality. Previous TB has been described as a determinant of early mortality in other studies, and as a marker of advanced disease; it is thus not surprising to find an association with early mortality in



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this study. The protective effect of tuberculosis at initiation however, is a new finding. It is possibly due to a prophylactic effect of TB treatment, protecting patients against incident TB during several months or years after treatment. Another explanation would be survivor bias. TB is associated with a two-fold increase of early mortality in univariate analysis. It is possible that patients who survive the first three months on TB treatment have unmeasured factors making them more likely to stay alive later on.

The independent association of male gender with mortality has been described previously in African cohorts. In this analysis, late but not early mortality was higher in men. Reasons usually put forward include that men tend to present later, and thus with more advanced disease, and that adherence is poorer in men. However, the association remained after adjusting for markers of disease severity and failure to suppress the viral load. Loss to follow-up and gender were not associated. Some studies have also suggested that the $CD4^+$ T-lymphocyte response to ART was slower in men than in women (Maskew, MacPhail et al. 2007). Another explanation is that before the year 2000 the male/female ratio for death was higher than 100 for adults between 15 an 64 years (Statistics South Africa 2005), implying that without HIV mortality is higher in young men in South Africa. This tendency, which had been partially reversed by higher HIV-related mortality in young women, could surface again after attenuation of higher female mortality by ART. Non-HIV related deaths could not be ascertained from the data in this cohort.

The fact that age was associated with the hazard of late death confirms findings of previous studies (Erikstrup, Kallestrup et al. 2007). This might be related to a longer time between infection and presentation, or to naturally increasing mortality with age.

Episodes of opportunistic infections on ART were all very strongly associated with the hazard of death. The associations were stronger for late than for early mortality, except for wasting syndrome (which didn't occur after 3 months) and Kaposi sarcoma. This does probably reflect that clinical failure on treatment is an even stronger risk factor for late death than opportunistic infections at baseline. This hypothesis was supported by the association of $CD4^+$ T-lymphocyte count and viral escape on ART with an increased hazard of death.

When looking at parameters close to the time of death, a diagnosis of WHO stage 3 or 4 defining opportunistic infection was present in 71% of early deaths, as opposed to 51%



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of late deaths. $CD4^+$ T-lymphocyte count at the visit before death was markedly lower in early (14 cells/μl) as opposed to late deaths (147 cells/μl). A quarter of late deaths had had a viral load above 400 copies/μl on ART on at least one occasion. Virological response during the early period was not assessed. These findings suggest that early deaths most often occurred in the presence of opportunistic infections associated with advanced immune suppression. This was also the case for about half of late deaths, partly as a possible consequence of virological failure. However, a significant number of late deaths occurred in patients without known opportunistic infections or immune suppression. In ART cohorts in Europe and the US a decrease of AIDS-defining illnesses as cause of death was described after the introduction of ART (Lewden 2003; Crum, Riffenburgh et al. 2006), and the proportion of deaths attributable to non-HIV related causes has increased since the introduction of ART. The most frequent causes included cardiac and liver disease, malignancies, obstructive lung disease, drug overdose, suicide, homicide and trauma. Nevertheless, HIV-related conditions remained the principal cause of death on ART. This study explores parameters close to the time of death but does not provide evidence on causes of death. More research is needed to determine whether the maturing of ART programmes in Africa is leading to a similar transition from HIV-related to non-HIV related deaths.

Strikingly, tuberculosis was present in almost half (49%) of the early deaths, and 42% of the total number of deaths, confirming the role of TB as the major cause of mortality among HIV-infected people in South Africa (Martinson, Karstaedt et al. 2007). Following tuberculosis, the opportunistic infections most frequently diagnosed at the visit before death were, in order of frequency, Kaposi sarcoma, chronic diarrhoea, and cryptococcal meningitis. In early deaths wasting syndrome was present in 6%. Chronic diarrhoea was more frequent in late deaths.

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## 8.3. FACTORS ASSOCIATED WITH LOSS TO FOLLOW-UP

Contrary to the study by Wools-Kaloustian, loss to follow-up was not associated with gender (Wools-Kaloustian, Kimaiyo et al. 2006). In some programs with passive follow-up, misclassification of deaths as losses to follow-up might bias the association of gender with loss, seen that gender is associated with mortality. Differences in

adherence levels between men and women are also likely to vary according to the context.

Interestingly, younger age was independently associated with late loss to follow-up, with patients between 14 and 24 years being markedly more at risk than patients older than 25 years. Age was not associated with early loss to follow-up, although there was a trend for patients in the 14 to 24 years age group to have a lower probability of being lost during the first three months. Adolescence is a well-known risk factor for poor adherence to chronic medication (Dobbels, Van Damme-Lombaert et al. 2005). These findings suggest that future research and appropriate strategies should focus on enhanced adherence support for younger patients on ART.

Lower baseline weight as well as low weight gain was associated with an increased risk of loss to follow-up. To our knowledge, this association hasn't been previously reported. As weight is a known risk factor for mortality, it is possible that this association is due to misclassification bias of deaths as lost to follow-up. However, baseline characteristics as well as other variables associated with loss to follow-up in multivariate analyses were different from those associated with mortality. The independent association of AIDS with a decreased risk of early loss to follow-up doesn't support the hypothesis of misclassification bias, and suggests that patients with more severe illness were more adherent, at least during the first months on ART. $CD4^+$ T-lymphocyte count wasn't independently associated with loss to follow-up, although in univariate analysis there was a trend of higher loss to follow-up in patients who had higher $CD4^+$ T-lymphocyte counts at baseline.

Tuberculosis and HIV encephalopathy on ART were associated with increased loss to follow-up in univariate analyses. Although not previously described, these associations are not surprising. Combined therapy for TB and HIV results in increased pill-burden and higher rates of adverse events, both known risk factors for poor adherence (Vervoort, Borleffs et al. 2007). HIV encephalopathy does often cause deterioration of the mental state, which is likely to result in lower adherence as well.

Failure to suppress viral load and loss of weight were also predictive of loss to follow-up and occurred more often in youth. Since these are variables associated with virological and clinical failure, it is plausible that patients with lower adherence levels are more likely to fail therapy as well as being lost to follow-up.

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The independent association of efavirenz at baseline with increased early loss to follow-up was an unexpected finding, and is conflicting with existing comparisons between nevirapine and efavirenz which showed no difference between the two drugs in terms of adherence (Manfredi, Calza et al. 2004). This is probably due to the complex relationship between efavirenz, the temporal periods, and clinical indication bias rather than to a true independent effect. An alternative hypothesis is that unrecognized central nervous system adverse events would lead to higher rates of loss to follow-up of patients on efavirenz. More research is needed to investigate the relationship between efavirenz and loss to follow-up in this setting.

The increased risk of early as well as late loss to follow-up in women who were exposed to prevention of mother to child transmission interventions is likely to be related to local circumstances. Many women from South Africa's poorer Eastern Cape Province migrate to the richer Western Cape during pregnancy to access better quality healthcare. The majority of these women return to their home some time after they have delivered. It thus probable that women who were started on ART after diagnosis during pregnancy were more likely to be originally from the Eastern Cape and returned home when feeling better. A proportion of these women might have accessed ART in the Eastern Cape as the national programme was expanded.

Finally, the independent protective effect against late loss to follow-up of previous tuberculosis at baseline suggests that patients who experienced severe disease before starting ART were less likely to default treatment later on. The protective effect of AIDS for early loss to follow-up can be read with the same interpretation.

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## 8.4. LIMITATIONS

### UNMEASURED CONFOUNDING, SECULAR TRENDS, AND SELECTION BIAS

This study is not randomized and as such, the usual limitations of observational studies apply: unmeasured residual confounding and selection bias. Self-selection into the study is likely to have occurred differently during the early years of the programme, when ART was new, as compared to the later years, when it had become part of a national roll-out programme. During the early years of the programme a higher proportion of patients were treatment activists, well-informed, and/or highly motivated. As ART became more widespread, patients became more representative of the general population. In addition, the programme evolved from a nurse-based, small scale pilot project, to giant clinics seeing up to 200 patients per day. As the standard ARV drug regimen did change over the years, year of initiation is potentially an important source of confounding, especially for the association between ARVs and the outcome. All multivariate analyses included year of initiation to attempt adjustment for these temporal trends. However, when comparing cohorts of different years, residual selection bias or unmeasured confounding might remain even in a multivariate analysis.

### INFORMATION BIAS, MISCLASSIFICATION, AND INFORMATIVE CENSORING

Misclassification of the outcome as well as some exposure variables is a potential problem, as in many observational studies based on surveillance data. Active tracing of patients who missed their clinic appointment for more than seven days has been in place since inception of the programme. However, after 2004 this system has been saturated, and an increasing number of patients lost to follow-up could not be traced. It is likely that some deaths were misclassified as lost to follow-up, or even as in care if they occurred during the last six months of the study. This would lead to an underestimation of the mortality rate, and could bias the measures of effect both towards the null and away from the null, if misclassification occurred differentially between exposed and unexposed. For example, if young patients were more likely to be lost to follow-up (which seemed to be the case) and less likely to die than older patients, this would bias the association between age and mortality away from the null. If young people had a true increased risk of dying, differential loss to follow-up would

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dilute the association. However, comparisons of baseline characteristics of patients lost to follow-up with patients who died suggest that little misclassification occurred. Patients lost to follow-up presented more similarities with patients in care than with patients who died; when differences were present, they were in the opposite direction. Comparisons of associations with death and loss to follow-up confirmed this. Deaths were associated with older age, low $CD4^+$ T-lymphocyte counts, and AIDS, whilst loss to follow-up was associated with younger age, higher $CD4^+$ T-lymphocyte counts, and the absence of AIDS at baseline. However, some variables, such as efavirenz and lower baseline weight, were associated with death as well as loss to follow-up, suggesting some level of misclassification of deaths.

Misclassification of individual opportunistic infections is also likely to have occurred, since data capturers were relying on clinicians' notes. Some opportunistic infections might not have been recorded in the notes, and patients who died a certain period after having defaulted from the clinic were less likely to have a diagnosis on their last clinical visit. However, this was more likely to be a problem for opportunistic infections on treatment than at baseline. The magnitude of the effect of opportunistic infections is such that it is unlikely to be entirely caused by misclassification.

An important infection that was missing from the analysis was pneumonia. Due to the absence of a clear case definition at the beginning of the study, this diagnosis was never recorded systematically. It is known from other studies that respiratory infections play an important role in mortality on ART. Patients who died in hospital were also less likely to have their diagnosis recorded. Infections necessitating hospitalisation for diagnosis, such as cryptococcal meningitis and pneumocystis jirovecii pneumonia, were probably underestimated as conditions present close to the time of death.

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## OVER-ADJUSTMENT

Although careful consideration of potential confounding variables was done, and it was attempted not to include variables that were on the causal pathway between exposure and outcome, some level of 'overlap' of associations was unavoidable. For example, previous tuberculosis is a risk factor for recurrence of TB, and thus TB at baseline could be considered as being on the causal pathway between previous tuberculosis and death. However, both variables were exposures of clinical interest, and tuberculosis needed to stay in the model as a potential confounder of the association of death with

efavirenz. Purposeful selection of variables and choice of the final model with the best fit, whilst keeping all the exposures of interest and potential confounders, was attempted to present associations that are the best reflection of the data.

## GENERALIZABILITY

The very high incidence of tuberculosis as well as HIV in a township setting are particular features of this study population that need to be taken into account if one considers generalizing the findings to other populations. The fact that the study was conducted in a non-research environment in a public sector programme improves its external generalizability to similar settings. However, the influence of programme specificities can't be excluded and similar studies from other sites are needed to verify the validity of this study's results.

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# 9. CONCLUSION

This is one of the first public sector cohorts in Africa to describe determinants of late mortality and loss to follow-up over a 5-year period. It exemplifies how enhanced surveillance can be used to advance knowledge on clinical as well as operational issues. One recommendation is that departments of health in countries with large ART programs should invest in a few sentinel surveillance sites with good monitoring infrastructure and resources in order to be able to inform the rest of the programme. Despite increasing losses to follow-up, the outcomes of the programme compare positively to other programmes in Africa.

This study contributed to identify characteristics of patients who are at particular risk of dying or being lost to follow-up, and from this a number of new strategies to improve retention in care and reduce early as well as late mortality can be proposed. Enhanced and adapted adherence support for youth, patients who start ART with high $CD4^+$ T-lymphocyte counts, and patients with tuberculosis or HIV encephalopathy are some of these potential strategies that could prevent the higher rates of loss to follow-up in these categories of patients. Closer monitoring of patients with low weight, severe disease, wasting syndrome, chronic diarrhoea, or Kaposi sarcoma during the first months on ART might reduce early mortality. More research is needed into the optimal

management of these conditions. Loss of weight or appearance of new opportunistic infections after the first months on ART warrants increased medical attention and further investigations since these patients are at higher risk of dying. Importantly, rates of both mortality and loss to follow-up were highest during the first months on ART. Enhanced adherence support interventions and close clinical monitoring during this period are needed to decrease excess losses to follow-up and high early mortality. Finally, the protective effect against late mortality of treatment for tuberculosis at baseline highlights the need for more research into a possible prophylactic effect and supports the exploration of isoniazid preventive therapy in patients on ART.

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