Title of the Research Project

Patterns and predictors of early and late mortality during HIV antiretroviral therapy in a military-based programme in South Africa: a cohort study

by

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Submitted in partial fulfilment of the requirements for the degree

Master of Science (Clinical Epidemiology)

In the Faculty of Health Sciences

University of Pretoria

Pretoria

October 2013

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Summary of dissertation

Title: Patterns and predictors of early and late mortality during HIV antiretroviral therapy in a military-based programme in South Africa: a cohort study

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Background

The patterns and predictors of mortality in patients receiving combination ART (cART) in the South African military are unknown. Although predictors of early mortality have been described in southern Africa, over 95% of those patients had initiated 3TC + NNRTI-based regimens. In this secondary analysis of Phidisa II, a completed, factorial RCT comparing lopinavir/r vs efavirenz, and D4T+3TC vs ZDV+DDI, 75% of patients initiated other regimens.

Few sub-Saharan studies have reported predictors of late mortality, and fewer have reported causes of mortality in cART-treated patients for periods longer than five years. We investigated the patterns and predictors of early and late mortality after extended follow-up of up to eight years.

Methods

The cohort initiated cART in the Phidisa II trial, and after 31 March 2008, a median follow-up of 24.7 months, transferred to an observational study. Randomisation was stratified by site. The primary end-points of the trial were all-cause mortality and progression of disease to AIDS. In the observational study, the follow-up schedule and event reporting continued as in the RCT. Randomised regimens were continued for as long as indicated.

For the current study, Kaplan-Meier survival estimates and mortality rates were calculated. Follow-up was censored at: 365 days post-randomisation for early mortality; and 31st December 2011 for late and overall mortality.

Baseline factors associated with mortality were assessed using Cox regression analyses, stratified by site. For late mortality, updated values of CD4+ count,

haemoglobin, and BMI were considered instead of baseline values. The final reduced multivariate models were obtained with backward elimination.

Results

Between 2004 and 2007, 1771 patients enrolled (mean age 35 years, 68% male, median CD4+ count = 102 cells/μL). The total follow-up was 8921 person-years, with median follow-up of 5.4 person-years. Of the 279 deaths, 151 occurred in the 1st year (death rate 9.1/100 py). The rate after 1 year was 1.8/100 py.

Tuberculosis was the most common cause of death, responsible for 12.6% of early and 22.7% of late mortality.

Factors predicting early mortality were: BMI<18.5 kg/m^2 (adjusted Hazard Ratio (aHR) 2.35, p-value <0.001), lower CD4+ count (p<0.001), WHO clinical stage 3/4 (aHR 1.84, p= 0.003), anaemia (aHR 2.87, <0.001), higher AST (aHR 2.06, p=0.006), and marriage (aHR 0.63, p= 0.009).

Factors predicting late mortality were: male sex (aHR 2.84, p=0.001), initiating ZDV+DDI (aHR 1.63, p= 0.032), low BMI (aHR 3.55, p<0.001), haemoglobin (aHR 0.75 per g/dL increase, p < 0.001), low CD4+ count (p=0.007) and viral suppression at month 12 (aHR 0.49, p= 0.007).

Conclusions and Recommendations

Mortality is highest early in the course of cART. Advanced HIV disease, and not being married, predicted higher early mortality. Poor response to cART at one year, male sex, and initiating ZDV+DDI, predicted higher late mortality. The unfavourable effect of ZDV+DDI on mortality has not been described in southern Africa before.

Closer follow-up and support of persons with advanced HIV disease, poor response to cART, males, and the unmarried, are recommended. The use of cART regimes recommended by WHO and national guidelines, is encouraged.

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AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency virus
(c) ART	(combination) Antiretroviral therapy
95% CI	95% Confidence Interval
CDC	United States Centers for Disease Control and Prevention
RNA	Ribonucleic acid
WHO	World Health Organization
PLWHA	People Living With HIV/AIDS
TB	Tuberculosis
RPR	Rapid Plasma Reagin (tests for presence of antibodies to T. pallidum)
ALT	Alanine transaminase
AST	Aspartate transaminase
PCR	Polymerase Chain Reaction
HBV	Hepatitis B virus
HepBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
LTFU	Lost to follow-up
SD	Standard deviation
IQR	Interquartile range
PMTCT	Prevention of mother to child transmission (of HIV)
SAMHS	South African Military Health Service
SANDF	South African National Defence Force
NIAID	National Institute of Allergy and Infectious Diseases

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

The Joint United Nations Programme on HIV/AIDS (UNAIDS) Global Report on AIDS for 2012, estimates that 34 million (95% CI: 31.4-35.9 million) people worldwide were living with HIV at the end of 2011. Of these, 23.5 million (95%CI: 22.1-24.8 million) were from sub-Saharan Africa, with 4.9% of adults from this region living with HIV.[1] South Africa has the highest burden of HIV worldwide, with an estimated 5.6 million (95%CI: 5.3-5.9 million) infected, and an estimated 17.3% (95%CI: 16.6-18.1%) of the adult population aged 15-49 years living with HIV.[2]

In 2004, HIV/AIDS was the sixth leading cause of death, responsible for 3.5% of deaths worldwide; whereas in low-income countries it was the fourth leading cause and was responsible for 5.7% of all deaths.[3] Although in 2010 the HIV/AIDS ranking was unchanged globally, HIV/AIDS was the leading cause of death in southern and eastern sub-Saharan Africa.[4] Additionally, although the number of AIDS-related deaths in sub-Saharan Africa declined by 32% during the period 2005 to 2011, the region still accounted for 70% of all AIDS deaths in 2011.[1]

In South Africa, HIV/AIDS was the leading cause of death in 2000 in all provinces except Western Cape, with almost 40% of premature mortality in South Africa attributed to HIV/AIDS.[5] HIV/AIDS decreased to the seventh leading cause of death in South Africa in 2010, contributing 3.4% of all deaths.[6]This decrease in HIV-related mortality continued in 2011, where 270 000 [95% CI: 240 000 – 300 000] of deaths in South Africa were attributed to AIDS, a 37% drop compared to 2005.[1,2]

Although combination antiretroviral therapy (cART) is estimated to have averted 250 000 to 350 000 deaths in low and middle-income countries in 2005, and continues to decrease AIDS-related mortality in sub-Saharan Africa, the age-specific mortality rates of HIV-infected individuals on cART are higher than those of the general population.[7,8,9,10] Mortality in HIV-infected patients on cART in sub-Saharan Africa, and South Africa in particular, is therefore a major public health concern.

This study aims to describe the patterns of mortality, and to identify patient characteristics predicting mortality in a cohort initiated cART and followed-up long-term, up to eight years, in a military health programme in South Africa.

Literature Review

Introduction

This section will give background information on the natural history of HIV; disease monitoring; key aspects of combination antiretroviral therapy (cART), for example, cART delivery at regional, national, and military settings; guidelines on cART initiation; the literature on patterns, and predictors of mortality in those treated with cART; and the South African Military Health Service's HIV treatment programme.

The *Pubmed* database was used to identify potentially relevant abstracts using combinations of the following search terms: *HIV, antiretroviral therapy, mortality, early mortality, late mortality, military, resource limited, and causes.* The full articles were sourced online.

Natural history of HIV infection

The Human Immunodeficiency Virus (HIV) primarily infects and depletes the CD4+ T Lymphocyte cells (CD4+ cells) which are vital in mounting an immune response against multiple disease-causing pathogens. Primary infection with HIV leads to the 'acute HIV syndrome' typically lasting six to 12 weeks, and is characterised by rapid depletion of the CD4+ cells and very high viral replication (measured as viral load; RNA copies/ml). The infected usually experience non-specific symptoms during this period and antibody tests for HIV may be negative (window period) whereas the individuals are highly infectious. Towards the end of this stage, as the host immune system is activated, it is able to control the viral replication resulting in a rapid decline of viral load and restoration of the CD4+ count.[11]

During the subsequent early asymptomatic 'clinical latency' phase, the viral load reaches a set point with viral replication continuing at a low rate and gradual CD4+ count decline. This phase generally lasts an average of 8-10 years with the individual being mostly asymptomatic.[12]

If still untreated, over 90% of those infected will progress to the symptomatic phase and ultimately develop the Acquired Immunodeficiency Syndrome- AIDS, a cluster of opportunistic infections and cancers resulting from the depressed immune system, causing significant

morbidity and mortality. Without antiretroviral therapy, survival time for those with AIDS is under one year in many developing countries, whereas around 85% of the untreated used to die within 3-5 years in developed countries.[13]

Disease monitoring

Monitoring of HIV disease progression is done clinically through clinical staging, immunologically by CD4+ count monitoring, and in selected settings, by viral load monitoring.

Clinical monitoring: In routine care settings in Africa, clinical monitoring utilises the WHO HIV clinical staging system. This staging system describes four clinical stages of HIV disease in adolescents and adults, ranging from stage 1 (asymptomatic or persistent generalised lymphadenopathy) to stage 4 (AIDS defining illnesses). The staging was first developed in 1990 and was revised in 2005 and 2007.[14] In other settings, the CDC staging is used, wherein: stage A indicates asymptomatic disease, and stage C, the worst, indicates the presence or history of an AIDS-defining illness.[15]

Immunological monitoring: The WHO and CDC staging systems both have immunological classification components. The WHO staging, outlines four bands of HIV related immunodeficiency (Table 1.1): no significant immunodeficiency, mild immunodeficiency, advanced immunodeficiency and severe immunodeficiency. The likelihood of disease progression to AIDS or death without cART increases with increasing immunodeficiency.[14] Clinical and immunological classifications are used to guide the initiation of cART, and prophylaxis against opportunistic infections, e.g. co-trimoxazole prophylaxis against *Pneumocystis jiroveci* pneumonia and toxoplasmosis. However, the CD4+ count is considered the most valuable and useful indicator of the stage of HIV disease and for predicting the disease outcome, and therefore the best guide for initiation of cART.[16]

CD4+ counts can be used to monitor response to cART.

Table 1.1: WHO immulogical classification for established HIV infection in persons older than 5 years.[14]

HIV-associated immunodeficiency	Absolute number/mm^3 or %CD4+
None or not significant	>/=500
Mild	350-499
Advanced	200-349
Severe	<200 or <15%

Virological monitoring: In patients not yet initiated on HIV treatment, viral load monitoring is only practiced by selected resource-rich countries. In some resource-limited settings like South Africa, viral load monitoring is the cornerstone of monitoring of response to cART, as it is in resource-rich countries. Viral load monitoring gives the earliest indication of success or failure of cART when compared to clinical and immunological monitoring. However, some resource-limited countries are still not monitoring cART virologically due to the relatively high cost of viral load testing when compared to CD4+ testing.[17]

Combination antiretroviral therapy and ART programmes

Combination antiretroviral therapy (cART) has had a dramatic effect on morbidity and mortality. Globally, in 2011 there was a 24% decline in AIDS-related mortality compared to 2005; 1.7 million AIDS deaths in 2011 compared to 2.3 million in 2005.In sub-Saharan Africa, AIDS deaths declined by 32% from 2005 to 2011, whilst in South Africa, there were 100 000 fewer deaths in 2011 compared to 2005.[1,2,7]

cART delivery in sub-Saharan Africa and in military-based programmes

The scale-up of antiretroviral therapy in low- and middle-income countries including the scale-up through the World Health Organisation's (WHO) 3 by 5 Initiative, is attributed with the dramatic change in AIDS-related mortality indicated above.[1,18] Launched in 2003, the initiative aimed to provide 3 million people worldwide with antiretroviral therapy by the end of 2005. This goal was finally realised in 2007.[18]

The 'Global Fund to fight AIDS, Tuberculosis and Malaria', launched in 2002, and the US 'President's Emergency Plan for AIDS Relief (PEPFAR)', launched in 2003, have

been instrumental in ART scale-up through funding (partially or wholly) of ART programmes implemented by governments and non-governmental organisations in sub-Saharan Africa. Prior to the national scale-up of ART programmes, non-governmental organisations, and university-sponsored research units implemented successful community based ART programmes, funded by donor organisations or by patients. There were also corporation-funded work-based ART programmes such as that of the Anglo-American group of companies in South Africa.[19]

African militaries, having long recognised the threat posed by HIV/AIDS, are actively developing a consensus on best practices for its prevention, treatment and care.[20] However, there is very limited published data that describe African military ART programmes in the literature. No articles describing the delivery and outcomes of an ART programme in African military non-research settings, were found. The only article accessed, described the assessment of the HIV/AIDS services at military health facilities in Zambia. Briefly, the Zambian Defence Forces operates a network of 54 health facilities, including three hospitals, which represent 16% of all health services in Zambia and serve military personnel and their families as well as surrounding civilian communities. They have dramatically expanded HIV/AIDS-related services including prevention of mother-to-child transmission of HIV (PMTCT) and access to cART.[21] This article does not evaluate the outcomes or impact of this ART programme.

South African National ART programme

In South Africa, free cART became available in the public sector in April 2004 under the Operational Plan for Comprehensive HIV and AIDS Care, Management, and Treatment (CCMT).[22]The HIV & AIDS and STI Strategic Plan for South Africa 2007-2011 flows from this Plan and from the National Strategic Plan of 2000-2005, and has 'Treatment, Care and Support' as the second key priority area after prevention of new infections. The goal was to offer the package of treatment, care and support to 80% of HIV positive people and their families by 2011.[23] At the end of 2011, cART coverage in South Africa was 73% (1.7 million on cART), falling below the target. The cART coverage increased to 83%, as 2.1 million PLWHA were on cART as at December 2012.[24] South Africa's ART programme remains the largest in the world.

Initiating cART

The WHO guidelines for the treatment of HIV in adolescents and adults (non-pregnant/non-lactating), have progressively recommended earlier cART initiation with each revision of the guidelines since 2006.[25,17,26] The 2006 revision recommended HIV treatment in those with CD4+ counts of 200 cells /mm^3 or less-irrespective of WHO clinical stage, in those with WHO stage 4 disease regardless of CD4+ count, and those with CD4+ of 201-350 cells /mm^3 and WHO stage 3 disease.[25] The 2013 revision, recommends cART initiation in the following: all patients with CD4+ count </= 500 cells/mm^3 regardless of WHO clinical stage; individuals co-infected with active TB disease regardless of CD4+ count or WHO stage; those co-infected with Hepatitis B with evidence of severe chronic liver disease; and partners with HIV in sero-discordant couples.[26]

From 2004 until April 2010, the South African national guidelines for antiretroviral therapy recommended cART only in those with CD4+ counts of 200 cells /mm^3 or less-irrespective of WHO clinical stage, and in those with WHO stage 4 disease regardless of CD4+ count.[27] These guidelines have evolved through three revisions[28,29,30] to now recommend cART in the following non-pregnant/non-lactating PLWHA: patients with CD4+ counts of </=350 cells/mm^3 regardless of WHO clinical stage, those with all types of active TB, and in those with WHO stage 3 or 4 irrespective of CD4+ count.[30]

World Health Organization and South African guidelines for the treatment of adolescents and adults both recommend that first-line cART for HIV-1 infection consist of three drugs, i.e. two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) - one being lamivudine or emtricitabine, and the other being tenofovir (a nucleotide RTI),zidovudine, or abacavir where tenofivir and zidovudine are contra-indicated; and a non-nucleoside reverse transcriptase inhibitor (NNRTI) such as efavirenz or nevirapine. Other nucleoside RTIs like stavudine (the mainstay of cART in Africa until recently) and didanosine, are no longer recommended due to their toxicity profiles.[17,28]

Unlike in resource-rich settings, protease inhibitors (PIs) including co-formulated ritonavir-boosted lopinavir, and boosted atazanavir (atazanavir and ritonavir given separately), are currently reserved for second-line cART following first-line treatment failure, first-line therapy when both efavirenz and nevirapine are contra-indicated, and in

first-line cART for HIV-2 infection (which is naturally resistant to NNRTIs), in sub-Saharan Africa.[26] This recommendation stems from the understanding that their use in first-line therapy essentially rules out second-line options in the setting of limited formularies within a public health approach.[25]

When cART is taken correctly, there is a rapid decline in the HIV viral load (HIV RNA), with at least a 2 $\log_{10}$ drop expected in the first 12 weeks and viral suppression by 6 months of therapy.[31] This leads to a certain level of immune reconstitution, evidenced by a rise in the CD4+ cell count and a significant drop in the risk of opportunistic infections such as tuberculosis, and cancers. The viral load rapidly rebounds when cART is stopped or taken at inadequate levels of adherence, necessitating life-long therapy at high levels of adherence.[31]

Mortality in cART-treated HIV: effects, patterns and predictors

HIV-related mortality, whether occurring prior or during cART, has had devastating effects on the economies of countries with generalised HIV epidemics such as South Africa. The age groups with the highest combined prevalence of HIV, the 25-49 year olds, have also exhibited the highest HIV-related death rates, under-fives excluded, yet they are the most economically active age groups in the country.[5,32]

Patterns of mortality

In sub-Saharan Africa, 8% to 26% of patients die in the first year of antiretroviral treatment, with most deaths occurring in the first few months of cART.[33] In a Cape Town cohort, the death rate in the first 4 months of cART was 19.1 deaths/100 person-years (100PYs), decreasing to 2.9 deaths/100PYs beyond 4 months and 1.3 deaths/100PYs beyond 1 year.[34] Similarly, in a large Johannesburg cohort of 7583 patients who contributed 161,000 person-months of follow up, overall mortality rate was low (2.9 deaths per 100 person years, 95% CI 2.6-3.2), but high in the first three months of cART (8.4 per 100 person years, 95% CI 7.2-9.9).[35]

Furthermore, mortality rates within the initial months of ART are disproportionately higher in African programmes compared with rates in other regions although immunologic and virologic responses are similar.[33] The ART–Lower Income Countries (ART-LINC) Collaboration compared outcomes of 4810 patients from 18 ART

programmes predominantly in Africa, with those of 22217 patients from12 HIV cohort studies from Europe and North America. This analysis found that early mortality following initiation of cART was several-fold higher (four times in the first month) among patients in resource-limited settings compared to that of patients treated in high-income settings, even after adjusting for baseline immunodeficiency.[36]

There is paucity of data on patterns of mortality in cART-treated patients in military settings, even in high-resource countries. Additionally, in all military HIV programmes, the majority of patients are male, whereas in the sub Saharan national HIV programmes, females predominate. These differences may result in different patterns of mortality in the sub-Saharan military HIV populations when compared to those of the general HIV populations.

The non-military HIV population that best compares to the military HIV population in terms of gender composition in Southern Africa, is the mining workforce. In the South African Anglo-American workplace HIV programme, among 15060 individuals (64% men, median baseline CD4 cell count 127 cells/mm^3 (IQR 58-199), median follow-up of 1.8 years, (IQR 0.5–3.0)); the mortality rate was 34 (95% CI: 28 to 41) per 100 PY for 0–3 months; 12 (95% CI: 9 to 14) per 100 PY for 4–6 months; 6.3 (95% CI: 5.1 to 7.8) for 7–12 months; 3.9 (95% CI: 3.2 to 4.8) for 13–24 months; and 3.3 (95% CI: 2.6 to 4.1) per 100 person-years for 25–48 months.[37] This pattern of very high mortality rates early after cART initiation is similar to that observed in other cohorts in the region, although the crude mortality rates in the workplace programme are higher than the reported rates for the Cape Town and Johannesburg cohorts.[34,35]

In 1600 participants who intiated cART after July 1995 in the U.S. Military HIV Natural History Study (NHS), a prospective observational cohort of consenting HIV-infected military personnel, the overall mortality rate was 1.52 deaths per 100 PY (95% CI 1.31, 1.75) after a median follow-up of 8.7 years. Cumulative mortality was 5% (95% CI 4%-6%) at 4 years, 10% (95% CI 9%-12%) at 8 years, and 18% (95%CI 15%-20%) at 12 years. These data are similar to those of the U.S national population on cART.[38]

Predictors of mortality

In limiting the impact of mortality in any ART programme, the identification and close monitoring of patients identified to be at risk for mortality, and or early correction of modifiable risk factors, is necessary. Support to patients at higher risk of mortality can be provided more efficiently-by concentrating the limited resources on those who need the support most.

Several socio-demographic and clinical factors have been associated with mortality in different settings but without much consistency between settings. In a systematic review of 18 published cohort studies containing 39 536 patients treated in sub-Saharan countries, low baseline CD4+ cell count was a strong independent risk factor for early mortality in all those with available data. The summary hazard ratio for the association between CD4+ count of less than 50 cells/µL (versus CD4+ >50 cells/µL) was 2.5 (95% CI, 1.9–3.2) in studies with data presented in this format. Baseline viral load was not an independent risk factor in any of the studies.[33] Symptomatic HIV disease (WHO stage 3 and 4) was an independent risk factor in some but not all of the studies, possibly reflecting differences in the accuracy of clinical staging or homogeneity of cohorts with respect to this variable. Conversely, WHO stage 4 disease was found to be a strong predictor of mortality in all studies reporting on this. The result was a summary hazard ratio of 2.2, (95% CI, 1.5–3.2) in three studies when patients with WHO stage 4 disease were compared with those with WHO stages 1-3.[33]

Adherence to cART was found to be an independent predictor where explored.[39,40] In a study by Nachega JB et al, among 6288 South African adults enrolled in a private-sector AIDS management programme, adherence <80% (as assessed by pharmacy claims) was associated with lower survival (adjusted HR 3.23; 95%CI: 2.37-4.39), and when medication adherence was divided into 5 strata with a width of 20% each, each stratum had lower survival rates than the adjacent, higher-adherence stratum.[39]

Low body mass index (BMI), anaemia, and male sex, were independent risk factors in some of the studies. Some authors have suggested that male sex predicts higher mortality due to differences in health-seeking behaviour or poorer treatment adherence.[33,39] However, an analysis of a cohort of 4383 HIV-infected adult men and 8459 women on ART in Tanzania which found that men had a significantly higher risk of

overall mortality [hazard ratio 1.19, 95% CI 1.05–1.30, P<0.001], found that non-adherence to care and advanced immunodeficiency at enrolment explained only 17% of the higher mortality in HIV-infected men. This suggests that there may be other behavioural and biological factors that may negatively impact treatment outcomes in men.[41]

In contrast to data from high-income settings, increasing age was a predictor of mortality in only one cohort in the systematic review.[33] The authors suggested that the lack of association with age may reflect the younger and narrower age distribution of patients receiving cART in Africa, or the relatively short duration of follow-up in these studies since immunologic recovery during the first 4 months of treatment is not age-dependent whereas long-term recovery is, as was shown by Bennett, De Gruttola and Marscner.[42] However, in a later study undertaken among 11153 patients with median age of 34 years (IQR: 29-41 years) enrolled in ART scale-up programmes in South Africa, Malawi, and Cote d'Ivoire, and followed-up for one year, those aged at least 40 years had 43% higher risk of mortality than younger patients (adjusted hazard ratio: 1.23-1.66).[43] The current study will offer an opportunity to study this association in depth, given the long follow-up period.

None of the cohorts analysed found baseline history of TB or TB disease activity to be independent risk factors of mortality during ART although TB was a strong predictor of mortality pre-ART.[33] This is in keeping with the findings from a previous study among the current study's population, conducted after median follow-up of 24.7 months, which showed that history of TB at ART baseline was not independently predictive of death. Incident TB during ART follow-up was in contrast, found to independently predict death even after adjustments for baseline risk factors, including CD4+ cell count and viral load: hazard ratio for death of 2.49 (95% CI 1.68–3.69).[44]

Furthermore, the systematic review of 18 cohort studies, and other studies, suggest that risk factors for mortality may alter during the course of ART.[33,45] Whereas mortality during the first four months of treatment in a Cape Town cohort of 927 was independently associated with baseline advanced WHO clinical stage, lower baseline CD4+ counts, and male sex, mortality beyond this time point was only associated with the updated absolute CD4+ cell count at 4 months and with failure to achieve viral load

suppression.[34] These data suggest that the key long-term determinant of mortality is the response to ART as also shown by more recent findings from a large cohort study of 15060 patients treated in a multi-site community and workplace HIV management programme in South Africa. Hoffmann et al found that the baseline characteristics of WHO stage, haemoglobin, CD4+ count, and TB symptoms were all associated with mortality during the first 12 months of cART but there was attenuation in hazard of mortality after 12 months. However, time-updated factors of CD4 count, body mass index, TB symptoms, anaemia, and HIV RNA suppression were strong predictors of death throughout the follow-up period.[37]

South African military HIV treatment programme

The prevalence of HIV infection in the South African National Defence Force (SANDF) has been recently reported as 8.5% by the Surgeon General of the SANDF.[46] Members of the SANDF and their families receive care from a network of sickbays, Healthcare Centres and three military hospitals of the South African Military Health Service (SAMHS).

Until 28 February 2013, the majority (>80%) of ART roll-out in the SAMHS was undertaken by Project Phidisa, a collaborative programme between the SANDF, the US Department of Defense and the US National Institute of Allergy and Infectious Diseases (NIAID). Phidisa has provided free cART to qualifying soldiers and their families at six research sites since February 2004, through the Phidisa II trial and then from April 2004, also through the U.S President's Emergency Plan for AIDS Relief (PEPFAR) for patients ineligible or not consented to the trial.[47]

Phidisa II is a completed, randomised, open-label 2x2 factorial trial which enrolled 1771 participants with advanced HIV disease and/or CD4+ cell counts < 200 cells/mm^3, who were either treatment-naïve or had <7 days of prior ART. The trial compared the safety and efficacy of four regimens: efavirenz (EFV) vs. ritonavir-boosted lopinavir (LPV/r) + [stavudine+lamivudine (D4T+3TC) vs. zidovudine+didanosine (ZDV+DDI)]. Participants were enrolled during the period February 2004 to December 2007, followed-up under the trial until March 2008,[48] and were then transitioned into an observational cohort study in April 2008. The participants continued to be followed-up in the observational

cohort study until 28th February 2013, after a strategic decision to stop research follow-up and transition patients to routine HIV care. Hence, this cohort provides data on participants followed up for substantial periods.

A relatively high rate of mortality was observed in Phidisa II, particularly in the first six months. However, mortality continued to be significant even beyond 36 months. At primary study closeout, after a median follow-up period of 24.7 months, 11.7% of the participants had died. Over half of the deaths (51.9%) occurred within 6 months of follow-up.[48] The patterns and predictors of mortality after extended follow-up of this cohort have however not been examined.

Defining the research problem

The identification of patients at risk of mortality, done pre-cART initiation and in the course of therapy, will assist in directing interventions and additional support to those likely to benefit the most. Additionally, findings on causes of mortality will direct a full spectrum of preventive interventions to mitigate against the most commonly identified causes.

Data on patterns, including causes of mortality, and predictors of mortality in Southern African cART treated patients over long-term periods exceeding five years, are scarce. The majority of publications have studied mortality patterns during shorter follow-up, typically maximum of five years after cART initiation.[33-35,37,44,49]

A large proportion of the Phidisa population is military and itinerant, and maybe different from other cohorts previously studied. However, to our knowledge, there are no published studies reporting patterns and and/or predictors of mortality among members of an HIV antiretroviral therapy military health programme in Africa, besides a previous sub-study of Phidisa II.[44] That study investigated the risk factors for incident TB, as well as the effect of incident TB on mortality in the current study population. However, the patients had only had follow-up for a short period, i.e. a median of 24.7 months.[44]

The Phidisa II cohort is among the largest in sub-Saharan Africa to have received boosted-PI based cART, and non-3TC based cART, as first-line therapy. Although the primary results showed equivalent survival and progression to AIDS in both factorial comparisons (LPV/r vs EFV, and D4T+3TC vs ZDV+DDI),[48] the regimen effects may now be different given the longer follow-up. There was a more robust recovery of CD4+

cell counts i.e.18 more CD4+ cells in LPV/r arms compared to EFV arms averaged over the follow-up period, despite similar viral response.[48]The clinical implications of the observed difference in immunologic response may become apparent after longer follow-up.

Additionally, due to the lower than anticipated number of primary endpoints (AIDS or death) available for the primary analysis, i.e. 320 instead of 635 estimated events, the primary study could not rule out a 37% difference in mortality risk between LPV/r and EFV, and between ZDV+DDI vs D4T+3TC.[48] There is now greater power to detect differences in the risk of later mortality between the regimen factors, and other possible predictors due to the accumulation of mortality events after longer follow-up.

It is anticipated that the findings of the current study will inform policies and interventions to reduce mortality among cART treated patients in this and similar settings.

Aim(s), hypothesis and objectives

Aim

We aimed to describe the patterns of mortality in patients initiated randomised cART in Phidisa II, by baseline and on-treatment characteristics, and to identify patient characteristics predicting early and late mortality in this population, now with extended follow-up of up to eight years.

Hypothesis

Early mortality is predicted by baseline socio-demographic and clinical factors such as: increasing age, male sex, not being married, lower level of education, low Body Mass Index, anaemia, low CD4+ count, ALT >100 IU/L, AST > 100 IU/L, self-reported use of traditional medicine, higher HIV RNA loads, WHO Clinical stage 3 or 4, history of TB (current or previous), treatment for other chronic conditions, past/current history of depression, peripheral neuropathy, HIV co-infection with Hepatitis B or C, and early enrolment (2004 and 2005) into the programme.

Late mortality is predicted by baseline factors such as: increasing age, male sex, not being married, lower level of education, self-reported use of traditional medicine, WHO Clinical stage 3 or 4, history of TB (current or previous), treatment for other chronic conditions, past/current

history of depression, peripheral neuropathy, HIV co-infection with Hepatitis B or C, early enrolment (2004 and 2005) into the programme, initiation with LPV/r vs EFV, initiation with ZDV+DDI vs D4T+3TC. The following factors, updated at month 12, predict late mortality: low Body Mass Index; low haemoglobin; low CD4+ count; failure to suppress HIV RNA to < 400 copies/mL.

Study objectives

i) Describe the extent and patterns of mortality, including its causes, overall, in the early (<=12 months) and late periods (>12 months) in the cohort that initiated cART in Phidisa II.

ii) Identify the socio-demographic and clinical factors predicting early and late mortality, respectively, in this cohort.

CHAPTER 2

METHODS

Study Design

This is an observational cohort study on prospectively collected data.

Study Setting

Six research sites of the South African Military Health Service, located in Pretoria, Mtubatuba, Cape Town, Mthatha, Bloemfontein and Phalaborwa.

Study population

One thousand seven hundred and seventy-one (1771) participants initiated on cART in Phidisa II, a randomized controlled trial, during the period February 2004 to December 2007, and followed-up until 31st December 2011.

Brief Phidisa II methodology

The eligibility criteria for Phidisa II were: uniformed SANDF personnel and family members who were eligible to receive services from the SAMHS, age >14 years, antiretroviral naïve (defined as ART for < 7 days), CD4+ T lymphocyte cell counts < 200 cells/mm^3 (or </=14% for patients post-splenectomy), and/or any history of or current AIDS defining illness. Patients with pulmonary tuberculosis (TB) were required to have a CD4+ cell count <200 cells/ml and have completed the intensive phase of TB treatment, to be eligible. Pregnant women were excluded. The following laboratory criteria were also required: haemoglobin > 9 g/dL (>8 g/dL for women), neutrophil count > 500 cells/µL, platelet count > 25000/µL and serum liver transaminases < 5 times the upper limit of normal range. Screening included viral hepatitis studies although co-infection with Hepatitis B or C was not an exclusion criterion. Screening eligibility was completed within 56 calendar days of randomisation. The study was approved by the SANDF and NIAID Institutional Review Boards. All participants provided written informed consent.[48]

Data collection and follow-up of Phidisa II participants

At randomisation, the following were collected: Medical histories, complete physical examinations, and laboratory tests, namely:plasma HIV RNA level, CD4+ cell count, full blood count and differential, ALT and AST. [48] Participants received pre-treatment drug teaching and adherence counselling, in 2 sessions, prior to randomisation.

Follow-up visits for data collection occurred monthly for the first 3 months and every 3 months thereafter. At these visits, interim histories were taken, selected symptoms were assessed and graded on a 4-point scale (mild to potentially life-threatening), adherence to study medication was assessed and reinforced by pharmacists. Adherence was self-reported on a Likert-type scale of 1, representing 100% adherence (all pills taken every day during the past 28 days) to 5, representing 0%, (none of the pills were taken during the past 28 days). A scale of 3 represented an estimated adherence level of 50%. CD4+ cell counts and HIV RNA levels were measured, and haematology and clinical chemistry (urea, creatinine and electrolytes, ALT and AST) tests performed. The lower level of detection of the PCR assays for HIV RNA changed from 400 copies/mL to 50 copies/mL during trial follow-up.

Participants had to collect their medicines on a monthly basis and only had vital signs and adherence assessments when these collections did not coincide with the protocol scheduled follow-up visits described earlier.

Single antiretroviral drug substitutions after toxicity were allowed, and at least two drugs were changed after treatment failure. All changes to ART, including clinician-directed changes and participant-initiated stoppages of at least 30 days, were recorded.

All grade 4 clinical events that occurred throughout follow-up were reported and coded according to the *Medical Dictionary for Regulatory Activities* (version 12.0).

The primary endpoints of death from any cause and AIDS defining illness (Progression of disease) were recorded on protocol event forms. The End Point Review Committee reviewed site reported AIDS-defining illnesses, masked to treatment assignment. AIDS events classified as confirmed or probable by the End Point Review Committee based on pre-established criteria were considered end-points in the primary analysis, as were all deaths irrespective of cause. A final visit was conducted to confirm end-point and vital status as of the primary study close-out date, 31 March 2008.[48]

Brief observational cohort methodology

Participants previously enrolled in Phidisa II provided additional written consent to continue follow-up under an observational study from 1st April 2008. Thereafter, the participants continued their 3-monthly follow-up visit schedules, e.g. if their last protocol visit prior to 31st March 2008 was month 36, their first visit under the observational study was month 39.

At odd-numbered visits, i.e. months 15, 21 etc. laboratory tests were the same as the Phidisa II monitoring tests. At even-numbered visits, i.e. 6-monthly; glucose, triglycerides and total cholesterol; Hepatitis B and C studies; and tests for sexually transmitted infections (STIs), namely, RPR for syphilis, and urine PCR tests for Neisseria gonorrhoea and Chlamydia trachomatis, were added to the battery of routine Phidisa II monitoring tests. Participants continued on their regimens for as long as was indicated with regimen changes dictated by standard-of-care practices.

The protocol events of death from any cause, progression of disease diagnosis, and grade 4 clinical events continued to be recorded within 3 days of site awareness and were captured centrally. AIDS events were now reviewed by the Medical Monitor for verification of diagnostic confidence, i.e. whether the diagnosis was confirmed, probable, or possible/empiric. All ART changes were recorded. Adherence assessments continued to be made at the clinics, but the adherence information was not captured centrally. Pharmacy refills were made 3 monthly, or more frequently for patients with recent changes in therapy or with poor virologic response.

Prevention and Follow-up of missed visits

To reduce missed visits, participants enrolled in all Phidisa protocols were followed-up closely. The visit schedules which were generated by the project's Coordinating Centre at study enrolment, enabled sites to schedule participants accordingly. Sites were encouraged to continually update the participant contact details at all visits, and to contact participants telephonically to remind them of their upcoming appointments within the week. If a participant missed their appointment, the site staff members had to contact the participant or their next of kin, at least three times before the visit window closed. Home visits were conducted where necessary.

Since most of the participants were members of the South African National Defence Force (SANDF), their whereabouts could also be traced via their work units. For participants who were still eligible for South African Military Health Service (SAMHS) benefits when they missed a visit, the SAMHS Health Informatics system could be accessed to check the member's vital status. At primary analysis, the Phidisa II study found that only 5% of participants could not have their vital status ascertained at study close-out, and were considered LTFU.[48] A similar percentage in the observational cohort study had unknown vital status at any time.

All study procedures and clinical management algorithms were detailed in the project's Manual of Operations (MOOP), allowing for standardisation across all six sites.

Measurements

Measurement tools

The primary measurement tools were the Phidisa II and observational cohort databases.

Measurement methods

a) Baseline data, which include socio-demographic characteristics, clinical and laboratory data, were obtained from the Phidisa II database.

b) Clinical and laboratory data from follow-up visits were extracted from the Phidisa II and observational cohort study databases.

c) The vital status of participants as at this study's close-out of 31st December 2011, were obtained from both databases.

d) The causes of death were extracted from protocol event forms in the databases.

Variables

Outcome variables

Failure: All-cause mortality at different time points

Overall mortality was defined as mortality at any time during follow-up.

Early mortality was defined as mortality within one year of initiating cART (up to 365 days since randomisation in Phidisa II).

Late mortality was defined as that occurring after the 365 days post-randomisation period.

***Loss to follow-up (LTFU)*:** *Early period*: Participants whose vital status was unknown at 365 days post-randomisation were considered early LTFU. Unknown vital status was defined as: not known to be have died by 365 days, AND did not honour a visit after 365 days, AND did not have a withdrawal date after 365 days, AND had no reportable event date after 365 days.

Late period: Participants known to be alive 365 days post-randomisation, and whose vital status was unknown at common close-out date of 31st December 2011, were considered late LTFU. Unknown vital status in the late period was defined as: not known to be have died by the 31st December 2011, AND did not honour a visit six months after the close-out date, AND did not have a withdrawal date six months after the close-out date, AND had no reportable event date six months after the close-out date.

Withdrawal date: This is the date at which the participant explicitly indicated their wish not to be followed-up or have their vital status ascertained, AND never honoured a visit after that date. The withdrawal of consent was captured on a specific form. Participants could withdraw and resume follow-up at a later stage.

Censoring

a) *Date last known to be alive:* The date of last visit, or withdrawal date, or date of reportable event other than death- whichever was the latest.
b) For the assessment of *early mortality*, follow-up was censored 365 days post-randomisation, or date last known alive- whichever occurred first.
c) For the assessment of *late mortality*, follow-up was left truncated at 366 days post randomisation, and right censored on 31st December 2011 or date last known alive, whichever occurred first.
d) For overall mortality: follow-up was censored on 31st December 2011 or date last known alive- whichever occurred first.
e) Withdrawals were censored on the date of their last withdrawal.

Exposure Variables

Early mortality: The following baseline characteristics were considered possible predictors of early mortality: age, sex, marital status (married vs others), level of education (completed at least high-school), body mass index, haemoglobin, CD4+ count, ALT >100 IU/L, AST > 100 IU/L, self-reported use of traditional medicine, HIV RNA load, WHO clinical stage 3 or 4, randomisation to LPV/r vs efavirenz (3rddrug), NRTI backbone (D4T+3TC vs DDI+ZDV), history of TB (current or previous), treatment for other chronic conditions, past/current history of depression that required medical intervention, peripheral neuropathy, HepBsAg, HCV antibodies, and year of enrolment (randomisation).

Late mortality

Updated characteristics: The following updated characteristics were considered possible predictors of late mortality: Any transfer between sites by 6 months, missed protocol visit in first 6 months of randomisation; month 12 haemoglobin, HIV RNA load at 12 months, CD4+ count at 12 months, incident TB (confirmed, probable or possible) in the first 12 months. Since the lower level of detection of the PCR assays for HIV RNA changed from 400 copies/mL to 50 copies/mL during trial follow-up, viral suppression was defined as viral load < 400 copies/mL for all analyses.

Baseline characteristics: The characteristics were as for early mortality, but excluding baseline haemoglobin, CD4+ count, HIV RNA, and transaminases (AST, and ALT), since it is clinically and epidemiologically plausible that the updated, rather than the baseline values of these will predict later mortality. Studies have shown that proximal/ updated levels of CD4+ count and haemoglobin,[37,50,51,52] and updated viral load and BMI are better predictors of subsequent mortality.[37,52]

Data Management and Statistical analysis

Data were extracted onto MS Excel, cleaned, and then imported into STATA. All analyses were done with STATA version 11.2 (College Station, Texas); p-values less than 0.05 were considered statistically significant.

Descriptive statistics of the cohort

The baseline characteristics of the whole cohort, and the baseline and month 12 updated characteristics of the cohort surviving beyond one year were described. Mean (standard deviation-SD) or Median (inter-quartile range-IQR) for continuous variables, as appropriate, and proportions were calculated for categorical variables. Kaplan-Meier curves to show the overall cumulative probability of mortality, and mortality rates (/100 person years) for the overall, first year of cART, and the period beyond the 1st year, were calculated.

Univariate analyses

Kaplan-Meier survival estimates with their 95%CI were used as exploratory graphical analysis of associations of exposure variables with mortality.

Pre-estimation evaluation of proportionality of hazards (PH) assumption was performed graphically using two methods: i) assessing for parallelism of the log-negative-log of the survival distribution plotted against the log of survival time for variable categories. Reasonably parallel curves satisfied the PH assumption; and ii) observed and expected Kaplan-Meier survival curves for a category- close plots indicate satisfaction of the PH assumption.[53] The assumption was considered violated when both graphical displays indicated so, and was not violated when at least one of the plots were in favour of the assumption. Factors violating the PH assumption were not considered for further modelling unless the factors had been described as predictors of mortality in the literature, or their association with mortality is clinically and epidemiologically plausible. Univariate Cox Proportional Hazards (PH) regression models were used to detect crude associations with mortality. Unadjusted hazard ratios (HR), their 95% (CI), and p-values were reported.

Multivariate analyses

Epidemiologically plausible interactions, e.g. between gender and BMI, marriage, or concomitant chronic medication; NRTI-backbone and Hepatitis B surface antigenaemia, were investigated. Multivariate Cox PH regression models, giving adjusted HR, 95% CI, and p-values were used to identify the independent predictors of mortality.

All covariates and interaction terms with p <0.25 in the univariate Cox regression models, were included in the full multivariate models. The multivariate assessment of variables with p>0.05 in univariate analysis reduces the risk of inadvertently excluding variables that have borderline associations with the outcome.[54]Final reduced models were obtained after backward elimination, i.e. after fitting the full model, the non-significant covariates were removed one at a time starting with those with the least significant Wald test p-value and a non-significant Likelihood Ratio (LR) test. The robustness of the reduced models was confirmed by the forward inclusion method.

For both univariate and multivariate regression models, analyses were stratified by the randomising site to control for residual site-level confounding. Factorial comparisons of randomised cART regimens were analysed in 'intention to continue' fashion, with disregard for cART changes or discontinuation.

Post-estimation, the global and covariate Schoenfeld residuals tests (uses test statistic or equivalent p-value) were used after fitting the final models; p<0.05 indicated violation of the PH assumption by that covariate. The Harrell's C concordance statistic was used to assess the fit of the final multivariate models.

Ethical considerations

This study was approved by the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria. The parent studies (Phidisa II and the observational cohort study) were approved by the SANDF and NIAID Institutional Review Boards.
The Phidisa II study started enrolling patients prior to the implementation of the national South African ART roll-out and the PEPFAR-funded programme at the research sites in April 2004. As a result, for the period January 2004- Mar 2004, the consent process for the trial highlighted that the patients reserved the right not to enrol in the study, but that the non-research SAMHS clinics did not provide cART. From April 2004, the patients were informed that they had a choice to enrol in the trial or access HIV care including cART, through the PEPFAR programme at the research sites, and then later at non-research SAMHS facilities once that became available. (see Informed Consent Appendices)

CHAPTER 3

RESULTS

Description of study population at enrolment

From January 2004 to 31 December 2007, 1771 patients were enrolled into the Phidisa II study. Site 001, the first of the 6 sites to be opened, enrolled the highest number, 919 (52%), whilst site 006 was the last site opened in 2007 and enrolled 28 patients (1.6%). The median year of enrolment was 2005, with 56.6% of patients enrolled by the end of 2005.

The baseline socio-demographic and clinical characteristics of the study population are shown in Table 3.1. The median age at baseline/randomisation was 35 years (IQR 32-38), and the majority of patients were men (68%), had at least a high school education (86%), and 62.3% were married.

Clinical characteristics

The median Body Mass Index (BMI) was 22.9 kg/m^2 (IQR 20.4-25.9), with 58.8% of the patients having a BMI within the healthy range of 18.5-25 kg/m^2; the mean haemoglobin (Hb) was 11.5g/dL (standard deviation, sd =1.5) for women and 13 g/dL (sd=2) for men, with nearly 10% of all patients having clinically significant anaemia of <10g/dL. Sixty-two patients (3.6%) had at least grade 2 elevated ALT (>100IU/L), whist 4.7% had AST levels >100IU/L.

The cohort had advanced HIV disease as indicated by a median CD4+ count of 102 cells/mm^3 (IQR 41-157); a median HIV viral load of 141000 copies/mL (IQR 53900-305000), with nearly 60% of patients having high viraemia above 100000 copies/mL; and 49.6% with WHO clinical stage 3 or 4 disease. Five hundred thirty-two (30%) of the patients had a history of past or current TB disease.

Hepatitis B co-infection, indicated by positive surface antigen, was detected in 101 (5.8%) of the patients. The patients were equally distributed to the randomisation treatment arms: 50% randomised to the ZDV/DDI NRTI arm (vs 50% to the D4T/3TC arm), whilst 50% were allocated to the LPV/r (vs 50% to the EFV) arm. (See Table 3.1)

Table 3.1: Cohort baseline characteristics

Characteristic	N	n (%)
Randomising Site	1771	
001		919 (51.9)
002		297 (16.8)
003		159 (9 .0)
004		215 (12.1)
005		153 (8.6)
006		28 (1.6)
Enrolment year, median(IQR)	1771	2005 (2004,2007)
2004		605 (34.2)
2005		396 (22.4)
2006		418 (23.6)
2007		352 (19.9)
Age, years	1771	
median (IQR)		35 (32,38)
mean (SD)		35.4 (5.5)
Age < 40		1414 (79.8)
Sex	1771	
Male		1204 (68.0)
Education	1771	
High school education or higher		1521 (86.0)
Marital status	1771	
Married		1104 (62.3)
BMI, median, kg/m^2	1743	22.86 (20.37, 25.85)
BMI category		
<18.5		173 (9.9)
18.5-24.9999		1024 (58.8)
>=25		546 (31.3)
Haemoglobin (Hb), mean (sd), g/dL	1717	12.5 (2.0)
Mean Hb (sd)-women	(553)	11.5 (1.5)
Mean Hb (sd)-men	(1164)	13 (2)
Hb category		
<10 g/dL		166 (9.7)
CD4+ count, median (IQR), cells/mm^3	1721	102 (41, 157)
CD4+ category		
≤50		501 (29.1)
51 – 100		352 (20.4)
101 – 200		710 (41.3)
>200		158 (9.2)
HIV RNA viral load, median (IQR), copies/ml	1730	141000 (53900,305000)
>/= 1 X 10^5		1,033 (59.7)
WHO Stage	1771	
1		684 (38.6)
2		208 (11.7)
3		652 (36.8)
4		226 (12.8)
Hepatitis B surface Antigen, Positive, %	1756	101 (5.8)
Hepatitis C Antibody	1756	
Positive, %		13 (0.7)
History$^+$ of TB, Yes,%	1771	532 (30)
Traditional medicine use, %	1768	183 (10.3)
ALT >100 IU/L,%	1719	62 (3.6)
AST >100 IU/L,%	1719	81 (4.7)
History$^{+\&}$ of Depression, %	1771	20 (1.1)
Concomitant chronic medicine, %	1771	169 (9.5)
NRTI backbone,%	1771	
ZDV+DDI		884 (49.9)
D4T+3TC		887 (50.1)
3rd Drug, %	1771	
Lopinavir/r		883 (49.9)
Efavirenz		888 (50.1)

BMI=Body Mass Index, Hb=Haemoglobin, ALT= Alanine Transaminase, AST= Aspartate transaminase, $^+$=past or current history, $^\&$=depression that required medical intervention

Follow-up

By 31 December 2011, total follow-up observed was 8920.73 person-years (py), median 5.42 py (IQR 4.21-6.88). During the whole observation period, 279 (15.8%) patients died, 148 (8.4%) withdrew from the study, and 93 (5.3%) were lost to follow-up (LTFU). More than half (54%) of all the deaths, i.e.151 (8.5%) of the whole cohort, occurred within the first year of therapy. There were 22 withdrawals and five LTFU by the end of the first year, meaning that 1593 patients were known to have survived beyond one year (365 days) after cART initiation.

Characteristics of cohort that survived beyond one year

Selected baseline and updated characteristics of the cohort that survived beyond one year, and were therefore at risk of late mortality, are shown in Table 3.2.

In the first year, the median BMI of this cohort increased by 1.5 units. The proportion of the cohort that was underweight reduced from 8.0% to 4.0%; whilst the proportion that was overweight or obese, increased by 14.7%. These changes were statistically significant, (Wilcoxon signed-rank test, p <0.001). The mean haemoglobin of the cohort significantly increased to 13.8g/dL, over the year, (paired t-test, p< 0.001).

Regarding CD4+ count, there were significant increases in the proportion with CD4+ count > 200 cells /mm^3 over the first 12 months (from 9.7% to 66.9%). The median increased by 143 cells over the year, (Wilcoxon signed-rank test, p <0.001).

At month 12, 62.2% of this cohort had HIV viral suppression of <400 copies/mL, and 4.1% had viral loads above 100000 copies/ ml, whereas 59% had viral loads above 100000 copies/ ml at baseline, (McNemar's test, p<0.001). In conclusion, the majority of patients responded well to cART during the first year of cART.

Table 3.2: Selected baseline and updated characteristics of cohort surviving at 1 year

Characteristic	**N**	**n (%)**	**Test, P_{value}**
Baseline BMI, median (IQR),kg/m^2 BMI category <18.5 18.5-24.9999 >=25	1570	23.1(20.7,26.0) 126 (8.0) 933 (59.4) 511 (32.6)	Wilcoxon signed-rank test P= <0.001
12 month BMI, median (IQR),kg/m^2 BMI category <18.5 18.5-24.9999 >=25	1440	24.7(22,27.8) 58 (4.0) 701(48.7) 681(47.3)	
Haemoglobin, mean(sd), g/dL Baseline Haemoglobin month 12 Haemoglobin Mean m12 Hb (sd)-women Mean m12 Hb (sd)-men	 1550 1447 968 479	 12.7(1.9) 13.8(1.7) 14.4(1.5) 12.6(1.4)	Paired t-test P<0.001
Baseline CD4+ count, median (IQR), cells/mm^3 CD4+ category <=100 101-200 >200	1553	108(48,159) 735(47.3) 667(43.0) 151(9.7)	Wilcoxon signed-rank test P<0.001
Month 12 CD4+ count, median (IQR), cells/mm^3 CD4+ category </=100 101-200 >200	1452	251(176,339) 95 (6.5) 385 (26.5) 972 (66.9)	
Baseline viral load, median (IQR), copies/ml Viral load category,% > 100 000	1562	136430(52725-302000) 918(58.8)	McNemar's test* P <0.001
Month 12 viral load, copies/ml Viral load >100 000 Viral load category <400 400-999 >=1000	1453	 59 (4.1) 904 (62.2) 190 (13.1) 359 (24.7)	
Incident TB by month 12,%	1593	138 (8.7)	-
Any missed visit in 1st 6 months,%	1593	315 (19.8)	-
Early transfer (in 1st 6 months),%	1593	84 (5.3)	-

P_{value} = p-value for comparison between baseline and month 12 values; *comparing % above 100000 copies/ml.

The proportions of the cohort that were diagnosed with incident TB during the first year, missed a protocol visit in the first six months of follow-up, or were transferred between Phidisa sites in the first six months, were 8.7%, 19.8%, and 5.3% respectively.

Patterns of mortality

Overall mortality

Figure 3.1 shows the Kaplan-Meier curve for cumulative probability of mortality through the 96 months' maximum period of observation.

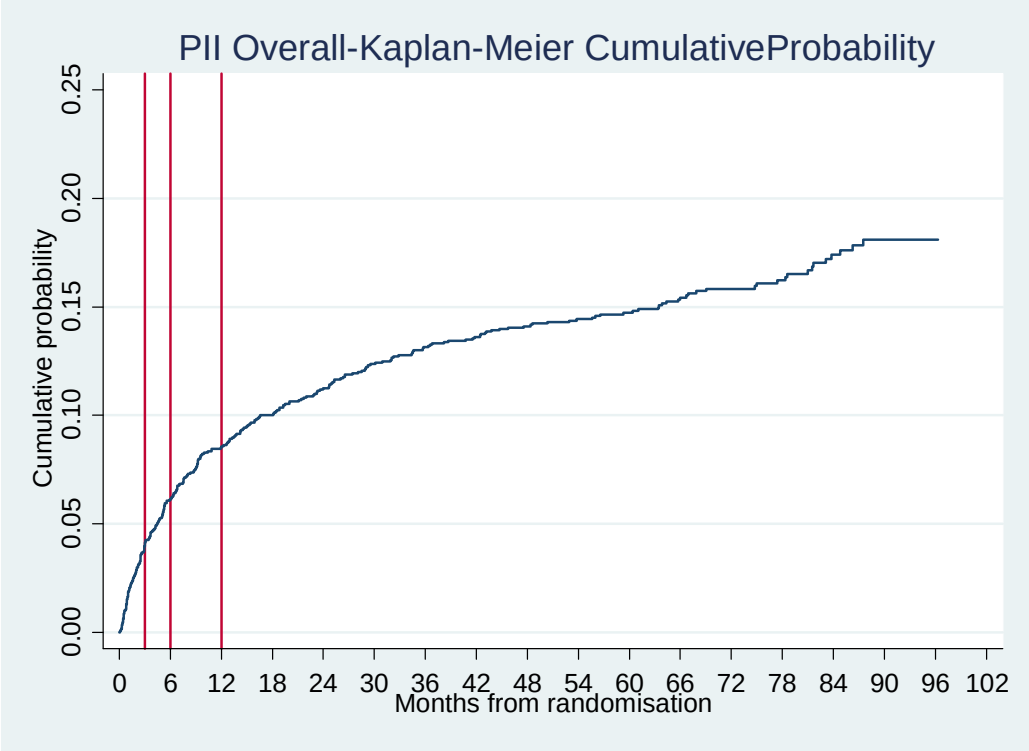

Fig 3.1: Kaplan-Meier Overall Mortality Cumulative Probability

The curve shows a sharp gradient in the first 12 months from randomisation/cART initiation, with 8.5 % of the cohort deceased by one year. The cumulative probability of mortality was 11%, and 15%, at two years, and five years respectively. The median time to death was 276 days from cART initiation (IQR: 88-842 days).

Table 3.3 below shows the follow-up, number of deaths, and mortality rate for the period up to one year and that beyond one year. The mortality rate was 3.13 per 100py (95% CI: 2.78-3.52) for the overall period; whereas it was 9.10 per 100py (95% CI: 7.76-10.67) for the period first year (early period), and was 1.76 per 100py (1.48-2.10) for the later period. The death rate in the first year was about five times that of subsequent years, [Rate Ratio (RR) =5.16 (95%CI 4.18-6.38)].

Table 3.3: Mortality rates: early, late and overall periods

Period	Person-time (py)	Deaths (n)	Rate (/100py)	95% CI
Early (</= 1year)	1659.22	151	9.10	7.76-10.67
Late (>1 year)	7261.51	128	1.76	1.48-3.10
Overall	8920.73	279	3.13	2.78-3.52

Early mortality

Among the 151 patients who died within the 1st year of cART initiation, the median time to death was 104 days (IQR: 39-198 days). As Figure 3.1 suggests and Table 3.4 shows, the mortality rate during the first six months was higher than in the latter six months in the 1st year of cART.

Table 3.4.: Mortality rates: early period

Period	Person-time (py)	Deaths (n)	Rate (/100py)	95% CI
0 – 6 months	851.87	109	12.80	10.60-15.44
> 6 months- 1 year	807.35	42	5.20	3.84-7.05
Total	1659.22	151	9.10	7.76-10.67

Most of the 151 early deaths occurred during the first six months of antiretroviral therapy, giving the high mortality rate of 12.80 per 100py (95% CI: 10.60-15.44). During the second six months of the 1st year, the mortality rate decreased to 5.20 per 100py (95% CI: 3.84-7.05). The risk of mortality in the first six months was therefore nearly 2.5 times that of the second semester, [RR =2.46 (95% CI: 1.74-3.47)].

Late mortality

One hundred and twenty-eight (8.0%) of the 1593 patients that survived beyond the first year, died during the subsequent seven year observation period ending 31 December 2011.

Table 3.5: Mortality rates after 1 year, by years on cART

Period	Person-time (py)	Deaths (n)	Rate (/100py)	95% CI
1 - 2 years	1547.21	46	2.97	2.22- 3.97
>2 – 3 years	1474.64	33	2.24	1.59 3.15
>3 - 4 years	1414.82	17	1.20	0.75 1.93
> 4 years	2824.84	32	1.13	0.80 1.60
Total (> 1 year)	7261.51	128	1.76	1.48 2.10

Table 3.5 shows the mortality rates per additional year on cART. The mortality rate decreased from 2.97 per 100py (95% CI: 2.22-3.97) in the 2nd year to 1.20 per 100py (95% CI: 0.75-1.93) in the 4th year. The mortality rate after 4 years of cART was the

lowest, at 1.13 per 100py (95% CI: 0.80-1.60). However, the death rate in the 4th year was similar to that of later years, [RR = 1.06 (95% CI: 0.59-1.91)]

Figure 3.2 shows the smoothed hazard (instantaneous rate) estimate of mortality from time of cART initiation. The hazards are highest early after cART initiation, then deteriorate steeply until 12 months, and are generally level between 12 months and end of follow-up.

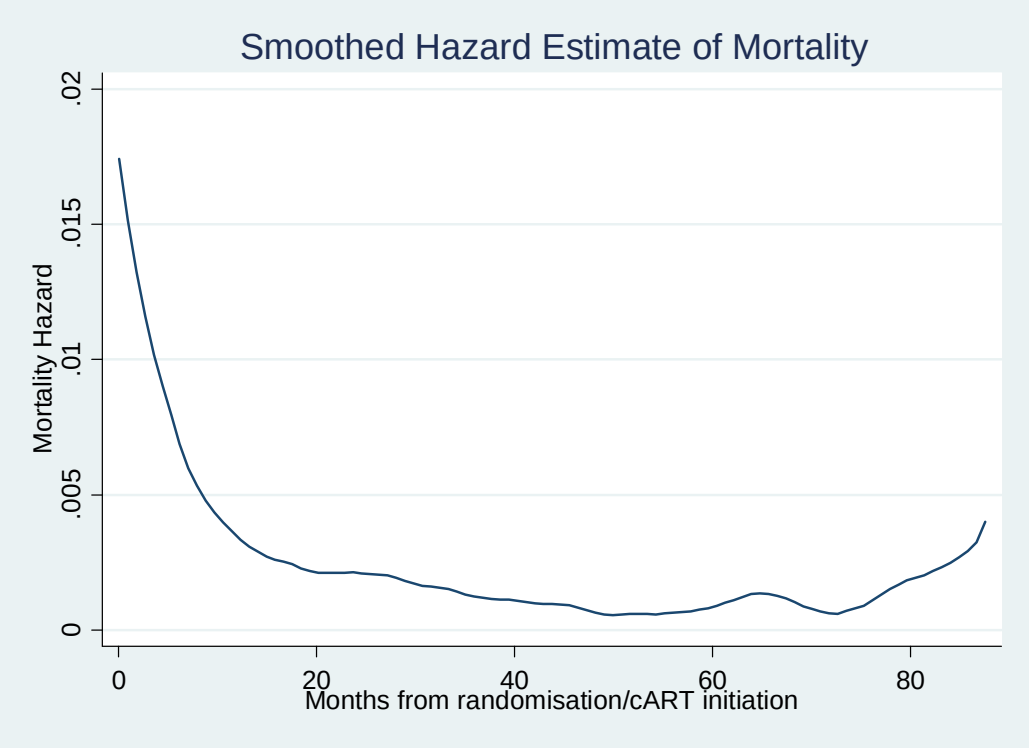

Fig 3.2: Smoothed Hazard Estimate of Mortality from cART initiation.

Causes of mortality

Ninety-four of all deaths (33.7%) were of unknown cause to the study team. There was no statistically significant difference in the proportions of unknown causes between the early and the later periods, (p=0.775).

Tuberculosis was the most common single cause of death during both periods of follow-up. Interestingly, there was a statistically significant higher proportion attributable to TB in later mortality, i.e. 22.7% compared to 12.6% in early mortality, (p=0.026).

In the early period, 25.8% of the 151 deaths were from WHO stage 3 or 4 opportunistic infections excluding TB, whilst in the later period, these infections were identified as causes in 14.1% of deaths, a statistically significant difference, (p=0.015).
AIDS-defining malignancies, e.g. Kaposi's sarcoma and cervical cancer, were identified as causes in 7 deaths and 1 death in the early and late periods respectively, showing a higher proportion of these causes in early mortality with borderline statistical significance, (p=0.074). Consequently, there were significantly more deaths attributed to WHO stage 3 and 4 conditions excluding TB (AIDS malignancy included) in early vs later mortality, 30% vs 15%, (p=0.002).
However, when all WHO stage 3 and 4 conditions (including TB, other opportunistic infections, and AIDS-defining malignancies) were considered as a group, there were 65 of 151, and 48 of 128 deaths in the early and late periods attributable to WHO stage 3 and 4 conditions respectively. These proportions were not statistically different, (p=0.347). (Table 3.6)

Table 3.6: Comparison of causes of mortality

Cause	**Early mortality (N=151)**		**Late mortality (N=128)**		**P**	**P**	**P**
	n	%	n	%			
Unknown	52	34.4	42	32.8	0.775		
TB	19	12.6	29	22.7	0.026		0.347
WHO 3/4 Opportunistic Infections- excluding TB	39	25.8	18	14.1	0.015	0.002	
AIDS-defining malignancy	7	4.6	1	0.8	0.074*		
Other malignancy	1	0.7	5	3.9	0.097*		
Unnatural causes	3	2.0	8	6.3	0.119*		
Diarrhoea	7	4.6	2	1.6			
Other infections	4	2.6	3	2.3			
Miscellaneous	19	12.6	20	15.6	0.492		
Total	**151**	**100**	**128**	**100**			

P= p-value from Pearson's chi-square test, unless specified otherwise. *Fischer's exact test

Predictors of mortality

The results of the assessments of independent risk factors (predictors) for early mortality and late mortality are presented separately below. All univariate and multivariate models were stratified by the randomising site.

Early mortality

Exploratory graphical analysis of associations

The Kaplan-Meier survival estimates with their 95%CI for selected baseline variables are found in Figs 3.3a-h and in Appendix 1. Baseline factors highly suggestive of having a crude association with early mortality were: enrolment year, education, marital status, BMI, WHO stage, haemoglobin, CD4+ count, viral load, and the transaminases (ALT and AST).

Factors not suggestive of association with early mortality were: sex-(although the survival estimates for males were lower, the 95%CIs for the male and female estimates overlapped suggesting a non-significant association), age, history of TB, concomitant chronic medication, history of depression, Hepatitis B antigenaemia, NRTI backbone, and randomisation to either 3rd drug (Lopinavir/r or Efavirenz).

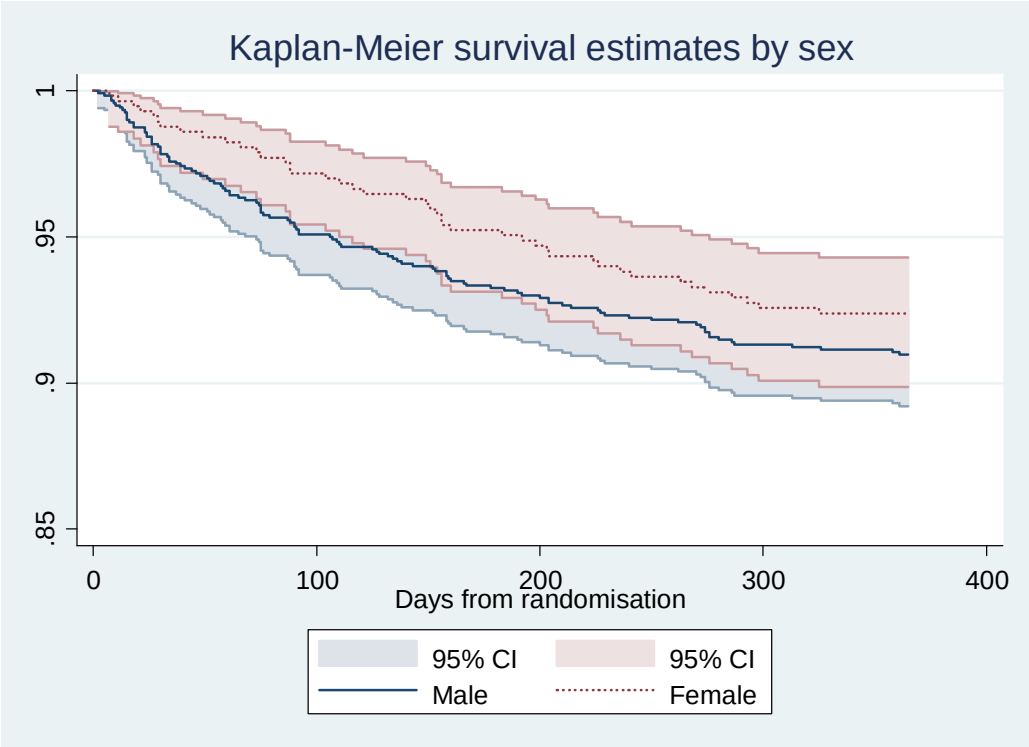

Fig 3.3a

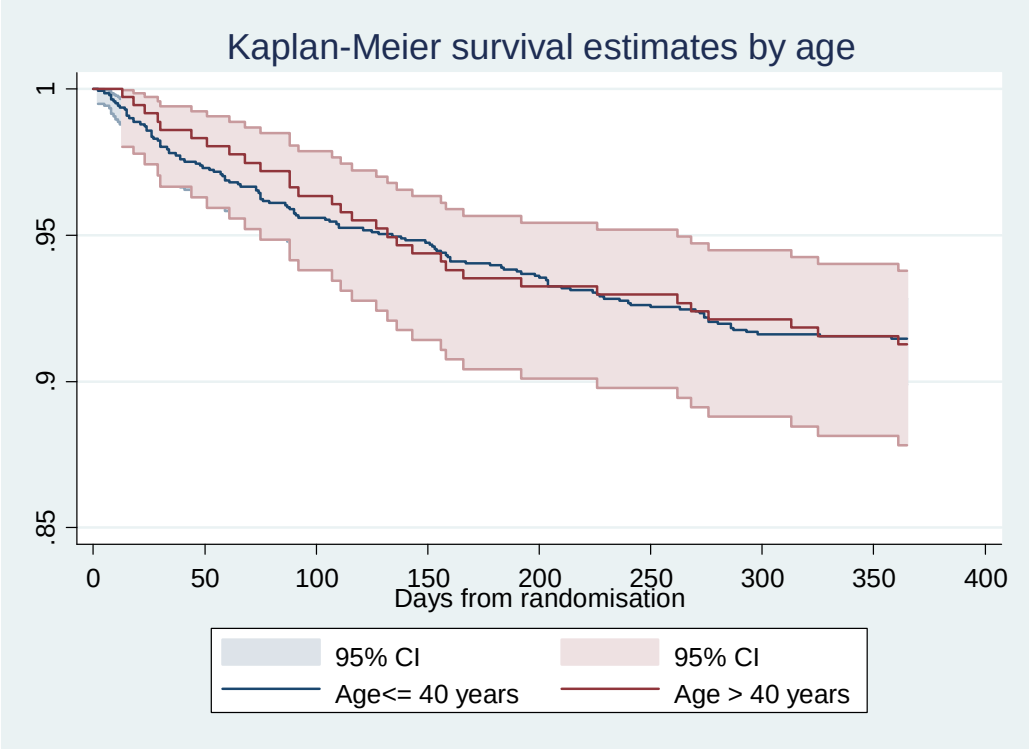

Fig 3.3b

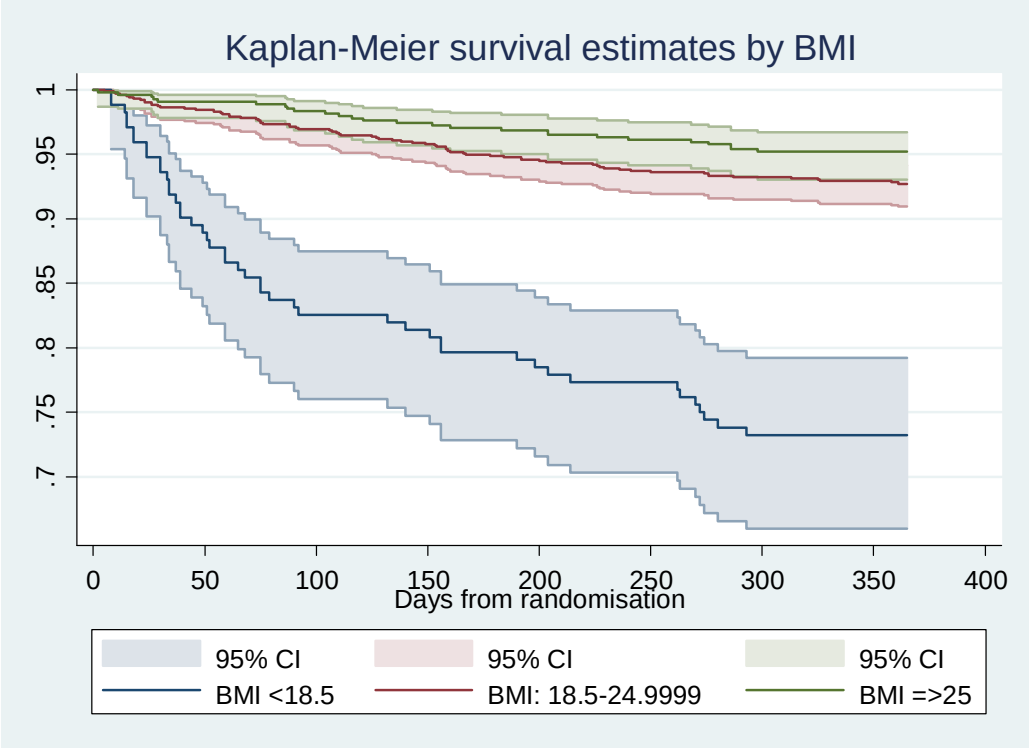

Fig 3.3c

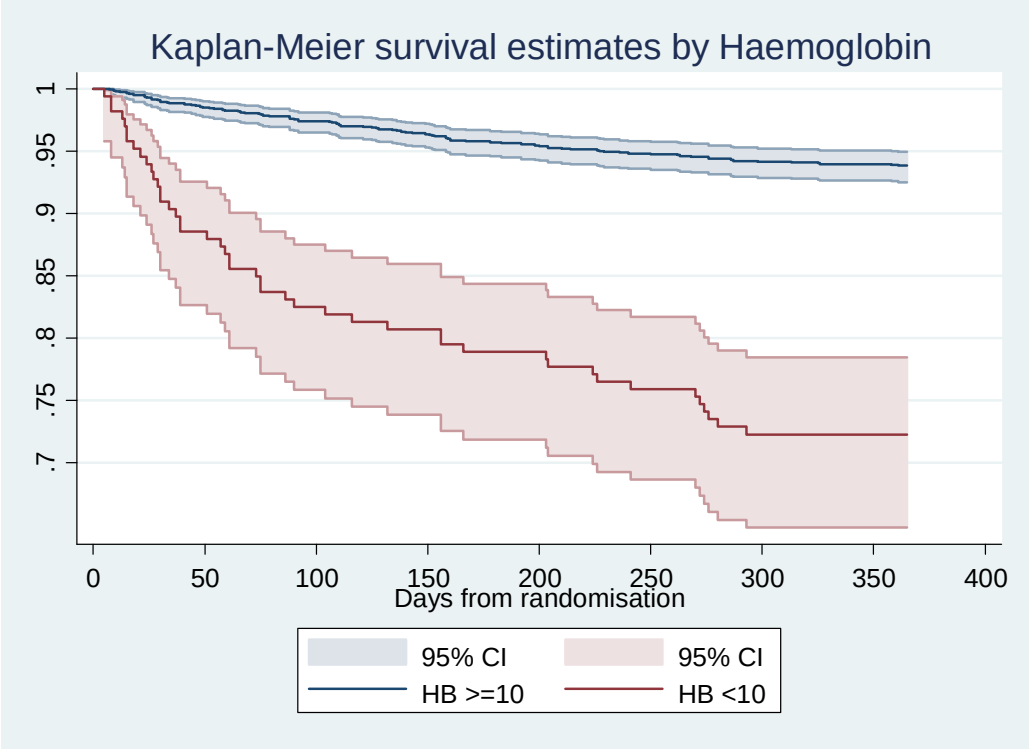

Fig 3.3d

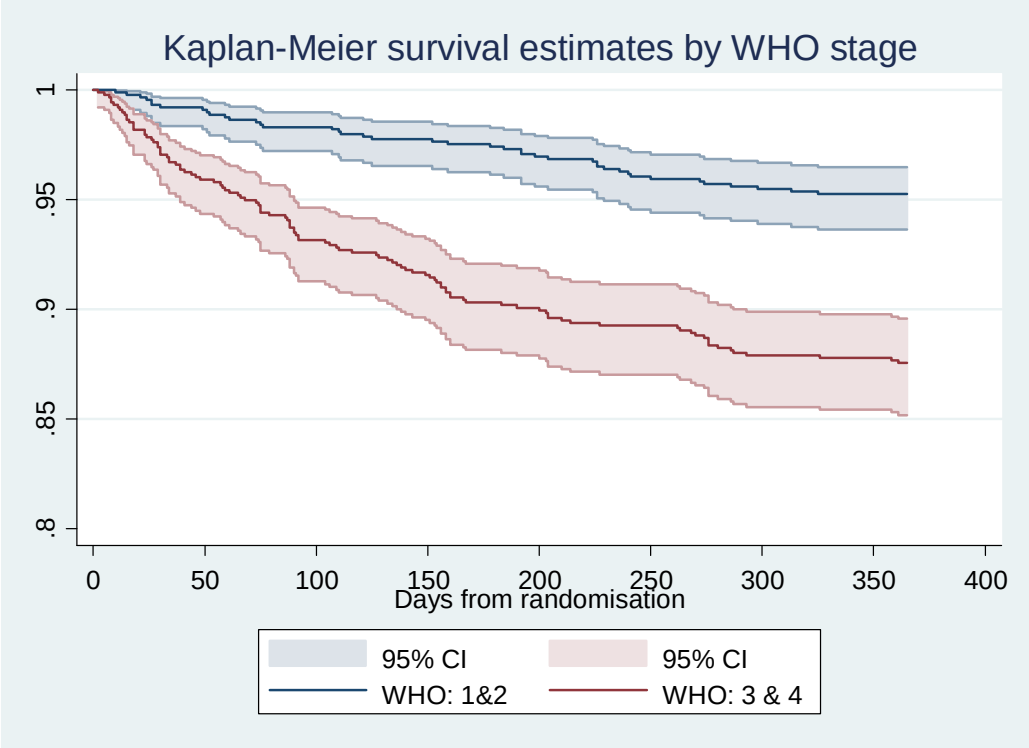

Fig 3.3e

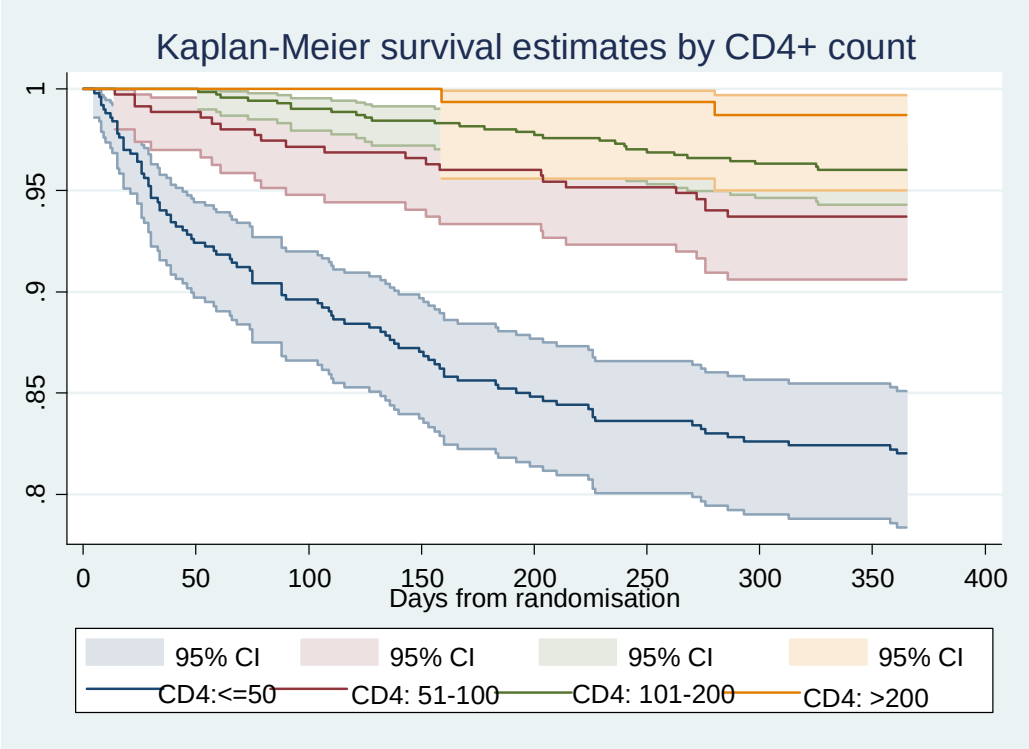

Fig 3.3f

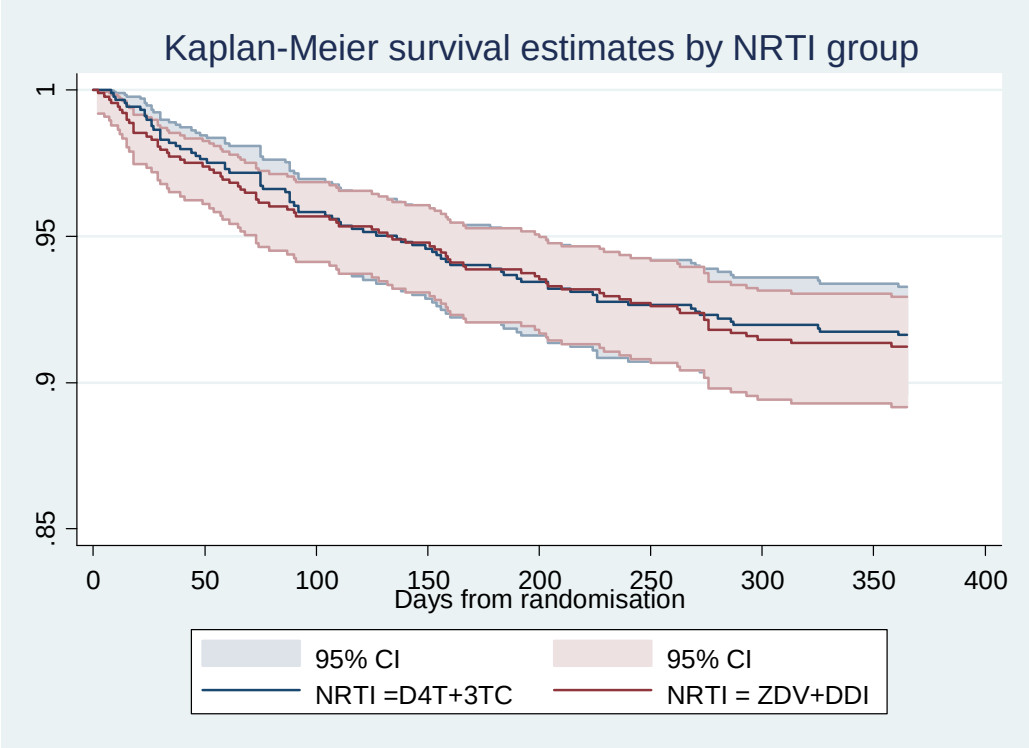

Fig 3.3g

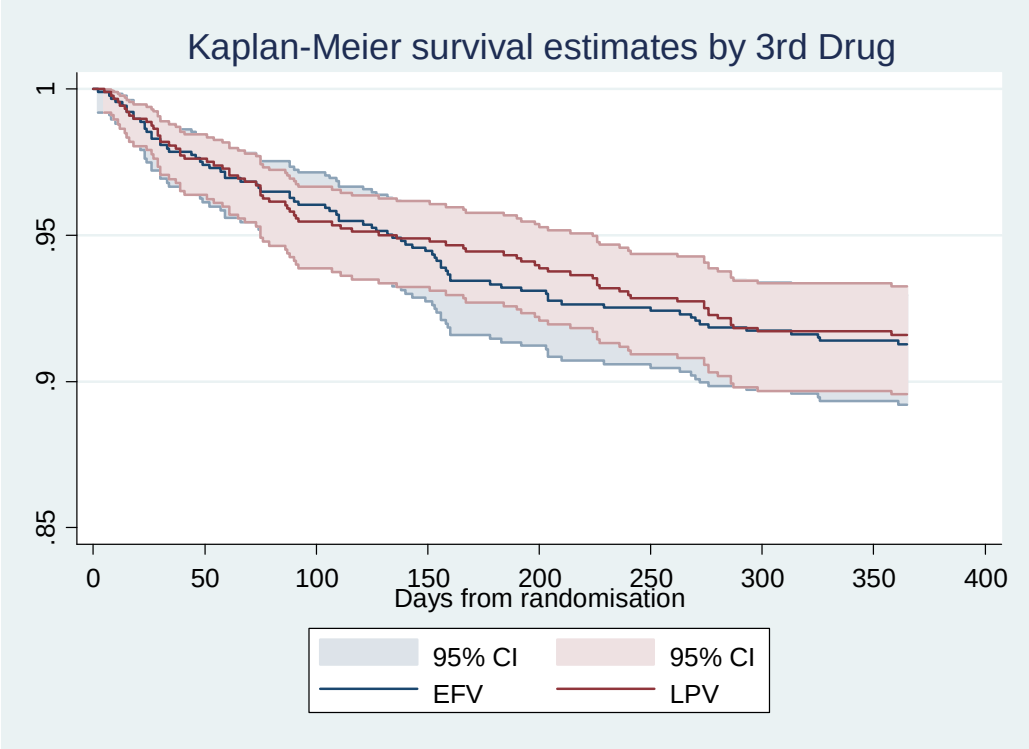

Fig 3.3h

Graphical assessments of proportionality of hazards

Appendix 2 contains the assessments, done pre-estimation, for selected variables. Only the 'education' variable suggested violation in the first year of follow-up, by both methods. This factor was thus not assessed further for its association with early mortality.

Univariate Cox regression

The following baseline characteristics were significantly associated with early mortality: enrolment early in the project, i.e. 2004 and 2005 (HR: 1.44, p=0.04); being married (HR: 0.70, p=0.033); BMI < 18.5 kg/m^2 (HR: 4.00, p <0.001); WHO stage 3/4 (HR: 2.95, p <0.001); higher CD4+ counts (ranging from 67% to 93% lower hazards when compared to counts </= 50 cells/mm^3, p <0.001); anaemia (HR: 5.1, p<0.001); HIV RNA viral load >/= 100000 copies/ml (HR: 1.82, p=0.001); AST above 100 IU/L (HR: 3.32 , p<0.001); ALT above 100 IU/L (HR: 2.39, p=0.006). (Table 3.7)

Sex, age, NRTI backbone, 3rd drug, Hepatitis B surface antigenaemia, having Hepatitis C antibodies, past/current TB disease, use of traditional medicines, being on

concomitant chronic medicines, peripheral neuropathy, and history of past or current depression, were not associated with early mortality. (Table 3.7)

Table 3.7: Baseline predictors of early mortality: Univariate and Multivariate regression

Variable	Univariate HR (95% CI)	Univariate P-value	Multivariate adjusted HR (95% CI)	Multivariate P-value
Enrolment year 2006 & 2007 2004 & 2005	 Referent 1.44 (1.02-2.03)	**0.040**	-	-
Female Male	Referent 1.22 (0.86-1.74)	0.264	-	-
Age(years)/unit ↑	0.99 (0.97-1.03)	0.794	**-**	**-**
Not married Married	Referent 0.70 (0.51-0.97)	**0.033**	Referent 0.63 (0.45-0.89)	**0.009**
BMI (kg/m^2) 18.5-24.9999 <18.5 >/=25	 Referent 4.00 (2.76-5.79) 0.67 (0.43-1.04)	**<0.001**	 Referent 2.35 (1.57-3.51) 0.87 (0.53-1.42)	**<0.001**
WHO stage 1 or 2 3 or 4	 Referent 2.95 (2.06-4.23)	**<0.001**	 Referent 1.84 (1.23-2.73)	**0.003**
CD4+ count(cells/mm^3) </=50 51-100 101-200 >200	 Referent 0.33 (0.20-0.52) 0.21 (0.14-0.32) 0.07 (0.02-0.27)	**<0.001**	 Referent 0.55 (0.34-0.89) 0.38 (0.24-0.60) 0.13 (0.03-0.53)	**<0.001**
Hb* >/= 10g/dl Hb* <10 g/dl	Referent 5.10 (3.58-7.26)	**<0.001**	Referent 2.87 (1.95-4.23)	**<0.001**
HIV RNA, copies/ml <=1x10^5 >1 x 10^5	 Referent 1.82 (1.26-2.63)	**0.001**	-	-
AST<=100IU/L AST >100 IU/L	Referent 3.32 (2.02-5.44)	**<0.001**	Referent 2.06 (1.23-3.46)	**0.006**
ALT<=100IU/L ALT >100 IU/L	Referent 2.39 (1.29-4.41)	**0.006**	-	-
EFV LPV/r	Referent 0.96 (0.70-1.32)	0.799	-	-
D4T+3TC ZDV+DDI	Referent 1.04 (0.76-1.44)	0.788	-	-
Hepatitis B sAg –ve Hepatitis B sAg +ve	Referent 1.43 (0.79-2.58)	0.242	-	-
Hepatitis C Antibody- Hepatitis C Antibody+	Referent 0.91 (0.13-6.51)	0.923	-	-
No TB history History of TB	Referent 1.20 (0.85-1.69)	0.305	-	-
No traditional Meds Traditional Meds	Referent 1.07 (0.65-1.77)	0.777	-	-
No peripheral neuropathy Peripheral Neuropathy	Referent 0.67 (0.34-1.33)	0.252	-	-
No other chronic meds Other Chronic meds	Referent 1.12 (0.66-1.88)	0.682	-	-
No depression history History of Depression	Referent 1.93 (0.61-6.06)	0.262	-	-

*haemoglobin

Examination for possible interactions

There was a trend towards interaction between sex and haemoglobin <10g/dL-(males with anaemia had 93% higher mortality hazard than males with higher haemoglobin and females at all levels of haemoglobin, p=0.095). Other tested interactions were not

statistically significant: NRTI-backbone and haemoglobin, (p=0.282); sex and BMI, (p=0.476); NRTI-backbone and Hepatitis B surface antigen, (p=0.707); ALT level and Hepatitis B surface antigen, (p=0.738); sex and concomitant chronic medication, (p=0.788); NRTI backbone and 3rd drug, (p=0.942); sex and marriage, (p=0.831).

Multivariate Cox Regression

The following variables were included in the full multivariate model: enrolment year, marital status, BMI, WHO stage, CD4+ count, haemoglobin, HIV viral load, Hepatitis B surface antigen, the sex*haemoglobin interaction term, and sex. AST and ALT were included in separate multivariate models to avoid collinearity between the two variables. The model with ALT was not subjected to backward elimination as high ALT had a non-significant association in the full model, (Hazard ratio: 1.55, p=0.182).

The following factors were still associated with early mortality after multivariate adjustment, and are thus independent risk factors/predictors of early mortality (Table 3.7): Marital status; married vs not married (adjusted HR (aHR): 0.63, p=0.009); low BMI < 18.5 kg/m^2 (aHR: 2.35, p <0.001); WHO clinical stage 3 or 4 vs WHO stage 1 or 2 disease (aHR: 1.84 times, p=0.003); CD4 +count-counts of 51-100, 101-200, and > 200, vs 50 cells/mm^3 or less (aHR: 0.55, 0.38, 0.13 respectively, p<0.001); anaemia (aHR: 2.87, p <0.001); and AST (aHR: 2.06, p=0.006).

Enrolment year and HIV viral load lost their statistical significance after multivariate adjustment, whilst Hepatitis B surface antigenaemia, and sex, maintained statistical non-significance. Furthermore, the gender*haemoglobin interaction term remained non-significant implying that the haemoglobin-mortality relationship is not modified by gender. (Table 3.7)

Post-estimation assessment of the Cox Proportional Hazards (PH) assumption

Although the pre-estimation graphical assessment of the 4-category CD4+ count variable did not suggest violation of the PH assumption, the post-estimation test was statistically significant for violation, (scaled Schoenfeld residuals test, p=0.006) for the comparison of the 101-200 cells/mm^3 category with the reference category (</= 50 cells/mm^3). Since CD4+ count is a known strong predictor of mortality, and was important in the final model as judged by the LR test, it could not be excluded from the

final model. The violation of the PH assumption indicates that the effect of this category of CD4+ count on mortality (when compared to the referent category) attenuates within the first year. Furthermore, it was the only comparison (of the three comparisons with the referent category) that violated the assumption.

Assessment of model fit

The Harrell's C concordance statistic for the final model was 0.787, indicating that by using the final model; the order of the survival times for pairs of patients was correctly identified 79% of the time. This is considered good predictive accuracy.[55]

Conclusion

Markers of advanced HIV disease at cART initiation, i.e. low CD4+ count, anaemia, low BMI, and advanced WHO stage; higher AST; and not being married, predict higher early mortality.

Late mortality

Exploratory graphical analysis of associations

Figs 3.4a-3.4h and Appendix 3, contain the Kaplan-Meier survival estimates with 95%CI, for the period beyond the first year, for selected variables. Male sex, missing at least one visit in the first six months, incident TB in the first year, baseline WHO stage 3 or 4, starting with ZDV+DDI, low BMI, lower haemoglobin, lower CD4+ counts, and high viraemia at month 12, were graphically suggestive of association with increased hazards of later mortality.

The estimates for marriage, Hepatitis B surface antigenaemia, enrolment years, age, the assignment to either 3rd drug (Lopinavi/r or Efavirenz), and inter-site transfer within six months of cART initiation, did not suggest significant associations with later mortality.

Pre-estimation assessment of the Cox proportional hazards assumption

The graphical assessments of proportionality of hazards for selected variables, are contained in Appendix 4. Most tested variables satisfied the proportional hazards assumption, except for 'history of TB', use of traditional medicines at baseline, and concomitant chronic medication- which all seemed to violate by both graphical methods. However, the 'history of TB' factor was included in univariate analysis as it is clinically

and epidemiologically plausible that it is associated with later mortality. Additionally, baseline CD4+ count and baseline anaemia (haemoglobin < 10 g/dL), violated the PH assumption, supporting the strategy to not consider them as predictors of late mortality. (results not shown)

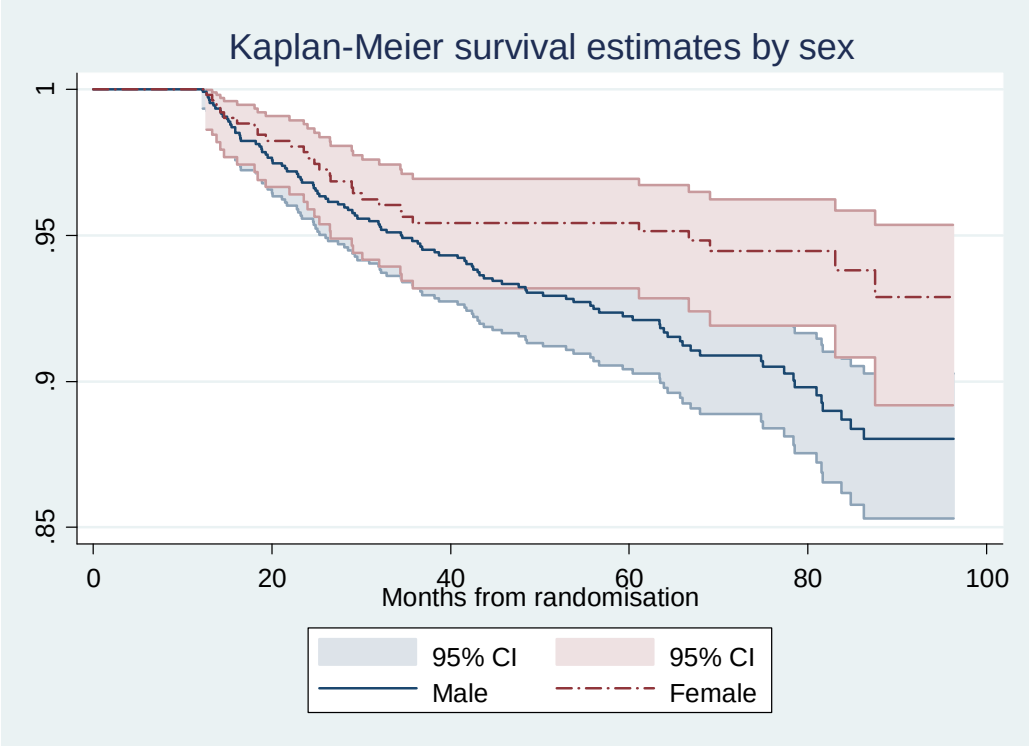

Fig 3.4a

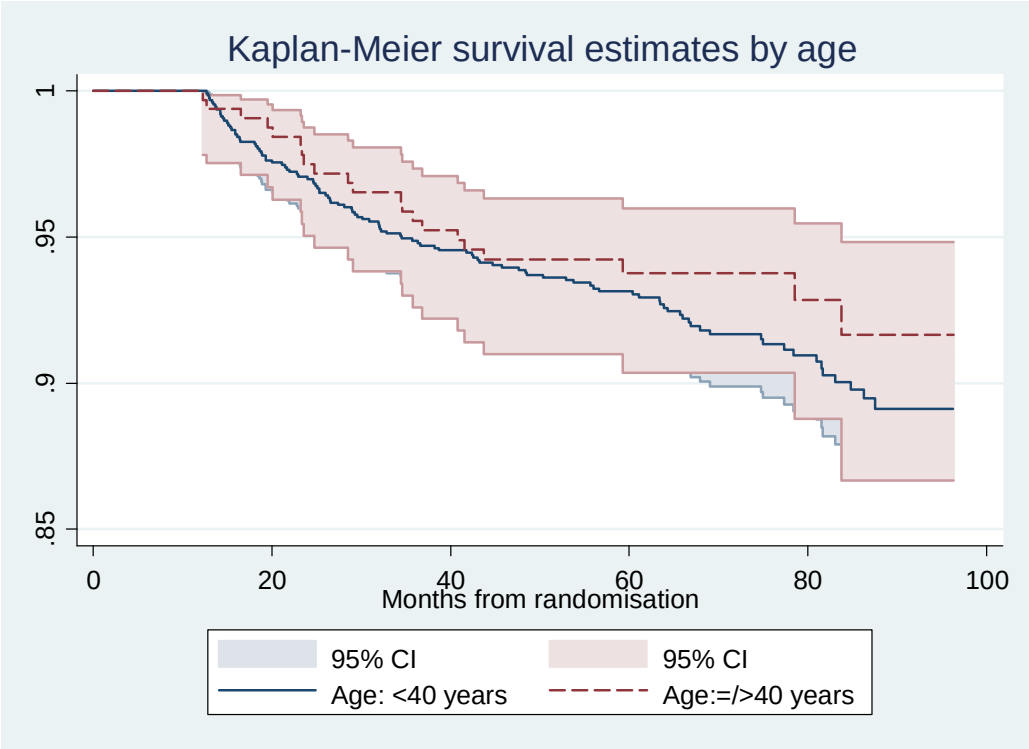

Fig 3.4b

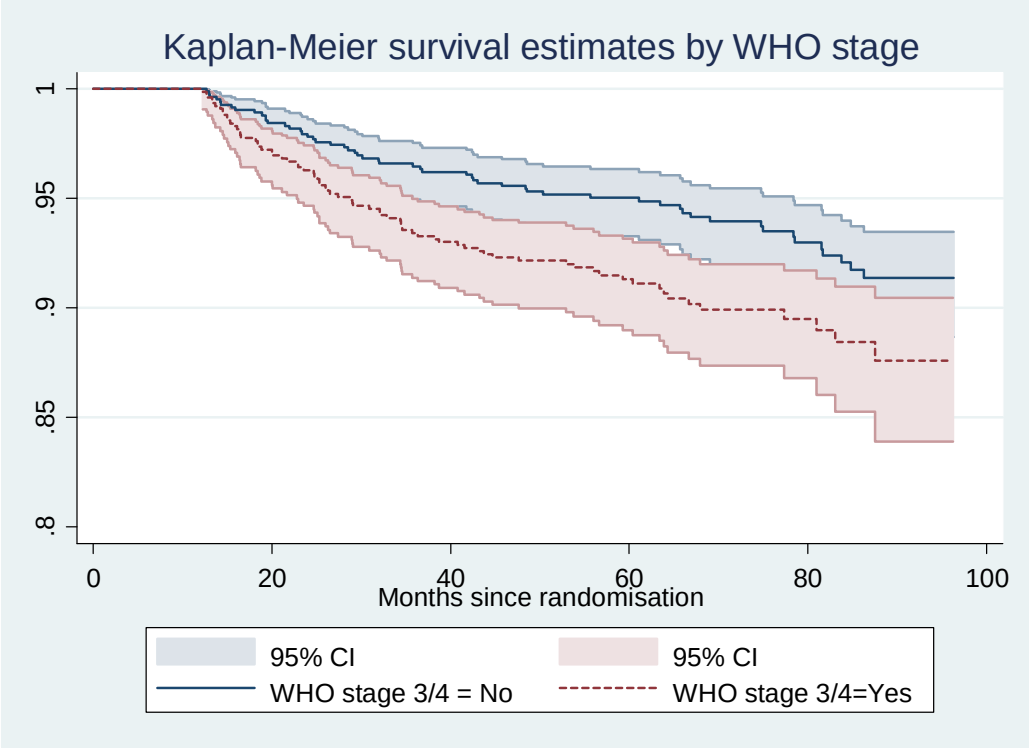

Fig 3.4c

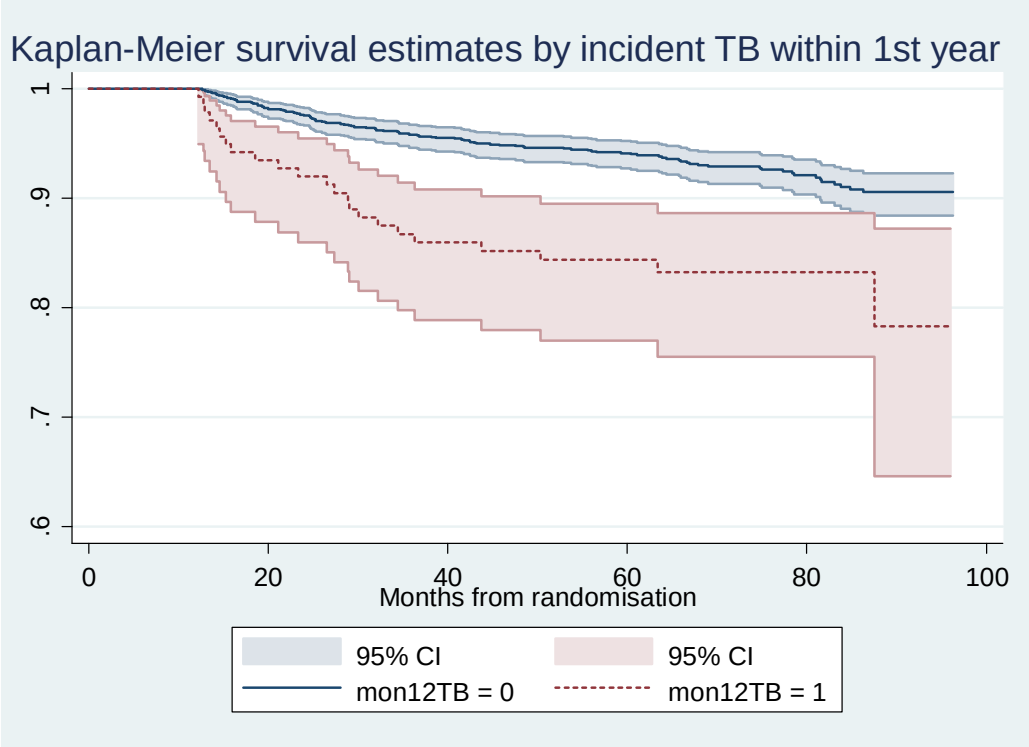

Fig 3.4d:

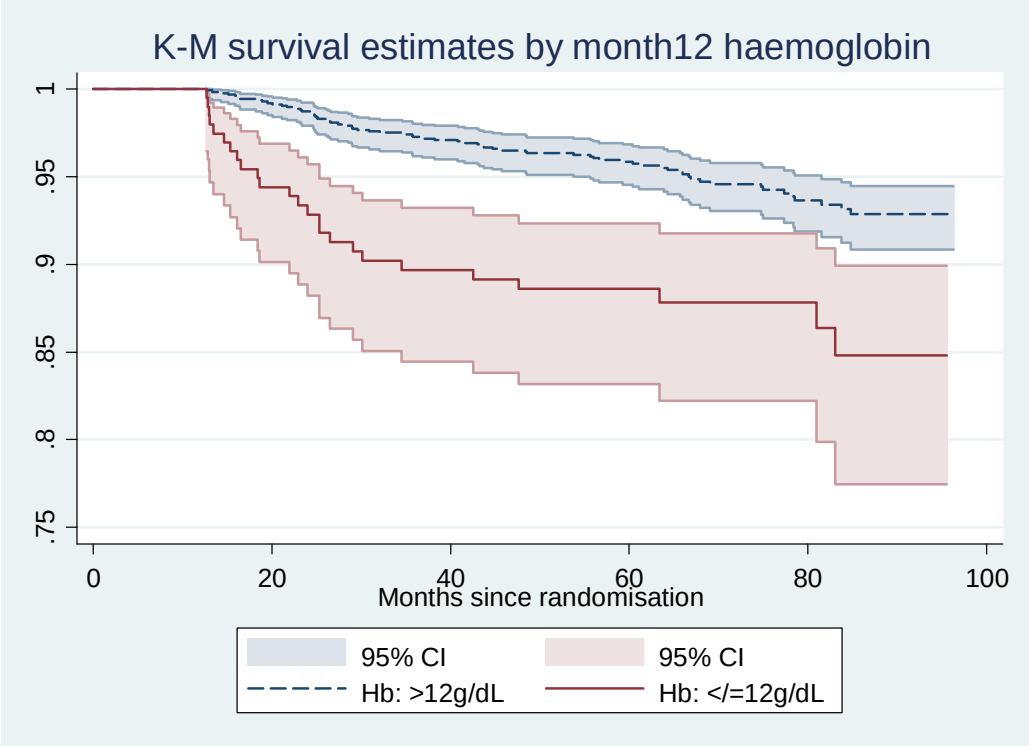

Fig 3.4e

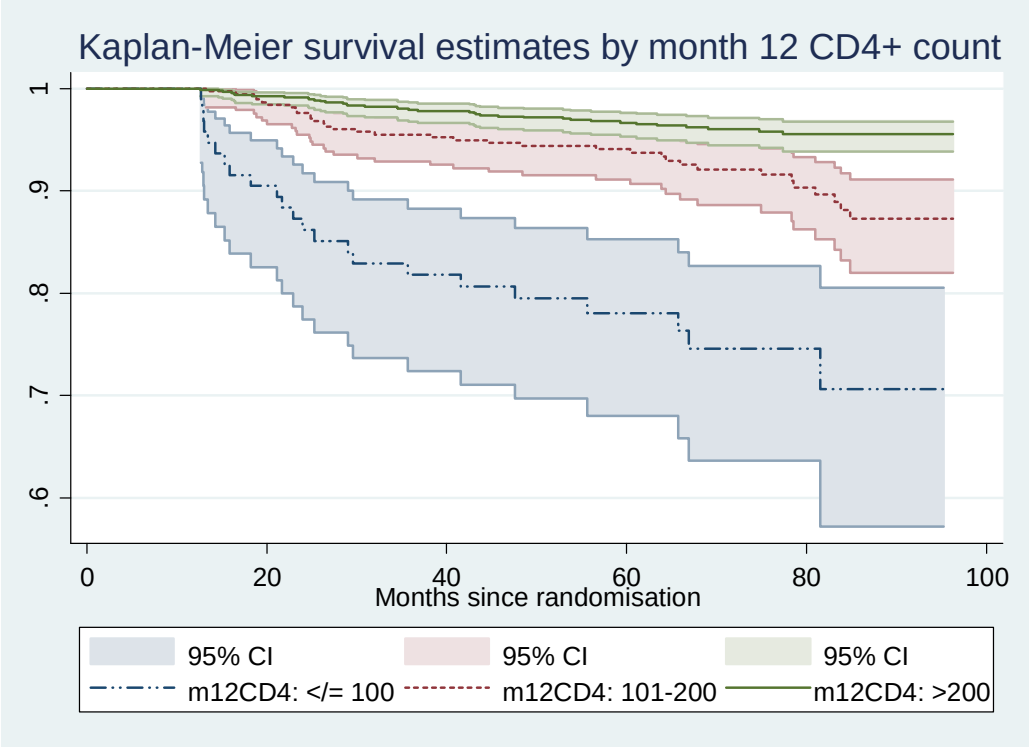

Fig 3.4f

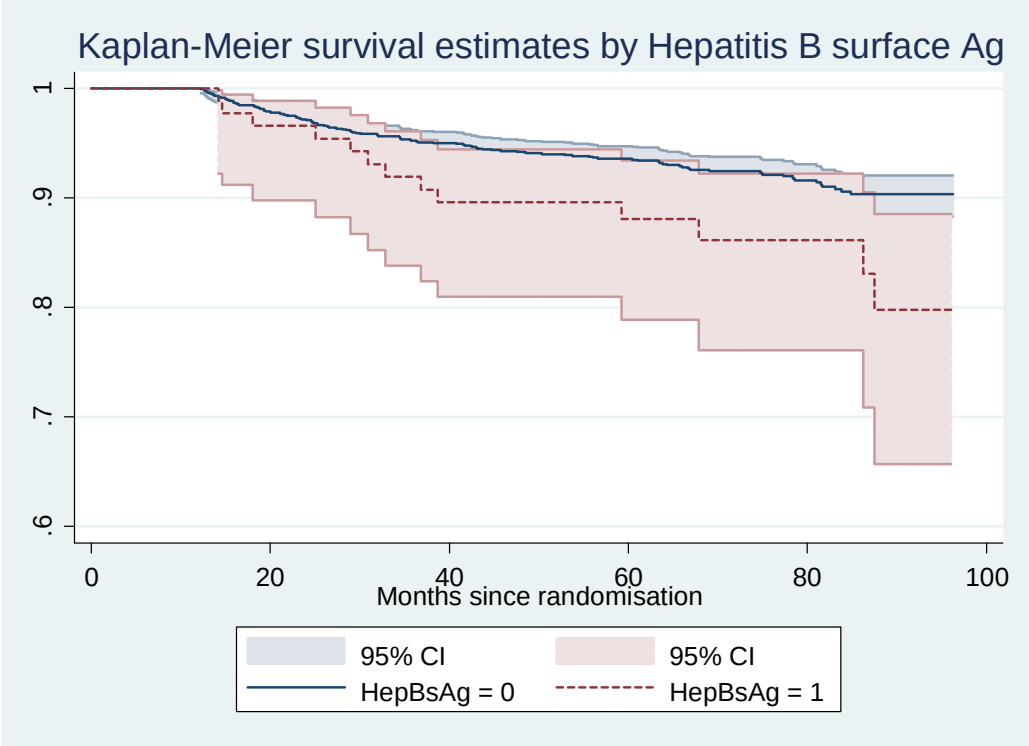

Fig 3.4g

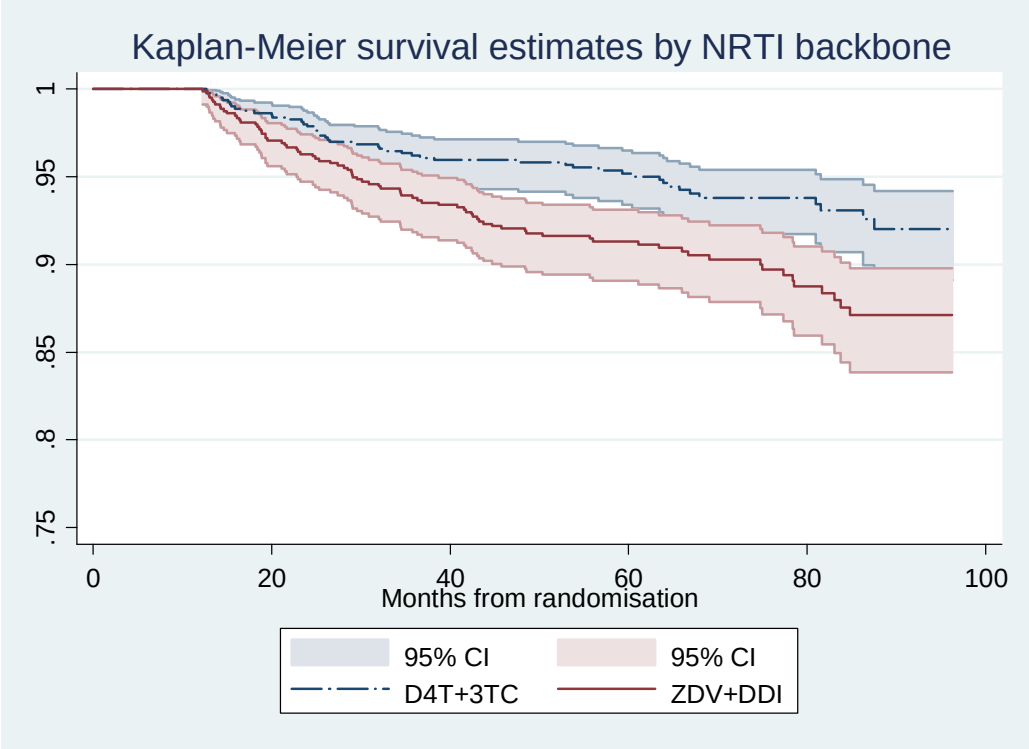

Fig 3.4h

Univariate Cox regression

Table 3.8 shows that the following had crude associations with later mortality: male sex (HR: 1.65, p=0.021); WHO stage 3 or 4 (HR: 1.72, p=0.003); peripheral neuropathy (HR: 1.88, p=0.017); starting with ZDV+DDI vs D4T +3TC (HR: 1.73, p=0.003); missing at least one visit in the first 6 months of starting cART (HR: 3.15, p<0.001); incident TB in the first year of cART (HR: 2.56, p <0.001); month 12 haemoglobin (HR: 0.72 per unit increase, p<0.001); month 12 BMI < 18.5 kg/m^2 (HR: 6.71, p<0.001); month 12 CD4+ count (mortality risk was 77% and 88% lower in the categories 101-200 and >200 cells/mm^3 groups respectively, when compared with CD4+ counts </= 100, p<0.001); month 12 viral suppression to < 400 copies/ml (HR: 0.31, p<0.001).

Examination for possible interactions

None of the tested possible interactions yielded statistically significant results: sex and month 12 haemoglobin, (p=0.408); sex and month 12 BMI, (p=0.931); sex and marriage, (p=0.887); sex and month 12 CD4+ count, (p=0.474); sex and month 12 viral load, (p=0.878); NRTI backbone and month 12 haemoglobin, (p=0.284); NRTI-

backbone and Hepatitis B surface antigen, (p = 0.629); NRTI backbone and 3rd drug, (p=0.52); history of TB and incident TB in the first year, (p=0.167).

Table 3.8: Predictors of late mortality: Univariate and Multivariate regression

Variable	Univariate HR (95% CI)	Univariate P-value	Multivariate adjusted HR (95% CI)	Multivariate P-value
Enrolment year 2006 & 2007 2004 & 2005	 Referent 1.42(0.94-2.15)	0.093	-	-
Gender Female Male	 Referent 1.65(1.08-2.51)	**0.021**	 Referent 2.84(1.58-5.13)	**0.001**
Age(years)/unit ↑	0.99(0.96-1.02)	0.541	-	-
<High School High school+	Referent 0.83(0.49-1.40)	0.485	-	-
Not married Married	Referent 0.72(0.51-1.03)	0.069	-	-
WHO stage 1 or 2 3 or 4	 Referent 1.72(1.20-2.45)	**0.003**	-	-
No peripheral neuropathy Peripheral neuropathy	Referent 1.88(1.12-3.16)	**0.017**	-	-
EFV LPV/r	Referent 0.96(0.68-1.34)	0.823	 -	 -
D4T+3TC ZDV+DDI	Referent 1.73(1.20-2.47)	**0.003**	Referent 1.63(1.04-2.54)	**0.032**
Hepatitis B sAg -ve Hepatitis B sAg +ve	Referent 1.77(0.99-3.16)	0.054	-	-
Hepatitis C Antibody- Hepatitis C Antibody+	Referent 1.24x e-14	1	-	-
No TB history History of TB	Referent 1.39(0.95-2.01)	0.087	-	-
No depression history History of Depression&	Referent 0.78(0.11-5.59)	0.805	 -	 -
No missed visit+ Missed visit+	Referent 3.15(2.2-4.49)	 **<0.001**	-	-
No transfer by month6 Transfer by month 6	Referent 0.72(0.29-1.77)	0.474	-	-
No new TB in 1st year New TB in 1st year	Referent 2.56(1.63-4.00)	**<0.001**	-	-
Month 12 BMI (kg/m2) 18.5-24.9999 <18.5 >/=25	 Referent 6.71(3.92-11.49) 0.54(0.33-0.87)	 **<0.001**	 Referent 3.55(1.95-6.45) 0.75(0.46-1.25)	 **<0.001**
Month 12 Hb (g/dl)/ unit ↑	0.72(0.64-0.80)	**<0.001**	0.75(0.66-0.86)	**<0.001**
Month 12 CD4+ count (cells/mm3) </=100 101-200 >200	 Referent 0.23(0.17-0.48) 0.12(0.07-0.20)	 **<0.001**	 Referent 0.75(0.40-1.40) 0.40(0.21-0.77)	 **0.007**
Month 12 HIV RNA copies/ml >=1000 <400 400-999	 Referent 0.31(0.20-0.47) 0.22(0.10-0.52)	 **<0.001**	 Referent 0.49(0.30-0.78) 0.43(0.18-1.04)	 **0.007**

+in first 6 months of therapy; &depression required medical intervention

Multivariate Cox Regression

The following variables were included in the full multivariate model to assess independent predictors of late mortality: enrolment year, sex, marriage, baseline WHO

stage, baseline peripheral neuropathy, randomised NRTI backbone, baseline Hepatitis B surface antigen, baseline history of TB, incident TB within the first year, history of TB*incident TB interaction term, missed visit in the first 6 months, month 12 values of BMI, haemoglobin, CD4+ count, and HIV viral load.

The following factors continued to have statistically significant associations with mortality: male sex, (aHR: 2.84, p=0.001); initiating with ZDV+DDI vs D4T+3TC (aHR: 1.63, p=0.032); month 12 BMI <18.5 kg/m^2 (aHR: 3.55, p <0.001); Haemoglobin (aHR: 0.75 per unit increase, p<0.001); month 12 CD4+ count (aHR: 0.4 and 0.75 for categories >200 and 101-200 vs </= 100 cells/mm^3 respectively, p=0.007); month 12 viral suppression to <400 copies/mL (aHR: 0.49, p=0.007).

Baseline WHO stage, peripheral neuropathy, missed visit in first 6 months and incident TB in the first year, lost statistical significance after multivariate adjustment. The marginally significant excess mortality in those with Hepatitis B surface antigenaemia (HR:1.77, p=0.054), became insignificant after multivariate adjustment. Enrolment year, marriage, 3rd drug, history of TB, and the history of TB*incident TB interaction, maintained statistically insignificant associations with late mortality.

Post-estimation assessment of proportionality of hazards assumption

The final model and all its component variables (sex, NRTI backbone, month 12 values of haemoglobin, BMI, CD4+ count, and viral load) favoured the PH assumption, (global scaled Schoenfeld residuals test, p=0.1395).

Assessment of model fit

The Harrell's C concordance statistic for the final model was 0.7795, indicating that by using the specified final model; the proportion of all usable patient pairs in which the predictions and outcomes are concordant is 78%. This is considered good prediction performance.[55]

Conclusion

In this cohort, male sex; initiating with ZDV+DDI; month 12 updated values of BMI, haemoglobin, CD4+ count; and lack of viral suppression to <400 copies/ml, predicted late mortality.

CHAPTER 4

DISCUSSION and CONCLUSION

The study has succeeded in providing a description of mortality patterns (including causes of mortality) and predictors of early, and then late mortality in a cohort initiated randomised cART, and followed-up for up to 8 years, in a military healthcare system in South Africa.

Patterns and causes of mortality

The pattern of mortality exhibited here, namely; very high crude mortality rates in the first six months of cART, followed by lower rates in months 7 to 12, and even lower mortality beyond the first year, is as described in all studies that have examined mortality in HIV-treatment programmes in sub-Saharan Africa. However, the crude mortality rates observed in the overall period and in the first year in our cohort were higher than those observed in most South African studies wherein baseline immunodeficiency was similar, but females were the predominant sex.[33-36] When compared to settings that linked vital status with the national vital registry such as the Khayelitsa cohort, there was a considerably lower mortality rate in our cohort, even though the Khayelitsha cohort was predominantly female.[49] However the cohort described by Hoffmann et al. was similar to our cohort in male-to-female ratio.[37] It is however unlikely that there was extensive under-ascertainment of death in those lost to follow-up in our cohort given the very low percentage LTFU, and the access to military information systems that allow identification of deceased members of the South African National Defence Force.

The hazard (instantaneous rate) of mortality was much lower and was nearly constant after one year, when compared to the first year in this study. This pattern in the hazard of mortality was also reported in the study by Hoffman et al.[37] In our opinion, this division of early vs. late mortality by the one-year mark, gives a better differentiation in patterns and predictors of mortality by follow-up period compared to the 3 to 6 months' cut-off examined in other southern African studies. [33,34,49,56]

In this study, the immediate causes of mortality were not determined in about a third of deaths, probably because of the research staff not being involved in the patients' terminal illness either due to the patients dying in non-military institutions or at home.

Like in most other sub-Saharan cohorts reporting causes of mortality in treated HIV patients, TB was the most common cause identified.[40,56,57] It is also the most commonly reported cause of death in the general South African population.[6]

It has been reported that the highest proportion of deaths attributable to TB occur early in the course of cART, usually within the first 6 months. A suggested reason is that a high proportion of the incident TB cases and deaths are a result of 'unmasking' Immune Reconstitution Inflammatory Syndrome (IRIS) of previously unsuspected active TB, particularly in the severely immunocompromised.[58] Paradoxically, in this study we found a higher proportion of TB-attributable deaths observed beyond a year compared to the first year of cART. A probable explanation is that the deaths attributable to TB in the first year were underestimated given the diagnostic difficulties associated with advanced HIV disease, increasing the probability of smear negative sputum and atypical chest X-ray appearance. This was also observed in a Senegalese study.[57] With the observed increase in CD4+ counts in those surviving beyond one year, the diagnosis of TB would have then became easier as the probability of smear positive sputum increases, and X-ray appearance approximates that of HIV negative patients. The underestimation of TB deaths in HIV positive patients was also shown in a post-mortem study from Kwa-Zulu Natal, which found that 45% of hospital deaths in non-TB suspects were TB culture positive.[59] Another reason for the observed higher TB deaths in late mortality could be the improved diagnostic rigour of TB in later years of the study through the 'sputum initiative'. Implemented from mid-2007, this initiative actively investigated for TB in all patients with at least one symptom suggestive of TB, including cough of at least three days' duration, using TB culture on all sputum samples collected.[60]

Tuberculosis deaths excluded, there were significantly fewer deaths attributable to other WHO stage 3 and AIDS-defining conditions, so called HIV-related deaths (e.g. cryptococcal meningitis, bacterial pneumonia etc.), in late mortality compared to early mortality. Similar patterns were reported in a Thai cohort and in the EuroSIDA cohorts.[50,61] In the Thailand study, wherein patients were followed-up for a maximum of 5 years, deaths defined as immunodeficiency-related, (i.e. possibly or probably HIV-related and AIDS-defining events), accounted for 92% of deaths within 6 months of cART initiation, and 56% of later deaths.[50] HIV-related deaths were still predominate

causes in later mortality. We observed a similar trend in our study, with AIDS-defining conditions and other infections accounting for 45% of later mortality.

Similar to the EuroSIDA study, the non-HIV-related and non-infectious deaths, (i.e. other malignancies, unnatural deaths, and miscellaneous causes), were constant over time in this study. In EuroSIDA, a large cohort of 12069 cART treated patients from Europe, Israel, and Argentina, the risk of death from AIDS defining diseases drastically decreased after 2 years of cART, whereas death from non-AIDS defining causes were constant after 2 years of follow-up.[61]

The few studies that described causes of mortality in cART-treated individuals in sub-Saharan Africa either did not compare causes by duration of cART or did not have long enough follow-up.[40,56,57,58] The study by Lawn et al described causes of early mortality in an ART programme in Cape Town with patients followed-up for a maximum period of 30 months, and found that the majority of deaths happened before patients initiated cART.[58] The data presented here therefore continue to show the strengths of this study.

Predictors of early mortality

Clinical predictors of high mortality in the early period (first year of cART) were WHO clinical stage 3 or 4, low BMI, low CD4+ count, anaemia, and high AST levels. Except for AST levels, which are not reported in most other studies sub-Saharan Africa, these findings are consistent with those of several other studies.[33,36,37,43,] These data confirm markers of advanced HIV disease at cART initiation as strong predictors of mortality in the first year.

The eligibility criteria for the trial excluded severely anaemic individuals from enrolling. This was designed to buffer the risk of worsening anaemia from zidovudine. The fact that in a population with a smaller range of haemoglobin levels at baseline - the aHR for the comparison of haemoglobin < 10g/dL and higher haemoglobin was 2.87 - indicates that the effect of anaemia (<10g/dL) on early mortality is strong. The absence of significant interaction between NRTI backbone and haemoglobin, (p=0.282), supports the use of the haemoglobin thresholds in the trial.Lower baseline CD4+ counts were associated with higher early mortality, but there was a strong suggestion that this effect was attenuated by the end of the year, as evidenced by violation of the proportional

hazards towards the end of the year. Of all the predictors of early mortality, CD4+ count was the only variable exhibiting this phenomenon in the study, suggesting that the updated CD4+ count at 6 months or 9 months may have been more appropriate for predicting mortality during the period 6 (9)-12 months, and then the month 12 levels be utilised for later mortality.

High AST levels above 100 IU/L, and not high levels of ALT, predicted early mortality. Since raised ALT is specifically associated with liver disease, and AST may arise from other sources including skeletal muscles and erythrocytes, AST seems to be a marker of general poor health in this situation. Additionally, AST levels > 100 IU/L may indicate alcohol abuse,[62] such that the increased mortality in those with higher AST may indicate an association between alcohol abuse and early mortality, probably mediated by poor adherence. Poor adherence to cART is a known predictor of survival, [39,40] whilst the independent association between alcohol misuse and poor adherence to HIV treatment, and poor HIV treatment outcomes, is supported by a recent systemic review of 11 cross-sectional, seven cohort studies, and two randomised controlled trials.[63]

Additionally, despite the exclusion of patients with ALT or AST above 5x the upper limit of normal (around 200IU/mL) from the trial, this result is generalizable to the standard-of-care of sub-Saharan Africa which recommends that cART be delayed when the serum transaminases are more than 200IU/mL. This reduces the risk of exacerbating hepatic injury from the often hepatotoxic NNRTIs used in first-line cART.[27-30]

In concurrence with other sub-Saharan studies , baseline viral load did not predict mortality in this study.[33,34,43,37,57] As the WHO guidelines for HIV treatment have consistently maintained, baseline viral load does not add further information to the 'when to start' question and the estimation of prognosis when CD4+ count and WHO clinical stage data are available.[17,25,26] For this reason, since 2009, the national South African ART guidelines no longer recommend the collection of viral load at cART initiation.[28-30]

This study also examined social factors such as marital status and level of education as possible predictors of mortality, with the hypothesis that these factors may have influenced health-seeking behaviour and therefore access to care for inter-current diseases, or adherence to cART and/or concomitant treatment, ultimately resulting in

differential risk of mortality. In this regard, marriage was strongly protective against early mortality in this cohort. This may reflect unmeasured confounding, i.e. it is likely that the kind of people who choose/manage to get married are also the kind of people who are more likely to be adherent or have better health-seeking behaviour. It is also plausible that the spouses, whether living with or apart from the patients during working days, were 'treatment buddies' that assisted with adherence and/or would have facilitated earlier consultation of health practitioners in case of clinical deterioration.

Predictors of late mortality

Immunologic and virologic markers of HIV treatment response, namely, updated values of CD4+ count and viral suppression, were strong predictors of later mortality in this study, as reported by others. All the studies reporting on the effect of updated CD4+ count have shown that the follow-up CD4+ count is a stronger predictor of subsequent mortality than the baseline CD4+ count.[3437,40,51,52] With regard to viral suppression, results have been mixed. In this study, viral suppression to < 400 copies/ml at one year, resulted in halving subsequent mortality, whereas in two studies, one conducted in Cape Town and the other in rural Uganda, viral suppression or time-varying viral load, was not associated with later mortality.[34,40] However in a large South African study, similar to the Phidisa II cohort in sex composition, failure to achieve or maintain viral suppression after 12 months of cART, increased mortality more than 5-fold.[37]

The extent of clinical disease represented by low haemoglobin or overt anaemia, and by low BMI at 12 months was also associated with subsequent mortality. The presence of the two signs at 12 months could indicate the presence of inter-current infections and serious conditions like malignancy. However, similar to Hoffmann et al, baseline advanced clinical HIV disease (WHO stage 3 or 4) did not predict mortality beyond the first year. The effect of baseline WHO HIV clinical stage on mortality therefore wanes with time.[37]

Similar to many other southern African studies, age did not predict mortality, even after long-term follow-up in this population. This may reflect the narrower age distribution in young patients receiving cART in this setting.[42]Nearly 80% of the cohort was younger

than 40 years, whilst the mean was 35.4 years with a small standard deviation of 5.5 years.

The unique feature of this cohort is that 75% of them started regimens not usually used as first-line cART in non-pregnant adults in Southern Africa. Half of the cohort started LPV/r-based therapy, usually reserved for second-line therapy or pregnancy in that era. We did not observe differences in mortality between the LPV/r vs and EFV arms after long-term follow-up in this large cohort. WHO and South African national guidelines recommending the use of efavirenz-based first-line cART regimens, which are much cheaper than boosted PI-based cART, are thus cost-effective.[26,30]

This study is the first in Southern Africa to describe the unfavourable effect of ZDV+DDI on later mortality. Four hundred and forty-four patients (25%) of this cohort started on ZDV+DDI+EFV, known to have been used in only one other setting in southern Africa, but in much fewer patients and for much shorter follow-up. It was one of six arms of the Tshepo study in Botswana, which aimed to evaluate several protease-inhibitor sparing cART regimens for use in southern Africa at the start of the scale-up of ART in this region.[64] In that study, with 650 participants randomised 1:2 to ZDV+DDI vs D4T/ZDV + 3TC arms, there was no excess mortality in the ZDV+DDI arms, but there were higher rates of virologic failure compared to the 3TC arms, after a median follow-up of 104 weeks. These findings are similar to those of the primary Phidisa II study, after a similar period of follow-up, i.e. a median of 24.7 months.[48] The excess in later mortality in the ZDV+DDI arms observed in the current study, does not seem to be mediated by the inferior increases in CD4+ count and higher viral loads observed in these regimens after 12 months of follow-up, as reported in the primary Phidisa study[42]; aHR for the NRTI backbone did not differ significantly in models excluding month 12 CD4+ count, viral load,or both.(results not shown)

In this predominantly male population, the majority of which had primary membership of a workplace healthcare scheme, males had higher late mortality than females. In contrast to other studies in sub-Saharan Africa, there was no excess mortality in the early period in this cohort.[34,37,41,43] In some of these studies, male excess mortality was observed throughout the follow-up period.[37,41] The differential in mortality risk by follow-up period in this cohort, may be attributed to observed differences in responses to cART

in the first year. Specifically, there was a higher proportion of females with CD4+ count above 200 cells/mm^3 at month 12 compared to males, 73% vs 64% (chi-square test, p=0.001), although proportions with viral suppression were similar, 64% vs 61% (chi-square test, p=0.318). At baseline, the proportions with CD4+ count </= 50 cells/mm^3 were similar, 28% for females vs 30% for males (chi-square test, p=0.148). Although we adjusted mortality risk by month 12 updated values of CD4+ count and viral load, and other factors, adjustment may not have been complete, resulting in the inferior immunologic responses reflected in differential risk by sex in later mortality. The person-time spent with CD4+ count less than 200 cells/mm^3 has been shown to strongly predict mortality.[65] However, poorer adherence in males during subsequent follow-up cannot be ruled out.

Contrary to a previous Phidisa II analysis, which showed that incident TB, analysed as a time-varying covariate, was associated with a 2.5- fold greater risk of death, incident TB in the first year, was not associated with higher mortality risk in subsequent years in the current study.[44] This difference may be due to the different analytical method employed, as the current analysis did not capture TB events that occurred after the one year cut-off.

Despite the high prevalence of HIV/ chronic Hepatitis B co-infection in southern Africa and Asia, there is paucity of data on the effect of chronic Hepatitis B on mortality in co-infected patients on cART. Most national public sector ART programmes in southern Africa have not included Hepatitis B testing in the routine package of care. Similar to the study findings among 692 Thai patients on cART, which showed no difference in mortality or CD4+ recovery at 48 weeks, we did not observe significant excess mortality among chronic Hepatitis B co-infected patients in the Phidisa II cohort after long-term follow-up.[66] A previous Phidisa II analysis, which studied the effect of lamivudine on HIV and HBV outcomes in HBV co-infected individuals, however reported excess crude mortality at median follow-up of 24.7 months, but did not adjust for other covariates.[67] The social factors, namely level of education, and marriage, were not associated with late mortality.

Limitations

First, the month 12 updated variables of haemoglobin, CD4+ count and viral load were missing in 9.2%, 8.8%, and 8.8% of patients respectively. This was due to a combination of missed visits and laboratory operational challenges e.g. difficult bleeds and clotted specimens-for visits honoured by the patients. Since only 3.7% of patients had missing BMI, we can assume that the majority of patients with missing laboratory values had honoured their visits. Although there is a chance that there was non-random distribution of these data if phlebotomy was especially difficult in the sicker patients, it is unlikely to have had a major impact (decrease the statistical power to detect differences in mortality risk) on the analyses for late mortality since less than 10% of patients had missing data, in concurrence with Bennett.[68]

For the assessment of predictors of mortality, the final adjusted models were obtained with backward elimination, which may be volatile. To minimise that risk, for epidemiologically plausible predictors that we assessed, variables with $p>0.05$ but $<p=.25$ in univariate analysis were also included in the full model. Additionally, both the LR test and the Wald test had to be statistically non-significant for a variable to be dropped from the model, as such we believe the results would be similar had $p>0.05$ been used for the Wald test.[54] Adherence was not considered as possible predictor in this study. First, the variable was not collected for the month 12 visits of the 228 patients enrolled in late 2007 (14.3% of those alive at one year), since observation under the trial and adherence data collection would have stopped on the 31st March 2008. Second, the primary study results showed that 95.3% and 95.6% of the 1242 patients assessed for month 12 drug adherence self-reported taking 'most or all of their prescribed pills' for the NRTI backbone and the 3rd drug respectively, yet the proportion with viral suppression of < 400 copies/mL at this visit was around 62%.[48] This indicates that adherence was probably over-estimated by the self-reports, a known major limitation of this measurement of adherence.[69] HIV viral suppression at month 12 was therefore used as a proxy indicator of good adherence during the preceding month. Third, the adherence questionnaire did not allow for much discrimination of the adherence measure, since the highest recordable adherence was 100% (all of pills taken), and the

next level was a subjective and non-specific 'most of the pills' category. (see Appendix 5)

Another limitation is that a third of causes of mortality were unknown. This raises the possibility that a higher proportion of the early deaths were from WHO stage 3 and 4 conditions, particularly TB, given the great extent of immunodeficiency as indicated by the low median CD4+ count at enrolment. Additionally, for later mortality, we cannot be certain of the proportion of deaths caused by non-communicable diseases.

Questions arising from this study

The excess later mortality in males is not fully explained by poorer adherence in the first year of cART. Additional investigations into possible adherence disparities in the later period and contributing causes, and into health-seeking behaviour after good early response to cART are also needed.

Conclusion

We have successfully presented a comprehensive description of patterns of mortality, and socio-demographic and clinical characteristics predicting high mortality in patients started on first-line therapy in the South African military health system, and followed-up long-term. Evidence from this study should assist in the formulation of targeted interventions in the most-at risk groups initiating cART in this setting.

The investigation into causes of death in this cohort indicates that most patients died from HIV-related causes in the first year, and so did a substantial proportion of those who survived the first year of cART. This suggests that patients initiating cART will benefit from the following: the implementation of all preventive measures against TB, and close monitoring of patients at highest risk of death, with prompt diagnosis, and treatment of all opportunistic infections and AIDS-defining malignancies. These measures need to be continued beyond the first year of cART.

Furthermore, early mortality is predicted by markers of advanced HIV disease. It is encouraging that the South African national guidelines now recommend priority cART initiation at CD4+ count less than 350 cells/mm^3 regardless of WHO clinical stage, WHO

clinical stage 3, and all active TB, regardless of CD4+ count. However, programmes need to ensure that most patients initiate cART as soon as possible after reaching eligibility criteria. Therefore, the early identification of HIV-infected persons, and their linkage to appropriate HIV care are paramount in reducing HIV-related mortality. Additionally, predictors of late mortality were identified as those indicating an initial response to cART, plus the initiating regimen, and male sex. Since the immunologic response is dependent on baseline immunodeficiency and adherence to cART, the strategy of initiating patients early, i.e. before significant deterioration, still applies here. The use of cART regimens proven to be potent, and recommended by WHO and national guidelines should be adhered to at all times. Last, males should receive additional support in ART programmes. Future research, whether quantitative or qualitative, into male long-term adherence to cART is required, and the findings need to be utilised to formulate appropriate interventions.

CHAPTER 5
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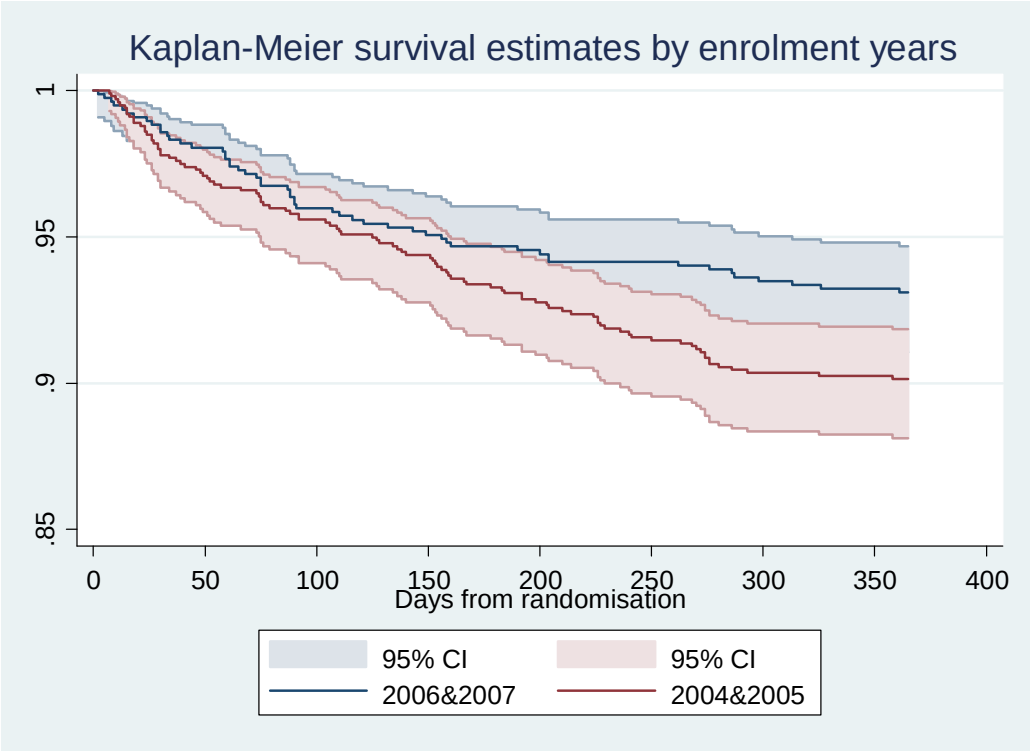
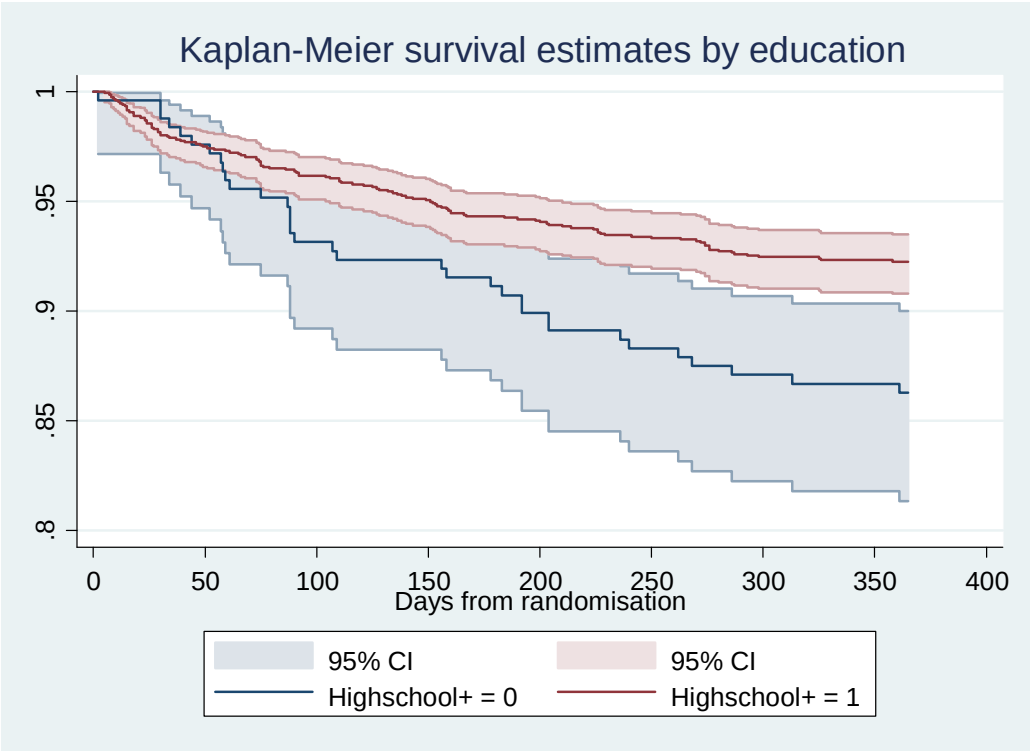
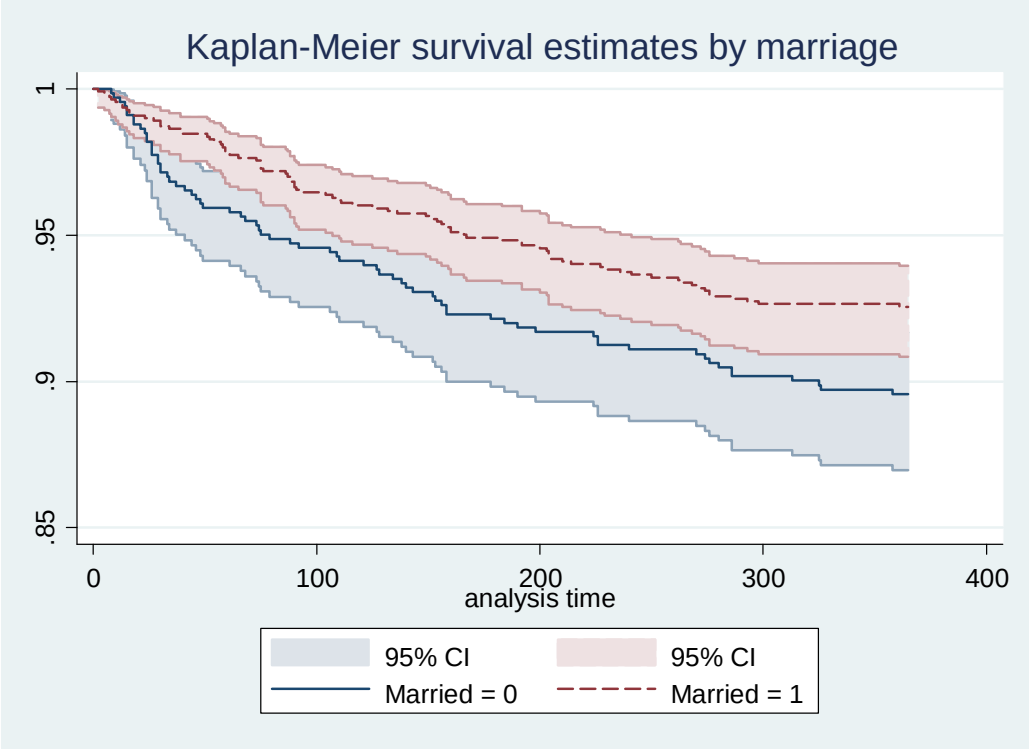
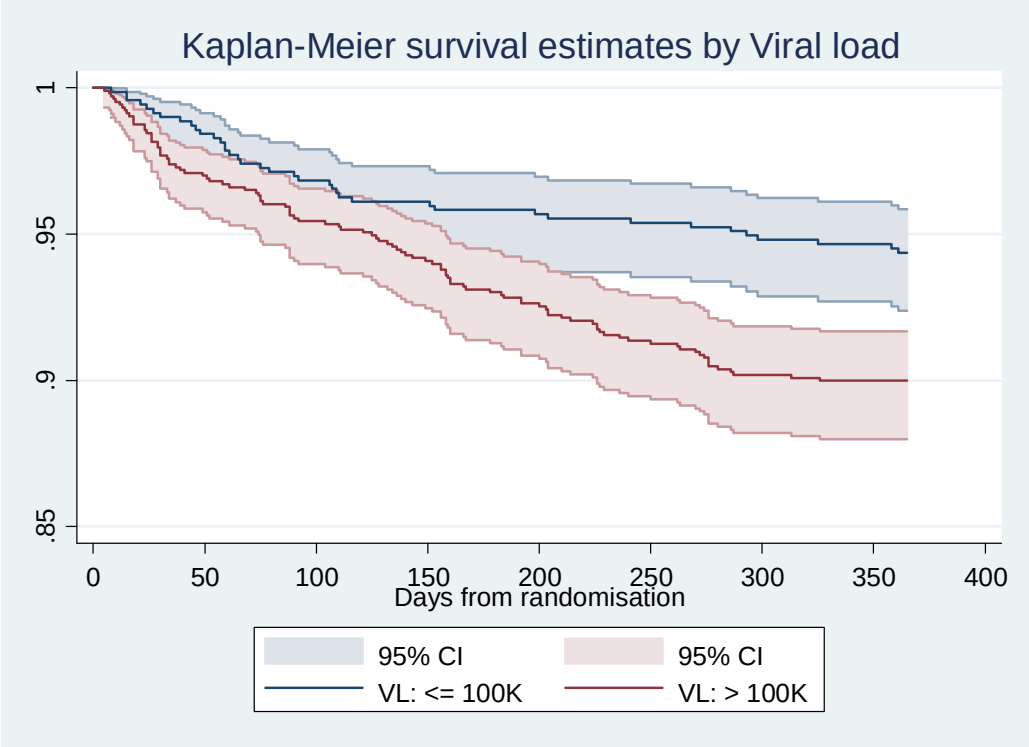
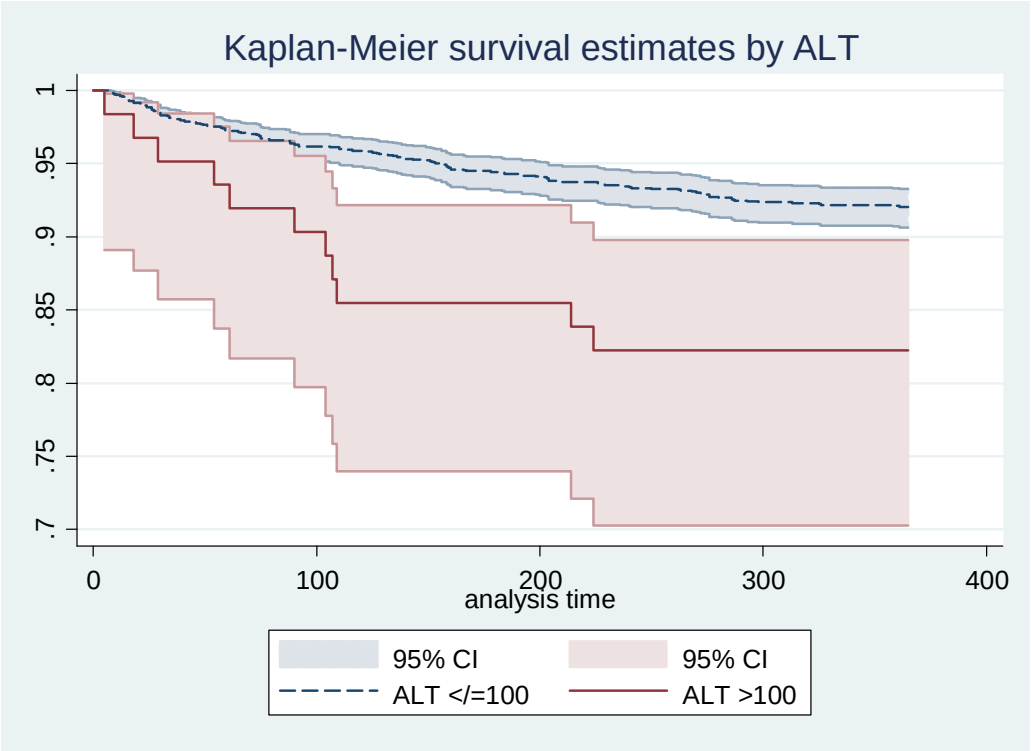
Appendix 1: Kaplan-Meier curves by selected variables: Early mortality

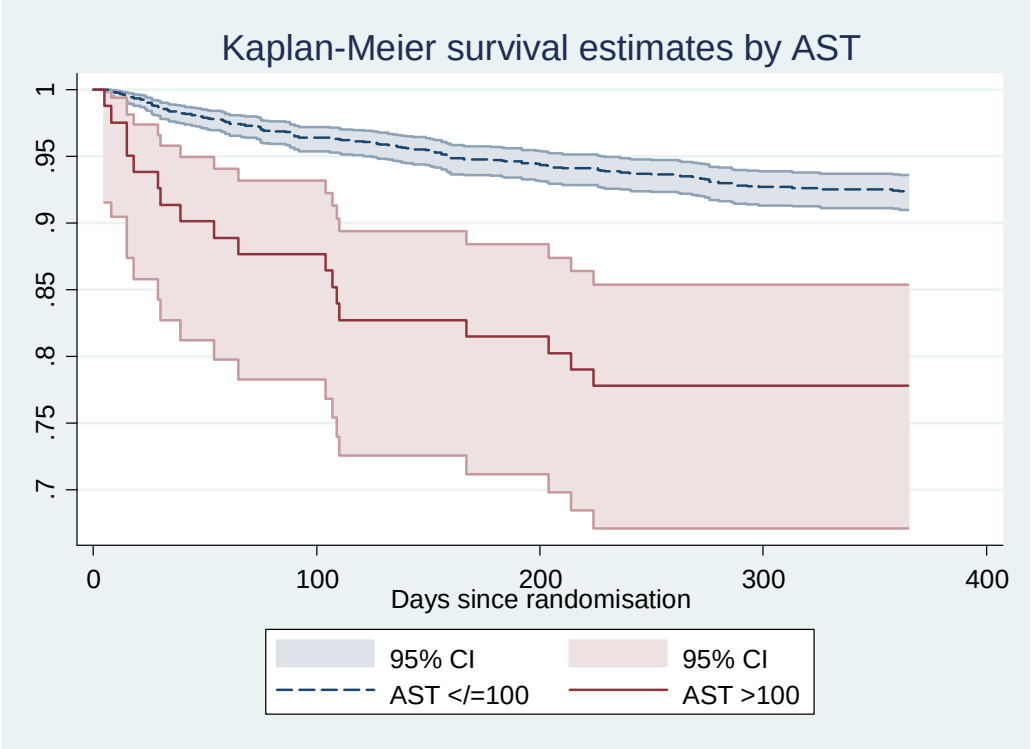


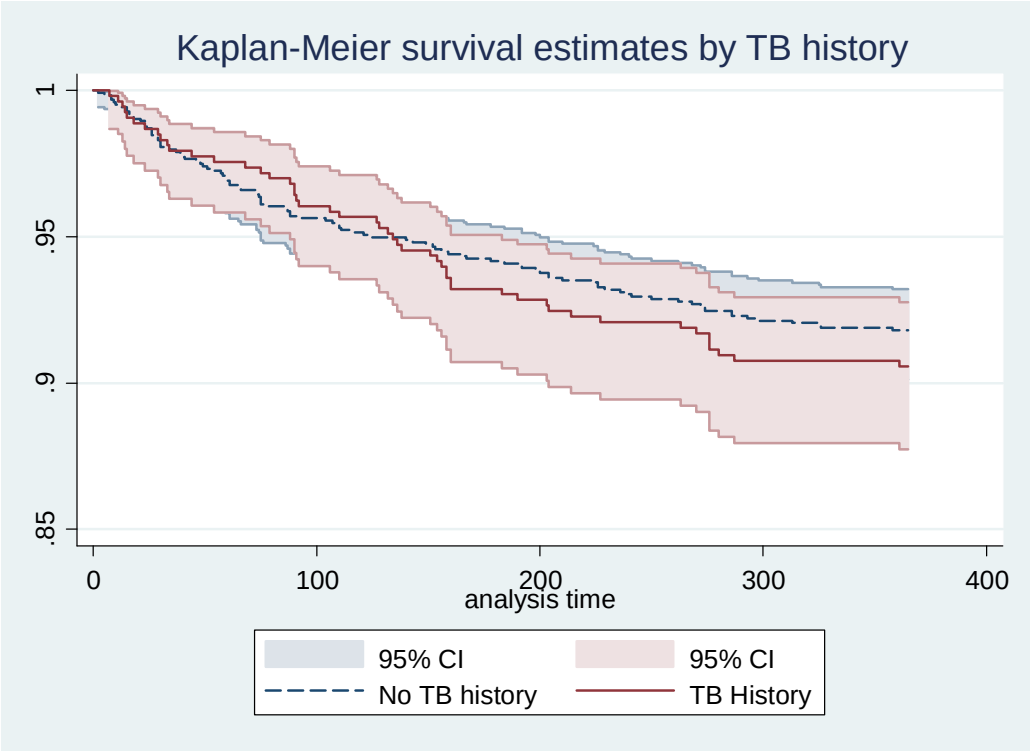


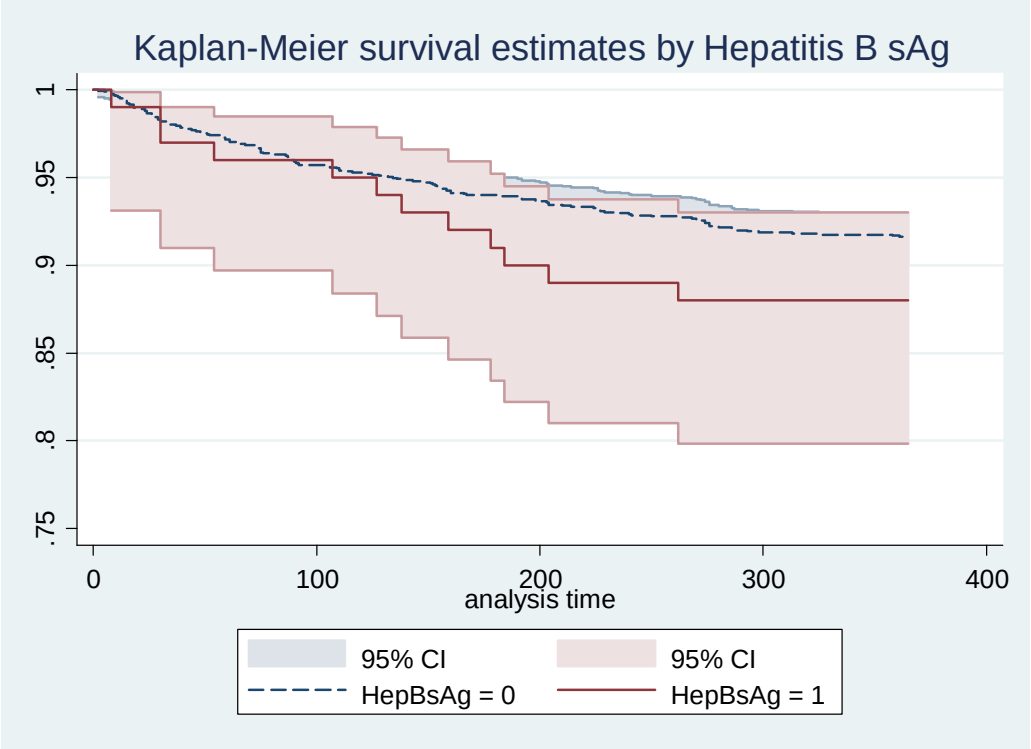

Kaplan-Meier survival estimates by marriage
1
.95
.9
.85
0
100
200
300
400
analysis time
95% CI
95% CI
Married = 0
Married = 1

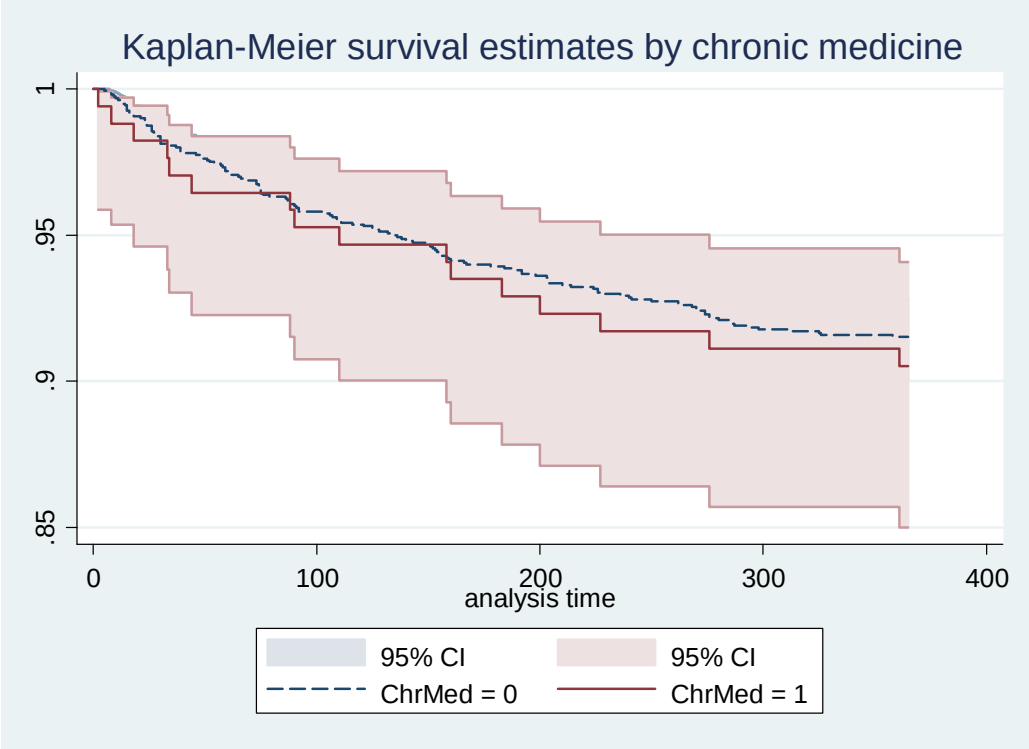

Kaplan-Meier survival estimates by Viral load
1
.95
.9
.85
0
50
100
150
200
250
300
350
400
Days from randomisation
95% CI
95% CI
VL: <= 100K
VL: > 100K

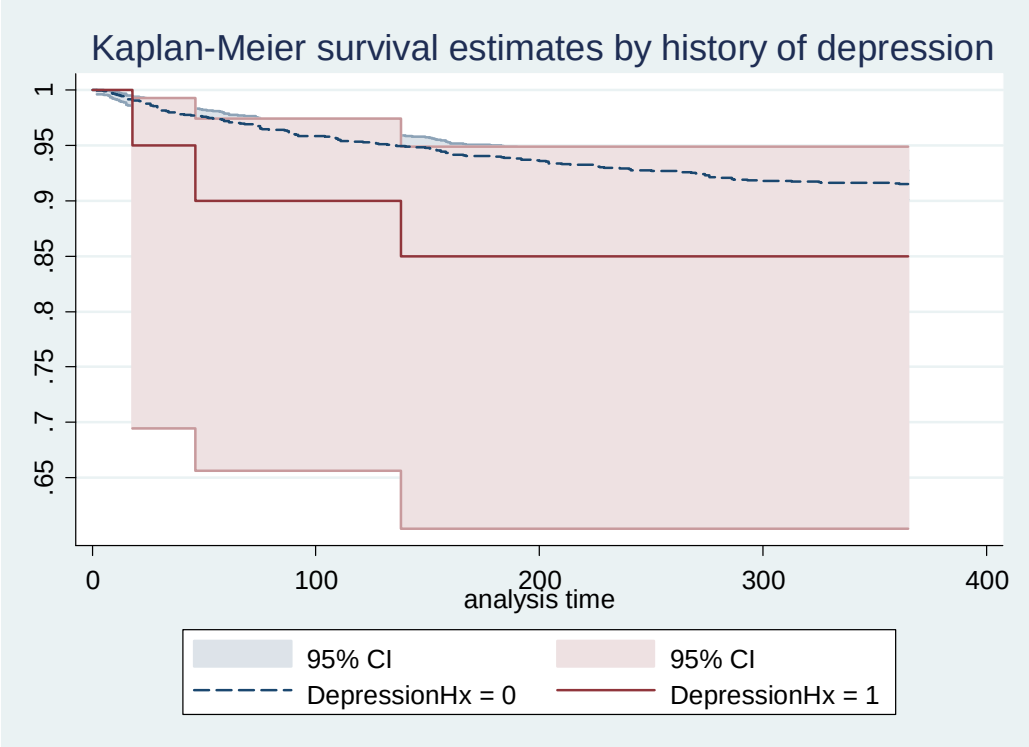

Kaplan-Meier survival estimates by ALT
1
.95
.9
.85
.8
.75
.7
0
100
200
300
400
analysis time
95% CI
ALT </=100
95% CI
ALT >100


Kaplan-Meier survival estimates by AST
1
.95
.9
.85
.8
.75
.7
0
100
200
300
400
Days since randomisation
95% CI
AST </=100
95% CI
AST >100

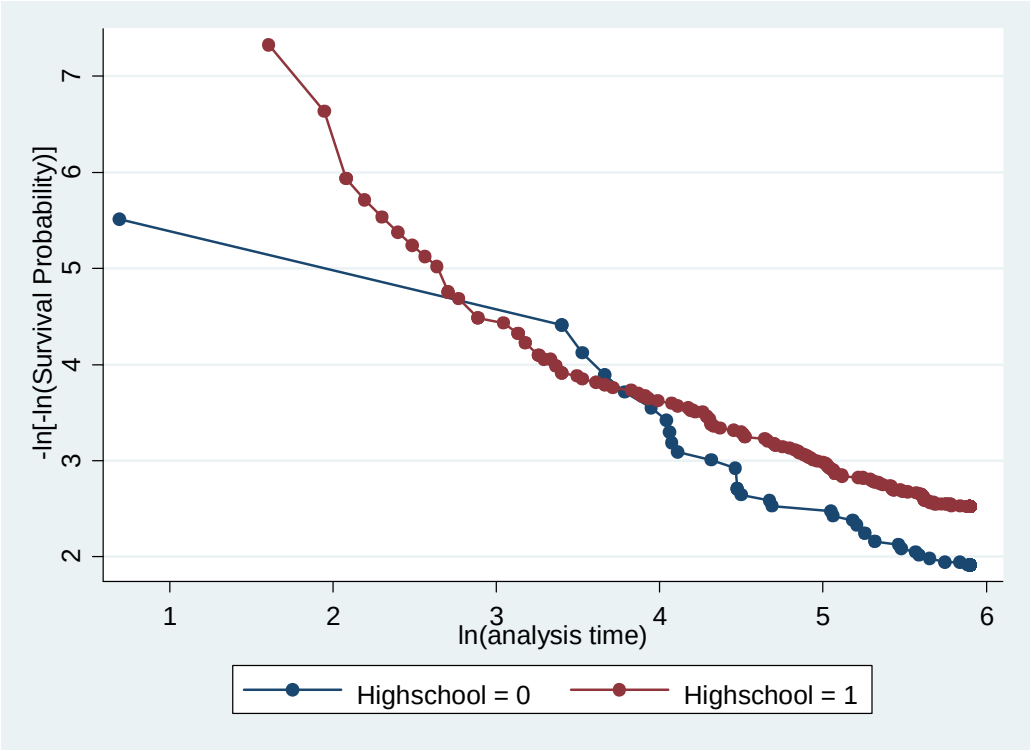

Kaplan-Meier survival estimates by TB history
1
.95
.9
.85
0
100
200
300
400
analysis time
95% CI
95% CI
No TB history
TB History

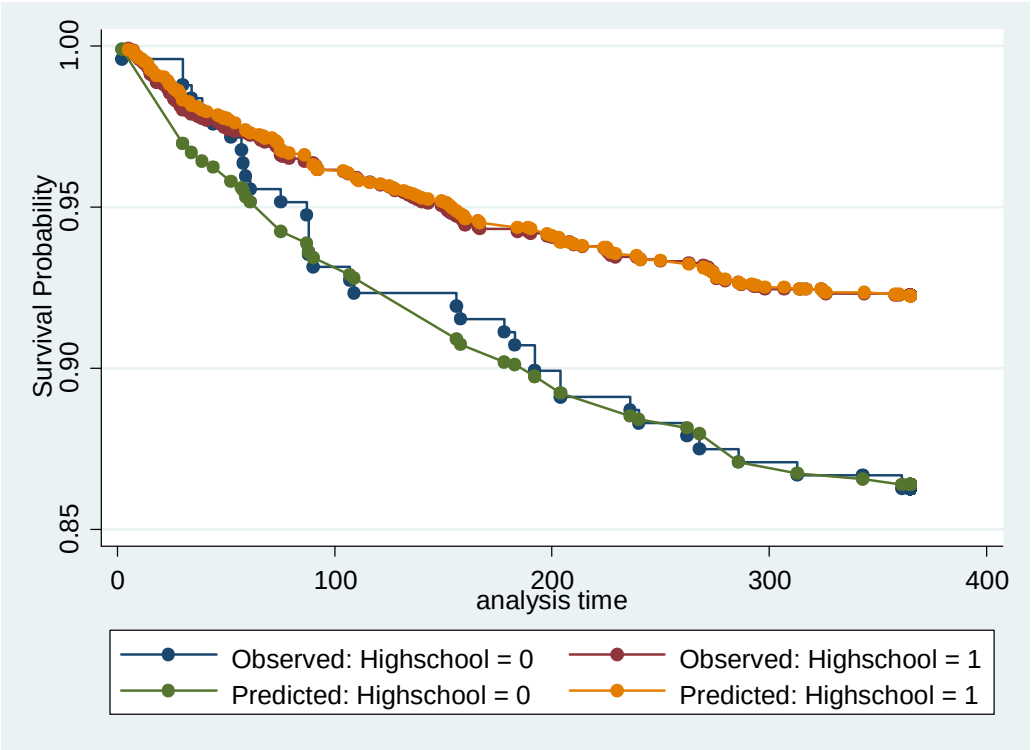

Kaplan-Meier survival estimates by Hepatitis B sAg
1
.95
.9
.85
.8
.75
0
100
200
300
400
analysis time
95% CI
95% CI
HepBsAg = 0
HepBsAg = 1

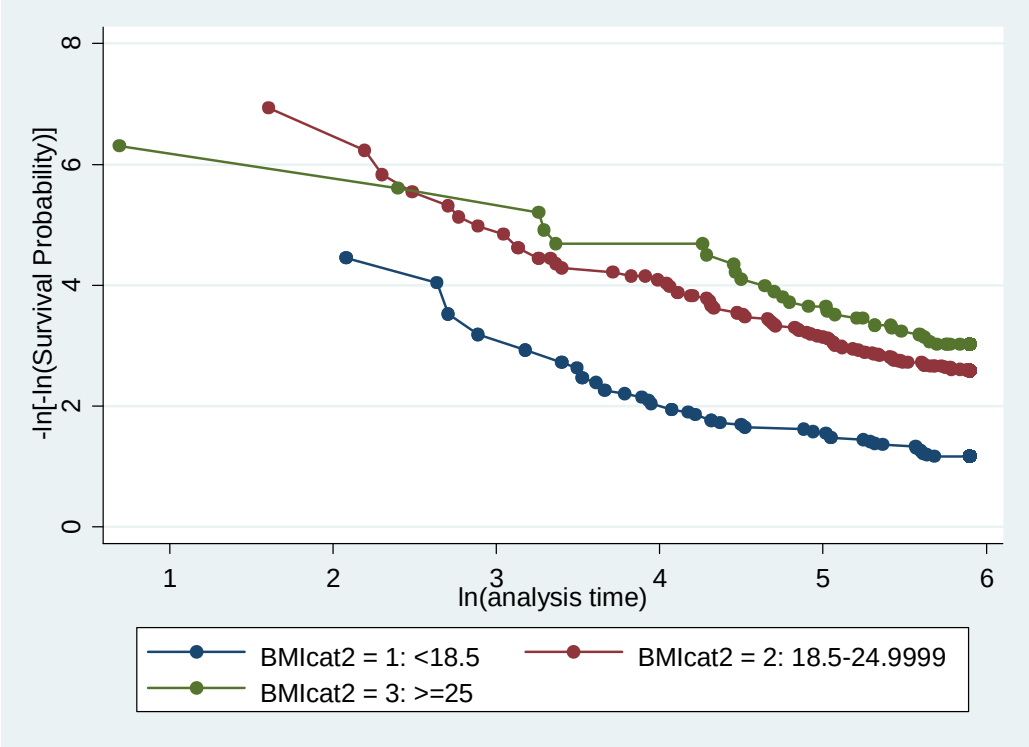

Kaplan-Meier survival estimates by chronic medicine
1
.95
.9
.85
0
100
200
300
400
analysis time
95% CI
95% CI
ChrMed = 0
ChrMed = 1

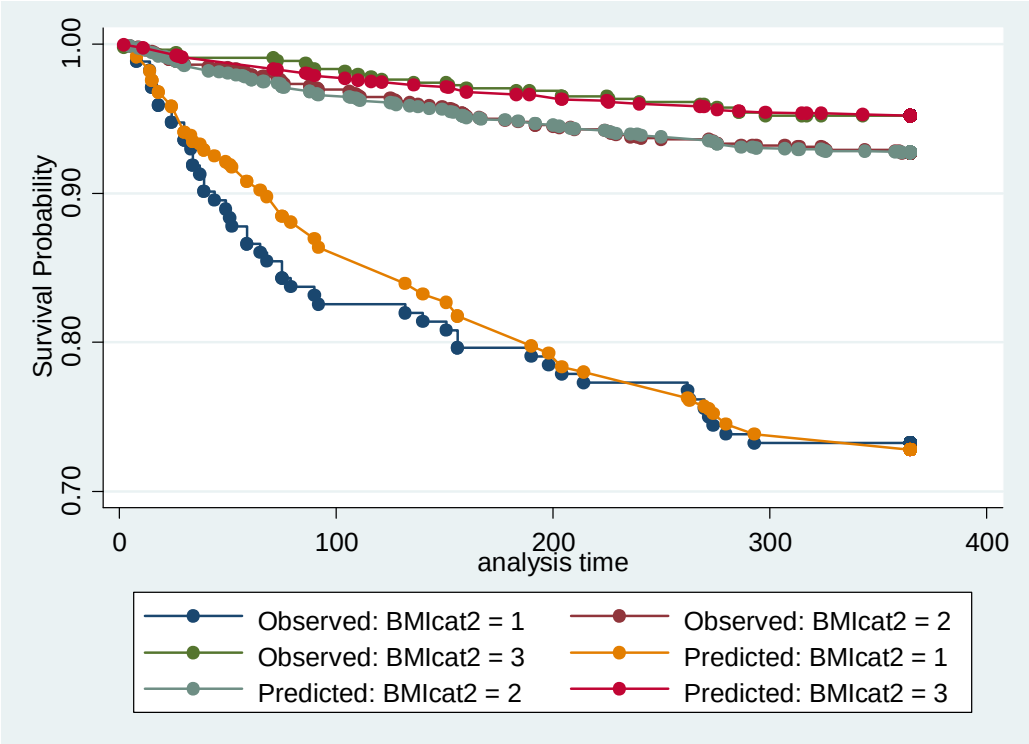

Kaplan-Meier survival estimates by history of depression
1
.95
.9
.85
.8
.75
.7
.65
0
100
200
300
400
analysis time
95% CI
95% CI
DepressionHx = 0
DepressionHx = 1

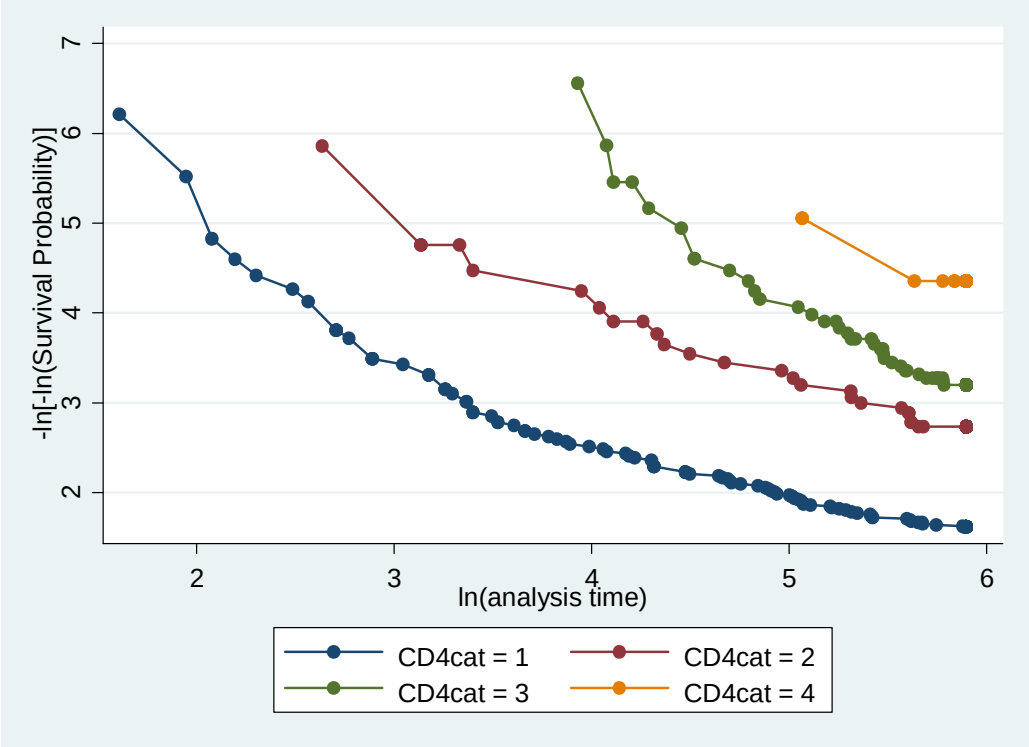
Appendix 2: Testing PH assumption—early mortality

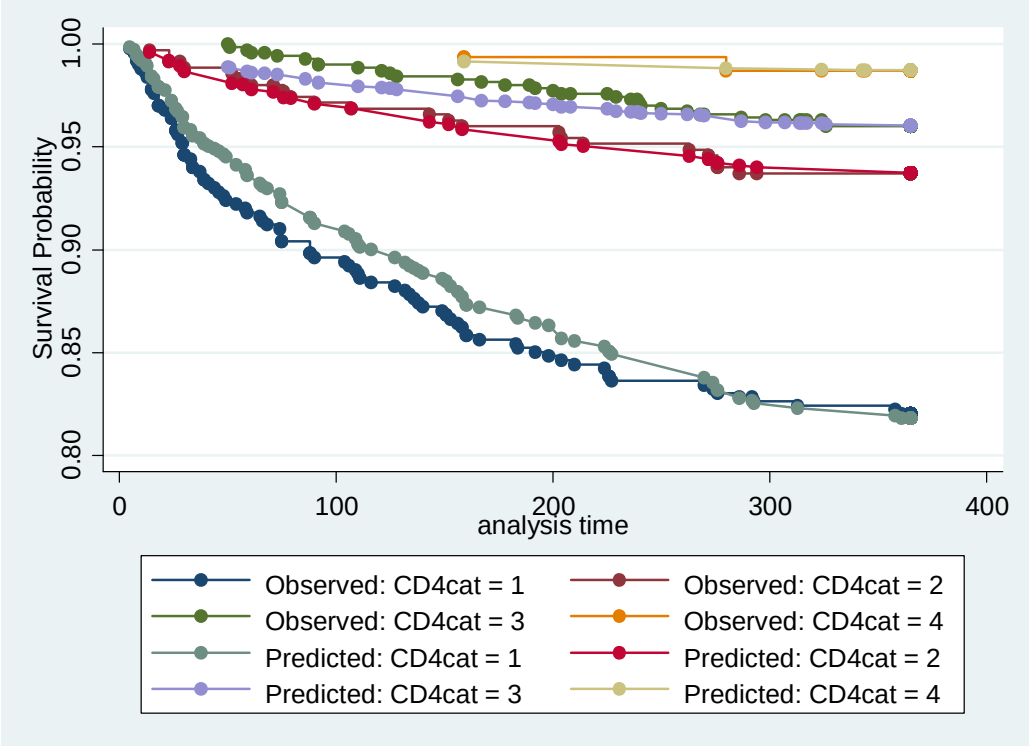




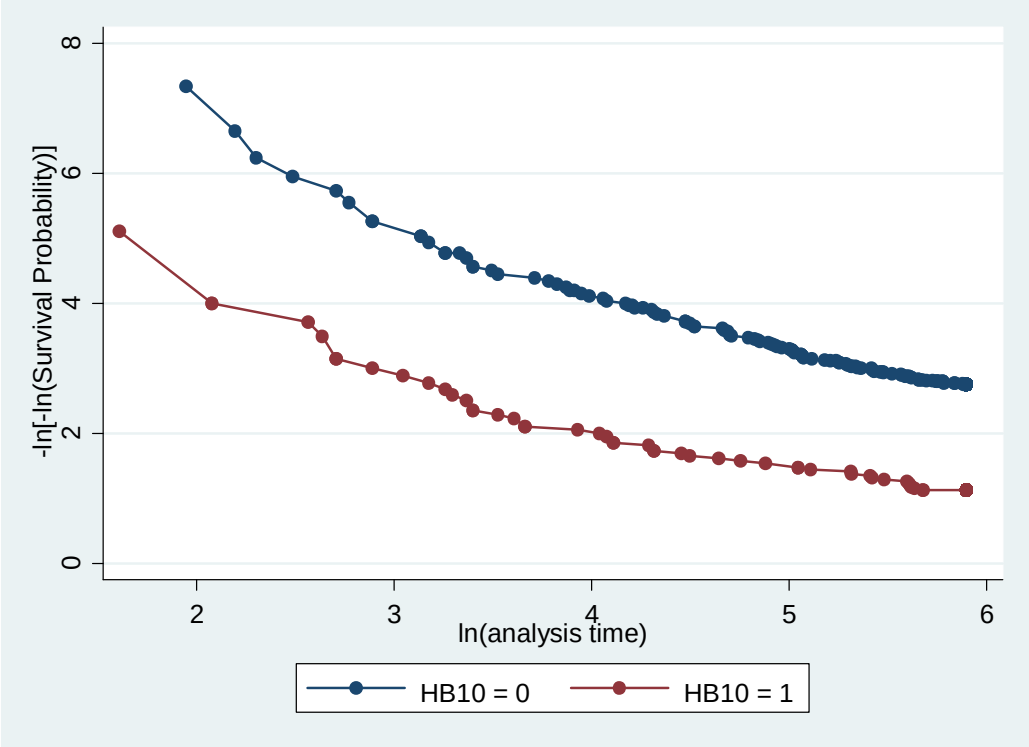

-ln[-ln(Survival Probability)]
0
2
4
6
8
1
2
3
4
5
6
ln(analysis time)
BMIcat2 = 1: <18.5
BMIcat2 = 2: 18.5-24.9999
BMIcat2 = 3: >=25

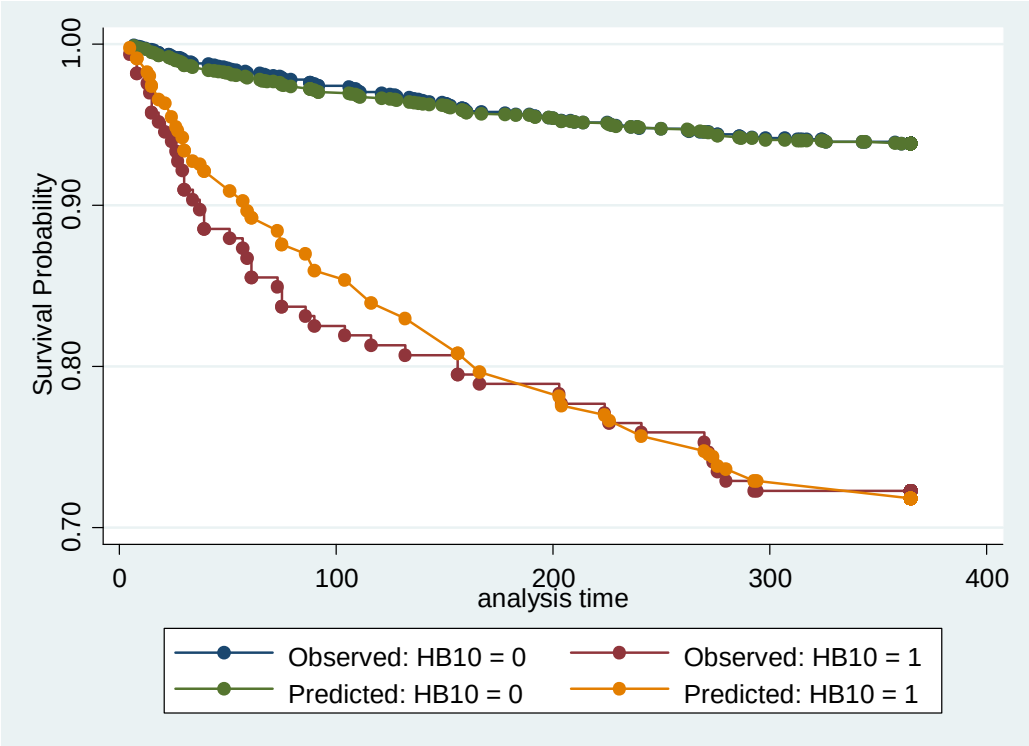

Survival Probability
0.70
0.80
0.90
1.00
0
100
200
300
400
analysis time
Observed: BMIcat2 = 1
Observed: BMIcat2 = 2
Observed: BMIcat2 = 3
Predicted: BMIcat2 = 1
Predicted: BMIcat2 = 2
Predicted: BMIcat2 = 3



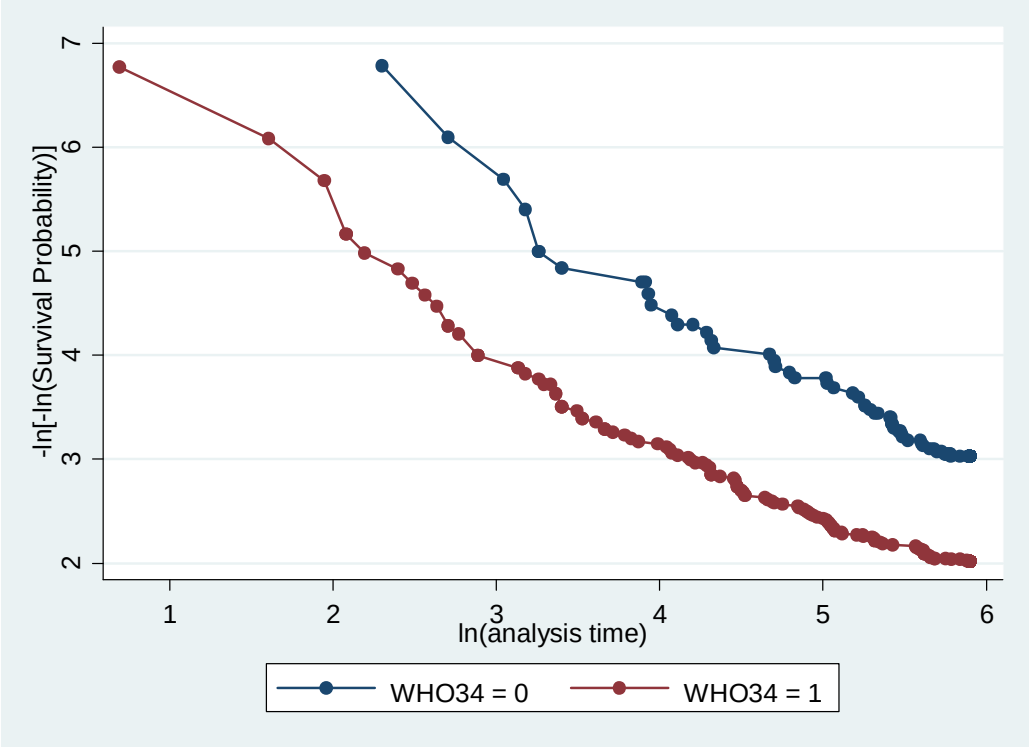


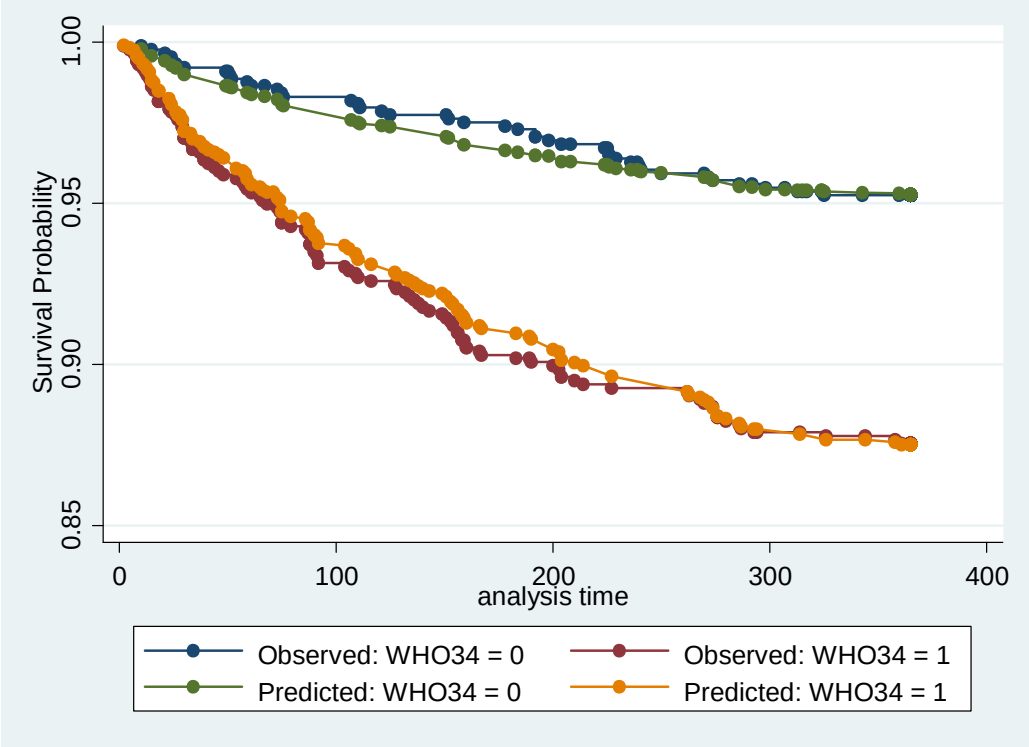
CD4cat: 1: </=50; 2: 51-100; 3: 101-200; 4: >200 cells/mm^3



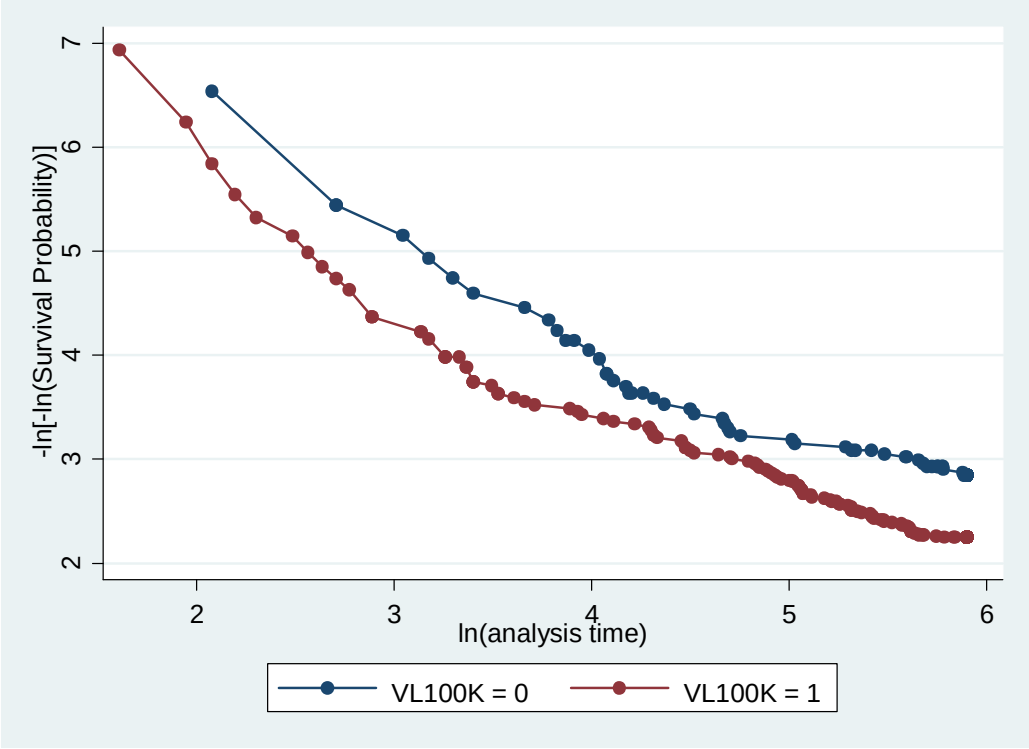


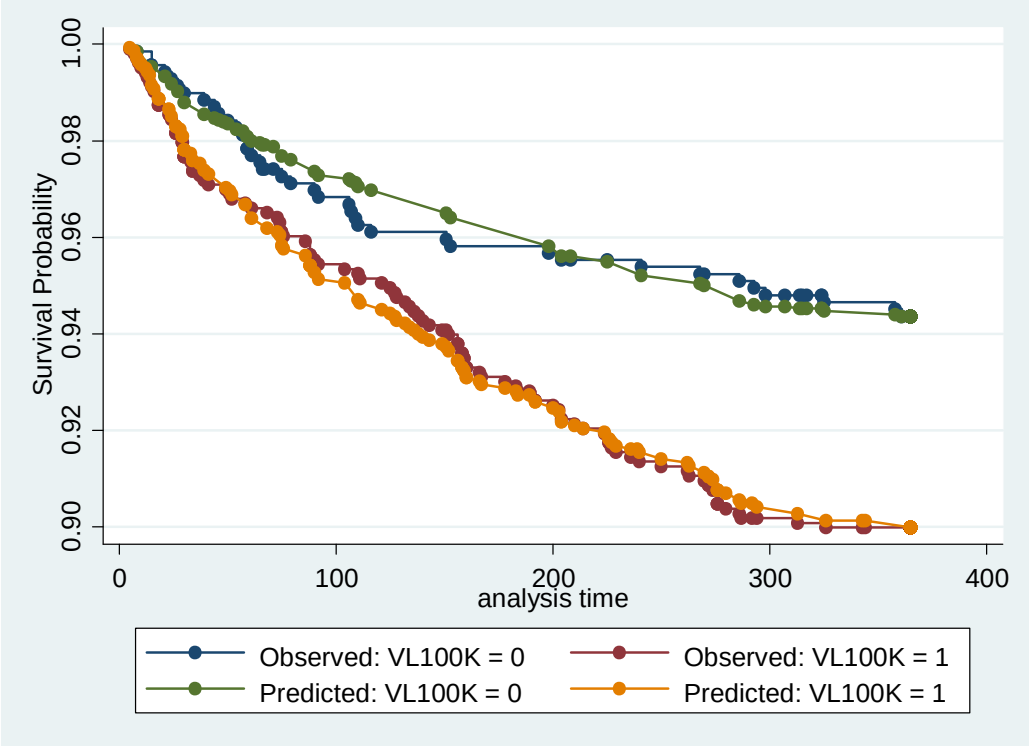
HB10=0: Haemoglobin >/=10g/dL; HB10=1: Haemoglobin ,10g/dL



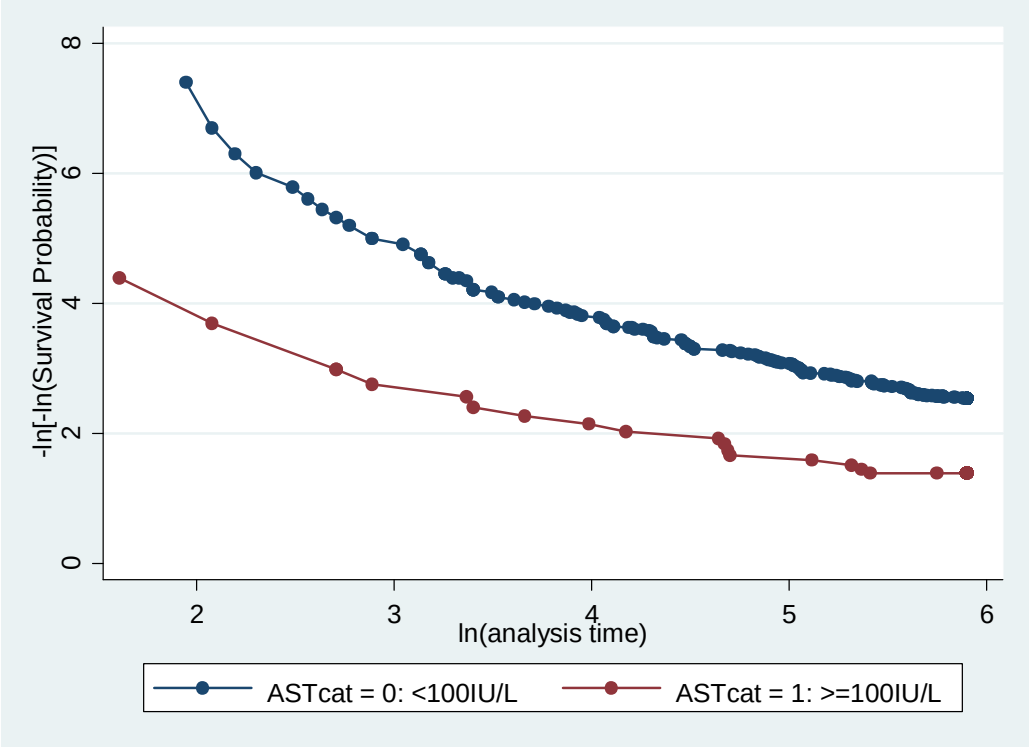


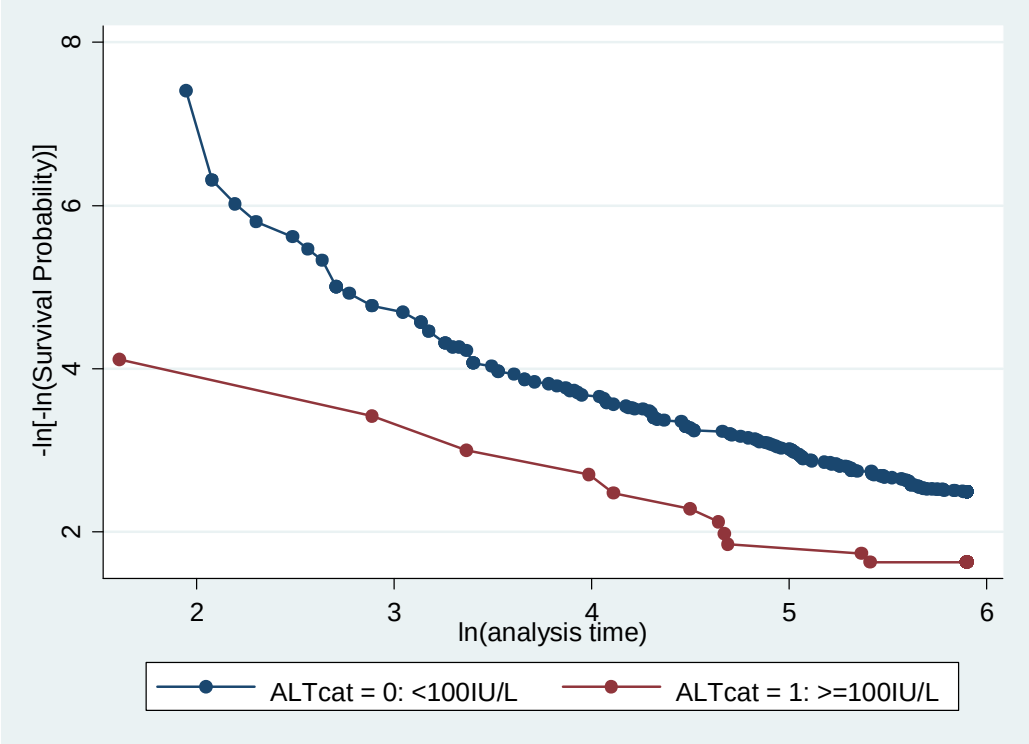
WHO34= WHO stage 3 or 4

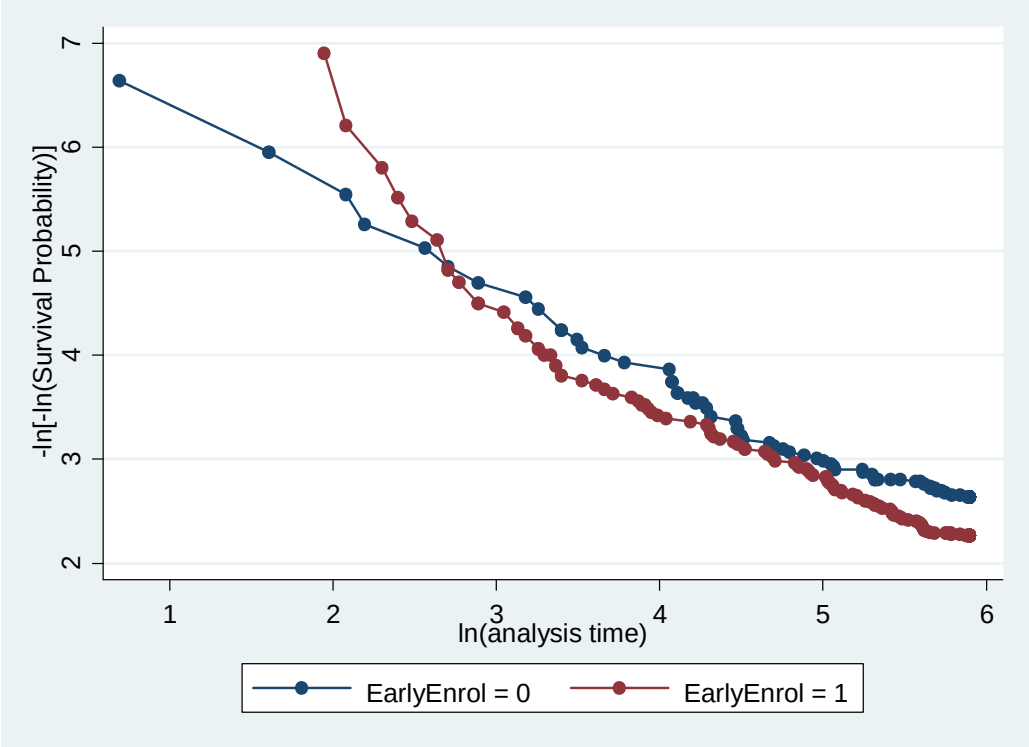


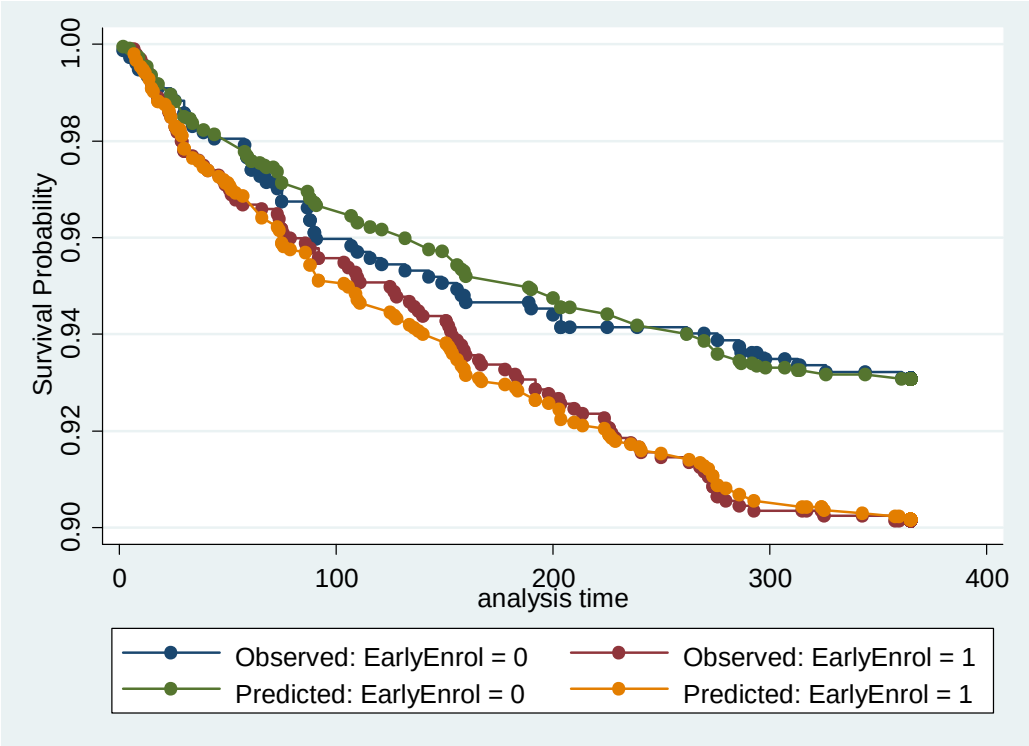


VL100K=0: Viral load </= 100 000 copies/mL; VL100K=1: Viral load > 100 000 copies/mL

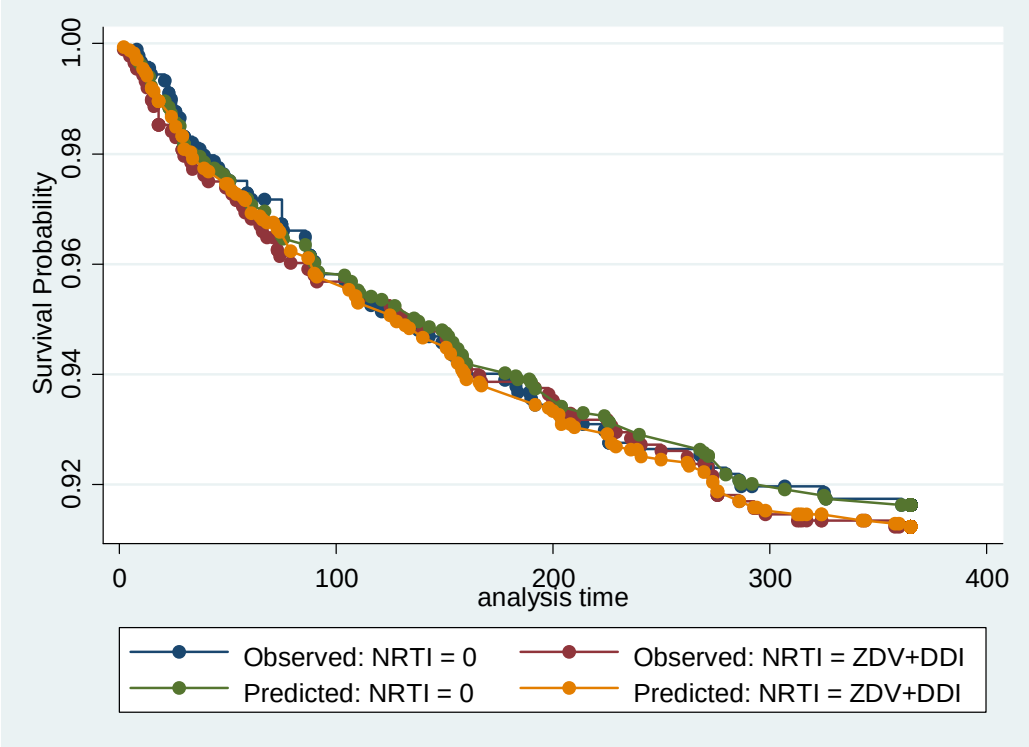

-ln[-ln(Survival Probability)]
0
2
4
6
8
2
3
4
5
6
ln(analysis time)
ASTcat = 0: <100IU/L
ASTcat = 1: >=100IU/L

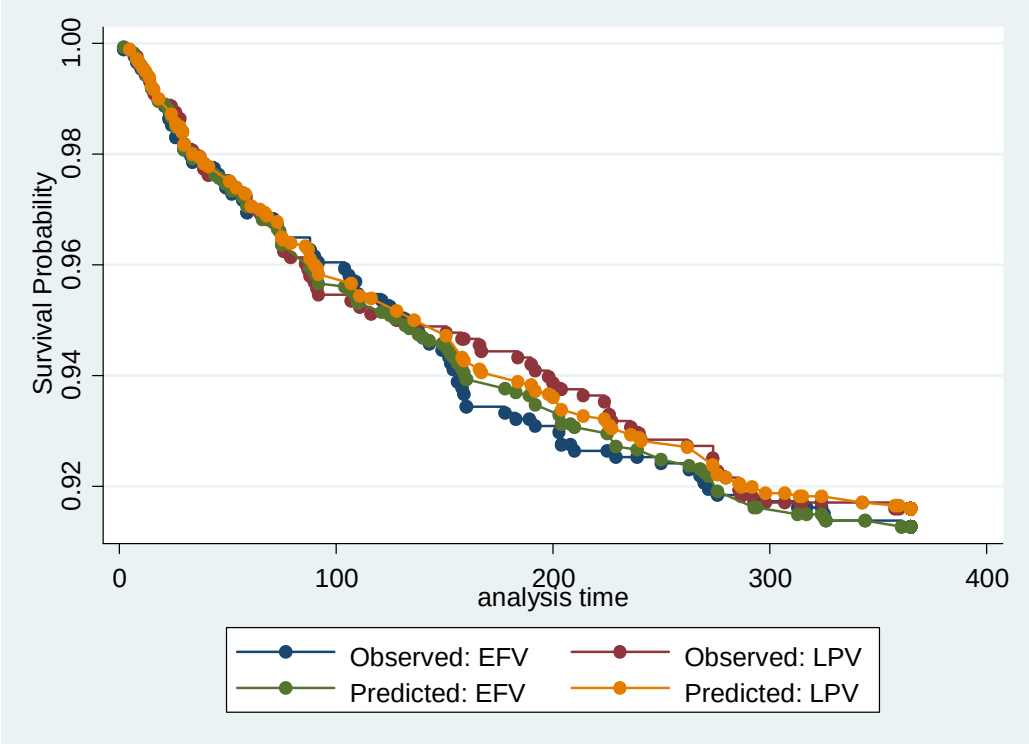

-ln[-ln(Survival Probability)]
2
4
6
8
2
3
4
5
6
ln(analysis time)
ALTcat = 0: <100IU/L
ALTcat = 1: >=100IU/L

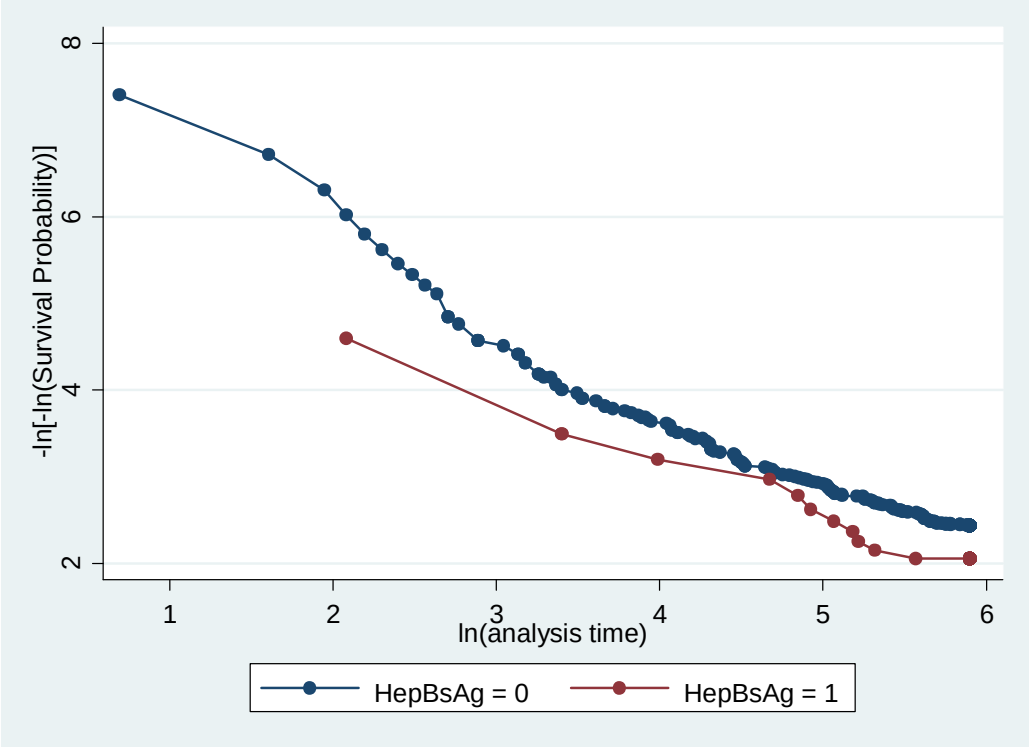




EarlyEnrol=1: enrolment in 2004 or 2005; EarlyEnrol=0: 2006 and 2007


Survival Probability
1.00
0.98
0.96
0.94
0.92
0
100
200
300
400
analysis time
Observed: NRTI = 0
Predicted: NRTI = 0
Observed: NRTI = ZDV+DDI
Predicted: NRTI = ZDV+DDI

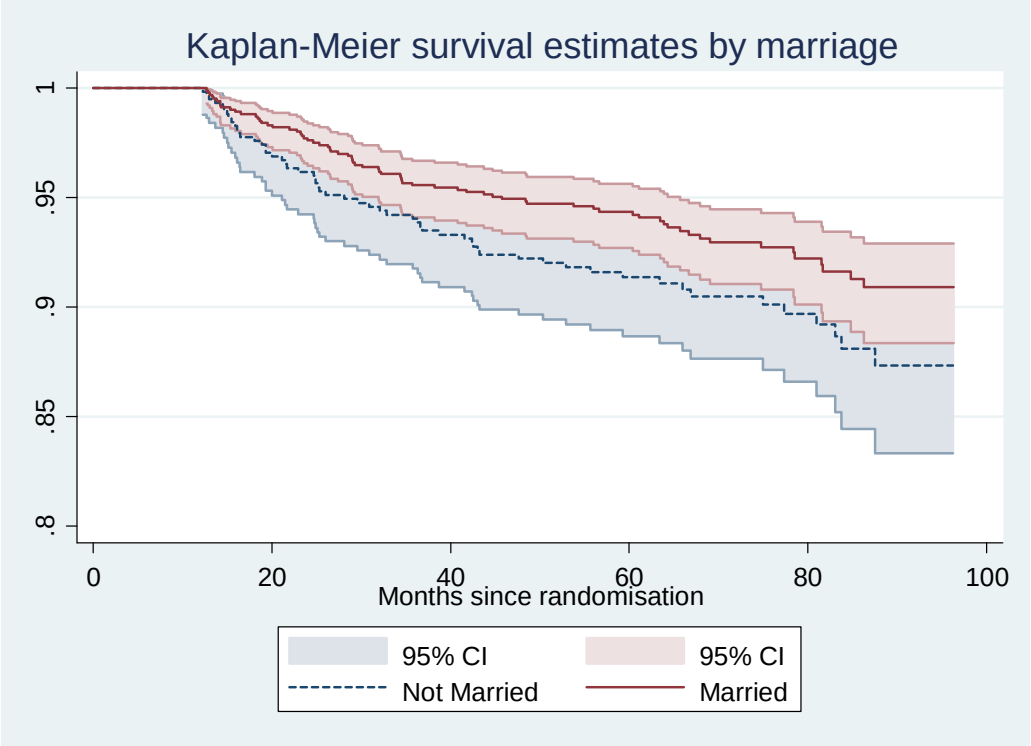

Survival Probability
1.00
0.98
0.96
0.94
0.92
0
100
200
300
400
analysis time
Observed: EFV
Predicted: EFV
Observed: LPV
Predicted: LPV

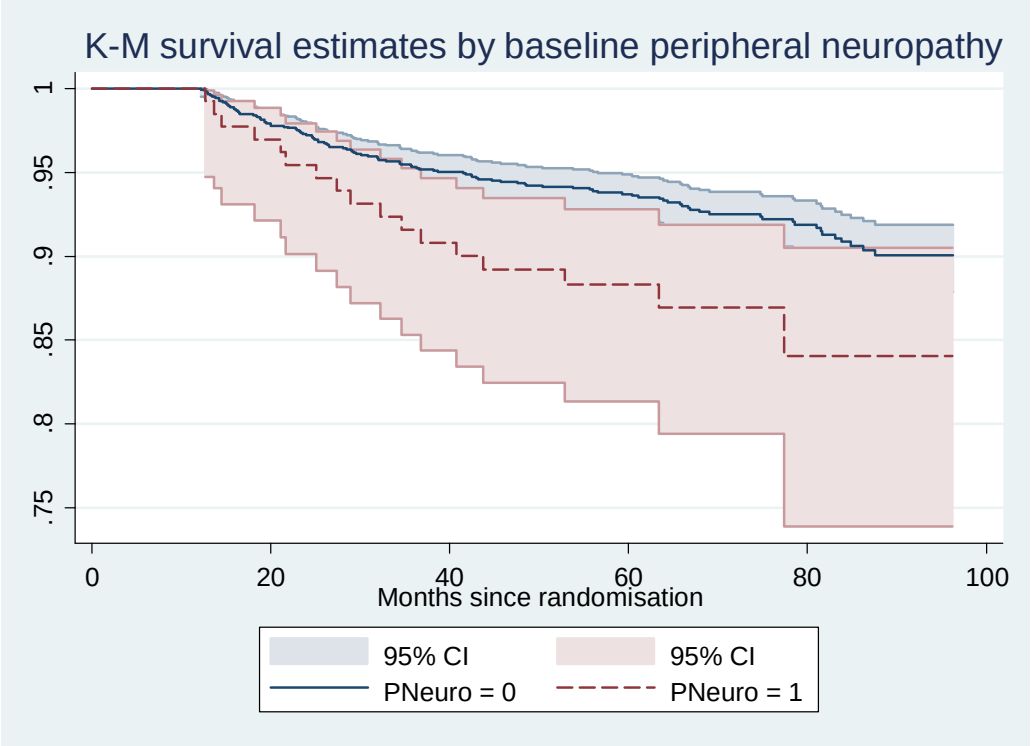


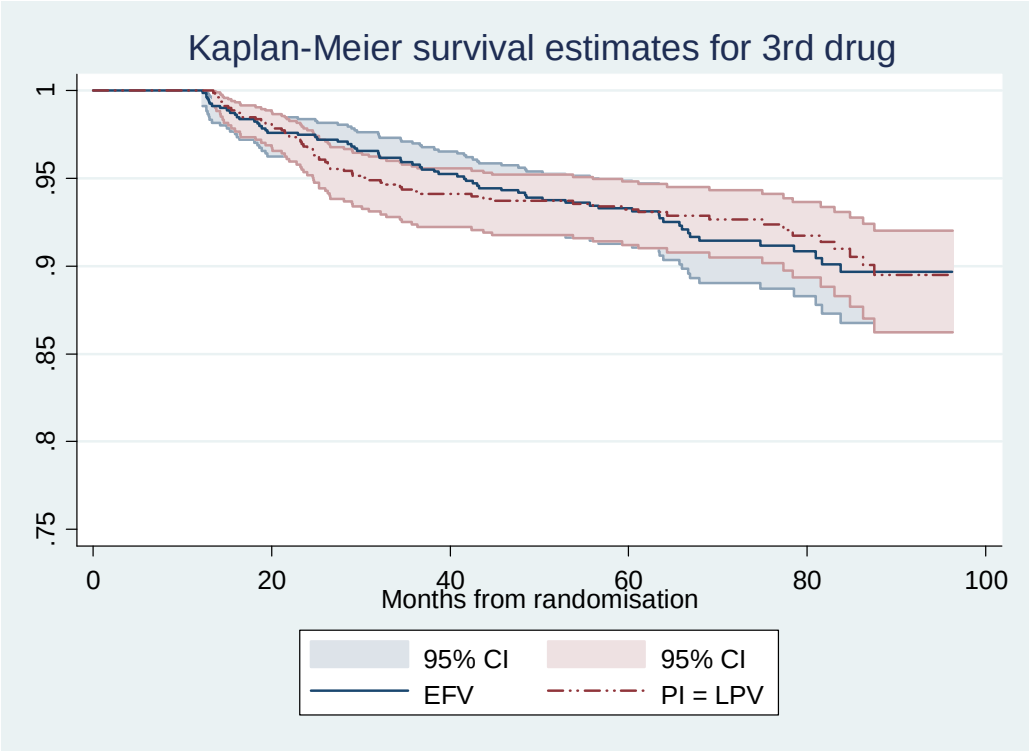
Post-estimation test of proportionality of hazards

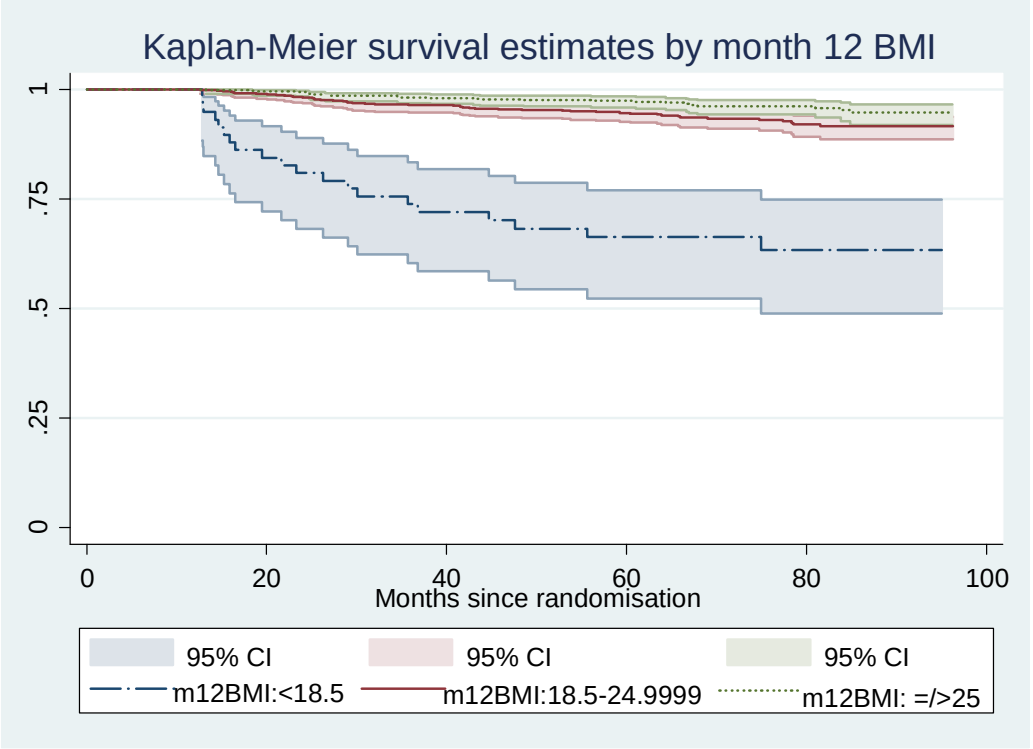
	rho	chi2	df	Prob>chi2
Married	0.12850	2.21	1	0.1370
WHO34	-0.05592	0.43	1	0.5103
1b.CD4cat	.	.	1	.
2.CD4cat	0.06420	0.61	1	0.4360
3.CD4cat	0.23488	7.56	1	0.0060
4.CD4cat	0.11352	1.77	1	0.1829
HB10	-0.02074	0.06	1	0.8038
1.BMIcat2	-0.06198	0.54	1	0.4618
2b.BMIcat2	.	.	1	.
3.BMIcat2	0.06503	0.62	1	0.4305
ASTcat	-0.13205	2.50	1	0.1135
global test		21.41	9	0.0110

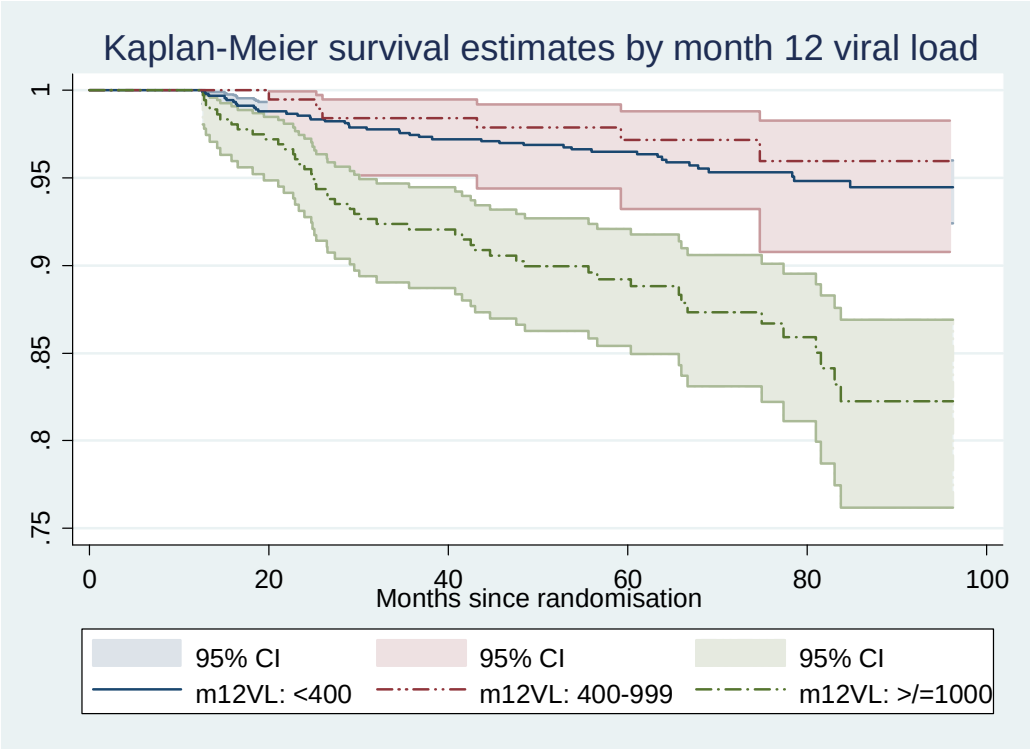
APPENDIX 3: Kaplan-Meier survival curves with 95%CI—late mortality

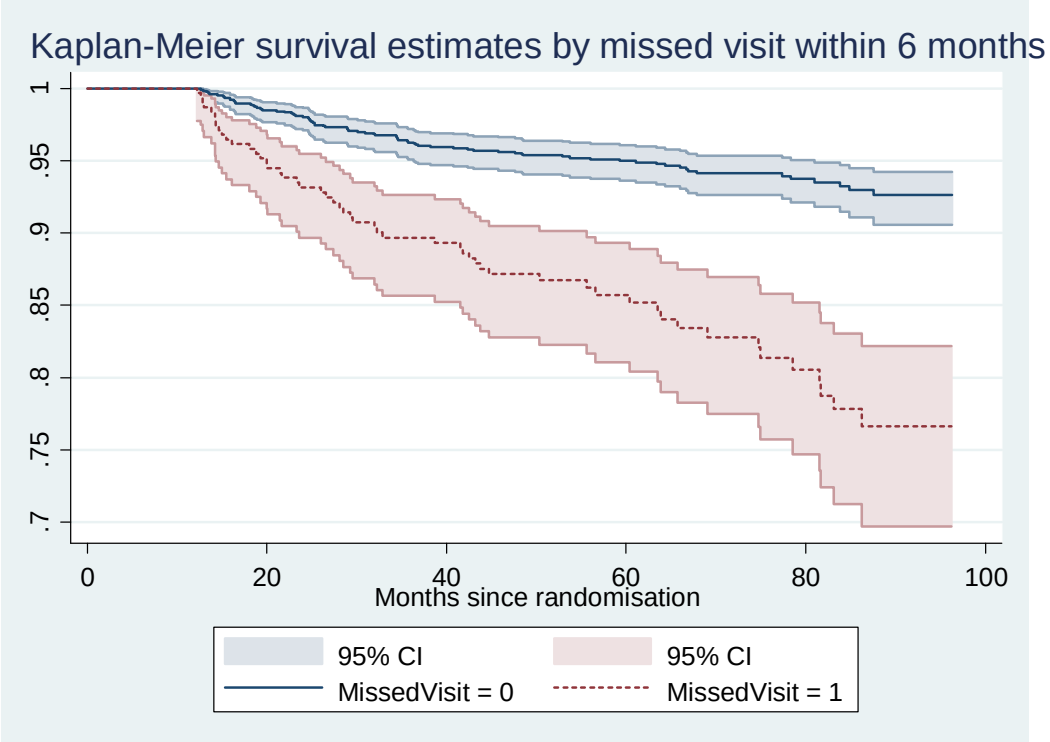


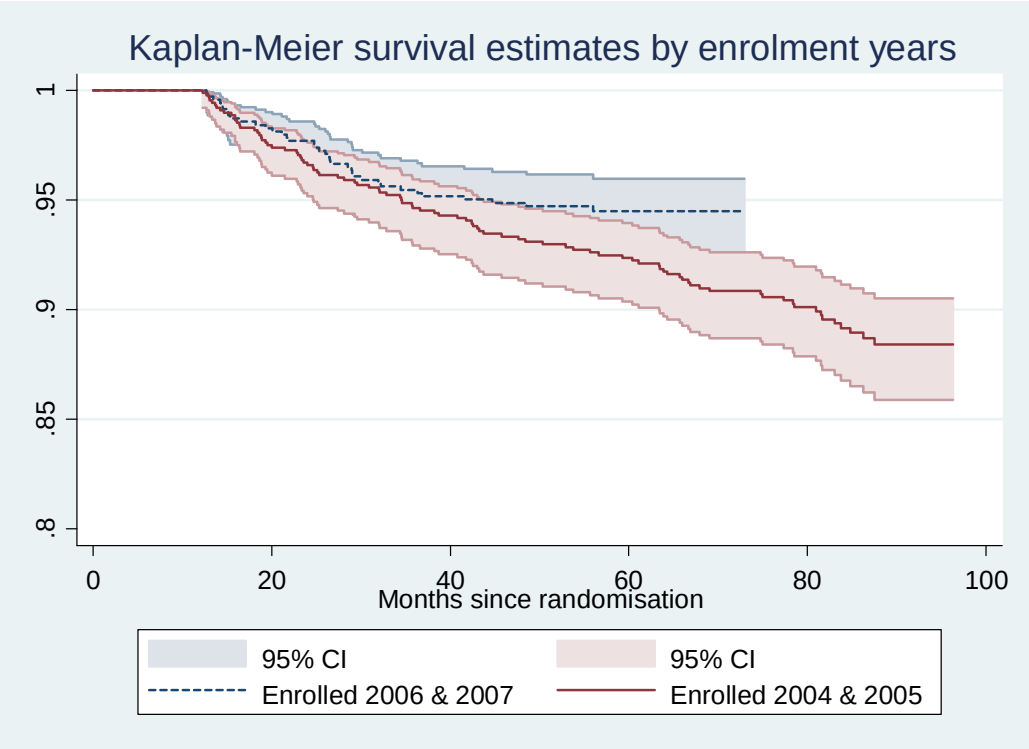


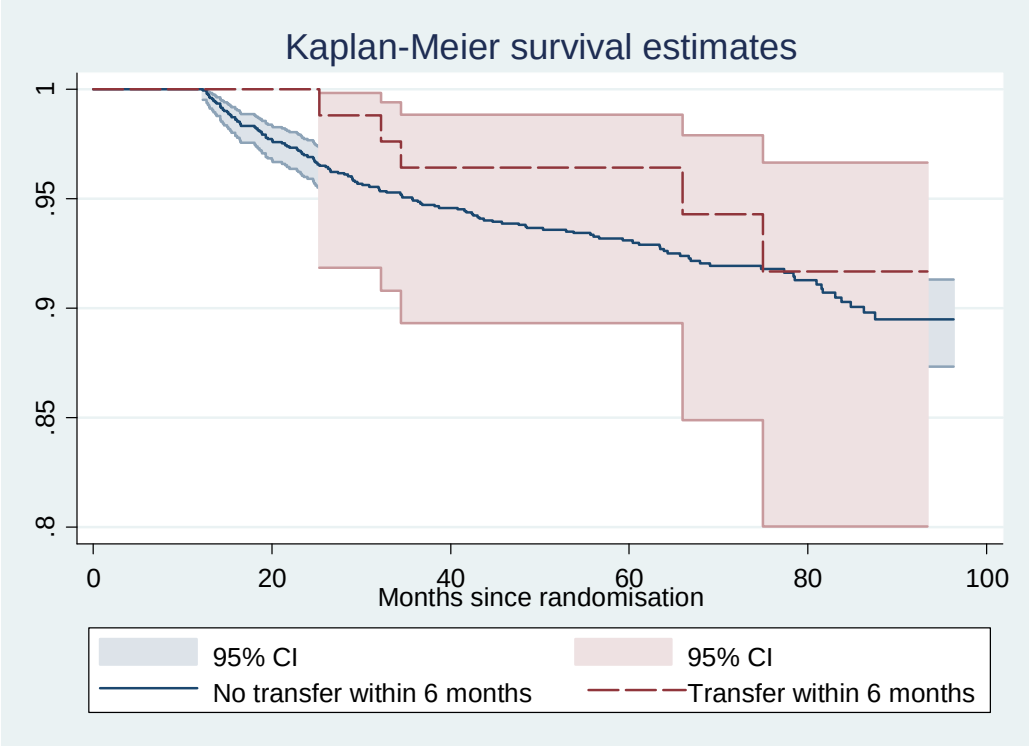

Kaplan-Meier survival estimates for 3rd drug
1
.95
.9
.85
.8
.75
0
20
40
60
80
100
Months from randomisation
95% CI
95% CI
EFV
PI = LPV


Kaplan-Meier survival estimates by month 12 BMI
1
.75
.5
.25
0
0
20
40
60
80
100
Months since randomisation
95% CI
95% CI
95% CI
m12BMI:<18.5
m12BMI:18.5-24.9999
m12BMI: =/>25


Kaplan-Meier survival estimates by month 12 viral load
1
.95
.9
.85
.8
.75
0
20
40
60
80
100
Months since randomisation
95% CI
m12VL: <400
95% CI
m12VL: 400-999
95% CI
m12VL: >/=1000

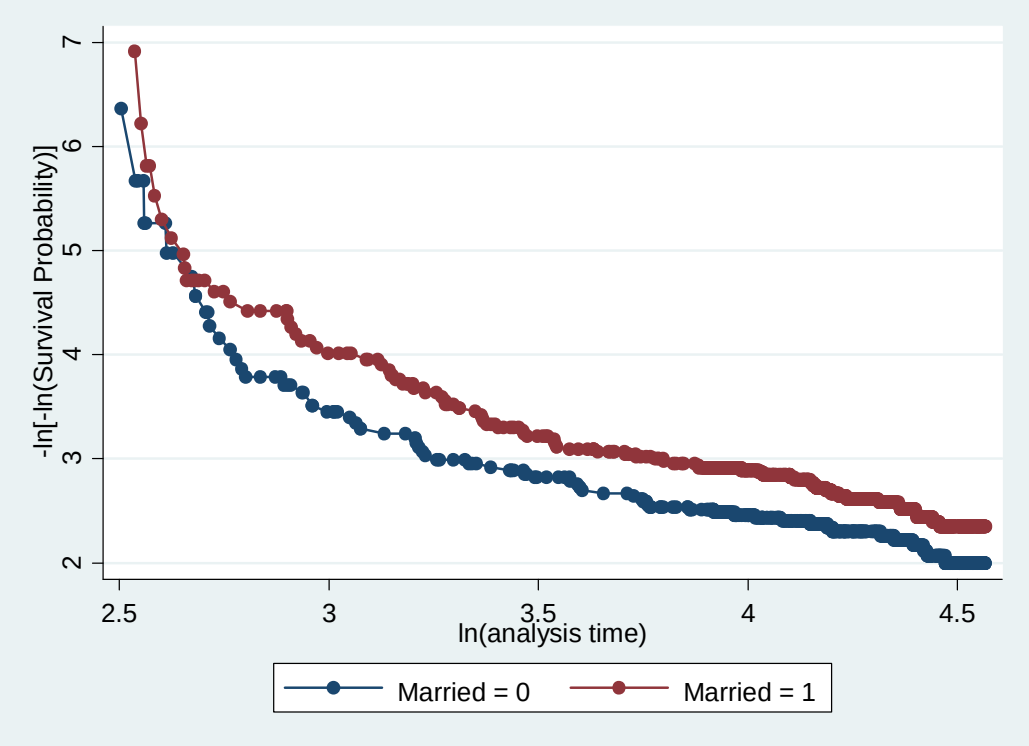

Kaplan-Meier survival estimates by missed visit within 6 months
1
.95
.9
.85
.8
.75
.7
0
20
40
60
80
100
Months since randomisation
95% CI
MissedVisit = 0
95% CI
MissedVisit = 1

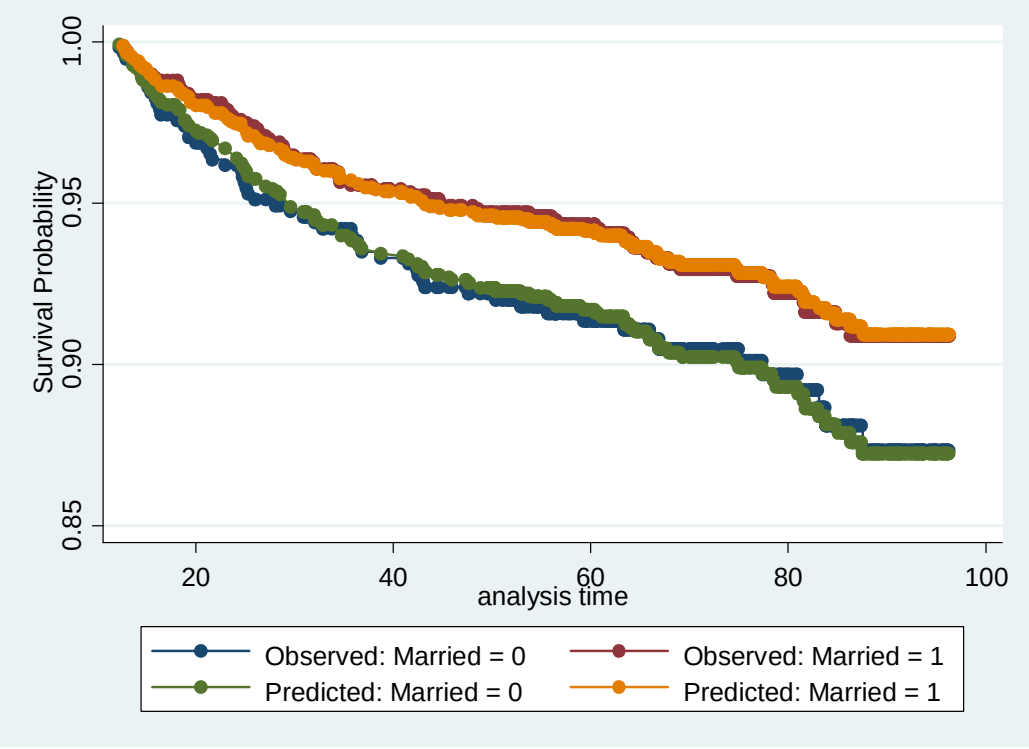

Kaplan-Meier survival estimates by enrolment years
1
.95
.9
.85
.8
0
20
40
60
80
100
Months since randomisation
95% CI
Enrolled 2006 & 2007
95% CI
Enrolled 2004 & 2005

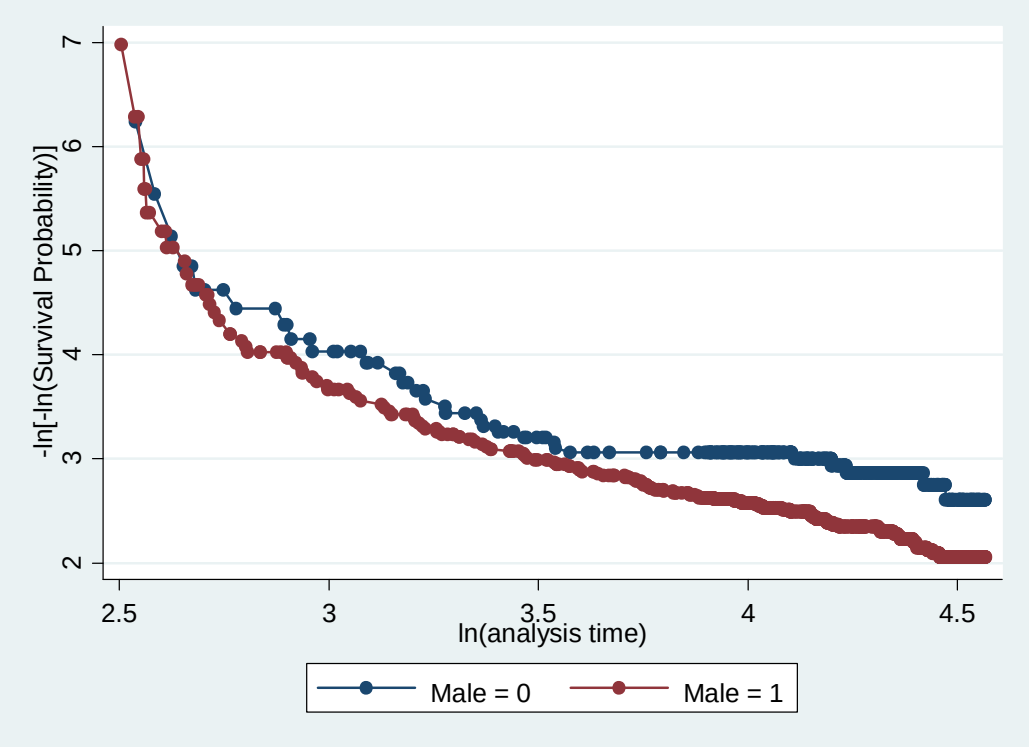

Kaplan-Meier survival estimates
1
.95
.9
.85
.8
0
20
40
60
80
100
Months since randomisation
95% CI
No transfer within 6 months
95% CI
Transfer within 6 months

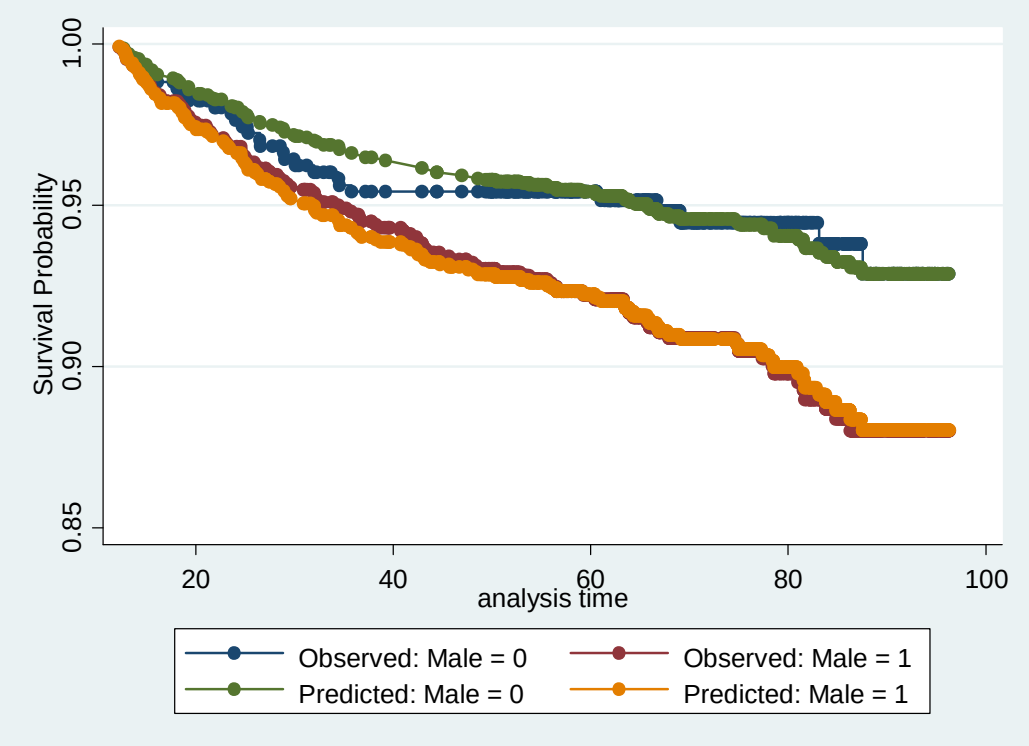
APPENDIX 4: Testing PH assumption: late mortality

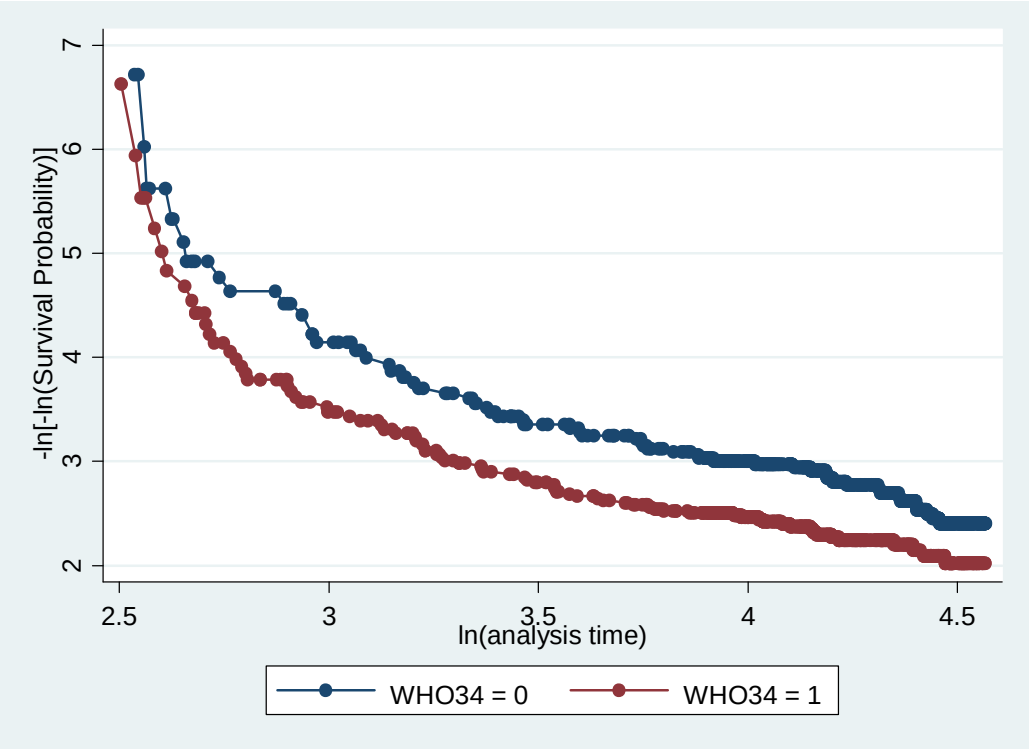
Baseline characteristics



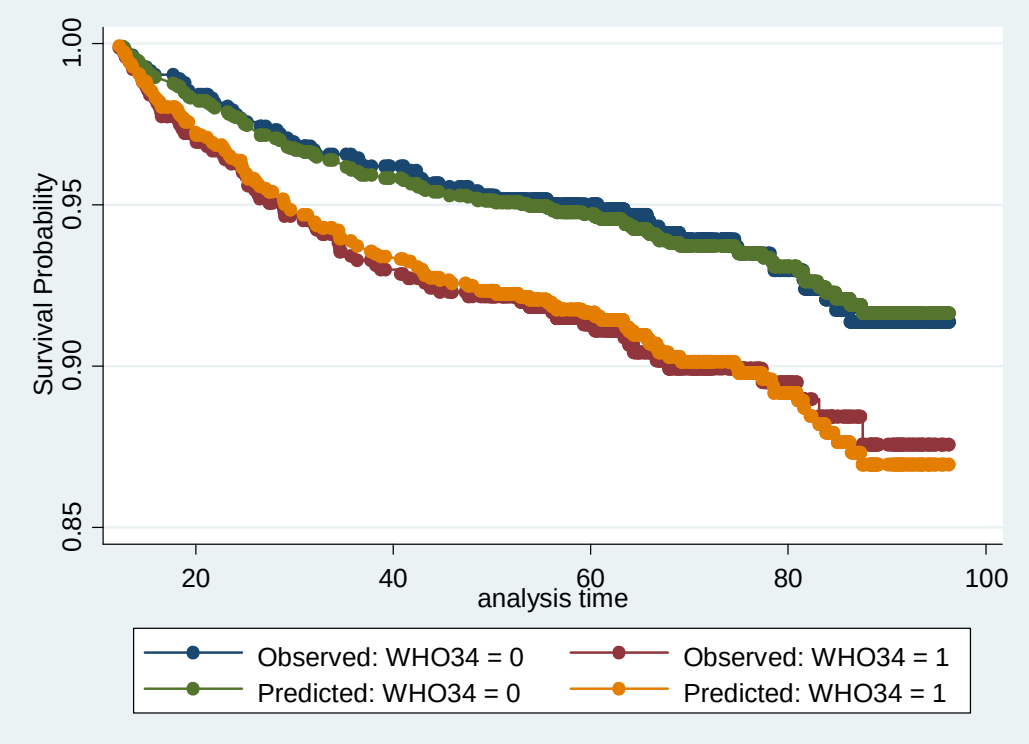


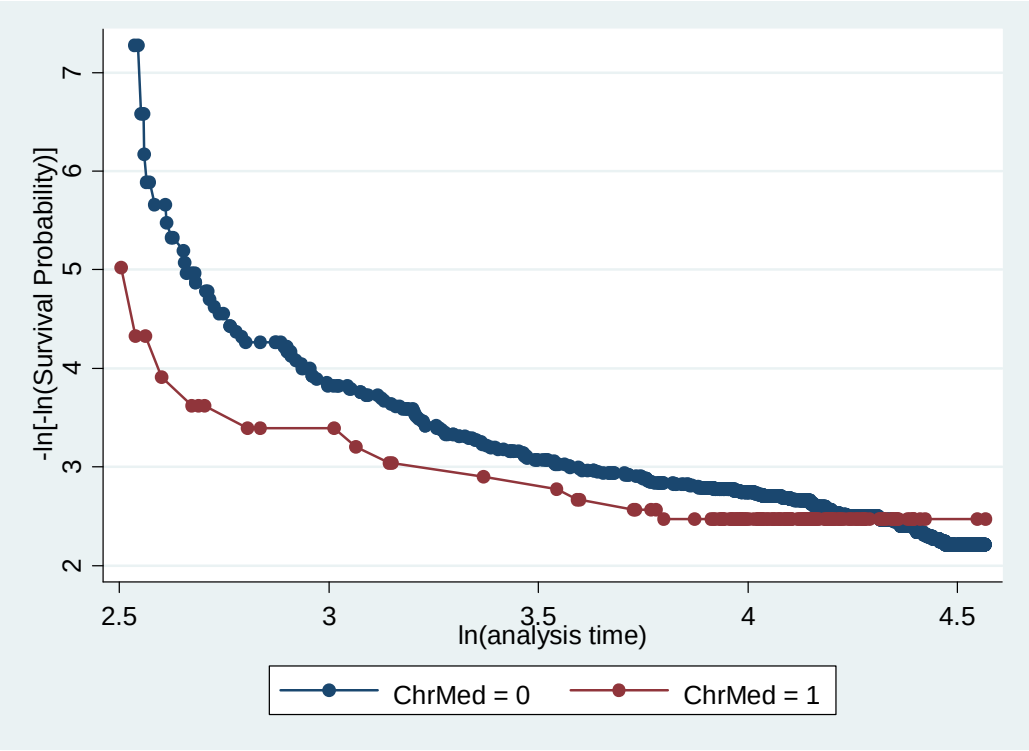

-ln[-ln(Survival Probability)]
2
3
4
5
6
7
2.5
3
3.5
4
4.5
ln(analysis time)
Male = 0
Male = 1

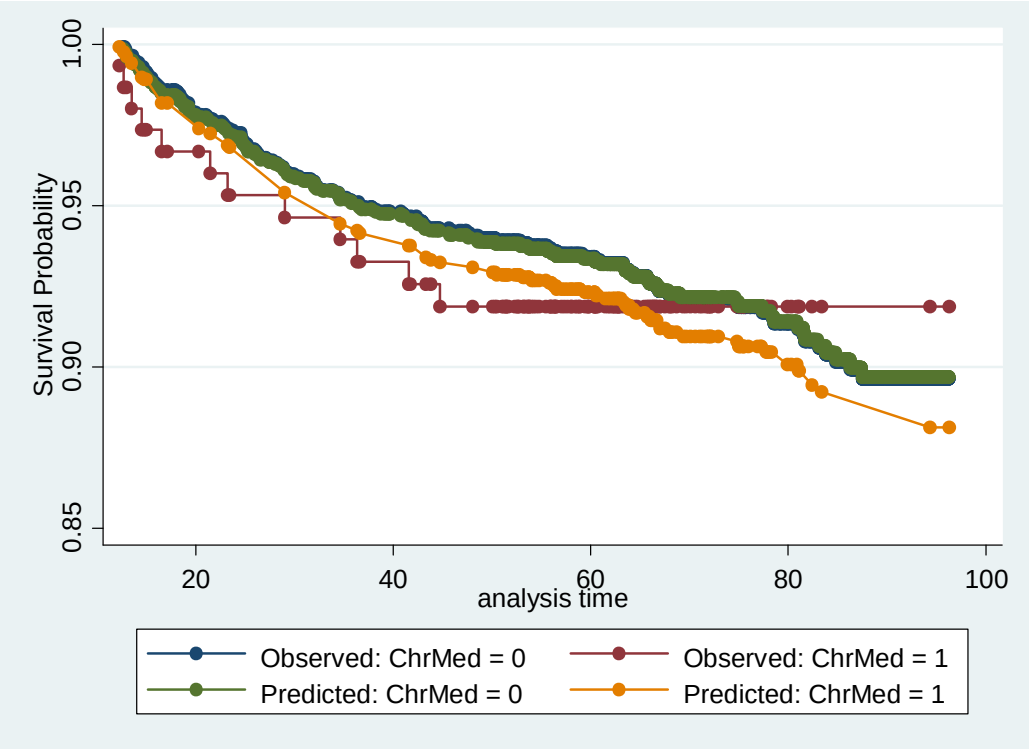

Survival Probability
0.85
0.90
0.95
1.00
20
40
60
80
100
analysis time
Observed: Male = 0
Observed: Male = 1
Predicted: Male = 0
Predicted: Male = 1



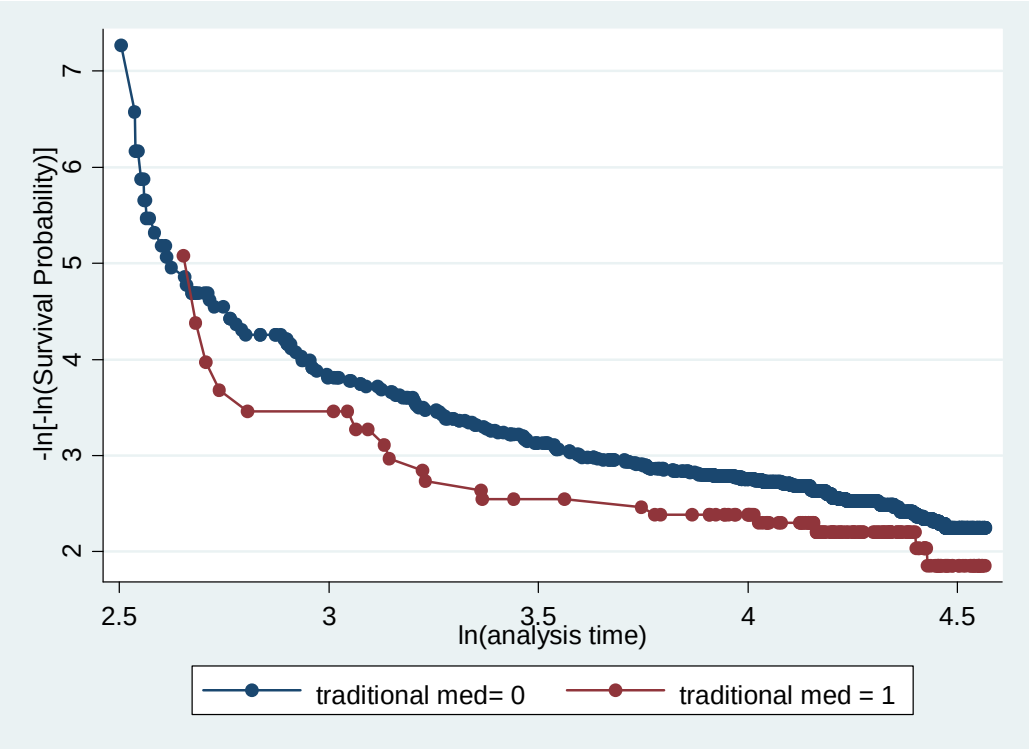
WHO34=WHO clinical stage 3 or 4

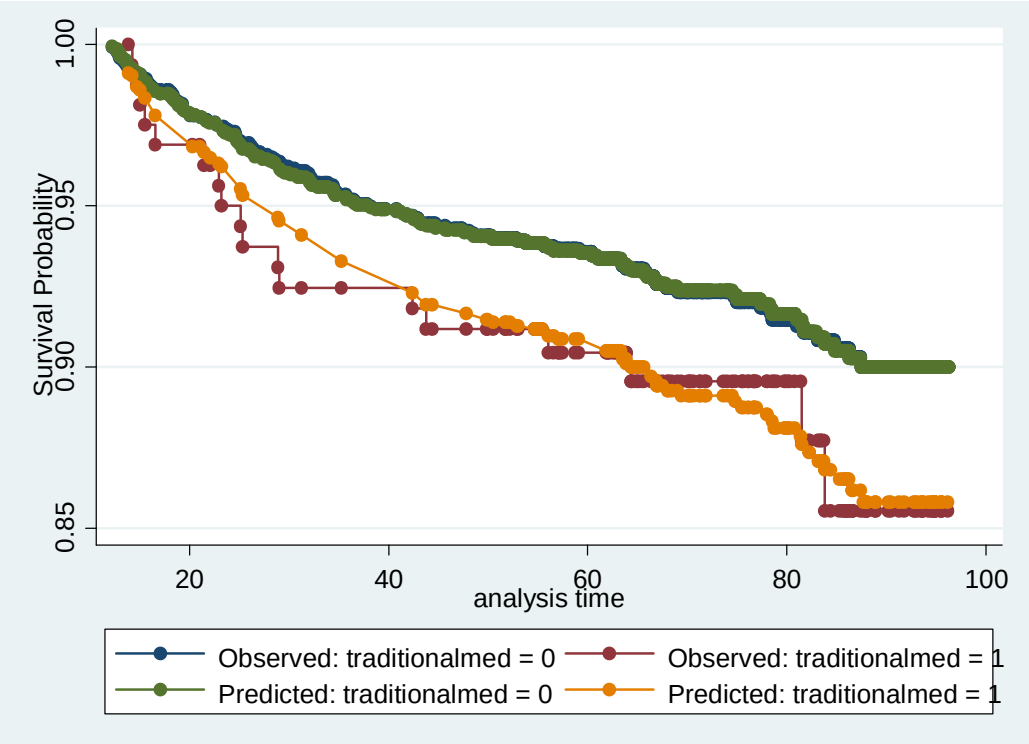


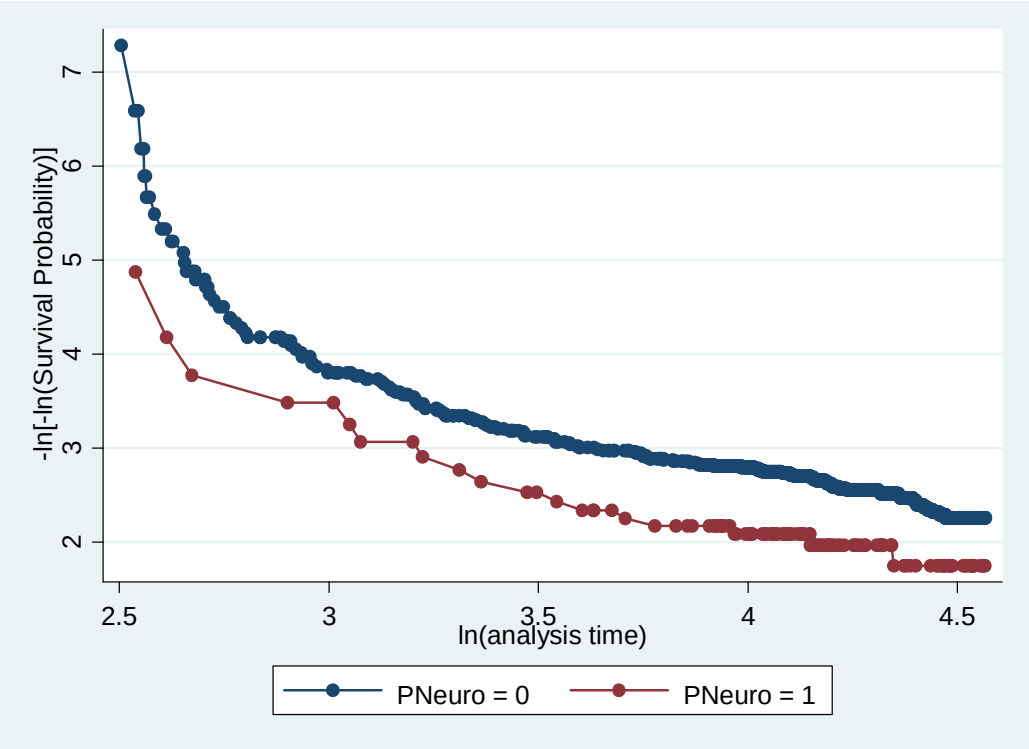




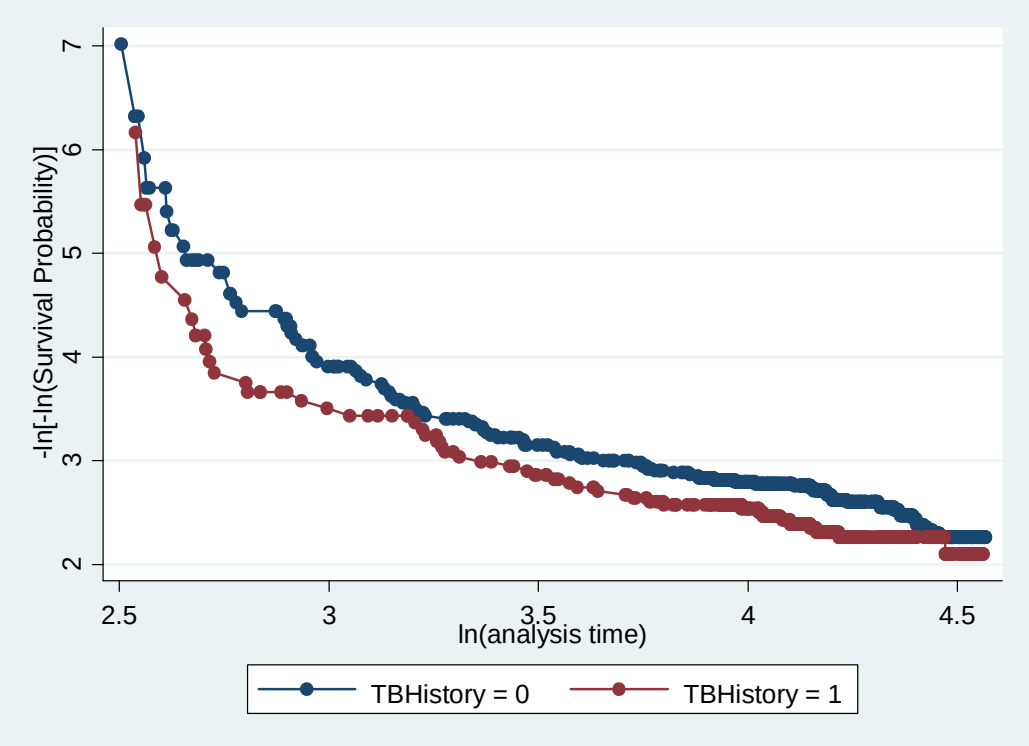
ChrMed= concomitant chronic medication

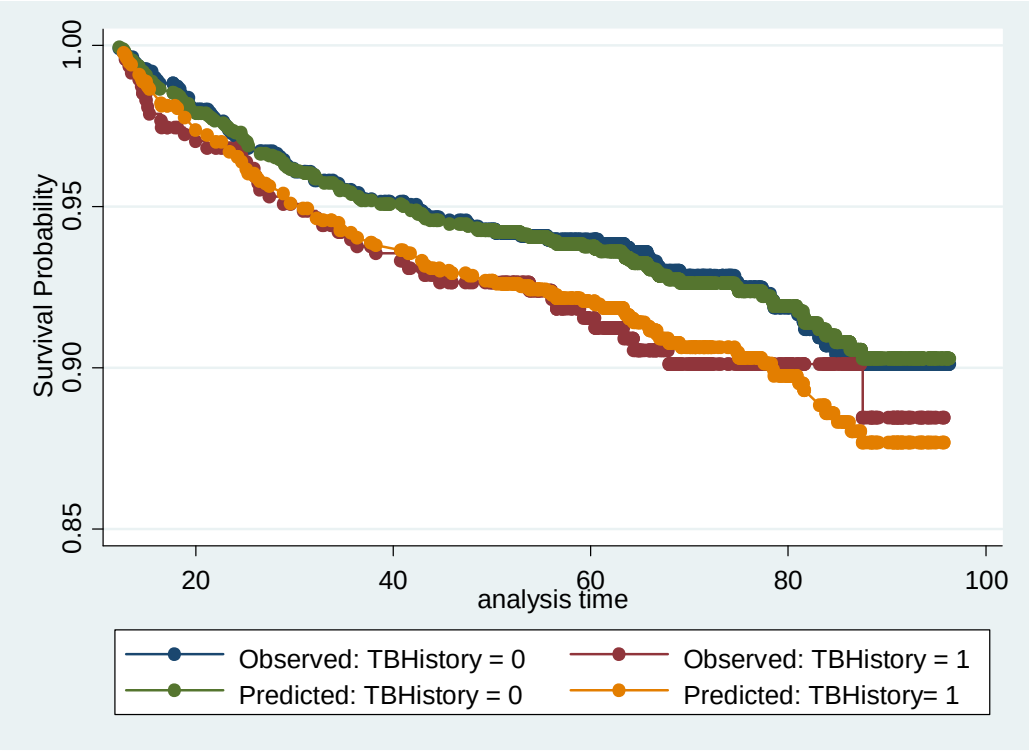

-ln[-ln(Survival Probability)]
2
3
4
5
6
7
2.5
3
3.5
4
4.5
ln(analysis time)
traditional med= 0
traditional med = 1

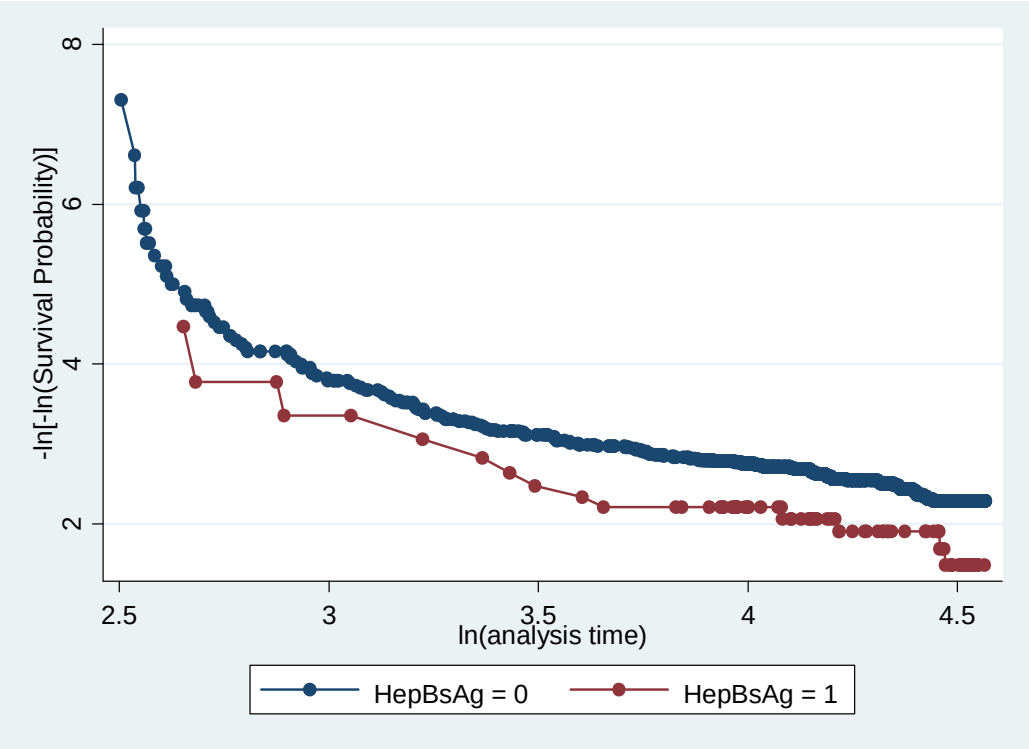

Survival Probability
0.85
0.90
0.95
1.00
20
40
60
80
100
analysis time
Observed: traditionalmed = 0
Observed: traditionalmed = 1
Predicted: traditionalmed = 0
Predicted: traditionalmed = 1



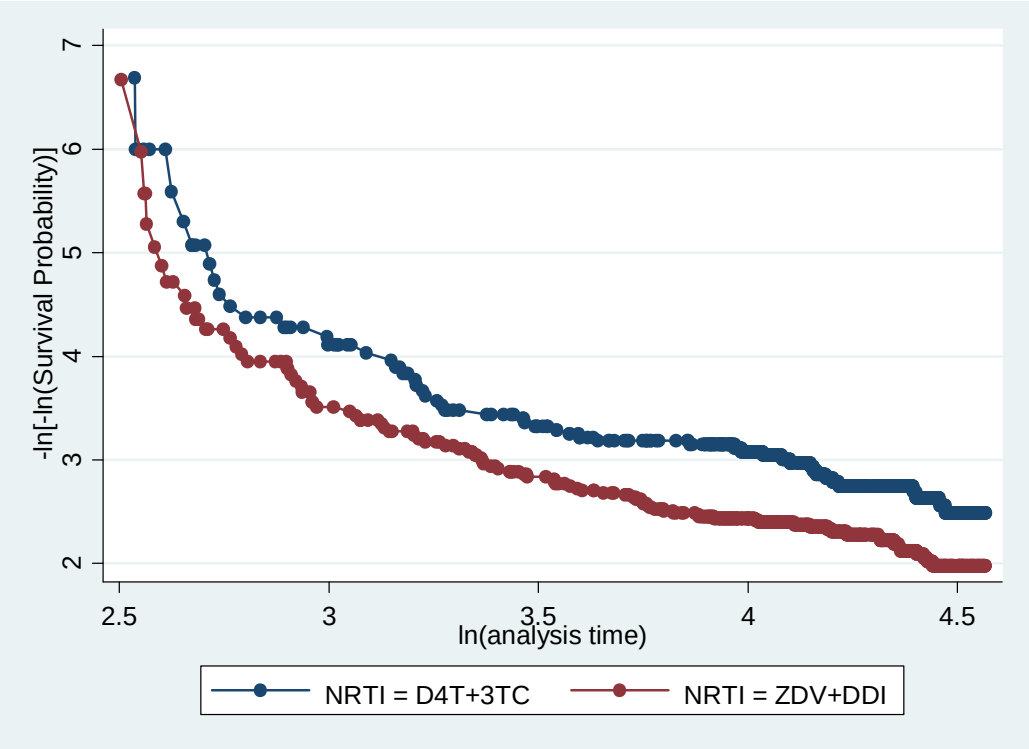
PNeuro= peripheral neuropathy at baseline

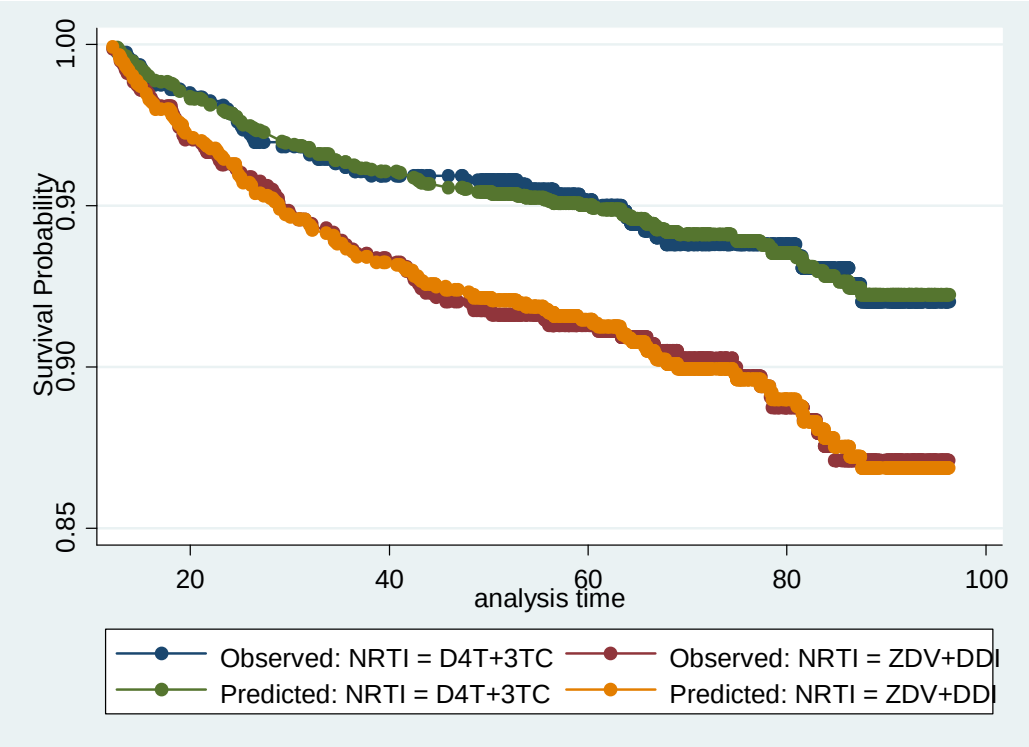




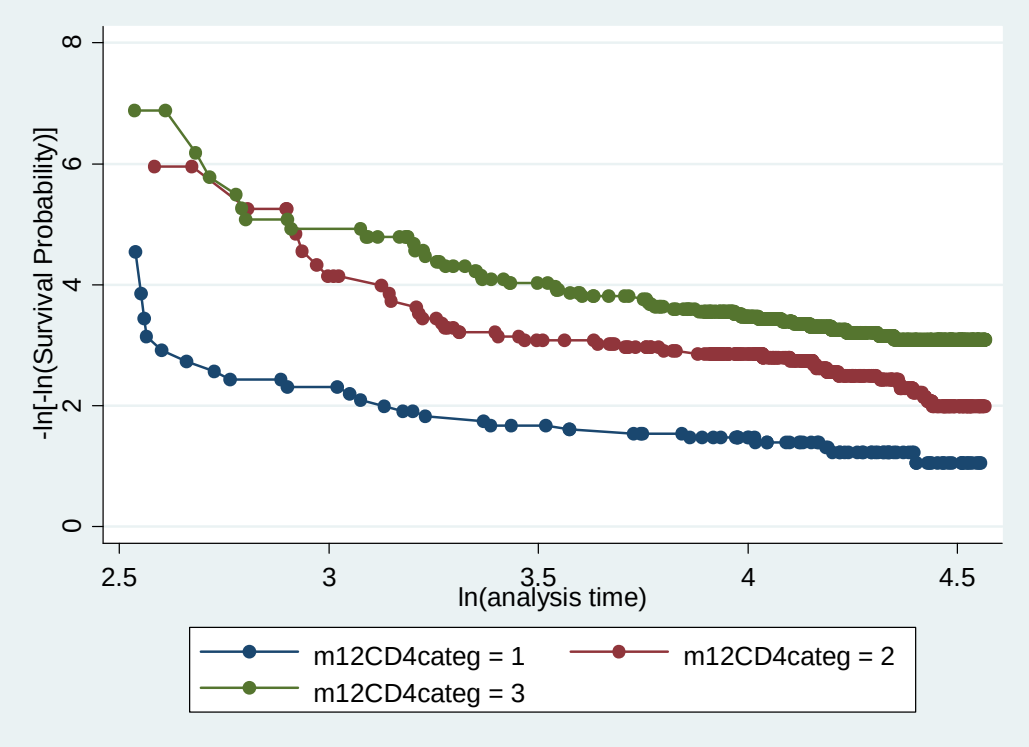


HepBsAg= Hepatitis B surface antigen positive

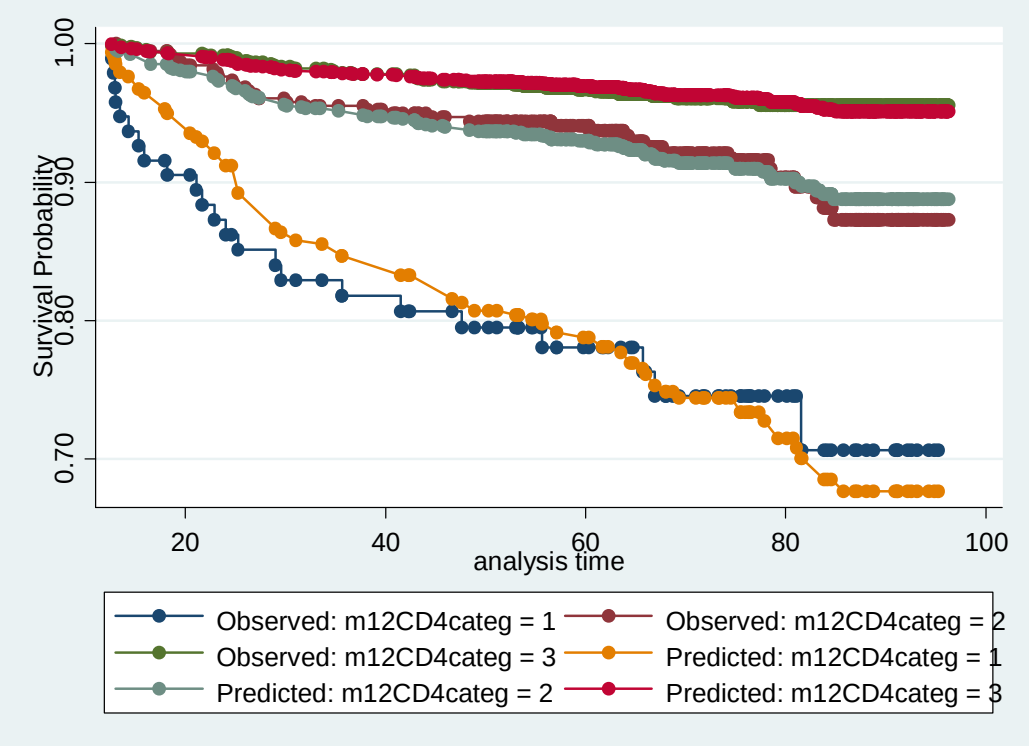

-ln[-ln(Survival Probability)]
2
3
4
5
6
7
2.5
3
3.5
4
4.5
ln(analysis time)
NRTI = D4T+3TC
NRTI = ZDV+DDI

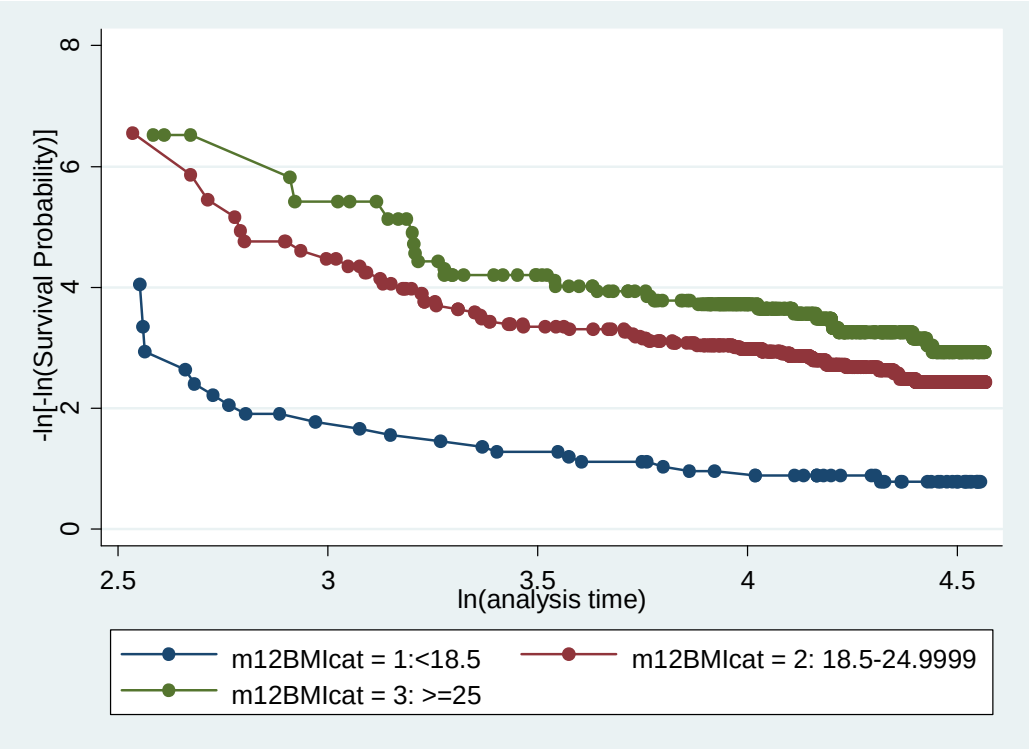

Survival Probability
0.85
0.90
0.95
1.00
20
40
60
80
100
analysis time
Observed: NRTI = D4T+3TC
Observed: NRTI = ZDV+DDI
Predicted: NRTI = D4T+3TC
Predicted: NRTI = ZDV+DDI

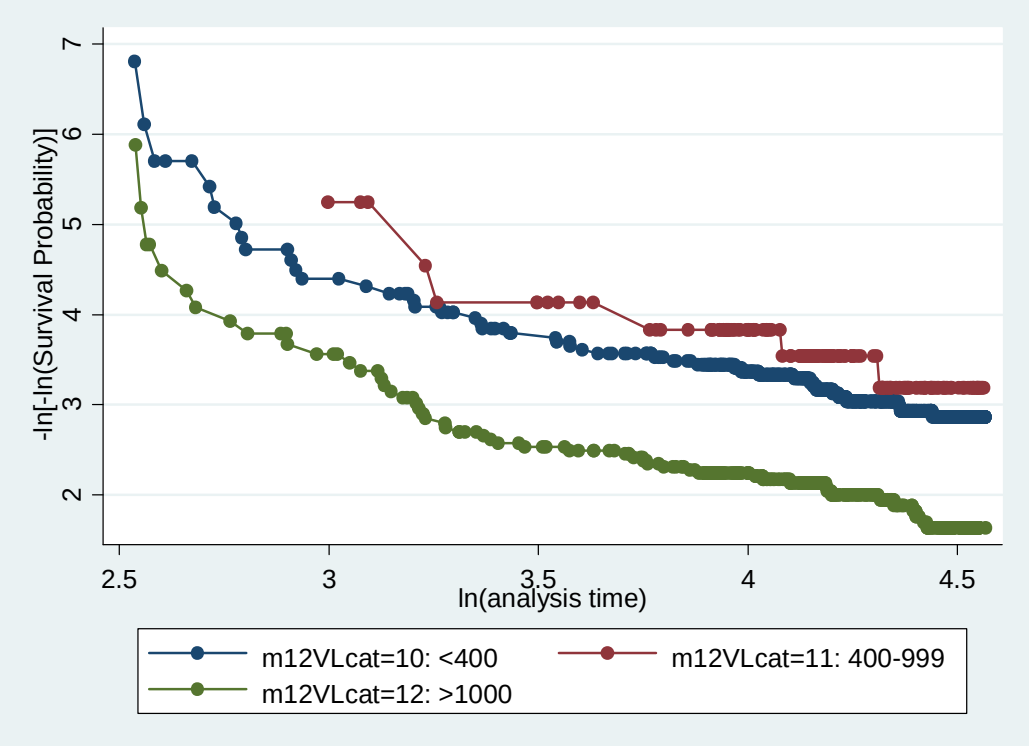
Testing PH assumption: month 12 values

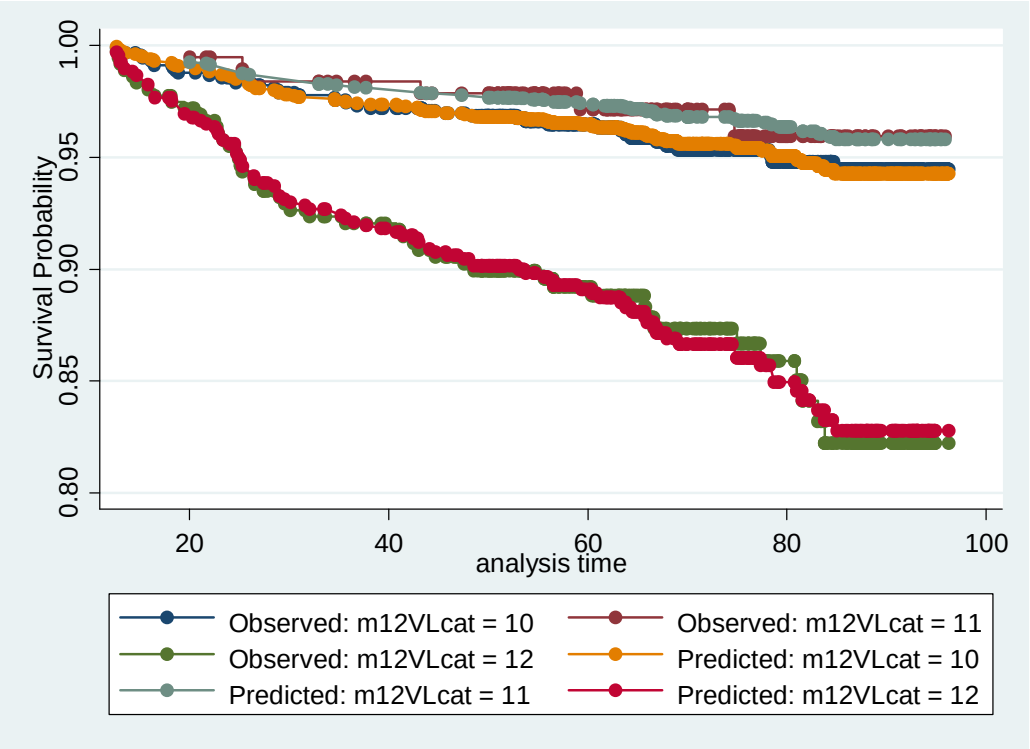


Month 12 CD4+ categories: m12CD4categ: (0/100 cells/mm^3=1)(101/200=2)(>200=3)

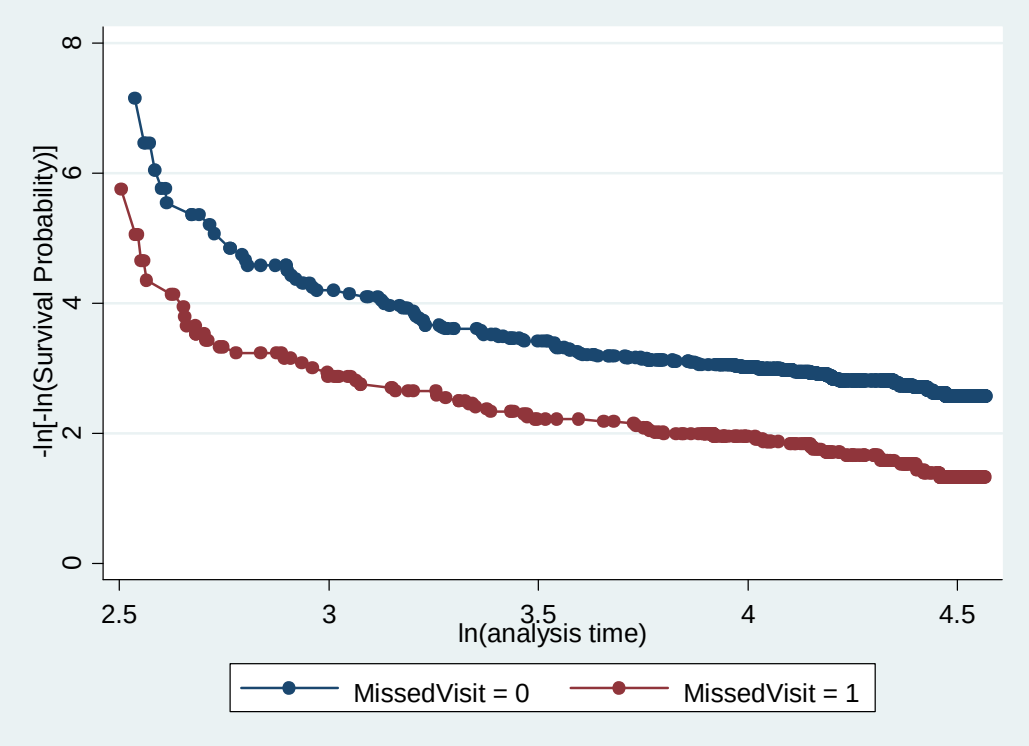



-ln[-ln(Survival Probability)]
0
2
4
6
8
2.5
3
3.5
4
4.5
ln(analysis time)
m12BMIcat = 1:<18.5
m12BMIcat = 2: 18.5-24.9999
m12BMIcat = 3: >=25

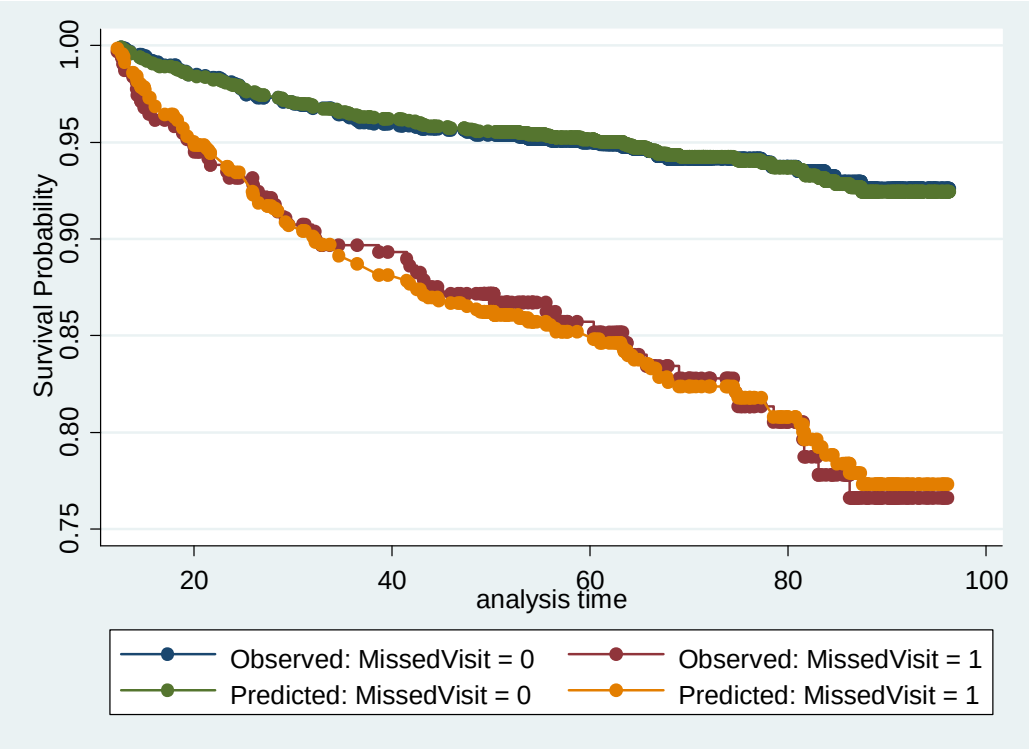

-ln[-ln(Survival Probability)]
2
3
4
5
6
7
2.5
3
3.5
4
4.5
ln(analysis time)
m12VLcat=10: <400
m12VLcat=11: 400-999
m12VLcat=12: >1000



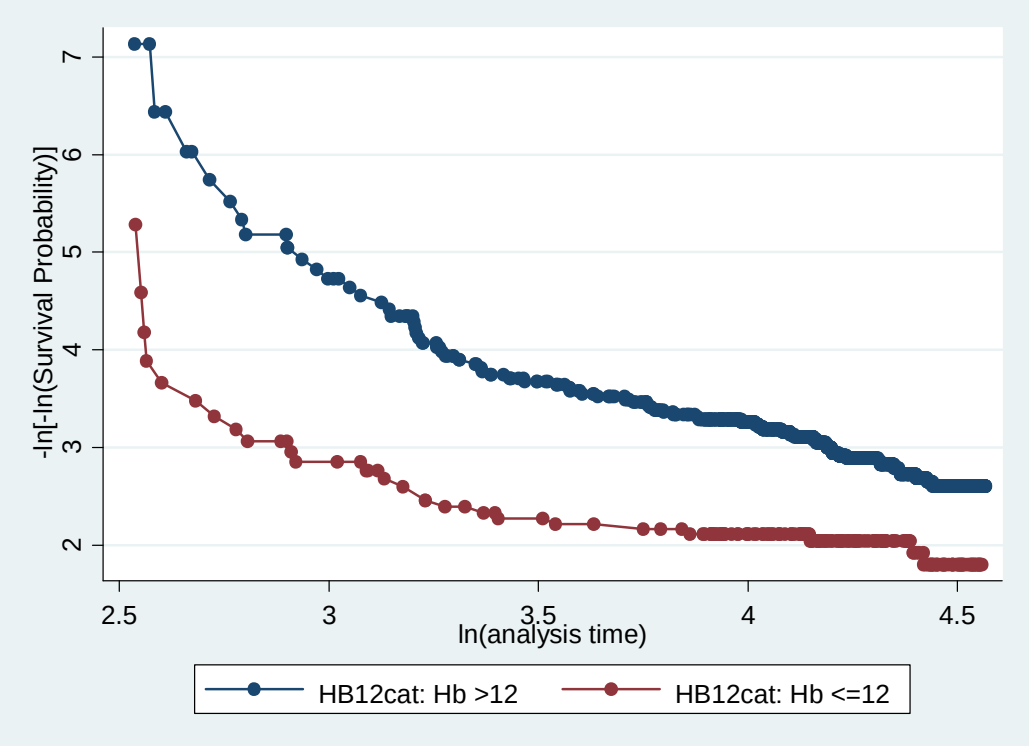
Month 12 Viral load: m12VLcat=10: <400 copies/mL; 11:400-999; 12:>/=1000



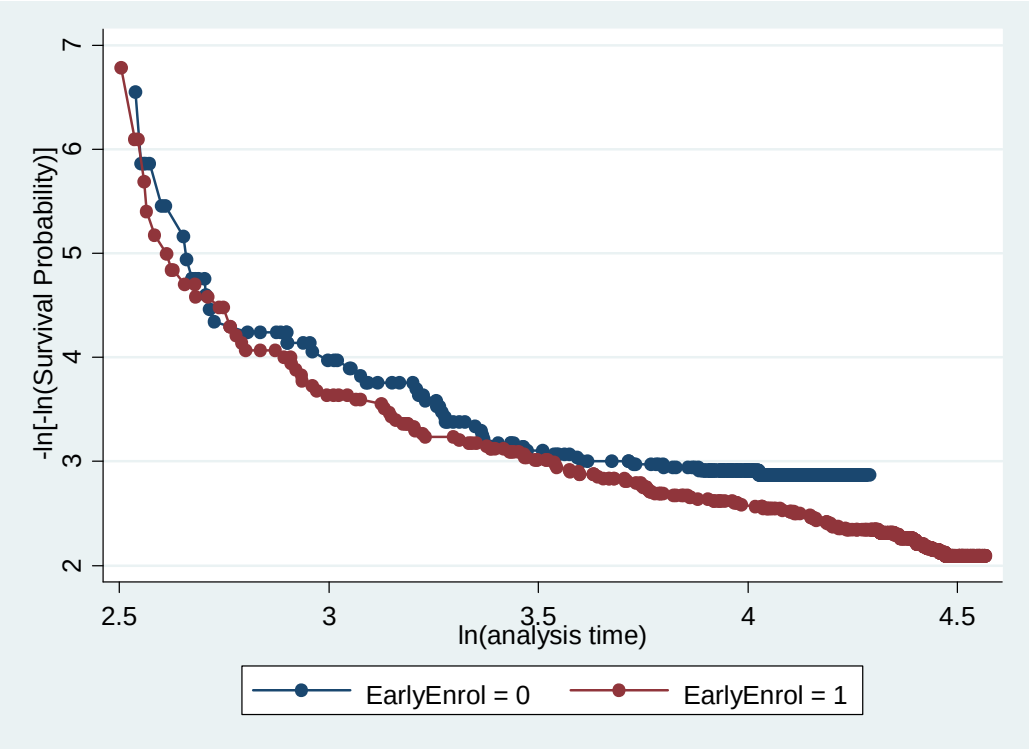
Missed Visit in the first 6 months

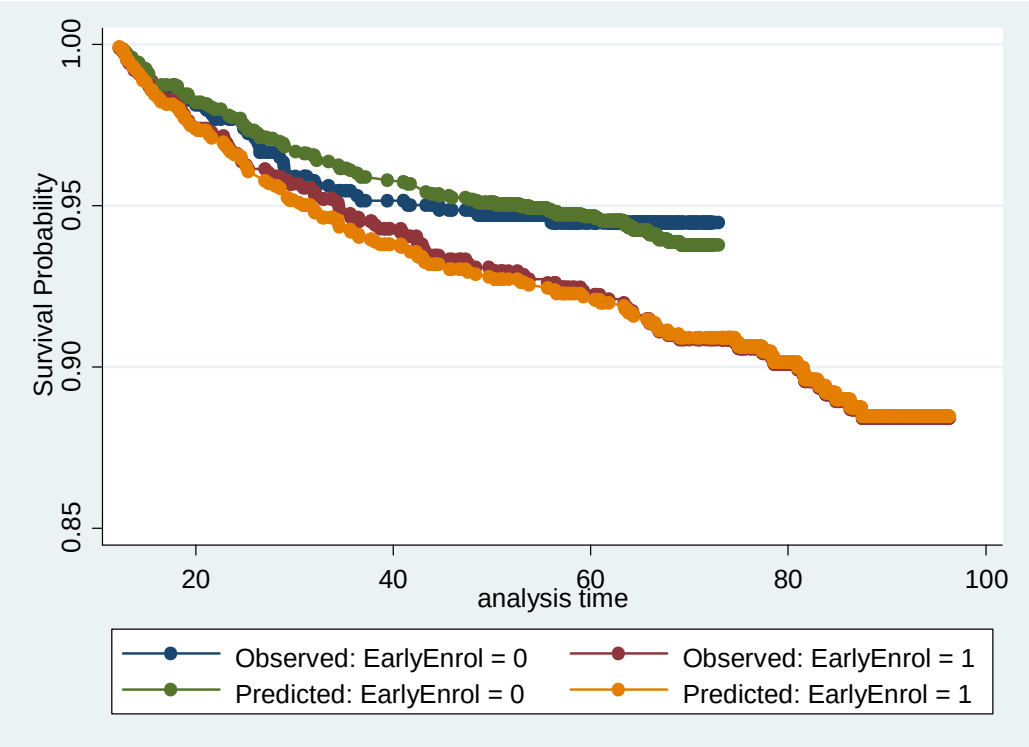


Missed Visit in the first 6 months

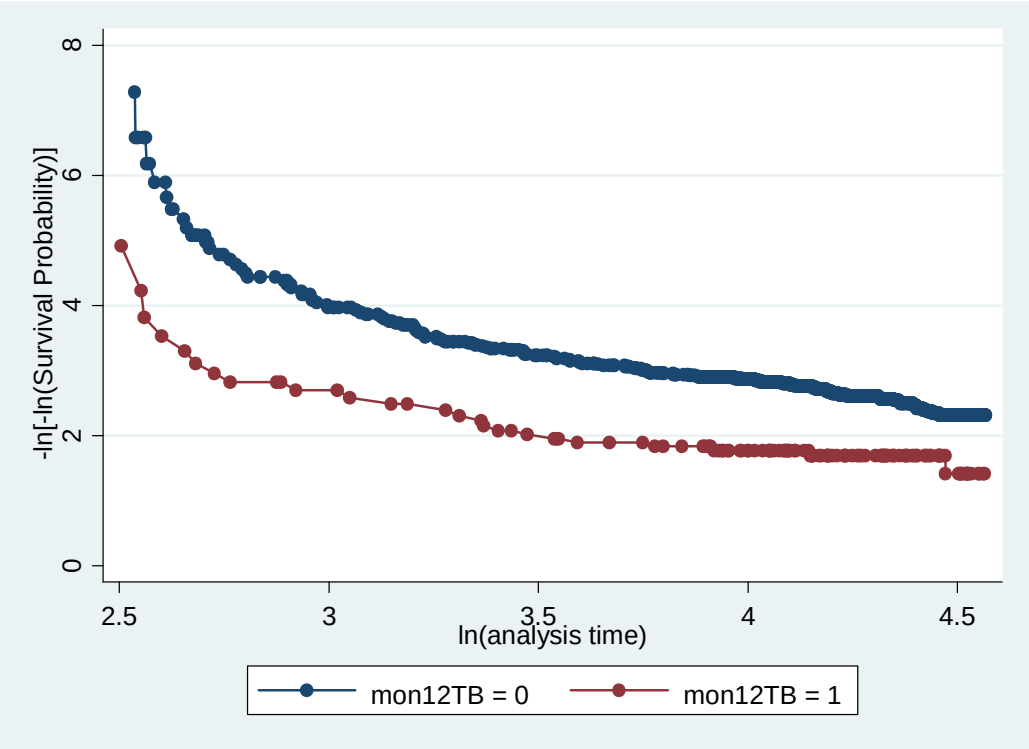


Month 12 haemoglobin





Early Enrolment into trial: 0=2006 and 2007; 1=2004 and 2005



Mon12TB= Incident TB within the first year of therapy.

Post-estimation test of proportionality of hazards

	rho	chi2	df	Prob>chi2
Male	0.16531	2.56	1	0.1093
NRTI	-0.00022	0.00	1	0.9983
m12Hb	0.09020	0.76	1	0.3839
1.m12BMIcat	-0.13154	1.28	1	0.2580
2b.m12BMIcat	.	.	1	.
3.m12BMIcat	0.11181	1.15	1	0.2835
1b.m12CD4~eg	.	.	1	.
2.m12CD4c~eg	0.09177	0.72	1	0.3970
3.m12CD4c~eg	-0.01550	0.02	1	0.8933
10.m12VLcat	-0.12171	1.25	1	0.2633
11.m12VLcat	0.01090	0.01	1	0.9215
12b.m12VLcat	.	.	1	.
Global test		13.54	9	**0.1395**