SURVIVAL AND RISK FACTORS FOR MORTALITY AMONG HIV/TUBERCULOSIS CO-INFECTED PATIENTS ON ANTIRETROVIRAL THERAPY IN A RESOURCE LIMITED SETTING

By

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DECLARATION

I certify that this dissertation is my original work and submitted for the degree of Masters of Science in Biostatistics program. It has not been submitted in part or in full to any University and/or any publication.

STUDENT

Signature_____ Date_____

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I, having supervised and read this dissertation, I am satisfied that this is the original work of the author in whose name it is being presented. I confirm that the work has been completed satisfactorily for presentation in the examination.

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DEDICATION

This work is dedicated to the Almighty God for his wisdom, power and grace, my wife Faith, my Mom and Dad who taught me the value of discipline and hard work and the entire family for their support and encouragement during my studies and subsequent writing of this thesis.

ABSTRACT

Background: Tuberculosis is the most common opportunistic infection and most frequent cause of mortality among HIV-infected persons in resource constrained settings and the number of patients with co-infection continues to grow rapidly.

Objective: To determine the survival and predictors of mortality among HIV/Tuberculosis co-infected patients on antiretroviral therapy at Wilkins Infectious Disease Hospital (WIDH), Harare.

Methods: A retrospective study in a cohort of 207 HIV/TB co-infected patients who presented to WIDH and started ART between 1 December 2004 and 1 March 2010 was carried out. A retrospective review of patient medical records was done. Kaplan-Meier method was used to construct survival functions, the log rank test was used to test equality of survivor functions across strata; we performed univariate and multivariate analysis and constructed a Cox-proportional hazards model to determine factors that determine survival in HIV/TB co-infected patients on ART.

Results: There were 45 (21.7%) deaths at the end of the study among whom 18 (40%) died in those who had extra-pulmonary tuberculosis and 27 (60%) in patients with pulmonary tuberculosis. The mortality rate was 9.8 deaths/100person years of follow-up. The cumulative mortality at 3, 6 and 12 months was 1%, 5% and15% respectively. Independent predictors of mortality were CD4 count<50cells/ul adjusted Hazard ratio [AHR] 2.37, 95% CI (1.158-4.856)], WHO stage four at baseline [AHR=2.69 95%CI (1.35-5.34)], cotrimoxazole use [AHR=0.29 95%CI (0.86-0.89)]. Haemoglobin was not found to be a risk factor.

Conclusion: Mortality was high in the first year relative to subsequent years. There was increased risk of death in patients co infected with HIV and TB who presented to the clinic with late stage disease as indicated by the WHO clinical stage criterion and low CD4 count at baseline and these were strong predictors of mortality. Collaboration of HIV/TB activities should be reemphasized and scale up of patients to access ART or effective treatment and control of TB among co infected patients. Increasing access of cotrimoxazole by patients on ART and interventions to identify patients before they develop these clinical markers will improve survival and increase benefits of therapy.

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LIST OF ACRONYMS AND ABBREVIATIONS

AFB	Acid Fast Bacilli
AIC	Akaike Information Criterion
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARVs	Antiretroviral Drugs
BIC	Bayesian Information Criterion
CBD	Central Business District
CCR5	Chemokine (C-C motif) receptor 5
CFR	Case Fatality Ratio
DF	Degrees of freedom
DOTS	Direct Observed Treatment Short course
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HIV/TB	The Intersecting Epidemic of tuberculosis and HIV
HR	Hazard Ratio
MOH	Ministry of Health
MTB	Mycobacterium Tuberculosis
OI	Opportunistic Infections
RNA	Ribonucleic Acid
SIV	Simian Immunodeficiency Virus
TB	Tuberculosis

UNAIDS	Joint United Nations Programme on AIDS
WHO	World Health Organization
WIDH	Wilkins Infectious Disease Hospital

DEFINITION OF TERMS

Aetiology: The study of causes or origins of disease.

AIDS: this refers to a progressive immune deficiency caused by infection of CD4+ T cells with the human immunodeficiency virus (HIV).

Co-infection: Infection with more than one virus, bacterium or other micro-organism at a given time. For example an HIV-infected individual may be co-infected with tuberculosis (TB).

Opportunistic infection: Any infection caused by a micro organism that does not normally cause disease in humans, occurs in persons with abnormally functioning immune system (as AIDS patients receiving immunosuppressive drugs).

Tuberculosis: is a common and often deadly infectious disease caused by *mycobacteria*, usually mycobacterium tuberculosis in humans. Tuberculosis often attacks the lungs but also affect other parts of the body.

Pulmonary tuberculosis: TB infecting the lungs, this is the most common form of active TB.

Epidemiology: The study of the frequency and distribution of disease in human population.

CD4 count: The number of helper T-lymphocytes in a patient's blood usually expressed as the number of cells per cubic millimeter.

Haemoglobin: is a protein found in red blood cells that carries oxygen from the lungs to the cells throughout the body.

HIV-1: The retrovirus isolated and recognized as the etiologic agent of AIDS.

Loss to follow up is defined as missing the last scheduled visit by ninety(90) days and above and all efforts have been made to try and contact the patients but have failed and his/her status is not known.

Survival rate: The percentage of people who are still alive after a particular length of time with a certain disease.

Survival function: is the probability that the time of death/failure is later than some specified time.

Right censoring: an observation is right censored it means that the information is incomplete because the subject did not have an event during the time that the subject was part of the study.

Left censoring: an observation is left censored if the event of interest has already occurred when observation of time begins.

Log rank test: is a test statistic that compares estimates of the hazard functions of the two groups at each observed event time.

Kaplan–Meier estimator (**product limit estimator**) estimates the survival function from life-time data. In medical research, it might be used to measure the fraction of patients living for a certain amount of time after treatment.

Non-parametric test: Distribution free methods which do not rely on assumptions that the data are drawn from a given probability distribution.

Regression: Regression is a statistical technique that is used to describe the relationship between two or more than two variables, or the study of dependent and independent variables.

Cox regression is a statistical technique that is used to determine the relationship between survival and several independent exploratory variables. Cox regression is useful for modeling the time to a specific event based upon the value of a given covariate.

Hazard function: In Cox regression, hazard is the event of interests occurring, that is the probability of the endpoint of an event of interest.

Covariate: In Cox regression, covariates are the independent variables. In Cox regression, covariates can be categorical or a dummy.

Median survival time: a period of time, often measured in months or years over which 50% of the patients are expected to be alive.

CHAPTER 1: INTRODUCTION

1.1 Background

Tuberculosis is an ancient disease which remains a major public health problem in much of the developing world. It has become a major public health burden in Asia and Sub-Saharan Africa. It is the most prevalent infectious cause of human suffering and death worldwide [1]. Increasing spread of Human Immunodeficiency Virus (HIV) causing Acquired Immunodeficiency Syndrome (AIDS) has become a major contributor in the increasing incidence of tuberculosis.

HIV fuels progression of tuberculosis infection to active disease in people infected with tuberculosis and HIV infected individuals co-infected with tuberculosis have an annual risk of 5-15% of developing active tuberculosis. This is in sharp contrast to the 5-10% lifetime risk of people who are infected with TB bacilli but who are not infected with HIV [2, 3].

TB and HIV co-infection is recognized as a major setback to both tuberculosis and HIV infection control programmes. HIV infection is a potential risk factor for tuberculosis and contributes to the development of TB from latent and exogenic re-infection [4,5]. Together HIV and TB form a lethal combination, each contributing to the other's progress, thus their effect is bidirectional. Correct diagnosis and treatment of TB help to reduce the burden of TB, given that infectious cases are detected and treated successfully. However, there are difficulties in achieving the goal of reducing the tuberculosis burden due to a number of challenges, such as difficulties in diagnosing tuberculosis in HIV infected patients due to unusual clinical picture with increase in smear negative acid fast bacilli (AFB negative) pulmonary tuberculosis disease, and atypical findings on chest radiography. This can contribute to over diagnosis or under diagnosis of smear negative disease leading to an increase in case

fatality ratio (CFR) if diagnosis is delayed [6]. The number of new TB cases has tripled in high HIV prevalent countries in the last two decades and has been declining since (WHO, TB/HIV Fact Sheet 2009).The twin problem should be simultaneously taken care of to stop the future pandemic. Treatment of TB through the direct observed treatment short course (DOTS) and providing combination ART to patients in resource limited settings can help reduce the impact of the deadly duo.

The ultimate goal of providing antiretroviral therapy is to delay progression to AIDS, improve quality of life and improve survival outcomes of patients. This however, has come under severe threat due to the dual influence of HIV and tuberculosis. HIV and TB are intricately linked to factors such as malnutrition, poverty, homelessness and overcrowding.

1.2 Aetiological agents and pathogenesis of tuberculosis and HIV infections

The aetiological agents of TB and HIV share some common characteristics, immunity to both is cell-mediated and both have long latent periods so that disease may only manifest after several years since acquiring infection. The aetiological agent of tuberculosis is *mycobacterium tuberculosis* (MTB) and the causative agent for HIV is a retrovirus [7].

1.2.1 Aetiology of tuberculosis

Tuberculosis was discovered by Robert Koch in 1985 and his findings on aetiology of tuberculosis were presented in the famous paper to the Physiological Society of Berlin. His description of the method of staining specimen slides, the method of inoculating the bacilli into animals was a major advance in the understanding of TB bacteriology, further to this it enhanced in finding the remedy for tuberculosis [8].

Mycobacterium tuberculosis belongs to the genus *Mycobacterium*. The genus is classified into the mycobacterium tuberculosis complex, which produces tuberculosis disease in humans and other non-

tuberculosis mycobacterium species which can produce disease in immunocompromised individuals. The mycobacterium complex includes the following *mycobacterium tuberculosis, M. bovis, M. africunum, M. macroti, M. avium and M. caneti* [7].

M. bovis is mainly responsible for infecting cattle and goats but can infect man. *M. microti* is infectious to some rodents but infection of humans is unclear and the recently discovered *M. caneti* can also infect humans. *Mycobacterium tuberculosis* can be separated from others by classification tests, it grows in culture media after two weeks, it is aerobic and sensitive to antituberculosis drugs [9].

1.2.2 Pathogenesis of Tuberculosis

Mycobacterium Tuberculosis (MTB), the causative organism of tuberculosis spreads almost exclusively by the respiratory route. A person with active pulmonary tuberculosis releases infectious droplets while coughing or sneezing. When a susceptible individual inhales droplets less than 10 microns in size, they will reach the alveoli (tiny air sacs) in the lungs, and seed a tuberculosis infection. Given a robust immune system, he does not progress to tuberculosis disease. Persons with latent tuberculosis infection are asymptomatic and do not spread tuberculosis to others. The only evidence of them having had tuberculosis infection will be a positive tuberculin skin test. Because of the progressive depression of the cell mediated immunity in patients with HIV disease, the immune system cannot hold the organism in check. Rapid multiplication occurs in multiple organ sites simultaneously. Patients with HIV disease may be unable to limit the multiplication of *Mycobacterium tuberculosis* after initial dissemination and thus HIV infected persons may have involvement of multiple sites. More commonly, HIV infected patients with dormant tuberculosis infection will have reactivation of the latent infection because of diminished cell mediated immunity [3, 10].

1.2.3 HIV infection Aetiology

The causative agent of HIV infection and AIDS is lentivirus of the retrovirus family. There are two human HIV viruses these are the HIV-1 and HIV-2. HIV-1 is the causative agent of most cases of HIV infection worldwide and HIV-2 is more confined to West Africa, in 1981 the first description of AIDS was made. The HIV virus was discovered and isolated in Paris at the Pasteur Institute. The virus was obtained from T lymphocytes of patients with lymphadenopathy [11, 12]. The Besthand Maryland centre developed a specific factor for culturing T lymphocytes which was essential for isolating retroviruses. Gallo and colleagues further proved the HIV virus as the cause of HIV and contributed to the development of HIV testing.HIV-1 and HIV-2 are both recognized to have zoonotic origins. HIV-1 has been accepted as having originated from simian immunodeficiency virus (SIV) from chimpanzees whereas HIV-2 is believed to have originated from sooty mangabeys[11,13].

1.2.4 HIV Pathogenesis

The HIV virus is formed of a core protein enclosed in gag protein, p24 and a two RNA structure. The viral structure also contains enzymes important for its replication, RNA reverse transcriptase and HIV specific protease. The viral envelope carries the g120 antigen which is important for interaction with CD4 protein in the T-cells. The virus attacks the CD4 T-cell and inflicts a decline in CD4 count. The CD4 contain receptors for HIV antigen, the chemokine receptors of CCR5 and CXCR4. HIV-infected macrophages attract T-cells and bring them in contact with the HIV virus [14, 15].

1.3 Epidemiology of HIV and TB

About a third of the HIV-positive population worldwide is co-infected with Mycobacterium tuberculosis. Globally, 9% of all tuberculosis cases in adults are attributable to HIV. Studies from Sub-

Saharan Africa have recorded HIV seroprevalence rates of 50 to 70% in patients with tuberculosis. In Asia the rate of HIV infection in tuberculosis patients has been lower. An HIV-positive person infected with *Mycobacterium tuberculosis* has a 50 - 60% lifetime risk of developing TB disease as compared to an HIV-negative person who has only a 10% risk [10].

WHO estimates that the largest number of new TB cases in 2008 occurred in the South-East Asia Region, which accounted for 34% of incident cases globally. However, the estimated incidence rate in sub-Saharan Africa is nearly twice that of the South-East Asia Region with over 350 cases per 100, 000 population [3].

An estimated 1.3 million people died from TB in 2008. The highest number of deaths was in the South-East Asia Region, while the highest mortality per capita was in the Africa Region at 48 per 100, 000 population against the world's 20 per 100, 000 population (WHO, Fact sheet 104).

Around one-quarter of deaths in people with HIV worldwide were caused by TB in 2007, around 500, 000 people with HIV died of TB in 2008 and there were 1.4 million HIV positive TB cases. Of the fifteen countries with highest incidence of TB in HIV-positive people, all but one is in Sub-Saharan Africa. In countries like Lesotho, Swaziland, South Africa, Zimbabwe, Namibia and Botswana the incidence of HIV-positive TB cases is above 400 cases per 100, 000 people. Seventy nine percent (79%) of HIV positive TB cases are estimated to occur in sub-Saharan Africa.

Zimbabwe is one of 22 high burden (severely affected) countries in terms of tuberculosis. The prevalence of TB in HIV infected patients was estimated at 270 cases per 100, 000 people in 2007 and the TB mortality rate in HIV positive people was estimated to be at 213 cases per 100, 000 people (WHO, 2009).

1.4 Impact of HIV on Tuberculosis

The HIV epidemic has the potential to increase the incidence of tuberculosis. This is mainly because HIV increases the risk of disease reactivation in people with latent tuberculosis and because HIV-infected persons are more susceptible to new tuberculosis infection [17]. These patients would add to the incidence of tuberculosis thereby leading to increase in new infections and re-infection. HIV is the most powerful risk factor for progression of tuberculosis infection to tuberculosis disease. Also, HIV-infected persons who become newly infected with *Mycobacterium tuberculosis* rapidly progress to active tuberculosis disease.

According to the Internet Journal of Pulmonary medicine volume 10 of 2008, TB is the most common opportunistic infection and a major cause of mortality among HIV-positive persons. It is the first manifestation of AIDS in more than 50% of cases in developing countries. HIV by itself does not cause multi-drug resistant tuberculosis, but fuels the spread of this dangerous condition by increasing susceptibility to tuberculosis infection and also accelerating the progress from infection to disease.

1.5 ART in TB-HIV co infected patients.

In 2002, Zimbabwe committed to an expanded effort to provide ART, access to ART remains limited, however because of insufficient financial and human resources. According to MOH, 100, 000 patients received ART in 2007 out of 480, 000 HIV-infected people in need of treatment. UNAIDS said only 15% of HIV infected people received ART in 2006. A total of 390, 000 (350, 000-440, 000) were in need of ART in 2006 though according to 2009 WHO recommendations for starting ART of CD4< 350, this was estimated to be at 640, 000 (600, 000- 690, 000). In 2007 there were 610, 000 (580, 000-670, 00) patients in need of ART according to the revised guidelines of 2009 [19]. In 2010 the need for

ART is expected to have declined and it is estimated that 503 678 adults would be in need. Of these patients approximately a third of them are expected to have TB.

The government of Zimbabwe started rolling out ART in April 2004 and the goals of ART are:

- Maximal and durable suppression of replication of HIV,
- Restoration and/or preservation of immune function,
- Reduction of HIV related morbidity and mortality, and
- Improvement of quality of life

Medical and psychosocial issues need to be addressed prior to initiation of ART. Patients should preferably begin the therapy before the CD4 cell count drops to or below 200 cells/mm^3. Initiation is recommended for patients with WHO clinical stages 3 and 4, further to this there should be demonstration of reliability by patient e.g. compliance with cotrimoxazole prophylaxis keeping appointments as this may give an indication of the likelihood of adherence to antiretroviral therapy [20].

Use of ARVs in patients with TB should be treated with caution; rifampicin (cornerstone of TB programme) interacts adversely with some antiretroviral agents such as protease inhibitors. The caution regarding rifampicin use together with nevarapine has been downgraded but it is still recommended to use Efavirenz. In patients with HIV-related TB and who are not yet on ART, treatment of TB usually takes priority. The optimal time to initiate ART is not yet clear though WHO recommends that in patients with CD4 cell count below 200cells/mm^3, ART should be started between two and eight weeks after start of TB therapy when patient has stabilized on TB treatment as early initiation of ART has been found to reduce mortality greatly and improve survival.

Management of co-infected patients is very tricky in resource limited countries and clinicians are faced with pressing questions in order to reduce mortality and improve survival in these patients, one such question is when to start highly active antiretroviral therapy (HAART) in patients who requires both anti-TB and ART. On one hand clinicians are understandably reluctant to delay initiation of HAART in patients with advanced HIV disease (particularly in patients with very low CD4 counts i.e. less than 100cells) because of concerns about occurrence of new opportunistic infections or AIDS related illness. On the other hand antiretroviral drugs and anti-TB drugs have overlapping toxicities, so concomitant HAART and TB treatment can confuse the clinical picture when signs of toxicity are evident. Further to this both clinician and patient may question patient's ability to adhere to 3 ARV drugs and 4 anti-TB drugs at the same time. HAART initiation in patients with active TB can also lead to an early paradoxical worsening of signs/symptoms associated with TB a condition known as immune reconstitution syndrome (The Medscape).

In light of these factors another possible approach as adopted in the Zimbabwe HAART guidelines in 2005 is to delay the initiation of HAART until the typical 2 months, 4 drug intensive phase of TB treatment has been completed, the patient has demonstrated adherence to TB medications and continuing of treatment requires only two drugs. WHO has also modified its guidelines in line with new evidence it is gathering and at the Vienna conference in 2010 the new guidelines were adopted [21].

Table 1: Guidelines for Initiating ART in HIV/TB co-infected Adults.

Target Population	2010 ART Guideline	2006 ART Guideline
HIV/TB co-infection ARV-naïve individuals	Presence of active TB disease irrespective of CD4 cell count	Presence of active TB disease and CD4<=350 cells/mm^3. ART initiation can be delayed if CD4>=200 cells/mm^3

Adapted from WHO, Antiretroviral therapy for HIV infection in adults and adolescents 2010.

In response to the dual epidemic internationally recommended TB/HIV collaborative activities have been postulated (Global Fund Fact sheet 7, 2009), these include,

1. Decreasing the burden of tuberculosis in People living with HIV/AIDS (PLWH)

To decrease the burden of TB in PLWH it was suggested that the following activities be implemented, promoting TB case finding, introduction of isoniazid preventive therapy and ensuring TB infection control in health care and congregate settings.

2. Decrease the burden of HIV in TB patients

Activities to reduce burden of HIV in TB patients were also brought to the attention of the health care providers and these include provision of HIV testing and counseling, communication of HIV prevention methods, introduction of cotrimoxazole preventive therapy, provision of HIV/AIDS care and support and provision of ART

1.6 Problem Statement

TB is the most common opportunistic infection and most frequent cause of mortality among HIV-infected persons in resource constrained settings and the number of patients with co-infection continues to grow rapidly. It is the first manifestation of AIDS in more than 50% of cases in developing countries [17]. Tuberculosis has reemerged as an important public health problem in Zimbabwe, following a stabilization of the TB rates in the early 1980s at approximately 50 per 100,000 people. Since 1987 there has been a drastic increase to 250 per 100,000, which is largely attributable to the human immunodeficiency virus (HIV) epidemic. In 2007 WHO reported that 1 in 4 deaths of HIV-infected patients were caused by TB infection. The high burden of deaths in people with HIV is especially alarming because TB is preventable. Mortality rates are high among HIV/TB co-infected patients who present for care with advanced disease. Improving outcomes from both diseases requires coordinated treatment programmes, understanding of survival patterns and risk factors for mortality among the co-infected patients. Despite adequate anti-tuberculous therapy and availability of ART, many individuals co infected with TB and HIV have an accelerated course of HIV disease and shortened survival.

Zimbabwe has not been spared either from this scourge and it is against this background that it is imperative to understand the risk factors for mortality among TB-HIV co-infected patients presenting at Harare's Wilkins Infectious Disease Hospital as well as their survival patterns so that targeted interventions can be made. Detection of risk factors related to early death in these patients could be of aid for clinical and administrative decision-making. These results can also be generalised to other places in the country with similar settings.

CHAPTER 2: LITERATURE REVIEW

2.1 Clinical Presentation of tuberculosis in HIV infected patients

The clinical presentation of tuberculosis in HIV infected patients varies depending on the severity of immunosupression. Clinical presentation of tuberculosis in persons with early HIV infection has been found to be similar to that observed in immuno-competent and HIV-negative patients. In immuno-competent patients, pulmonary tuberculosis is the most common form of tuberculosis encountered and accounts for about 80% of the cases. While extra pulmonary tuberculosis accounts for only 20% of the cases of tuberculosis in HIV-negative patients, it accounts for 53-62% of cases in HIV-positive patients. The most common extra pulmonary site is the lymph node. However, neurological, pleural, pericardial, abdominal and virtually every body site can be involved in HIV-positive patients. In studies reported from India, extra pulmonary tuberculosis constituted 45 to 56% of all the cases of tuberculosis in persons with AIDS [23, 24]. Further, extra pulmonary tuberculosis by itself was not associated with decreased CD4 but patients with a combination of pulmonary and extra pulmonary tuberculosis had significantly lower CD4 counts.

Antiretroviral therapy is lifesaving in patients with advanced HIV infection, but the magnitude of benefit in HIV-infected patients receiving tuberculosis treatment remains uncertain, and population-based data from developing countries are limited. In developing countries, many HIV-infected persons frequently receive the diagnosis of HIV infection or AIDS after first having TB diagnosed at a health facility. The proportion of HIV-infected TB patients who die during TB treatment is high: an estimated 6%–39% die during TB treatment in sub-Saharan Africa (WHO report 2005). Deaths occurring in the first few months after TB diagnosis are more likely TB related, whereas deaths occurring later are more likely to be attributable to other HIV-related illnesses [25].

2.1.1 Related Studies on TB and HIV

From a study done in Thailand (ART during tuberculosis Treatment) in 2004 it was observed that, physicians often do not prescribe ART to HIV-infected TB patients because of concerns about drug-drug interactions, overlapping toxicities, immune reconstitution syndrome, and pill burden. Expert groups and the World Health Organization (WHO) also recommend that public health programs make treatment of TB the first priority and ideally begin ART after TB treatment is tolerated and CD4+ T-lymphocyte (CD4) count is measured. In the same study results indicated that, in univariate analysis restricted to the 290 patients with an outcome of cured, completed treatment, failed treatment, or died, several factors associated with death during TB treatment were analyzed. For all TB patients', having an unknown CD4 count was associated with increased likelihood of death, and receiving cotrimoxazole was associated with reduced mortality but a further multivariate analysis showed that cotrimoxazole use was not associated with mortality while an unknown CD4 count remained a risk factor [26].

Weight gain within six months of starting antiretroviral therapy resulted in better survival and improved clinical outcomes, in particular among the most severely malnourished, at district clinics in Lusaka, Zambia. Failure to gain weight six months after the start of ART increased the chance of death tenfold when compared with those who had gained over ten kilogrammes. The authors also cite analyses of patient outcomes in Zambia, Malawi and Tanzania which showed a low BMI (malnutrition) at the start of ART to be an independent predictor of early death [27].

In another study done in Thailand on survival rate and risk factors of mortality among HIV/tuberculosis co-infected patients with and without antiretroviral therapy it was found that survival rates at 1, 2, and 3 years after TB diagnosis were 96.1%, 94.0%, and 87.7% for those who were receiving ART. Cox proportional hazard model showed that gastrointestinal TB and multidrug

resistant TB were associated with higher mortality rate (P<0.05) and also patients who delayed ART≥6 months after TB diagnosis had a higher mortality rate than those who initiated ART<6 months after TB diagnosis (p= 0.018, hazard ratio=2.651, 95% confidence interval=1.152-6.102). Initiation of ART within 6 months of TB diagnosis was associated with greater survival.

In a recent prospective study, survival experiences in South African cohort on TB treatment according to time of HAART initiation [31], there was no significant difference in risk of death if antiretroviral treatment was initiated within two months of beginning TB treatment or more after two months after beginning TB treatment. However baseline CD4 cell count, BMI and haemoglobin level at time of ARV initiation were significant risk factors for mortality regardless of time of ARV initiation. .

According to the Aids map, 2010, SAPIT, an ongoing randomized clinical trial is examining whether there is any difference in outcomes according to whether ART treatment is initiated quickly after starting TB treatment-within two weeks or delayed for two months in order to overcome potential problems of drug toxicity, drug interactions and immune reconstitution syndrome (IRIS). One arm of this study in which patients were randomized to receive ARV treatment after completion of course of TB treatment, was halted after an interim analysis showed that patients in this arm were more likely to die than those who started ART during their course of TB treatment. This cohort also provides interim indication that the condition of patient in early weeks of TB treatment is probably more important than interval between starting TB treatment and starting ART in predicting patient outcome that is more likely.

One thousand six hundred and twelve (1612) ARV-naïve patients receiving TB treatment at Themba Lethu clinic, the outpatient ART clinic of Helens Joseph Hospital in Johannesburg were recruited and followed up for 12 months after ART initiation with primary outcome being all cause mortality, of

these 165 (10.2%) of patients died, 84(50.9%) of which were in the group who initiated ART within two months of starting TB treatment and 81(49.1%) in the group who initiated ARVs at least two months after TB treatment initiation. There was no statistically significant difference found between the two groups.

The multivariate analysis for mortality showed that, patients with a baseline CD4 count of less than 100 cells/mm^3 were 3.16 times at risk of death as compared to those with a CD4 count above 100 cells/mm^3 (p=0.002), those with haemoglobin level of less than 10 g/dL at baseline were also found to be 2.52 times as likely to die in comparison to those with haemoglobin level above 10 g/dL (p=0.001). Further to this patients with BMI of less than 18.5 kg/m^2 were twice at risk of death than those with a BMI above 18.5 kg/m^2 (p=0.002). Poor immune response at six months (CD4 response less than 50 cells/mm^3 from baseline CD4 count) was found in 39% of the patients. The results say the authors support recent recommendations for ART initiation in patients with TB while still taking TB treatment.

2.2 Justification

Tuberculosis is the most frequent major opportunistic infections in people infected with HIV and the risk of TB is dramatically increased in HIV infected patients as a result of higher probability of either primary progression or reactivation of latent infection (Jon F, 2004). It is important to understand the survival rates and possible risk factors associated with death in this group of patients. To date there has been limited clinical data in Zimbabwe regarding survival rates among HIV/TB co-infected patients and this study also seeks to model the prognostic factors that are related to death among these patients which have not been modeled in our settings.

2.3 Research questions

What is the mortality rate of TB/HIV co-infected patients on ART at WIDH?

What are the survival patterns of HIV/TB infected patients at WIDH according to gender, marital status, age group, site of TB, baseline Haemoglobin, baseline CD4 count and time between TB treatment and ART initiation?

What are the factors associated with survival among HIV/TB patients on ART at WIDH?

2.4 Study Objectives

Broad objective

To determine the survival and predictors of mortality among HIV/TB co-infected patients.

Specific objectives

- To determine mortality rate of TB/HIV co-infected patients on ART.
- To estimate time in months from ART initiation to death of HIV/TB co-infected patients.
- To determine the factors associated with mortality among HIV/TB patients.
- To identify baseline factors associated with mortality in HIV/TB patients.
- To compare survival experiences of HIV/TB co-infected patients according to gender, marital status, age group, site of TB, baseline haemoglobin, baseline CD4 count and time between TB treatment and ART initiation.
- To model the time to death of TB/HIV co-infected patients using Cox-proportional hazard model.

CHAPTER 3: METHODOLOGY

3.3.1 Study Design:

Retrospective Cohort study was carried out and medical records of patients on antiretroviral therapy with HIV/TB co-infection at Wilkins Infectious Disease Hospital were reviewed for the period 1 December 2004 and 31 August 2010. Records of TB patients who attended the OI/ART clinic during this period were selected randomly reviewed and information of interest was extracted. HIV/TB patients who started ART between 1 December 2004 and 1 March 2010 were recruited, had their records retrieved and reviewed. All patients were followed up to 31 August 2010 and the outcome for each patient was recorded as either of the following (death, transferred out, lost to follow up, stopped ART or alive).

3.1.2 Study setting:

The study was done at Harare city Wilkins Infectious Disease Hospital (WIDH) OI clinic which is situated close to the Central Business District (CBD) of the city but more to the North western side. Provision of ART started in April 2004 at WIDH O.I clinic as one of the first public hospitals to start ART in Zimbabwe. Prior to that, patients were treated only for opportunistic infections. It serves patients from within the suburbs of Harare and surrounding areas ideally those areas within a radius of 40km from the city and to date it has enrolled about 30 000 patients. There is a separate OI/ART clinic and TB clinic and a patient on ART with TB receives treatment from the respective clinic.

3.1.3 Study population:

Adult and adolescent female and male HIV/TB co-infected patients on ART enrolled at Wilkins hospital OI clinic and were on TB treatment at ART initiation.

3.1.4 Participant Inclusion and Exclusion criteria

Inclusion criteria:

Female and male HIV/TB co-infected patients aged fifteen years or more at time of ART initiation and started ART at WIDH OI clinic between 1 December 2004 and 1 March 2010. The patients must have been diagnosed with TB before initiating ART and also on TB treatment upon initiation of ART.

Exclusion Criteria:

HIV/TB co-infected patients on ART less than fifteen years of age and started ART at Wilkins hospital OI clinic between 1 December 2004 and 1 March 2010.

HIV infected patients on ART without tuberculosis at ART initiation, HIV/TB co-infected patients who develop TB after ART initiation and HIV/TB co-infected patients who were enrolled before 1 December 2004 and after 1 March 2010 and patients who were not on TB treatment were excluded.

3.1.5 Sample Size:

Using PS Power and Sample Size Program for sample size calculation Version 3.0, January 2009, assuming a significance level α=0.05, power β=0.80, a hazard ratio of 2.651 and median survival time of 51.5 months [32] among patients who initiate ART early after starting TB treatment the minimum sample size required is **n=140.** Factoring in attrition rate of 10% and a design effect of one the sample size required becomes n=**154.**

3.2 Data sources

Records of HIV/TB co-infected patients on antiretroviral therapy between 1 December 2004 and 1 March 2010 were used.

The national Monitoring and Evaluation (M/E) tools that is the ART register, pharmacy register, OI/ART registration form, and patient notes were used. A retrospective review of all these records was done and information on covariates of interest was extracted for the period under study. Pharmacy register was used to construct a sampling frame as the ART register did not record information on TB, all records of patients listed as on TB treatment were reviewed if the inclusion and exclusion criteria listed above was met. ART register was used to obtain information on age, gender, baseline weight and baseline clinical staging and the same information was looked for in OI/ART registration to check for inconsistencies. The OI/ART registration form was further used to obtain information on marital status, employment status, baseline CD4 count, baseline haemoglobin, pneumonia, herpes zooster and site of TB. Patient notes were used to obtain information on subsequent weights, follow up CD4 counts, date of TB treatment and other OIs. Also used were laboratory tests results which were attached to patient notes, they were used to obtain information on CD4 count and haemoglobin.

Routinely collected Data

Wilkins Infectious Disease Hospital OI clinic medical records of patients on ART are kept in paper based format though they are in a process of setting up a computerized database. The OI/ART clinic routinely collects information on socio-demographic variables, information on allergies, past medical history, TB treatment if any and type of TB when patient comes to register with the clinic. Information on counseling session is also recorded and where the patient was tested for HIV. Also recorded is ART regimens given, amount of drugs taken and laboratory tests done and the results.

Patients are enrolled by CD4 count and it is supposed to be repeated after every 3 months and if there is need, this is done as a way of monitoring immunologic function of patients. Patients were seen at an interval of one month or two months depending on stability and availability of drugs. Weight is supposed to be recorded at each clinic visit which is usually done every month when patient comes for resupply of drugs. Patients who are on TB treatment are requested to do an HIV test and if found positive they are referred to the OI/ART clinic for management of HIV and conversely TB screening is done for patients enrolling for ART.

3.2.1 Study Variables

Outcome measure: Time in months from initiation of TB treatment until death or end of follow up**.**

Table 2: Independent Variables

Variable	**Measure**
Age	Years at ART initiation
Gender	Male or Female
Marital status	Married, single, widowed or divorced
Education level	None, Primary, Secondary or Tertiary
Employment status	Employed, Not-employed
WHO stage	Clinical staging,1(asymptotic), 2(mild) 3(advanced) or 4 (severe).
Weight at baseline	Kilogrammes
CD4 count	Cell count/mm^3
Hemoglobin	grammes/dl
Site of TB	Pulmonary, extra pulmonary
Cotrimoxazole use	Yes, No
Time between TB treatment and ART	Months from TB treat to ART
Herpes Zooster	Yes, No
Pneumonia	Yes, No
Malnutrition	BMI

3.2.2 Data management

We created a data entry screen with all variables of interest (shown in Table 2) in EPI-INFO Version 3.5.1(Centers for Disease Control and Prevention, Atlanta). The data entry screen had a section for demographic variables (age, gender, marital status and employment status), baseline and follow up clinical characteristics (WHO stage, CD4 count, Site of TB, date started TB treatment and weight). A variable status was created to collect information on the outcome of the patient at the end of the follow up period (31 August 2010) which was classified as either of the following, Alive and on ART, transferred out, Stopped ART treatment, dead and lost to follow up. Further in analysis the variable status was divided into two categories dead and not dead (included Lost to follow up, Alive, transferred out and stopped ART). Status was the outcome variable in the study and indicated whether the event of interest had occurred, death was coded as 1 and not dead as 0 for analysis in STATA Special Edition Version 10 Software (Stata Corporation, College Station, Texas, USA).

A review of patient notes, OI/ART registration form and the ART register was done and information on the variables of interest was captured electronically in Epi-Info. Data was checked for errors and inconsistencies. Wilkins Hospital kept a separate database for those on TB treatment at Beatrice Infectious Disease Hospital, so information regarding TB treatment and status was extracted from patients notes kept at Wilkins' OI/ART clinic. Data was imported into STATA for further cleaning and coding. Missing data was checked for during data capturing and in analysis variables with missing data were removed if the observations did not meet the required 154 for minimum sample size. All statistical analysis was done in STATA 10 and for each analysis a Log file and a do file were saved.

3.3 Statistical methods/Data analysis

Methods for survival analysis were used in the analysis of the data (Appendix A1-A6).

3.3.1 Exploratory Data Analysis

Univariate Analysis.

Time at risk will be time from TB treatment start till the ending date of entry. Survival time will be measured as time from starting TB treatment until death from any cause or last date of follow-up contact, transfer out or stopped antiretroviral therapy. All these observations will be considered censored at various times depending on the event. Patients were censored on the date of any one of the following events, whichever occurred first:

1. if the patient was lost to follow-up, the date of the last contact the patient had with the clinic
2. if the patient was transferred to another health institution, the date of transfer,
3. if the patient stopped ART treatment, the last date of drug re-supply plus two months, and
4. if the patient was alive and on treatment at the end of the follow-up, 31 August, 2010.

Summary statistics, that is, frequencies, mean (standard deviation), median (inter-quartile range), proportions will be used to describe patient characteristics. The Kaplan-Meier method (Appendix A2-A3) will be used to estimate overall survival curve from the observed survival times and in comparing survival curves for categorical variables.

To test for equality across strata to explain whether there is difference in survival experiences between groups, we used the non-parametric Log-rank test (Appendix A4) and Chi-square Wald statistic and its

associated p-value were calculated to test for significance. For continuous variables we use a univariate Cox proportional hazard regression which is semi-parametric.

3.3.2 Model Building

Backward stepwise multivariate analysis was done using the Cox proportional hazard model. We started with all predictors in the model, the variable that was least significant (highest p-value) was removed and the model was refitted. Each subsequent step removed the least significant variable in the model until all remaining variables in the model had individual p-values smaller than 0.05.

Backwards elimination allows all the variables in the model at once and considers predictive capability of variables as a set since it is possible for a set of variables to have considerable predictive capability even though any subset of them does not. All predictor variables in data set are variables that could be relevant to the model.

When removing a variable with the highest p-value we checked whether the magnitude of association of any other variable left in the model changed by more than 15 percent. If magnitude of association of any variable would have changed by more than 15 percent we would retain the variable in the model and treat it as a possible confounder and continue with the stepwise regression. Fifteen percent was chosen to allow for residual confounding. We used the Information criteria, Akaike Information Criterion (AIC) and Bayesian information criterion (BIC) to choose the best model in case of variables that were collinear. A minimum AIC/BIC strategy is used for selecting among two or more competing models. Fit is measured negatively by -2*ln(likelihood); the larger the value, the worse the fit. Given two models fitted on the same data, the model with the smaller value of the information criterion is considered to be better. Collinearity was assessed through plotting of the correlation matrix for the independent variables.

3.3.3 Model Diagnostics

Diagnostic of the model was done by testing the assumptions of the model. If the assumptions of the model hold then the model adequately describes the data and the results will be valid. The key assumptions of the Cox proportional hazard model are;

1. The proportional hazards models assume that the hazard ratio of two people is independent of time and it is valid only for time independent covariates. This means that the hazard functions for any two individuals at any point in time are proportional. In other words, if an individual has a risk of death at some initial time point that is twice as high as that of another individual, then at all later times the risk of death remains twice as high.
2. Censoring is independent of event of interest. In a survival study, one should ensure that patients are not removed from the study just before they die.
3. There is a log-linear relationship between the independent variables and the underlying hazard function.

The proportional hazard assumption is the pillar upon which the Cox proportional hazard model is built and was tested using plots of the Schoenfeld and scaled Schoenfeld residuals. The Chi square global test was also used to verify this assumption as the residual plots alone might not be enough to diagnose this.

3.4 Ethical considerations

Permission to carry out the study was sought from The Director Harare City Health, Dr S. Mungofa and Medical Officer in charge of OI at Wilkins Hospital Dr H Bara and the study protocol was approved by Chairman Department of Community Medicine, Prof S.Rusakaniko. Confidentiality was assured and maintained throughout the study, ART numbers were used as patient identifiers and no patient names were used. No informed consent was required as this study was a review of patient records and did not deal directly with human participants.

CHAPTER 4: RESULTS

4.1 Overview of results

This chapter presents results of quantitative findings from the study. The section covers the following: Demographic and baseline characteristics and associated mortality, comparison of survival experiences according to gender, marital status, CD4 count, age, WHO stage, baseline haemoglobin, site of TB and time between TB treatment and ART start. Also findings on factors associated with mortality and steps in Cox-proportional hazard model building are covered in this section. Finally we do model diagnostics to check for the violation of the proportional hazard assumption.

A total of 207 patients on ART at WIDH who fulfilled the criteria for HIV/TB co infection were recruited into the study and their total follow up time was 456.88 person years.

At the end of the study period 105(50.7%) were alive and on ART, 46(3.3%) transferred out, and 11(5.3%) lost to follow up. There were 45(21.7%) deaths after starting ART. We observed that the majority of the patients transferred were to local clinics in the Harare city while others were transferred to other provinces within the country and a few to South Africa. All transfer out were written on cover page of patients' notes indicating place where patient was transferred to.

4.2 Baseline demographic and clinical characteristics

Table 3: Baseline demographic characteristics and associated mortality of HIV/TB patients on ART during follow up period.

Characteristic	N=207 (%)	Deaths (%)
Gender		
Female	131 (63.3)	28 (21.4)
Male	76 (36.7)	17 (22.4)
Marital Status		
Married	103 (49.8)	23 (22.3)
Divorced	24 (11.6)	7 (29.2)
Widowed	50 (24.2)	9 (30.0)
Single	30 (14.5)	6 (12.0)
Employment Status		
Employed	92 (44.4)	18 (19.6)
Not-Employed	115 (55.6)	27 (23.5)
Age (years)		
15-24	12 (5.8)	3 (25.0)
25-34	67 (32.4)	13 (19.4)
35-44	85 (41.1)	21 (24.7)
45+	43 (20.8)	8 (18.6)

Majority of the patients recruited in this study were female 131(63.3%) as shown in Table 3. Most of the participants were not employed 115(55.6%) and about half of them were married 103 (49.8%) with the remaining half being either widowed 50 (24.2%), divorced 24 (11.5%) or single 30 (14.5%).

The median age of the HIV/TB co infected patients was 37 years [interquartile range (IQR) 32-43] and most patients 85 (41.1%) were aged 35-44 years. This suggests that most patients in this setting presented to the clinic for treatment at a relatively old age or this could be that treatment preference is given to those with old age as compared to young age.

A total of 167 (80.7%) had pulmonary TB with only 40 (19.3%) having extra-pulmonary TB and all were on TB treatment at ART start and were on Efivarenz based ART regimens (Table 4.0 below)

however information on the TB drugs they were taking was not recorded. The majority of 147 (78.2%) patients were in WHO clinical stage III at ART start and presented to the clinic with CD4 count less than 200 cells/µ*l* median 98 cells/µ*l* [IQR 45-155 cells/µ*l*]. Mean haemoglobin at baseline was 10.35 g/dl± SD(2.35) and mean weight was 55.5kg±11.50SD (n=80). Height measurements were not recorded so BMI could not be calculated.

Table 4 shows that most patients 130 (62.8%) started ART 8 weeks (two months) after commencing anti-TB treatment with a few patients (6.8%) starting ART within two weeks.

A few patients, 14 (6.8%) were co-infected with pneumonia, 64 (30.9%) with herpes zooster and 6 (3.0%) had both pneumonia and herpes zooster. Almost all 197 (95.2%) were on cotrimoxazole prophylaxis. Those who were not on cotrimoxazole had reacted to it and were given another drug in place of cotrimoxazole.

Table 4: Baseline Clinical Characteristics and associated mortality of HIV/TB patients on ART

Characteristic	N=207 (%)	Deaths (%)
CD4 cells/µl[a]		
<50	58 (28.2)	26 (44.8)
50-100	47 (22.8)	11 (23.4)
101-199	78 (37.9)	4 (5.1)
≥200	23 (11.2)	3 (13.0)
Haemoglobin[b]		
Normal(Above 10)	112 (56.3)	22 (19.6)
Moderate(7-10)	76 (38.2)	17 (22.4)
Severe (<7g/dl)	11 (5.5)	4 (36.4)
WHO Clinical Staging[c]		
Stage III	147 (78.2)	22 (15.0)
Stage IV	41 (21.8)	16 (39.0)
Time between TB treatment and ART start		
≤ 2weeks	14 (6.8)	3 (21.4)
>2-8 weeks	63 (30.4)	42 (21.8)
>8 weeks	130 (62.8)	
Tuberculosis Site		
Pulmonary	167 (80.7)	27 (16.2)
Extra-pulmonary	40 (19.3)	18 (45.0)
Pneumonia		
No	193 (93.2)	42 (21.8)
Yes	14 (6.8)	3 (21.4)
Herpes zooster		
No	143 (69.1)	27 (18.9)
Yes	64 (30.9)	18 (28.1)
Cotrimoxazole		
Did not receive	10 (4.8)	3 (30.0)
Received	197 (95.2)	42 (21.3)

[a]1 value missing (n=206). [b] 8 values missing (n=199). [c]19 value missing (n=188)

4.3 Survival analysis

During a median follow up time of 20.7 months (maximum 73.3 months) there were 45 deaths (28 female and 17 male) which represents a mortality proportion of 21.7%.The cumulative mortality at 3,6 and 12 months was 1%, 5% and15% respectively. The overall probability of surviving up to 73 months was 72.3% with the probability of surviving for 12, 24 and 36 months after starting TB treatment at 86%, 80% and 76% respectively.

The mortality rate was 9.8 deaths/100person years of follow-up.

4.3.1 Overall Survival experience for the Cohort

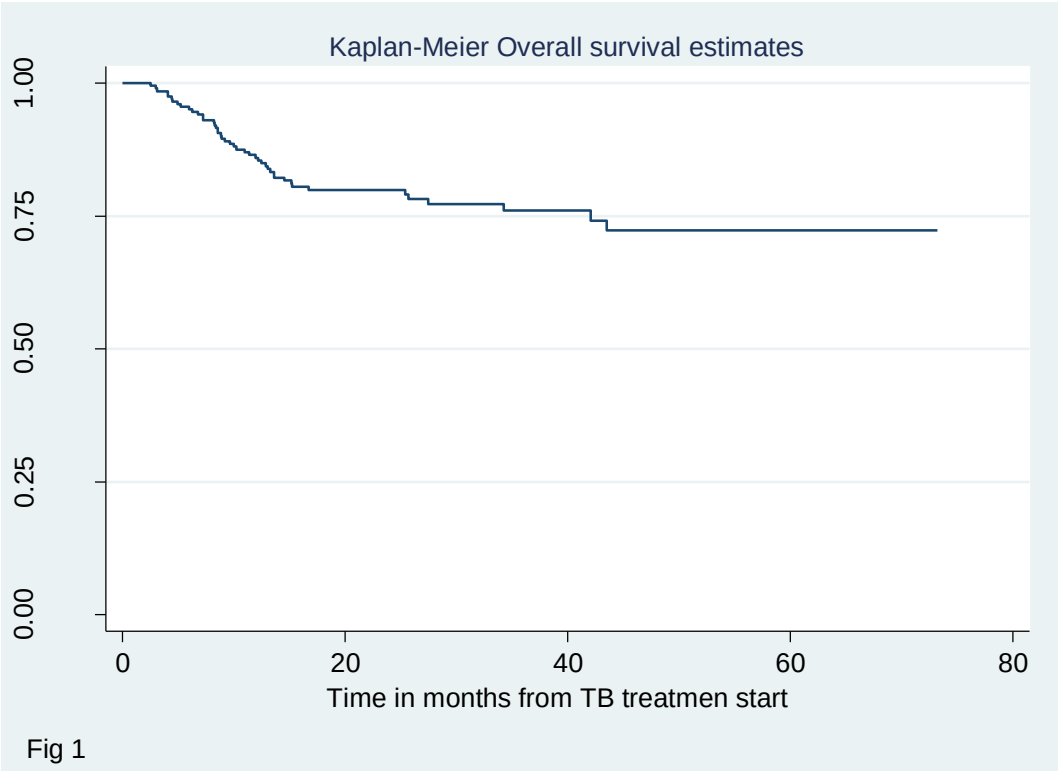

Fig 1

Fig 1 shows that the overall survival curve falls very sharply in the early months (about 20 months) after starting TB treatment this could suggest that the hazard of death is very high in the first twenty months for the HIV/TB co-infected patients. From forty months onwards the curve looks almost constant which suggest that the risk of death is constant over time after reaching just above forty

months. This curve however does not cut the point where survival probability=0.5 suggesting that the median survival time for this cohort is not defined.

4.3.2 Comparison of survival experiences between different groups of HIV/TB co-infected patients

This section shows Kaplan Meier curves for comparison of survival patterns between different groups. The univariate hazard ratios for each stratum are also shown in each graph as well as the p-value for the Log-rank Chi Square test of difference in survivor functions. Survival experiences for HIV/TB co-infected patients were compared according to baseline socio-demographic and clinical characteristics.

Comparison of survival experience by Gender

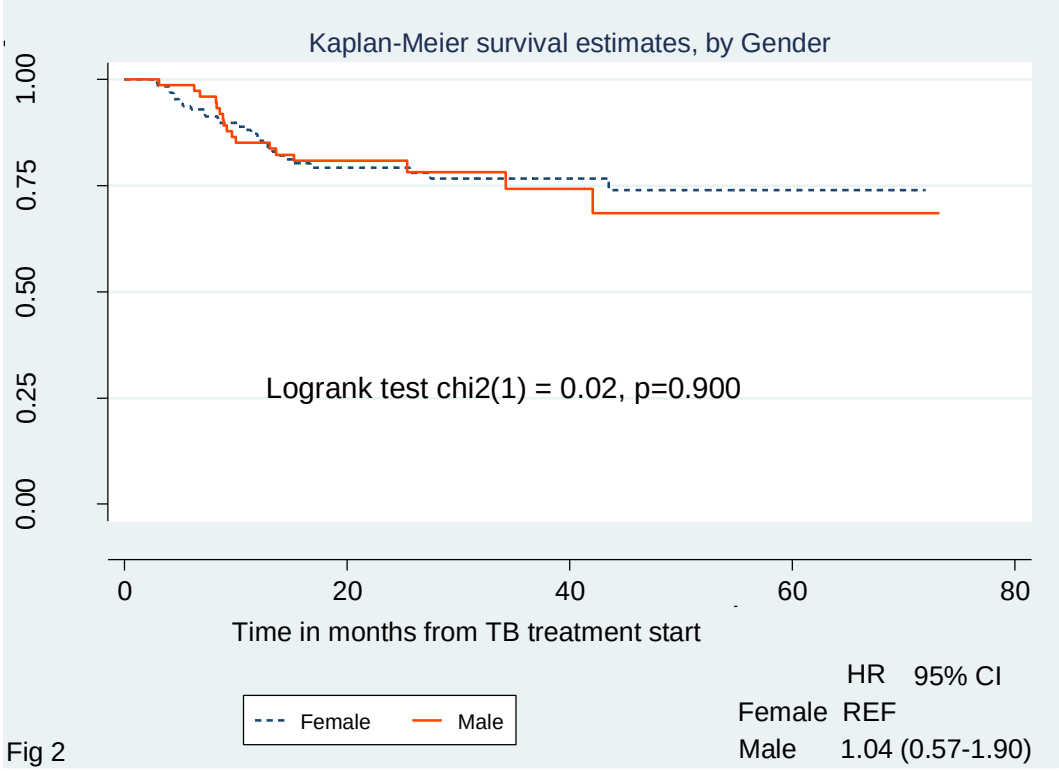

Fig 2

Survival curves for males and females are almost similar in Figure 2, suggesting little difference in survival rates between males and females and this is supported by the p-value of 0.900 from the log

rank test of equality across strata for the variable gender and this suggests no significant difference between survival times for females and males. Also the Hazard Ratio [HR=1.04, 95%CI (0.57-1.90)] suggests that gender had no significant effect on survival.

Comparison of survival experiences by baseline CD4 cell count.

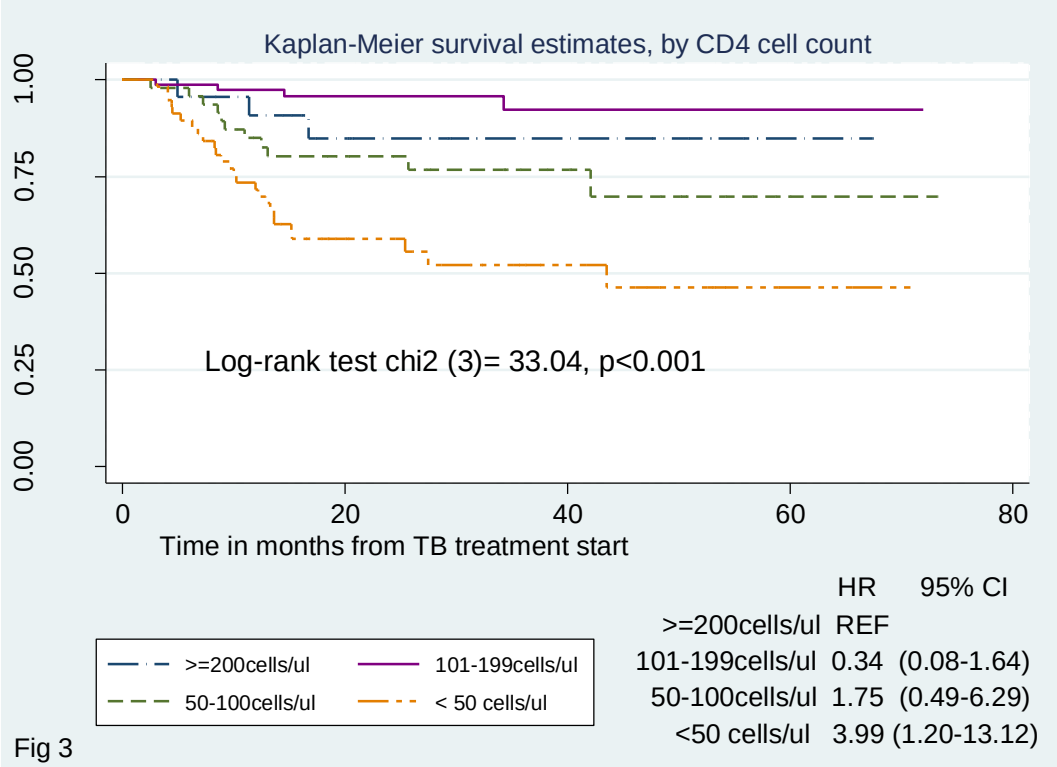

Fig 3

Survival curves look different for the different levels of CD4 count (Fig 3), the curve for those with CD4 less than 50cells is very steep suggesting that death rate is high in this group as compared to the other groups. The p<0.001 indicates that there is a significant difference in survival experiences according to baseline CD4 count of the HIV/TB co-infected patients with those with CD4 count <50 cells being worse off as compared to those withCD4 above 50 cells and the difference is more pronounced with increase in time. CD4 count<50 was found to be a risk factor of mortality with those with CD4 count<50 having a 3.99 times risk of death as compared to those with CD4 cells above 200,

95%CI (1.20-13.12). The other levels of CD4 count however, were not found to be significant as shown in Fig 3 above.

Comparison of survival experience by marital status

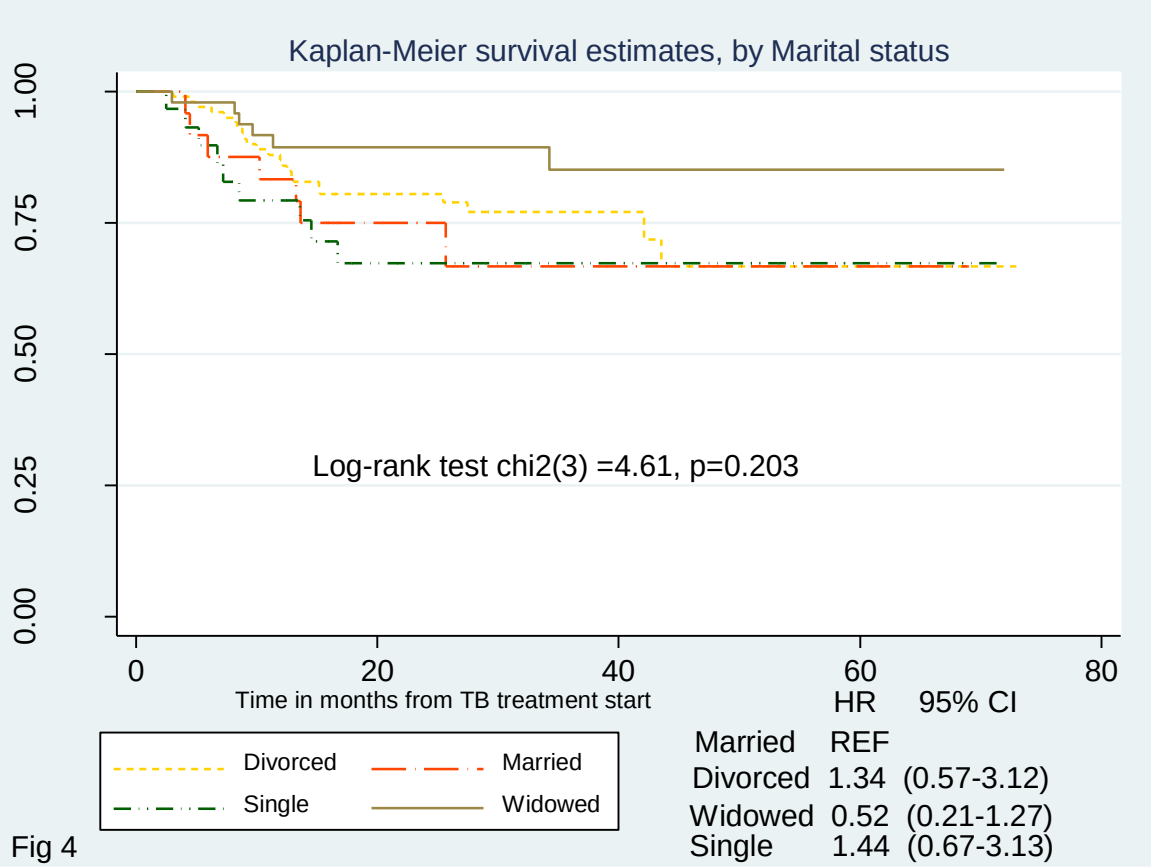

Fig 4

Survival curves according to marital status (Fig 4) appear the same the married, divorced and single but the curve for the widowed looks different. The =0.203 for the Log-rank test however suggest that there is no significant difference in survival over time according to one's marital status at ART initiation. With reference to the married the hazard ratios for the divorced, widowed and single are not significant as shown in Fig 4 since the confidence intervals include one.

Comparison of survival experience by age group

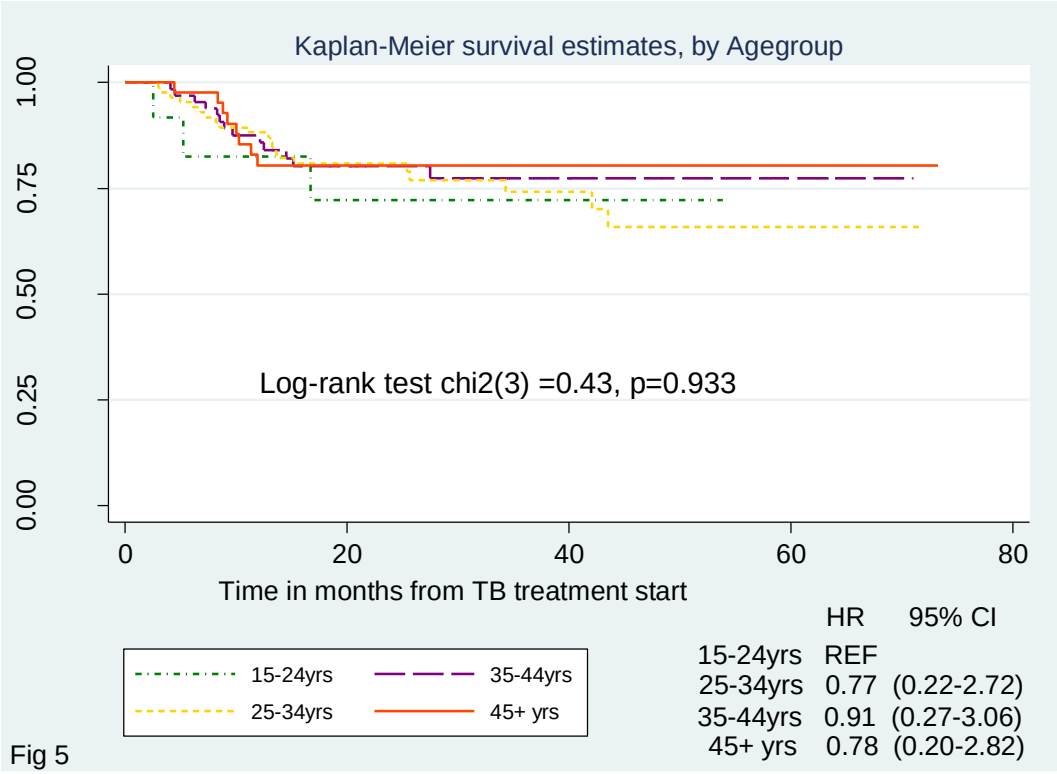

Fig 5

Fig 5 shows the survivor functions by different age groups and the curves look similar at all levels except in the late stages. The p=0.933 for the log-rank test suggest that there is no significant difference in survival by age. The hazard ratios for other age groups compared to the 15-24 years shows a trend for a protective effect however the effect is not significant as shown by the 95% confidence intervals. This implies survival does not differ according to age at baseline in this group of patients.

Comparison of survival experiences by employment status.

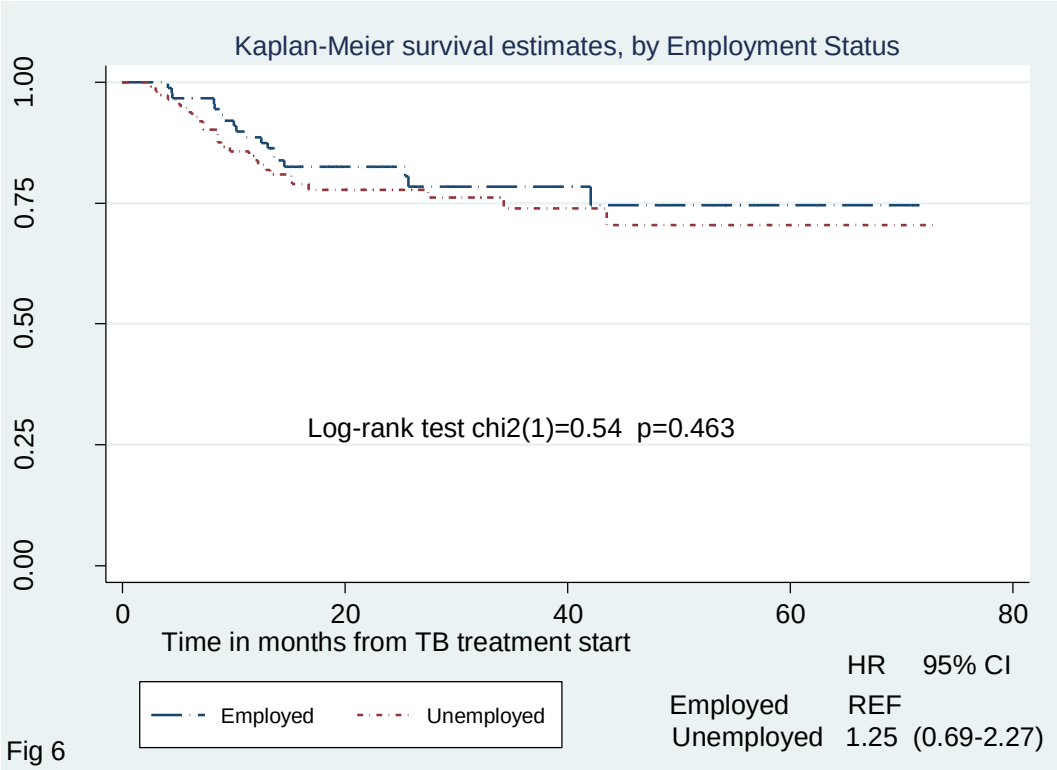

Fig 6

Fig 6 shows that survivor functions for those who were employed at baseline look almost similar to those who werenot employed. The log rank test p=0.463 suggests that there is no difference in the two survival curves.Those who were unemployed were 1.25 times at risk of death as compared to the employed however this was not significant implying one's employment status at baseline had no significant effect on survival.

Comparison of survival experiences by site of tuberculosis.

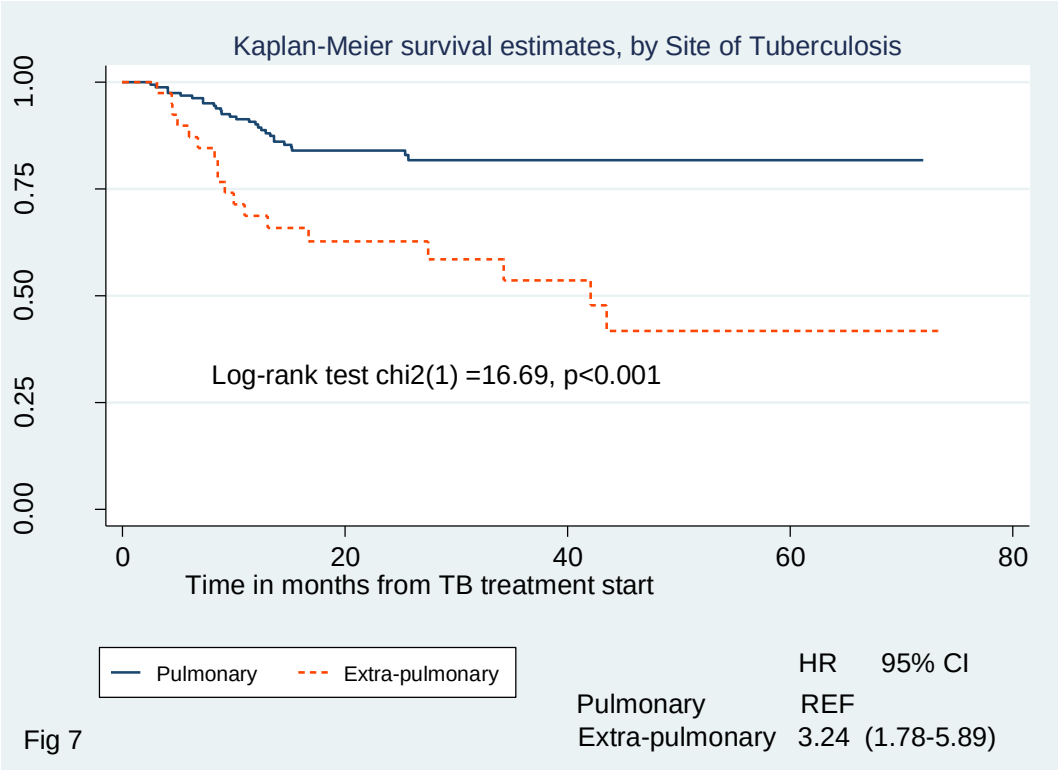

Fig 7

Survivor functions for site of tuberculosis look different (Fig 7) throughout the follow-up time with the curve for those with extra-pulmonary tuberculosis decreasing at a faster rate as compared to those with pulmonary TB. The survival rates between the two groups appear different and more pronounced after about 20 months from TB treatment start and this is confirmed by the log-rank test with a p-value<0.001 which is statistically significant, thus death is experienced earlier in those with extra-pulmonary TB as compared to those with pulmonary tuberculosis. Patients with extra-pulmonary TB had an increased risk of death as compared to those with pulmonary TB [HR=3.24, 95%CI (1.78-5.89) and this was statistically significant thus confirming that site of TB baseline had effect on survival of HIV/TB co infected patients on ART.

Comparison of survival experiences by haemoglobin levels

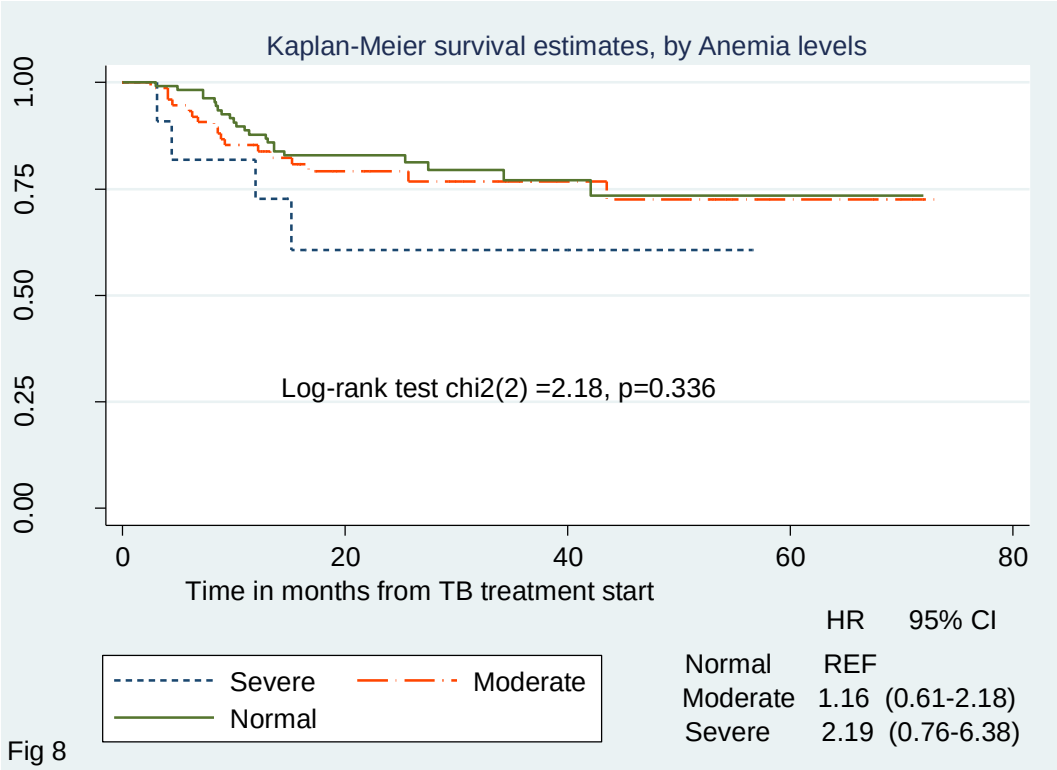

Fig 8

In Fig 8 the survival curves according to the levels of anemia looks different. The curve for those with moderate and normal anemia levels looks different as compared to those with severe anemia; however the log-rank test had a p-value of 0.336 which suggest otherwise. Thus the survival rate between the three groups is not statistically significant implying survival experiences are the same regardless of anemia (measured by levels of haemoglobin). Also the hazard ratios for those with moderate and severe anemia suggest an increased risk of death of 16% and 119% respectively as compared to those with normal levels, but however this is not significant.

Comparison of survival experiences by time between TB treatment and ART start.

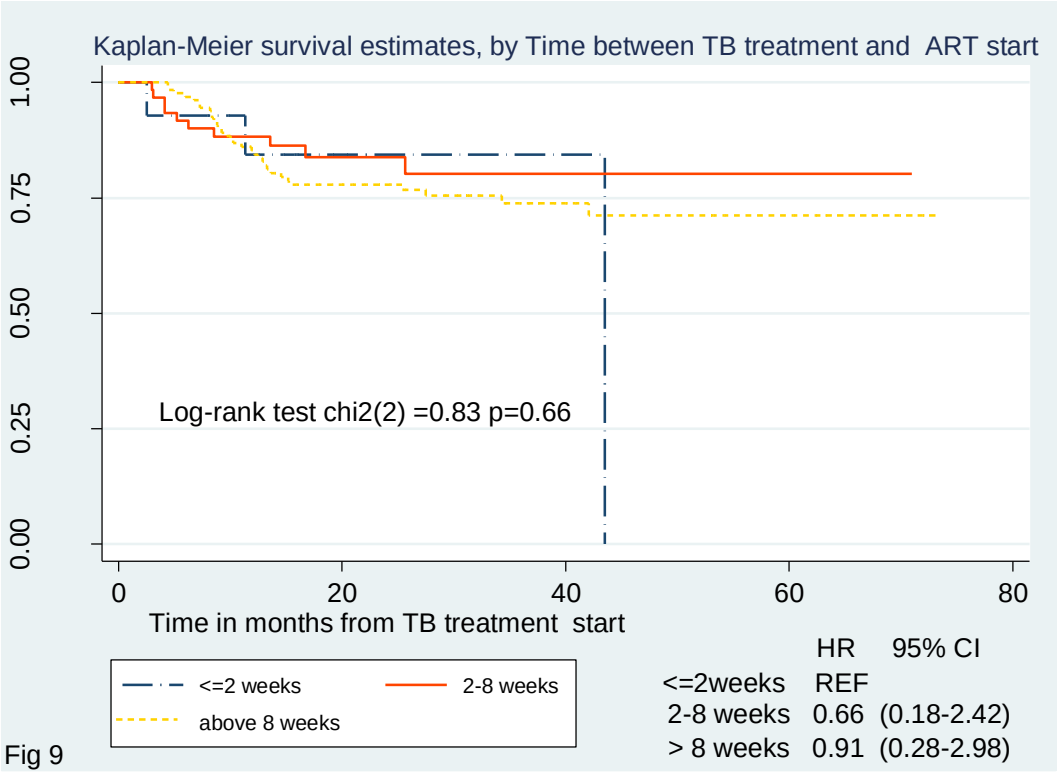

Fig 9

The survivor functions for time between TB treatment and ART (Fig 9) are similar. This suggests that survival experiences are the same in the three groups. The log-rank test for equality of survivor functions further supports this p=0.66. Thus those who started ART within two weeks after commencing TB therapy have same survival experiences with those who started ART 2-8 weeks and two months after commencing TB therapy. This is supported by the HR where it can be seen that there is no association between survival and time between TB treatment and ART for this group of patients.

Comparison of survival experiences by baseline WHO clinical staging

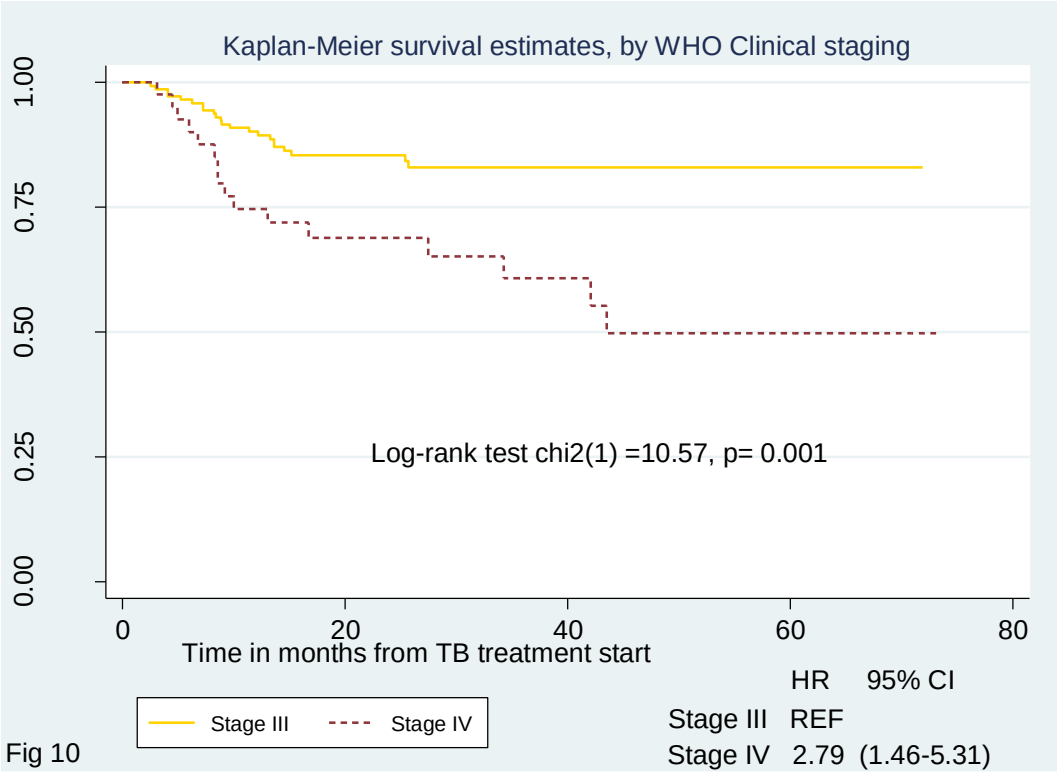

Fig 10

Figure 10 shows that there is a difference in survival curves according to the baseline WHO clinical staging of the patients. The curve for the group with WHO clinical staging IV falls sharply earlier compared to those in stage III giving an impression that those in stage four have worse survival experiences than those in stage three at baseline and a log-rank test equality across strata p=0.001 indicates that there is a significant difference in survival between these two groups of HIV/TB co-infected patients. Patients categorized in WHO clinical staging IV at baseline had a 2.79 risk of death as compared to those in stage three [HR=2.79,95%CI (1.46-5.31)] and this was significant.

Comparison of survival experiences according to pneumonia presence.

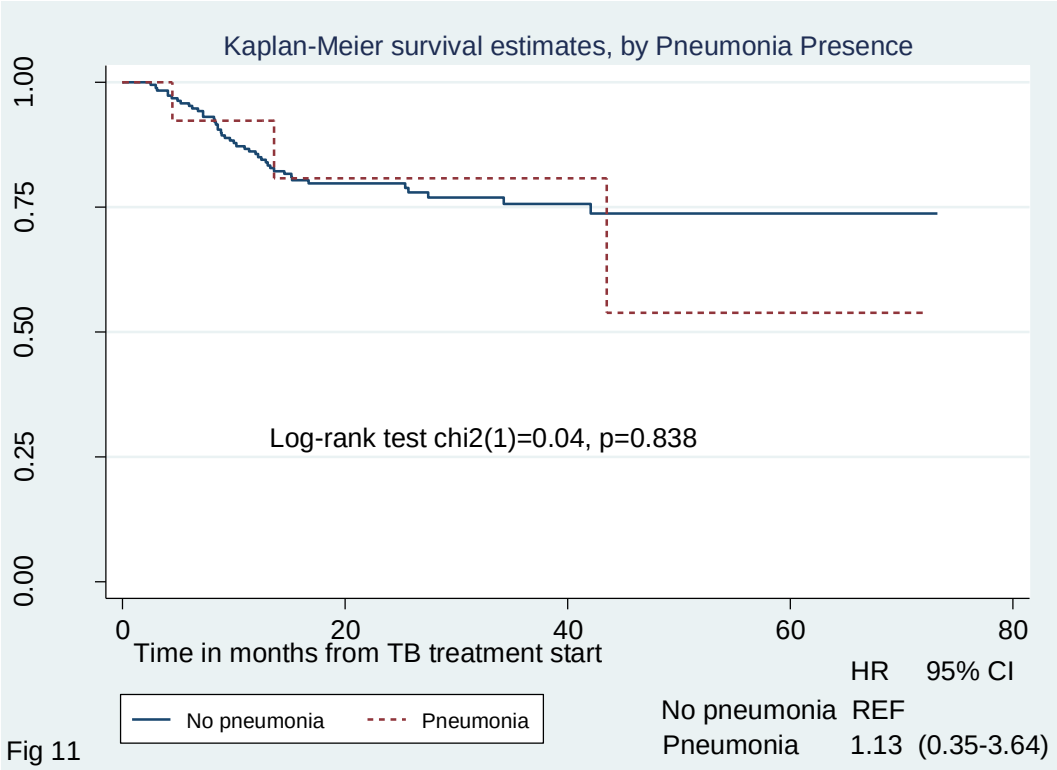

Fig 11

Patients co-infected with pneumonia were 1.13 times at risk of death but this was not significant 95% CI (0.35-3.64) as shown in Fig 11. The survival functions appear the same throughout except after 40 months from TB treatment start where there is a marked difference however, the log rank test p=0.838 suggest that overall the survival experiences of patients co-infected with pneumonia are no different with those without pneumonia.

Comparison of survival experiences according to presence of herpes zooster.

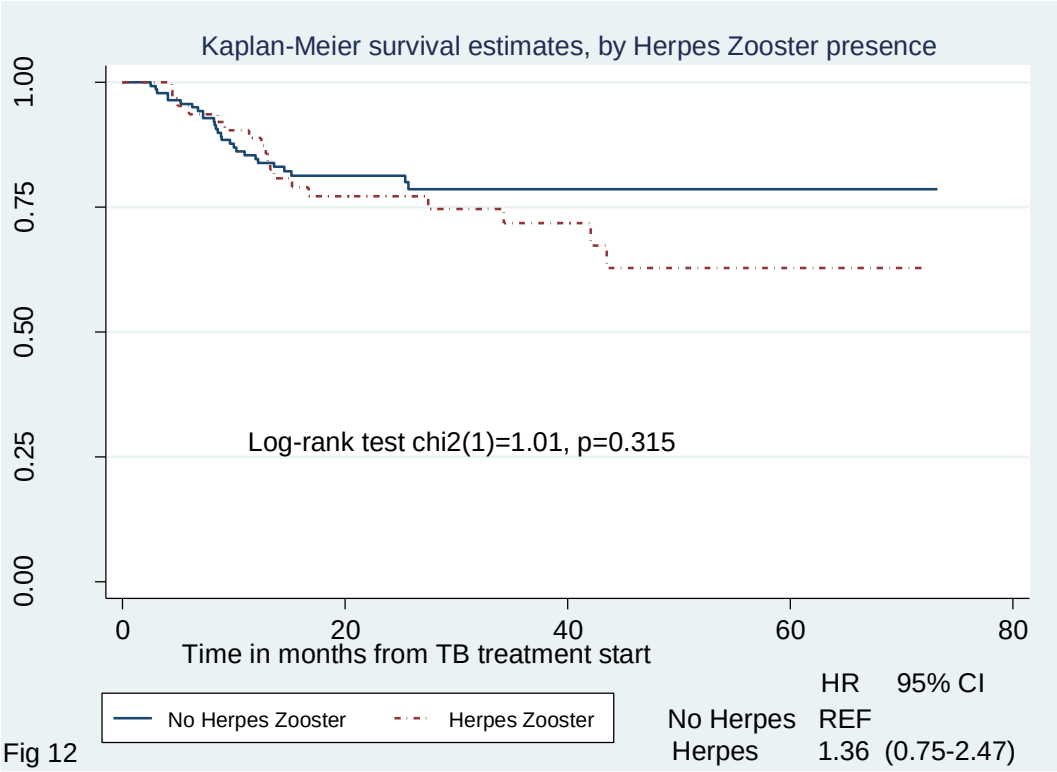

Fig 12

In Fig 12, patients with and without herpes zooster had similar risk of death as shown by the insignificant p= 0.315 for the Log rank test for equality across strata and the univariate hazard ratio which is insignificant though the survivor functions appear different as time increases.

Comparison of survival experiences according to whether cotrimoxazole was received.

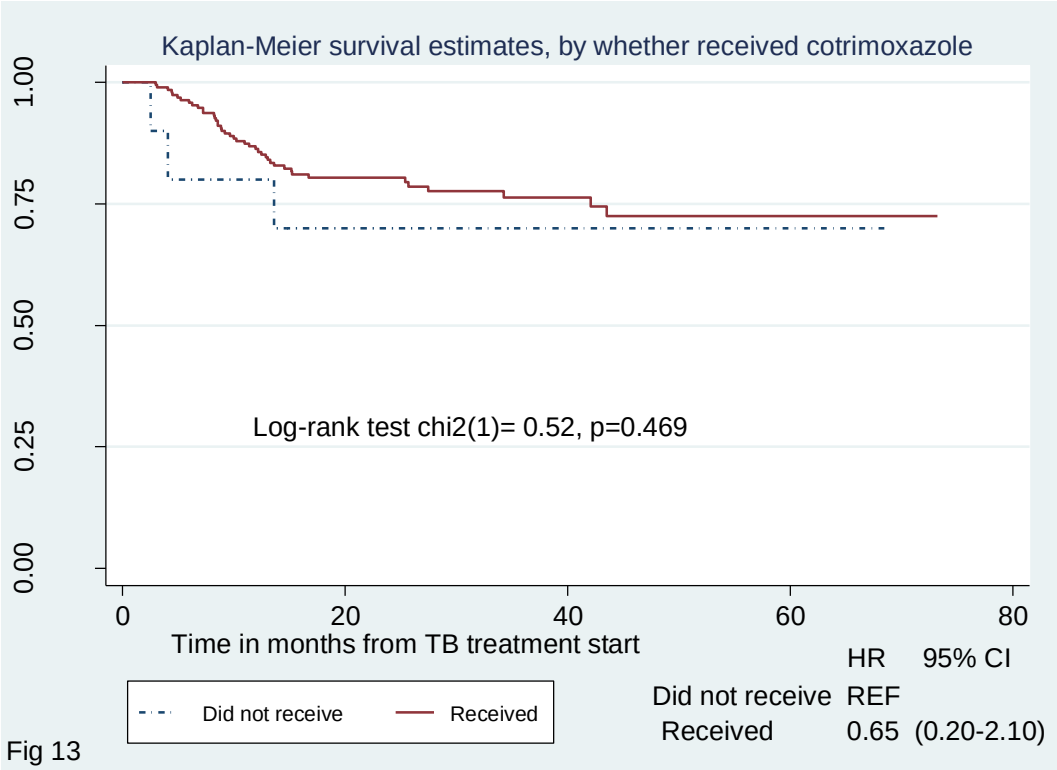

Fig 13

Fig 13 shows that the survival experiences of those who received cotrimoxazole at ART initiation and those who did not were almost similar and this is further supported by the log rank test p=0.469. Also the univariate hazard ratio though showing a protective effect of 35% it was not significant as indicated by the 95% confidence interval.

4.3.3 Multivariate Analysis

Further after doing the univariate analysis, backward stepwise multivariate analysis was done to find the independent predictors of mortality among the patients on ART Table 4.3.1 using the Cox-proportional hazard regression. Site of TB and WHO stage were found to be collinear with a correlation of 0.95 and we only included one of the variables in the model as one predicted the other.

We used the one with the smaller value of the Akaike information criterion (AIC) which we found to be WHO clinical staging.

Table 5: Cox-regression multivariate analysis of factors for mortality among HIV/TB co-infected patients at WIDH.

Characteristic	Adjusted HR (95% CI)	P-value
CD4 cell count (cells/μl)		
≥200	1	
101-199	0.30 (0.096-0.938)	0.038
<50	2.37 (1.158-4.856)	0.018
Marital Status		
Married	1	
Single	2.42 (1.1-5.34)	0.028
WHO clinical stage		
III	1	
IV	2.69 (1.35-5.34)	0.005
Cotrimoxazole during treatment		
Did not receive	1	
Received	0.29 (0.86-0.89)	0.047

From Table 5 a CD4 count of <50 cells is a significant predictor of survival among this group of patients. The risk of death was 2.37 times in those with low CD4 cont as compared to those with CD4 above 200 holding other factors constant. A high CD4 count at baseline CD4 of 101-199 cells reduced the risk of death by 70% in HIV/TB infected patients [HR=0.30 95%CI (0.096-0.938), p=0.018]. Being single is a significant predictor of survival [HR=2.42, 95%CI (1.1-5.34)]. This factor however, was not found to be significant in the univariate analysis. WHO clinical staging at baseline had a significant effect on survival (Table 5). It can also be said that those who received cotrimoxazole had a

71% reduced risk of dying as compared to those who did not receive cotrimoxazole at baseline [HR=0.29, 95%CI (0.86-0.89)]. This factor was not significant in the univariate analysis and this could be due to confounding.

After identifying the independent predictors of survival among the among HIV/TB co-infected patients at WIDH we went on to write an equation that tries to explain the hazard of death as a function of the independent factors that determines survival in this group of patients (see Appendix A7 for the coefficients and model fit statistics). The equation is;

In [h (t)/h_o(t)] = 0.863(CD4 cells <50)+0.885*(Single)+0.988*(WHO stage IV) -1.237*(Received Cotrimoxazole) -1.21*(CD4 cells 101-199)*

4.4 Assessing Survival Analysis (Model diagnostics)

Table 6: Test of proportional-hazards assumption

Variable	Chi-square	Df	p-value
CD4 101-199cells/µ*l*	0.19	1	0.660
CD4 <50cells/µ*l*	0.23	1	0.629
Single	1.05	1	0.305
WHO stage IV	0.97	1	0.325
Cotrimoxazole	0.58	1	0.448
global test	**3.59**	**5**	**0.609**

All the p-values are not significant at 0.05 levels

The above hypothesis test for the proportional hazard assumption for the variables we have included in the model, thus from Table 6 the p-values for all the variables are not significant which means that we cannot reject the null hypothesis that the hazard is proportional. The global test check on the same hypothesis for all variables combined thus it also support proportional hazard p=0.609. Fig14-Fig 18 below are plots to test the proportional hazard assumption and a plot which is parallel to the y=0 line satisfies the proportional hazard assumption.

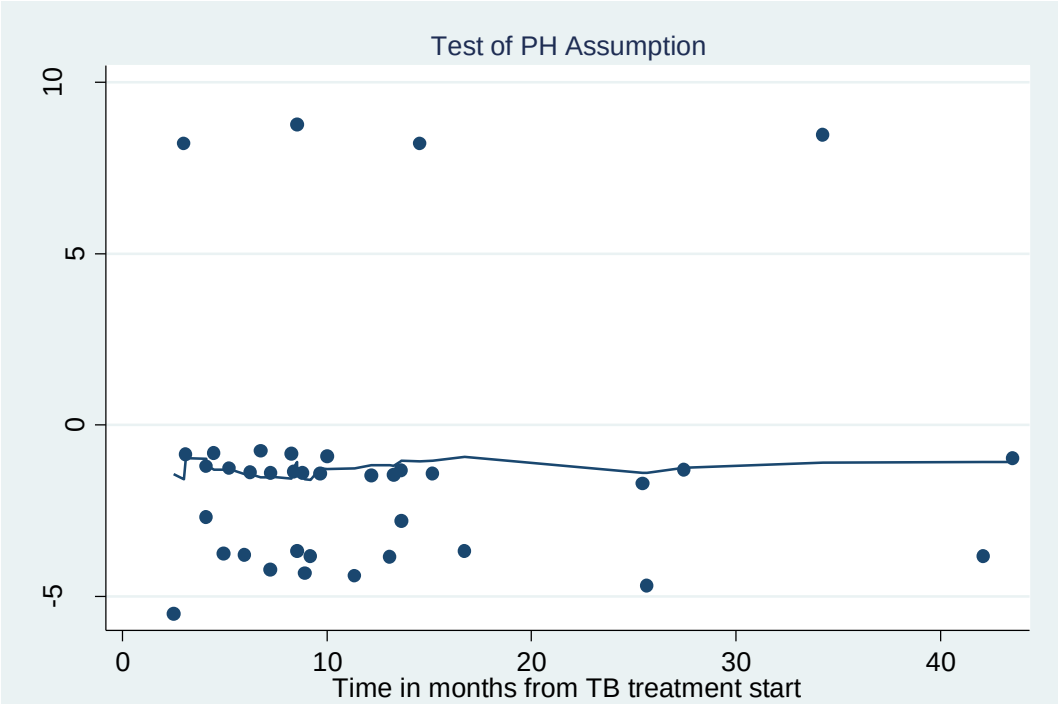

Fig 14

From the plot it can be seen that the line is almost parallel to the y=0 line which implies that the variable CD4 count 101-199 satisfies the proportional hazard assumption.

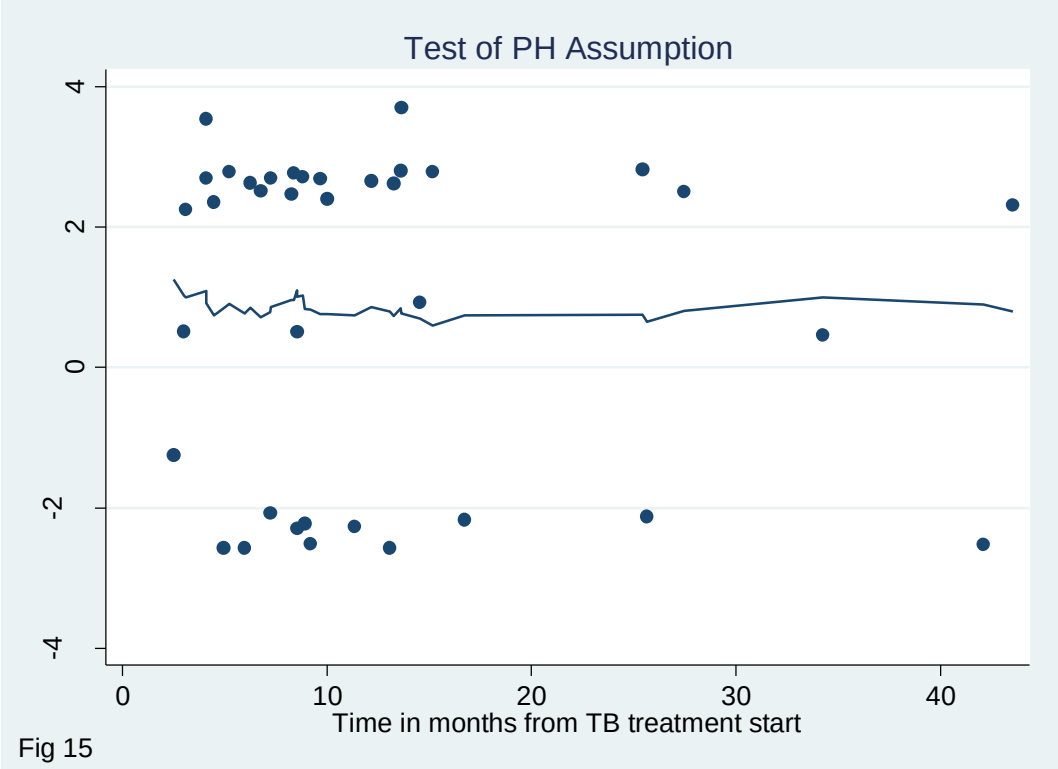

Fig 15

From the plot it can be seen that the line is almost parallel to the y=0 line which implies that the variable CD4 count <50 cells satisfies the proportional hazard assumption

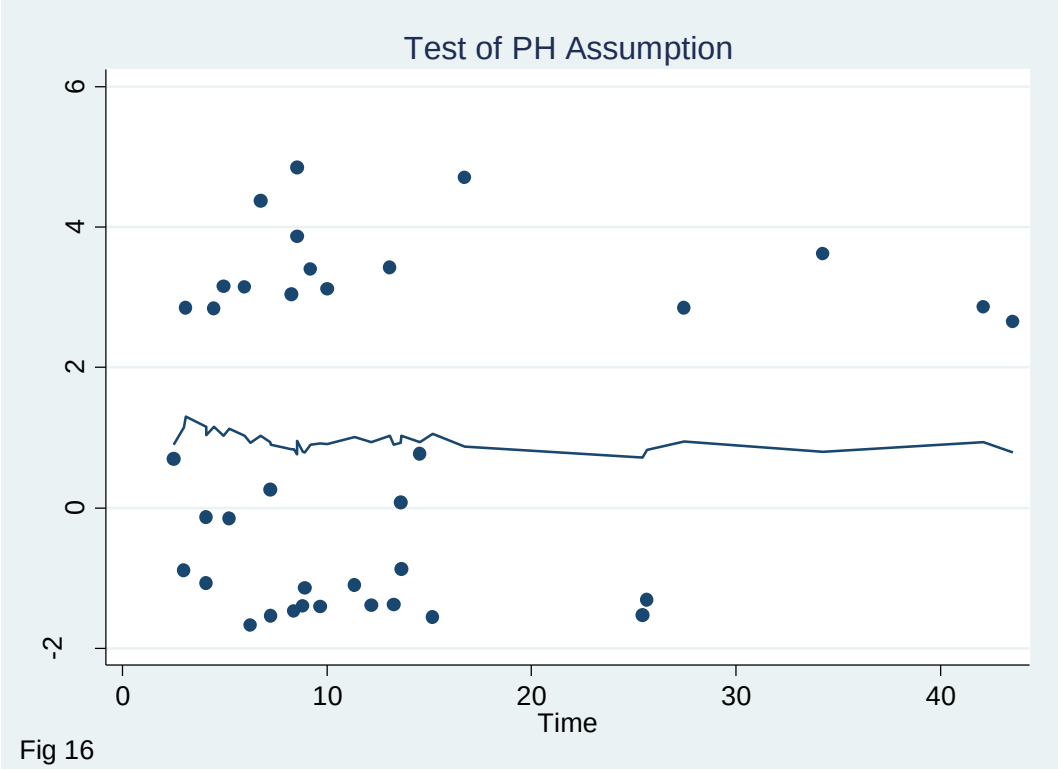

Fig 16

From the plot it can be seen that the line is almost parallel to the y=0 line which implies that the variable WHO stage satisfies the proportional hazard assumption

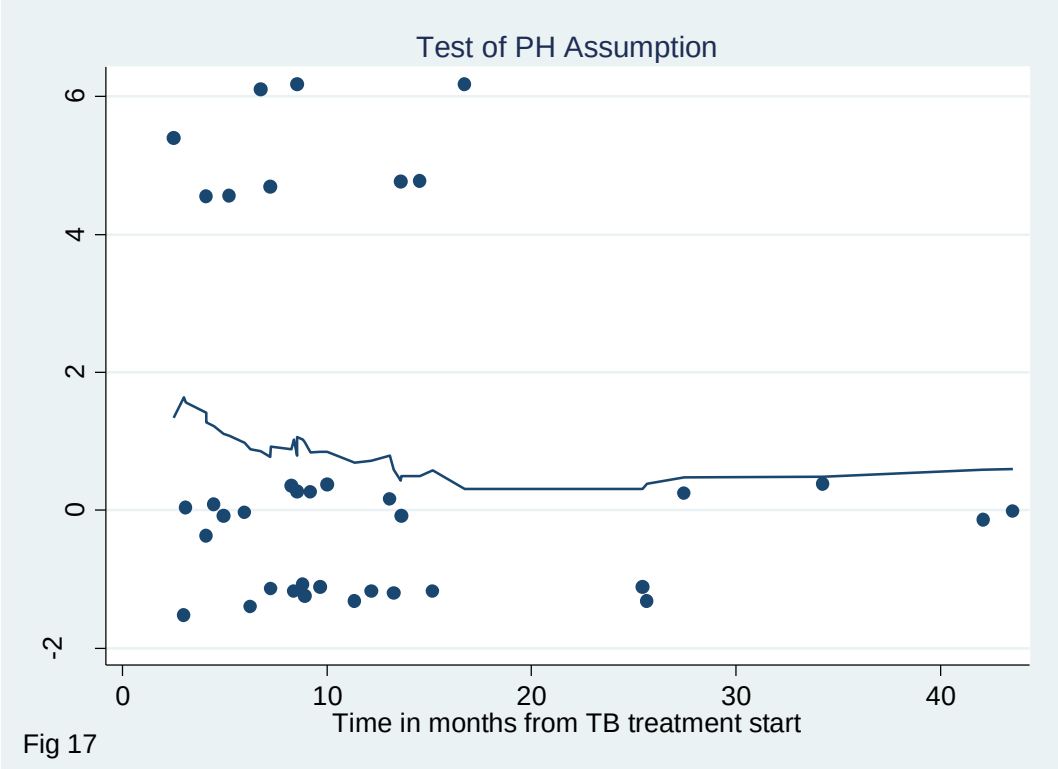

Fig 17

From the plot it can be seen that the line is almost parallel to the y=0 line though it seems not for the earlier values which implies that the variable single satisfies the proportional hazard assumption

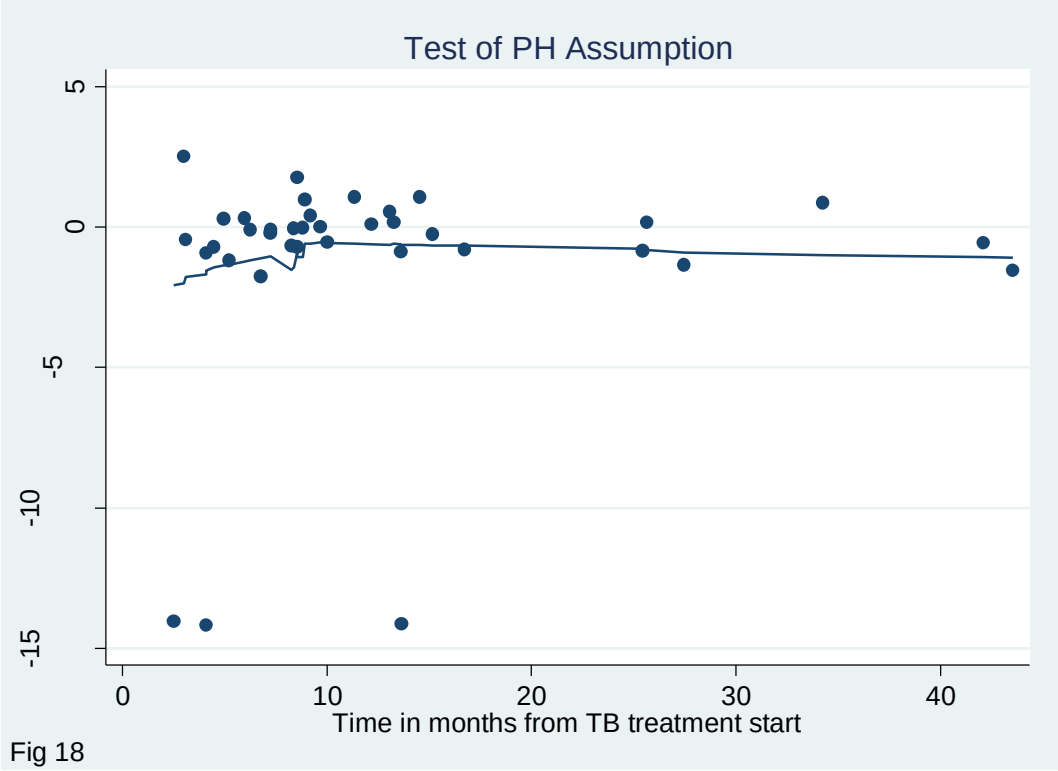

Fig 18

From the plot it can be seen that the line is almost parallel to the y=0 line which implies that the variable cotrimoxazole satisfies the proportional hazard assumption.

CHAPTER 5: DISCUSSION AND CONCLUSIONS.

4.1 Discussion

In this study, we have reported survival and risk factors for mortality among patients co-infected with TB and HIV who started ART at WIDH between 1 December 2004 and 1 March 2010. The majority of the study participants were female as compared to males (Table 3). This could be due to the health seeking behavior of women. Women who get ill are more likely to go to the clinic more quickly as compared to males. About half of the patients were married and a considerable proportion was divorced. This could be due to the death of the spouse due to HIV. This is in consistent with a study done in Somalia where half of the patients were married [31]

The overall survival rates at 1, 2, and 3 years after starting TB therapy was 86%, 80%, and 76%respectively. This indicates that the survival rate of HIV patients co-infected with TB is greatly reduced in the first year and decreases gradually thereafter. This could be because in the first year patients on TB treatment might not have stabilized on TB drugs or possible drug-drug interactions, overlapping side effects, immune reconstitution syndrome and high pill burden might lead to worsening of the outcome in the first year [33]. These rates are however comparably lower than those found in a study done in Thailand where the survival rates at 1, 2 and 3 years were 96.1%, 94.0%, and 87.7% [27].This could be due to differences in the sample sizes in the two studies. This study had a smaller sample size (207) as compared to the one done in Thailand which had 1003 patients.

The mortality rate of HIV/TB patients at WIDH was 9.8/100 person years of follow up. This mortality is relatively high as compared to one found in a study done in South Africa where a mortality rate of 5.4 per 100 person-years was found. This could be attributed to the difference between the health systems of the two countries where the South African system is better than the one in Zimbabwe in

terms of treating and managing patients. Also during the period for the study Zimbabwe experienced a shortage in health staff due to economic challenges and this could explain this difference in mortality rates.

In this study most of the patients presented to the clinic with advanced disease and low CD4 count with more than half the patients having a CD4 count of less than 100cells, median 98 cells/ul IQR[45-155cells/ul]. Patients in low income countries usually present to clinics when they feel the disease is too severe as they fear or cannot afford to pay for the user costs charged at clinics and hospital. The risk of death was increased in HIV/TB co-infected patients who present to clinic when CD4 is low and also with advanced disease as their outcomes would have already been compromised before start of treatment and these are the same patients at risk of IRIS if TB and ARV drugs are taken concurrently. This is consistent with studies done in South Africa showing that those with CD4 count,100 cells at baseline had an increased risk ,however this study further showed that those with CD4 count between 101-199 cells had a 70% reduced risk of death as compared to those withCD4>200 cells [HR,0.30 (0.096-0.938)] this could be due to selection bias or misclassification of study participants as this group had the largest number of participants as compared to other groups yet it had observed deaths which were almost similar to the one observed in CD4>200cells group thus resulting in a protective effect of CD4 count between 101-199 cells. Other studies have documented reduced risk of patients who initiate ART when their CD4 count is above 100cells as compared to below 100cells but this study explored the effect of CD4 count at four different levels [29]. In studies done in Thailand with categories similar to this study it was found that CD4 count 101-199 had no effect on survival but still found CD4<50 cells as a risk factor [30] . This needs to be explored further in prospective studies since this variable was not significant in the univariate analysis so it might also be a group effect of

variables that made it to be significant. This also means that survival experiences differed by levels of CD4 count.

Site of TB was a significant risk factor in the univariate analysis with those with extra-pulmonary TB being at more risk of death as compared to those with pulmonary disease. This could be explained by the fact that those with extra-pulmonary had severe disease at ART initiation as compared to the group with pulmonary who had progressed to advanced disease but not as severe as the later. However, when we did a multivariate backward stepwise regression we found that TB site and WHO clinical staging predicts each other as they had a high linear correlation thus we dropped one variable from the multivariable analysis based on the minimum AIC and BIC strategy. This can be true since if when we do clinical staging of disease those with extra-pulmonary disease are classified as stage four diseases while those with pulmonary TB can either be classified as stage three or four depending on the other co-morbidities.

WHO clinical staging was also found to be a significant predictor of survival both in the univariate and multivariate analysis. This is highly likely since staging is based on progression of disease so those with advanced disease (stage) are more likely to die as compared to those with less disease severity. This is one of the advantage of this study that it managed explore the effect of WHO clinical staging at baseline.

In the multivariate analysis taking cotrimoxazole was found to be significant though it was not in the univariate analysis. Cotrimoxazole protects AIDS patients against a wide range of infections that commonly occur in Thailand, including *Pneumocystis jirovecii* and *Toxoplasma* [30]. Initially there were concerns about use of this drug in resource poor countries but clinical trials done in countries such as Zambia, Cote d'voire and Malawi in patients with and without tuberculosis have shown that the use of the drug is feasible and safe and have beneficial effects which include 25-46% reduction in

mortality. However, this study showed a greater risk reduction of mortality of 71% and it could be due to the difference in sample size. Also patients who reacted to cotrimoxazole might have already developed severe disease upon ART initiation or other conditions that predisposes them other than those that we could study. Other studies were not able to demonstrate a survival benefit of cotrimoxazole in patients receiving ART and this was attributable to bias, misclassification, or small sample size. Further studies are needed to evaluate the survival benefit of cotrimoxazole in HIV-infected TB patients in WIDH. Pneumonia and herpes zooster were not found to be significant risk factors in this study.

Those who were single were also found to be at risk as compared to the widowed or those who were divorced

Haemoglobin was not found to be associated with survival. However, Studies done in South Africa and Asia have shown that haemoglobin level at baseline is one of the single most predictors of death [27, 29]. This could be due to the fact that the patients who presented to this clinic had almost homogeneous levels of haemoglobin so that its effect could not be seen.

Time between start of TB treatment and ART was not associated with survival and this is consistent with studies done in South Africa and South East Asia where there was no difference in survival experiences in those who started ART within 2 months after starting TB treatment and those who started ART after 2 months [27, 29]. However we did not explore the effect of those who started ART 6 months after starting TB therapy.

The demographic variables gender, employment status and age were not found to be associated with mortality and this means that all groups have equal chances of survival and thus when interventions are done no one group should receive special attention based on these results.

4.1 Conclusions

This study has demonstrated the substantial increased risk of death in patients co-infected with HIV and TB who present to the clinic with late stage disease as indicated by the WHO clinical stage criterion and the CD4 count at baseline. Patients with advanced disease by clinical criteria, those who were single and CD4 cell<50 cells were at high risk. Thus further collaboration of HIV/TB activities should be reemphasized and scale up of patients to access ART or effective treatment and control of TB among co infected patients. Regular checks of CD4 count should be done in order to monitor immunological function of those not on ART. This study has also demonstrated receiving cotrimoxazole during treatment reduces the risk of mortality therefore improving survival thus increasing patients who access cotrimoxazole is beneficial. However, further large prospective studies are recommended since they would likely reduce some of the effect of bias and missing data. The mortality rate of 9.8 per 100 person-years in HIV/TB co-infected patients on ART was high.

5.3 Limitations of the Study

Some patients' files were incomplete and we lacked information on many variables such as CD4 count, weight at baseline and follow up weights, education level, marital status, Site of TB, WHO stage and date TB treatment started and soon.

Due to the above problem variables like weight, follow up CD4 count BMI could not be well explored since there was no measurement of height taken and weights were missing for the majority of participants. Immunologic response at six months could not also be assessed in the model because we lacked follow up CD4 counts so records with fairly complete information were included in the study and this could have introduced selection bias. Also we depended on reported information in the patient

notes to determine site of TB instead of laboratory notes for diagnosis and this can have introduced misclassification bias.

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APPENDICES

A1: Survival Analysis

Survival analysis is another name for time to event analysis. It pertains to a statistical approach designed to take into account the amount of time an experimental unit contributes to a study period, or the study of time between entry into observation and a subsequent event. In survival analysis, originally the event of interest was death and the analysis consisted of following the subject until death.

The use of survival analysis today is primarily in the medical and biological sciences, however these techniques are also widely used in the social sciences, econometrics, and engineering. Events or outcomes are defined by a transition from one discrete state to another at an instantaneous moment in time. Examples include time until onset of disease, time until stock market crash, time until equipment failure, time until death either of patients or laboratory animals and so on. In this study a patient's time was observed from TB treatment to death or until observation is censored.

Survival analysis is well suited for dealing with incomplete data. Medical intervention follow-up studies are plagued with late arrivals and early departure of subjects. Survival analysis techniques allow for a study to start without all experimental units enrolled and to end before all experimental units have experienced an event. This is extremely important because even in the most well developed studies, there will be subjects who choose to quit participating, who move too far away to follow, who will die from some unrelated event, or will simply not have an event before the end of the observation period.

There are certain aspects of survival analysis data, such as censoring and non-normality, that generate great difficulty when trying to analyze the data using traditional statistical models such as multiple

linear regression. The non-normality aspect of the data violates the normality assumption of most commonly used statistical model such as regression or ANOVA, etc. Observations can be right or left censored. Most data used in analyses have only right censoring. Right censoring techniques allow subjects to contribute to the model until they are no longer able to contribute (end of the study, or withdrawal), or they have an event.

In survival analysis subjects are followed over time and observed at which point in time they experience the event of interest. It often happens that the study does not span enough time in order to observe the event for all the subjects in the study, due to a number of reasons. The common feature of all of these examples is that if the subject had been able to stay in the study then it would have been possible to observe the time of the event eventually. Censoring techniques enable researchers to analyze incomplete data due to delayed entry or withdrawal from the study. This allows each experimental unit to contribute all of the information possible to the model for the amount of time the researcher is able to observe the unit.

A2: Kaplan-Meier Survival Plot

This tool is used to visualize the Kaplan Meier survival and recurrence rate for a cohort. You can partition the data based on a single variable and compare the survival functions. The significance of difference in the Kaplan Meier survival rates for a cohort can be tested using the log-rank test.

A3: Interpretation of Kaplan-Meier Curves

Vertical axis represents estimated probability of survival for a hypothetical cohort, not actual % surviving. Precision of estimates depends on number observations; therefore, estimates at left-hand side are more precise than at right-hand side (because of small numbers due to deaths and dropouts).

Curves may give the impression that a given event occurs more frequently early than late, because of high survival rate and large number people at beginning.

A4: Log Rank Test

Comparison of two survival curves can be done using a statistical hypothesis test called the log rank test. It is used to test the null hypothesis that there is no difference between the population survival curves (i.e. the probability of an event occurring at any time point is the same for each population). The log rank test statistic compares estimates of the hazard functions of the two groups at each observed event time. It is constructed by computing the observed and expected number of events in one of the groups at each observed event time and then adding these to obtain an overall summary across all time points where there is an event.

A5: Cox regression

This model was proposed by Cox (1972) and has also come to be known as the Cox regression model. The Goal is to compare two or more groups (treatments), adjusting for other risk factors on survival times (like Multiple regression). The log rank test is used to test whether there is a difference between the survival times of different groups but it does not allow other explanatory variables to be taken into account. Cox's proportional hazards model is analogous to a multiple regression model and enables the difference between survival times of particular groups of patients to be tested while allowing for other factors. In this model, the response (dependent) variable is the 'hazard'. The hazard is the probability of dying (or experiencing the event in question) given that patients have survived up to a given point in time or the risk for death at that moment. This multivariate tool is used to identify factors that have a significant impact on the outcome. The survival or recurrence function provides information about the risk of death or recurrence of a disease for a cohort. In Cox's model no

assumption is made about the probability distribution of the hazard. However, it is assumed that if the risk for dying at a particular point in time in one group is, say, twice that in the other group, then at any other time it will still be twice that in the other group. In other words, the hazard ratio does not depend on time. The model can be written as:

$$\mathbf{Inh(t) = lnh_0(t) + b_0X_0 + \cdots + b_pX_p}$$

Where h(t) is the hazard at time t

t; x1, x2 · · · xp

are the explanatory variables; and h0(t) is the baseline hazard when all the explanatory variables are zero. The coefficients b1, b2 · · · bp

are estimated from the data using a statistical package. The assumption that the proportional hazards stay constant over time can be validated by looking at a graph showing the logarithm of the estimated cumulative hazard function. The assumption is equivalent to assuming that the difference between the logarithms of the hazards for the two treatments does not change with time, or equally that the difference between the logarithms of the cumulative hazard functions is constant. Test for effect of variable xi, adjusting for all other predictors:

H_0: $\beta_i = 0$ (No association between risk of event and xi)

H_A: $\beta_i \neq 0$ (Association between risk of event and xi)

A6: Interpreting a Cox Regression model

Interpreting a Cox model involves examining the coefficients for each explanatory variable. A positive regression coefficient for an explanatory variable means that the hazard is higher and thus the time to event is shorter. Conversely, a negative regression coefficient implies a better prognosis for patients with higher values of that variable. For explanatory variables which are categorical (for example, WHO stage, TB site, Cotrimoxazole), the regression coefficient refers to the increase in log hazard if the covariate WHO stage changes from three to four.

A7: Cox-proportional hazard model constructed in this study

The general form **is $Inh(t) = lnh_0(t) + b_0X_0 + \cdots + b_pX_p$** which can be written as

$In\ [h(t)/h_o(t)] = b_0X_0 + \cdots + b_pX_p$

Where **$In\ [h(t)/h_o(t)]$** is the hazard ratio.

Coefficients shown in table 7 below were used to obtain the equation that explains the hazard ratio as a function of the explanatory variables for HIV/TB patients on ART at Wilkins Infectious disease Hospital in this study.

Table 7: Cox regression Model coefficients

```
. stcox  _ICD4count_2 _ICD4count_4 _IMaritalSt_3     _IN8WHOStag_2 N23Cotrimox, schoenfeld(sch*) sc
> aled(sca*) mgale(mg) basesurv(surv) nohr

         failure _d:  DeathStatus
   analysis time _t:  Time_Months

Iteration 0:   log likelihood = -182.14772
Iteration 1:   log likelihood =  -165.8867
Iteration 2:   log likelihood =  -165.0094
Iteration 3:   log likelihood =  -165.0009
Iteration 4:   log likelihood =  -165.0009
Refining estimates:
Iteration 0:   log likelihood =  -165.0009

Cox regression -- Breslow method for ties

No. of subjects =          187                     Number of obs   =       187
No. of failures =           37
Time at risk    =  5115.800014
                                                    LR chi2(5)      =     34.29
Log likelihood  =    -165.0009                      Prob > chi2     =    0.0000
```

_t	Coef.	Std. Err.	z	P>|z|	[95% Conf.	Interval]
_ICD4count_2	-1.205936	.5824147	-2.07	0.038	-2.347447	-.0644239
_ICD4count_4	.8634015	.3657412	2.36	0.018	.1465619	1.580241
_IMaritalS~3	.885414	.4030643	2.20	0.028	.0954224	1.675406
_IN8WHOSta~2	.988089	.3507991	2.82	0.005	.3005354	1.675643
N23Cotrimox	-1.236973	.6225476	-1.99	0.047	-2.457144	-.0168022

Where _ICD4count_2 =101-199 cells/µ*l*,

_ICD4count_4 = CD4 <50 cells/µ*l*,

_IMaritalS~3= Single

_IN8WHOSta~2= Stage iv,

N23Cotrimox= receiving cotrimoxazole.