The Effect of Tuberculosis Infection on the Body Composition of HIV positive Adult patients' on HAART in Johannesburg South Africa.

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DECLARATION

I, Caroline Govathson declare that this research report is my own work, compiled under the supervision of Dr. Charles. S. Chasela and Ms Kate Shearer. The report is being submitted to the University of the Witwatersrand in partial fulfilment of a degree of Master of Science in the field of Epidemiology and Biostatistics. There are no prior submissions of this material to other institutions for academic purposes.

Signature

C.Govathson

Date: 2014 November 10

DEDICATION

I dedicate this report to my husband Lovemore Simbarashe Mandimika, whose love, support and continued encouragement gave me strength and determination. I am so blessed to have a partner like him. I also dedicate it to my parents who have been a constant source of support, inspiration and encouragement. Without their sacrifice, this report would not be possible.

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Lastly all glory goes to God, through whom all things are possible.

ABSTRACT

Background:

Both HIV and tuberculosis (TB) have been documented to have detrimental effects on the nutritional status of those infected and nutritional status is a strong predictor of disease progression and survival. Body composition measures can be used as a proxy for nutritional status and takes into account body fat, muscle and water. It constitutes Fat Mass(FM), Fat Free Mass (FFM), Total Body Water (TBW), Extracellular Water (ECW), Intracellular water (ICW), Daily Energy Expenditure (DEE), Basal Metabolic Rate (BMR), phase angle and BMI which can be analysed as separate outcomes. Its use in evaluation of nutritional status has been reported to give more accurate results than the use of weight alone. We compared body composition measures and changes over a 12 month period in patients with HIV alone to patients with HIV and TB.

Objective:

This study aimed to determine the effect of tuberculosis co-infection on body composition in HIV-infected patients over the first 12 months of treatment.

Design and Methods:

We conducted a secondary analysis of data from the Low–Cost Monitoring of HIV in Resource-Limited Settings (LCM) study to investigate changes in body composition measures and clinical measures from antiretroviral therapy (ART) initiation between TB positive (TB+ve) and TB negative (TB-ve) patients. Patients had to have TB (positive smear and culture) at ART initiation or been diagnosed one month before or after initiation. Body composition measures were at baseline, 6 months and 12 months. Continuous variables were compared using the Wilcoxon rank sum test and multiple regression analyses using longitudinal data Generalised Estimating Equation models to evaluate the relationship

between TB and body composition measures among HIV-infected patients, controlling for CD4 count, viral load, socio-economic factors and other clinical characteristics. Participants who provided three repeated body composition measures (baseline, 6 months and 12 months) were included in the multiple regression analyses. To account for the missing data, a sensitivity analysis was conducted using Multiple Imputation- Generalised Estimating Equations (MI-GEE). We analysed the data using imputed values for all missing information including both predictor and response variables. Multiple imputations provided complete sets of data by filling all missing data.

Results:

A total of 345 participants were evaluated at baseline. Of these, 36 (10.43%; 95%CI: 7.4%-14.15%) participants had TB. Patients in the TB+ve and TB-ve groups were similar in baseline demographic characteristics. TB+ve patients had significantly lower median fat mass (FM) than TB-ve patients (TB+ve: 19.40kg; IQR 11.95-27.35 vs. TB-ve: 11.90kg; IQR 7.20 - 21.75; p-value=0.003) at baseline. Differences in FM by TB status at baseline were more pronounced in females than males. Females without TB had significantly lower FM than those with TB (23.60kg vs. 16.80kg) with a p-value=0.024. This difference, however, was not significant in males (11.95kg vs. 9.5kg).

At baseline, TB+ve participants had a statistically significant lower median CD4 count (113 cells/mm^3; IQR: 63-199) than TB-ve patients (207 cells/mm^3; IQR: 116-279), with a p-value<0.001, TB+ve patients also had a significantly higher median log viral load (5.20; IQR: 4.87-6.00) than TB-ve patients (5.00; IQR: 4.60-5.40) with a p-value=0.006. Although not statistically significant, changes in clinical and nutritional measures were larger in TB+ve compared to TB-ve patients. At 6 months TB+ve patients demonstrated a greater increase in body mass index (BMI) (1.60% vs. 2.20%; p-value=0.950) and FM (5.10% vs. 13.80%; p-

value=0.610) than TB-ve patients. A similar trend was observed for CD4 count at 12 months but not for FM (19.14 vs. 42.3; p-value = 0.302). The sensitivity analysis showed similar results at 6 months with statistical significance, TB+ve patients demonstrated a greater increase in BMI (2.21% vs. 4.20%; p-value=0.044), FM (6.50% vs. 23.0%; p-value <0.001), and CD4 count (70% vs. 113%; p-value <0.001) compared to those without TB.

In all GEE models; there was no association between TB and any of the body composition measures after adjusting for possible confounders. However, baseline CD4 count, viral load, education, employment and gender were all associated with body composition amongst HIV-infected patients in both crude and adjusted models. The sensitivity analysis, however, showed that TB+ve patients had a decreased FM compared to TB-ve (-5.967kg; p-value = 0.041). There were no other significant associations found.

Conclusions

Patients with HIV/TB co-infection have lower BMI and FM at baseline than patients with HIV-infection alone. This association at baseline is more apparent in females but males have lower nutritional measures and immunological indicators regardless of TB status. Malnourished status in HIV/TB infected patients has been found to be associated with severity of TB amongst those infected. Although we restricted the time of TB diagnosis to within one month of ART initiation, this analysis did not include data on treatment status, or severity of TB infection. This could have contributed to lack of significance for some body composition measures. Although not statistically significant, patients with HIV/TB co-infection experienced greater changes in nutritional status compared to patients with HIV alone. Over the three visit periods, gender, education, CD4 count, viral load and employment were associated with body composition measures of HIV-infected patients.

Key Recommendations

Integration of HIV/AIDS and TB programs is important to detect TB cases early and intervene. Strategies to improve outreach activities to encourage males to test often and early could result in earlier detection of HIV and TB which could improve overall nutritional status of patients. Larger changes in FM, BMI and CD4 count amongst those with TB indicates that treatment of TB co-infected individuals with ART and TB medication improves the nutritional and immunological status of such patients. Further studies should be conducted with information from a larger population including TB severity and TB treatment status. This could provide a better explanation of the relationship between TB and body composition measures amongst HIV-infected individuals.

ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ART `	Antiretroviral Therapy
ARVs	Antiretroviral drugs
BCM	Body Cell Mass
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CI	Confidence Interval
DXA	Dual-energy X-ray absorptiometry
d4T	Stavudine
ECM	Extra Cellular Mass
ECW	Extra Cellular Water
EFV	Efavirenz
FFM	Fat Free Mass
FM	Fat Mass
GEE	Generalised Estimating Equations
HAART	Highly Active Antiretroviral Therapy
HE^2RO	Health Economics and Epidemiology Research Office
HIV	Human Immunodeficiency Virus

IQR	Interquartile range
LCM	Low cost monitoring
MI	Multiple Imputation
MICE	Multiple Imputation by Chained Equations
QIC	quasilikelihood under the independence model criterion
QICu	An approximation of QIC for variable selection
TB	Tuberculosis
TBF	Total Body Fat
TLC	Themba Lethu Clinic
UN	United Nations
WHO	World Health Organisation

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GLOSSARY OF TERMS

Bioelectrical Impedance: The measure of a body's resistance to the flow of an electrical signal passed through the body.

Body Cell Mass (BCM): The total mass of all the cellular elements in the body which constitute all the metabolically active tissue of the body

Body Weight: Sum of Fat mass (FM) and Fat Free Mass (FFM). This can be divided into three compartments i.e. Body Cell Mass, Extracellular Mass and Fat Mass.

Body Mass Index (BMI): The level of adiposity calculated using a person's weight and height. It is calculated as weight in kilograms divided by height in meters squared

Basal Metabolic Rate (BMR): Rate of energy expenditure of a body while at rest to maintain vital functions.

Daily Energy Expenditure (DEE): The number of calories a body burns at rest during an average day determined by the number of cells producing oxidative energy

Dual-energy X-ray absorptiometry (DXA): An X-ray technique used to measure body composition, including proportion of fat and bone mineral density.

Extracellular Mass (ECM): Tissue found outside the cell that provides the body structure and support and transport.

Extracellular Water (ECW): Fluid outside the cell that circulates outside the body. ECW is included in extracellular mass.

Fat Mass (FM): Functions as a major source of energy for the body. Fat stores help maintain body temperature and protect organs from trauma.

Fat Free Mass (FFM): FFM is made up off all the components of the body that are not fat. It includes skeletal muscle, bone and water.

Impedance: The total resistance of a biological conductor to alternating current

Intracellular Water (ICW): Body's fluid found inside the body cell mass and contains large amounts of potassium, magnesium and phosphate ions. Changes in ICW reflect changes in BCM. An increase in ICW usually means an increase in BCM.

Phase Angle: Phase angle is based on total body resistance and reactance. Lower values appear to be consistent with cell death. Higher values appear to be consistent with greater BCM and less morbidity and mortality.

Total Body Water (TBW): Expressed as a percentage of weight and includes intracellular and extracellular water.

Quasilikelihood under the independence model criterion (QIC): QIC is a goodness of fit index that is used for GEE model fit evaluation. The evaluation of choosing the best model of QIC and QICU is the one with the smallest value.

Quasilikelihood under the independence model criterion (QICu): Approximation of QIC that can be used for variable selection.

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CHAPTER 1: INTRODUCTION

1.1 Background Information

HIV/AIDS persists as a global pandemic with 35.3 million people globally living with HIV, of which 25.0 million people live in Sub-Saharan Africa(1). The relationship between HIV/AIDS and nutrition is well established and in both directions (2-4). The impact of HIV/AIDS on health and nutrition in countries greatly affected by the disease has been overwhelming (5). The nutritional impact is increased because HIV-related infections, like tuberculosis (TB), have severe implications on nutritional status that normally result in loss of appetite, weight loss and wasting (5, 6). This is a problem as HIV-related TB remains a serious challenge for the response to HIV/AIDS. In Sub-Saharan Africa, a large proportion of HIV-infected individuals also have TB as the occurrence of active TB in HIV-infected is more than eight times higher than in the HIV-uninfected (7). Of those with HIV-infection an estimated 30-50% will develop active TB (8) and TB is responsible for the largest number of deaths among those living with HIV/AIDS (1).

South Africa has the highest number of people currently living with HIV/AIDS (6.4 million) in the world and TB is still one of the most common opportunistic infections in those infected (1). A total of 330,000 of the country's' estimated 520,000 new TB cases in 2011 were related to HIV (1). South Africa not only has the problem of the dual-epidemics of HIV/AIDS and TB but it also has a problem with food scarcity which leads to the problem of malnutrition. HIV-related illness and death has also reduced the capacity of households to earn an income as people may be unable to work (9). Many patients become too sick to work which affects their ability to afford food. The resulting poor nutrition has detrimental effects on their health status increasing their risk of morbidity, hospitalisation and mortality (5, 10, 11). Nutritional status can be measured by the use of body composition measures which is a

measure of the amount of fat, bone, water and muscle in the human body. The components that constitute body composition are Fat mass (FM), body cell mass (BCM), extracellular mass (ECM) and total body water (TBW). Depletion of body weight, fat mass (FM) and fat free mass (FFM) are major symptoms of the advanced stages of HIV-infection. These body composition measures are proxies for nutrition which is a strong predictor of survival in HIV-infected persons (12). TB on its own is associated with wasting, loss of weight and FFM, and is thought to be a primary cause of HIV associated wasting in co-infected individuals because it worsens the wasting seen in HIV-infection (13). This suggests that patterns and severity of nutritional deficiency in individuals with HIV/TB co-infection are likely to be different from those with HIV alone or TB alone. Patients with co-infection may be at great risk of extreme wasting and this may be a major contributing factor to increased mortality in this group of individuals (13, 14).

Poor nutritional status has been associated with increased morbidity, mortality and poor immunological response to ART in HIV-infected individuals (15, 16). Malnutrition increases the immune suppression seen in HIV/AIDS which increases the risk of opportunistic infections (16) and may also interfere with a patient's response to antiretroviral therapy (ART) when initiated. Opportunistic infections like TB have an effect on nutritional status and can affect food intake absorption and metabolism. This produces problems of infection, malnutrition and weakening of immune system (16) which leads to increased hospitalisations in HIV-infected individuals (10). This not only has implications on patients' health but on the health care system as well.

The clinical management of HIV-infected individuals should include nutritional assessment as good nutritional levels can help improve treatment outcomes, quality of life and overall health of patients (17) and poor nutrition further weakens the body's immune system and accelerates disease progression (5). Interventions such as nutritional supplementation have

been shown to increase body weight and correct changes in FM and FFM in HIV–infected patients (18). The presence of TB in these patients is likely to further complicate the management of HIV-infected individuals as TB co-infection may increase the nutritional effects of HIV alone resulting in increased risk of poor response to treatment, morbidity and death (19).

Nutritional status in management of patients with HIV and/or TB is assessed by routine measurement of body weight (5). However weight on its own provides inadequate information about the nutritional status of patients with HIV and/or TB and can be misleading (5). Kotler et al. showed that measurement of weight alone was not enough to identify losses of body cell mass (BCM) and FFM which studies have shown to be predictors of morbidity and mortality in those infected (11). FFM has also been shown to affect treatment outcomes among both TB- and HIV-infected people (20) and to be a predictor of mortality in HIV-infected individuals in other research (21). Assessment of nutritional status through body composition measures like FM, FFM and BCM is therefore necessary to obtain a true reflection of the nutritional and health status in HIV-infected patients (8).

Bioelectrical impedance analysis (BIA) is one of the tools that can be used in the determination of nutritional status in HIV-infected individuals and has been recommended as a method for measurement of body composition (22).Bioelectrical impedance is measured when an electrical current is passed through the body. The rate at which the electric current passes through the body is dependent on body composition. BIA determines the electrical impedance/resistance of body tissue which provides an estimate of total body water (TBW) (22). Specific components of weight loss that are important to the development of adverse outcomes like FFM, FM, BCM and other body composition measures can then be determined(18). This study used BIA to examine the impact of TB on body composition measures amongst HIV-infected patients with TB and without TB.

1.2 Statement of problem and Justification

South Africa has the largest HIV/TB co-infection burden in the world and TB is still the foremost cause of death amongst HIV-infected individuals in South Africa (1). Poor nutritional status in people with HIV/TB co-infection is likely to be worse than in HIV or TB alone (23, 24). Studies have shown that poor nutritional status of patients, specifically loss of FM and FFM, has been associated with adverse outcomes, including hospitalisation and death, in both HIV-and TB-infected individuals (3, 6, 11). This has led to increasing recognition of the importance of nutritional assessment using body composition measures in HIV/AIDS management. In response to this, the South African National Guidelines on Nutrition recommend that total body weight, body composition, and body weight distribution be measured as a part of routine health care in persons with HIV (25).

In South Africa, both TB- and HIV-infections occur among populations that already suffer nutritional deficiency (25). Trends of body composition in this population are likely to be different due to availability of nutritional food, different dietary patterns, and differences in HIV management practices (26). Most studies on body composition changes have been conducted in resource-rich places and the results from them may not be generalizable to the South African population (10, 27). While few studies have been conducted in other parts of Southern Africa (16, 28), studies on body composition in HIV and TB co-infected patients in South Africa are lacking. The shortage of information from the South African setting makes it difficult to make policy-level and individual decisions for the HIV-infected South African population.

The relationship between malnutrition, TB and HIV-infection needs to be further investigated, especially in South Africans because of the prevalence HIV/AIDS, TB and malnutrition in this population (18). Studies that have been conducted on body composition

and HIV/TB co-infection have mostly been cross-sectional and have not measured change in body composition over time, especially in the era of ART (14, 16, 28, 29). The use of ART is associated with its own changes in body composition (5, 27) which may make body composition measures difficult to interpret in HIV-infected individuals on treatment. Thus, studies of body composition in patients on ART are important to measure response to treatment.

Linkages between HIV/TB and nutrition in resource limited settings are important to improve outcomes of patients. This study will provide information on characteristics of patients that are most at need for initiatives to improve nutritional status in HIV-infected individuals. Limited health resources demand that interventions be targeted towards those who need them most and our study will provide information that will inform these decisions

1.3 Literature Review

This section is a narrative of the literature that was reviewed for this research. The areas reviewed include: HIV and malnutrition

1.3.1 HIV and Malnutrition

The relationship between HIV and nutrition has been studied by researchers since the beginning of the HIV/AIDS pandemic and poor nutritional status has been determined to affect HIV/AIDS and vice-versa (9). Measures of weight and BMI have been used to assess nutritional status in HIV management (30), however, in a study of 33 AIDS patients, Kotler et al. showed that HIV was associated with greater percentage losses of body composition measures than weight (10). Thus, they concluded that weight alone was not enough to assess nutritional deficiencies in HIV-infected patients and body composition measures like BCM, FM and FFM were important to provide a better picture of nutritional status of patients (11).

In another study, Kotler et al. used body composition analysis to examine the extent of tissue wasting in patients with AIDS defining illnesses. They observed body composition changes in HIV/AIDS patients similar to those in HIV-uninfected, malnourished patients (11). The authors suggested that loss of body cell mass (BCM) and other body composition measures could influence the course of the disease and suggested that preventing depletion of BCM could prolong survival (11).

Likewise, Suttmann et al. showed that decreasing BCM and FFM in HIV-infected patients was associated with a poor prognosis (31). They showed that BCM was independently associated with death in HIV-infected patients and suggested detailed nutritional assessment as part of HIV/AIDS management. Analysis from the Tufts Nutrition for Healthy Living study that described weight loss and wasting in HIV disease during the time of ART showed that losses in weight, BCM and FM were all significant predictors of mortality in patients with HIV wasting syndrome (32).

There is considerable evidence showing that there is an association between malnutrition and adverse clinical outcomes in people living with HIV (11, 31, 33). While the studies described above were conducted in resource-rich settings, this relationship has also been investigated in some low-resource countries. A study in Rwandan women did not show any significant differences in body composition in HIV-infected individuals compared to HIV-uninfected individuals (34).The authors suggested that this was a result of malnutrition being common in the cohort indicating that malnutrition was endemic in the general population (34). Malnutrition could be a population-level problem in resource limited settings. A study in Malawi investigated TB patients with and without HIV-infection. They found that both HIV-infected and uninfected patients with TB were malnourished with no significant difference in their nutritional status (14). This suggested that TB may be the main cause of malnutrition not HIV (14). The above studies indicate that different environments may show differences in associations between malnutrition and HIV.

Patients receiving nutritional interventions have been seen to show improvements in body composition measures compared to those receiving placebo (18). This suggests that nutritional interventions could result in better treatment outcomes and reductions in morbidity and mortality, in HIV-infected individuals (11, 18).

1.3.2 TB and nutritional status

Malnourished people are more likely to develop TB and TB exacerbates malnutrition in those infected (6, 25). In low-resource countries where there is the highest burden of TB, the impact of TB on nutritional status is even bigger at population level (25). TB has been found in studies to be associated with serious wasting and body mass depletion, increasing the risk of mortality (3, 20, 35). The relationship is bidirectional and malnutrition has been shown to exacerbate the clinical manifestations of TB whilst TB in itself causes malnutrition (24).

1.3.3 TB/HIV co-infection

Studies conducted in HIV/TB co-infected patients have shown that survival rates in patients with HIV/TB co-infection are worse than of people with HIV alone (23, 29, 36).HIV-infected individuals are at greater risk of developing TB (37) and TB has been recognised as a major co-factor in HIV wasting (13) with some studies suggesting that TB is the main factor in the wasting in HIV/TB co-infected individuals (36). Co-infection of HIV and TB is thought to exacerbate the malnutrition seen in TB- or HIV-infection on its own (3).

A study conducted in Singapore among TB-infected men noted that the presence of HIV co-infection did not alter the magnitude or distribution of the body composition (FM and FFM) changes in patients with TB wasting (33). However, a study conducted in Malawi found that HIV-infection was associated with decreased FFM in patients with TB (19). Other studies have looked at the impact of TB on body composition measures in HIV-infected individuals. Paton et al. looked at the impact of TB on body composition in HIV-infected men and found that the nutritional status of HIV-infected men with TB was significantly worse than HIV-

infected men without TB co-infection (6). Van Lettow et al. also found that both HIV-infected and HIV-uninfected adults with TB were extremely malnourished compared to patients without TB or HIV-infection. This showed that TB is the main cause of malnutrition in co-infected individuals(28).Some studies have investigated gender differences in the effect of HIV on body composition in individuals with TB and found that the differences in body composition were by gender and not necessarily HIV status(38, 39). Females have been seen to have larger values for FM and males generally had larger values of FFM (38, 40).

Confounding factors

Some studies did not take into account the influence of confounders like socio-economic status and smoking and alcohol status (16, 29). It has been suggested that the setting of the study plays a major role in the results on the relationship between HIV, TB and malnutrition (26). The results from countries where malnutrition is endemic are likely to be different to those from countries where malnutrition is not a problem in the general population. A study amongst TB-infected patients in Tanzania investigated the association between gender, smoking and socio-economic status with body composition and found that all three were associated with nutritional status amongst these patients (40).

CONCEPTUAL FRAMEWORK

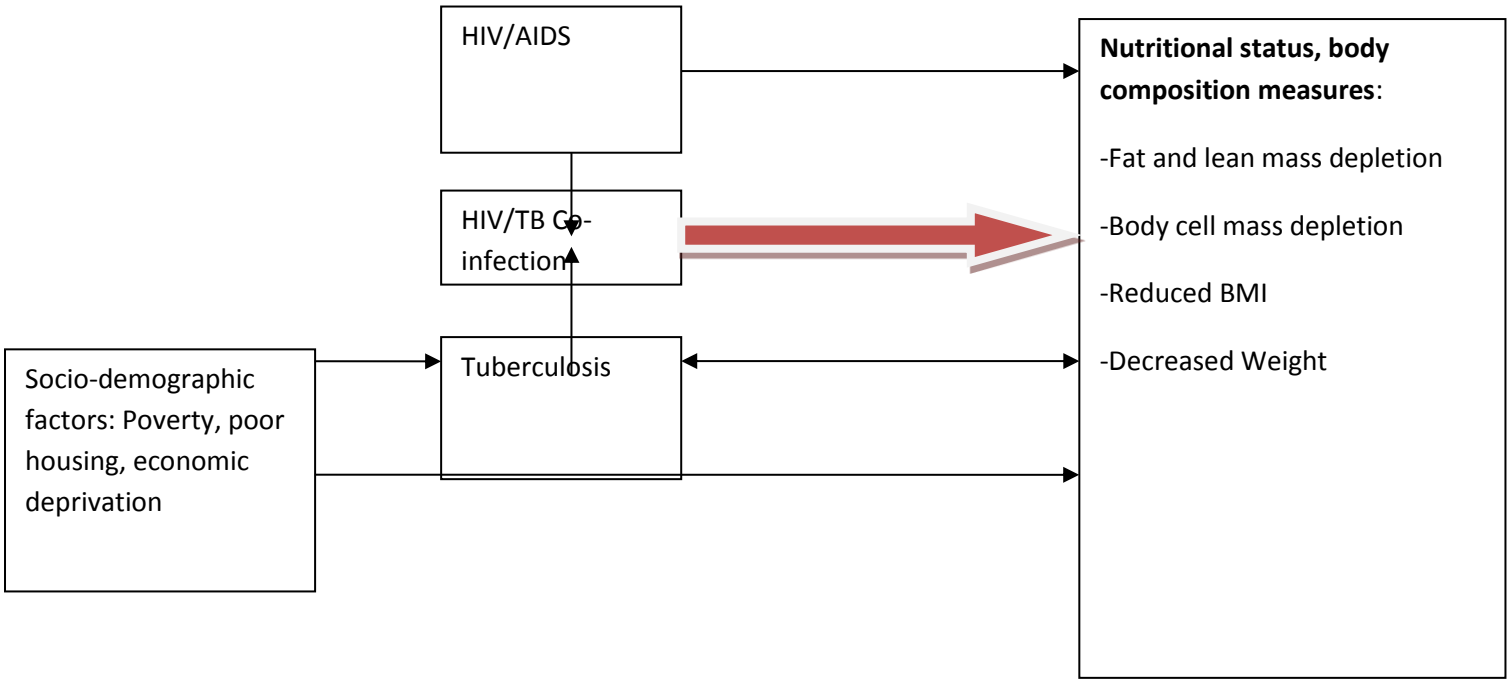

FIG 1.1: Conceptual Framework. Relationship between TB, malnutrition and HIV/AIDS

The conceptual framework (fig 1.1) above shows the relationship between HIV/AIDS, TB and nutritional status as measured by body composition measures. We created the framework by looking at relationships as seen in the literature (6, 29, 40). The relationship between TB and nutritional status is bi-directional and TB can be seen as a major co-factor in HIV-associated wasting. Factors like socio-demographic and behavioural factors (smoking, employment status) may also have an effect on body composition measures and may also be directly associated with TB.

1.3.4 Bioelectrical Impedance Analysis (BIA) and body composition

The term body composition refers to compartments of the body that constitute person's weight. Body composition may be represented as a three compartment model with FM, FFM

and water. It can be measured using a range of techniques that include dual-energy X-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). The BIA model is however based on a four compartment model for body composition, FM, body cell mass (BCM), extracellular mass (ECM) and total body water (TBW). FFM is the sum of BCM and ECM, whilst TBW is the sum of extracellular water ECW and Intracellular fluid (ICF) (41). Phase angle is marker calculated using reactance and resistance obtained from BIA (42), BIA provides a better assessment of FFM and FM and has been used in epidemiological studies (43).

Body mass index (BMI)

BMI is used in many settings to determine nutritional status in HIV-infected individuals. It is a ratio between weight and height and the World Health Organization (WHO) suggests the following classification values: underweight ($<18.5 kg/m^2$), normal ($18.5\text{-}24.9 kg/m^2$), overweight ($25\text{-}29.9 kg/m^2$), obesity ($30\text{-}39.9 kg/m^2$) and morbid obesity ($\geq 40 kg/m^2$) (44). As a measure of nutritional status it has been used as a predictor of outcomes in HIV-infected individuals for several years. Benova et al. investigated 1090 HIV/TB co-infected patients and found that patients with HIV/TB co-infection presented at baseline with lower BMI than patients with either TB only or HIV only (45). In addition, the authors found that remaining severely underweight or moving to a lower BMI increased the risk of mortality (45).

Phase Angle

Phase angle measures the overall wellness of an individual and is calculated from total body resistance and reactance (2). Lower phase angle have been found to be associated with cell death and lower BCM (2, 42, 46). Improvements in phase angle have linked to improvements in BCM resulting in better outcomes including survival in patients with HIV (2, 46).A study amongst 539 HIV-infected adults in Uganda found that in Sub-Saharan Africa, co-infection with pulmonary TB and HIV is associated with lower phase angle (32).

Fat mass (FM) and fat free mass (FFM)

Fat mass (FM) is responsible for storage of energy in the body and FFM is the combination of all tissues and cells that are not fat. FFM is the difference between weight and FM (46). The two measures have been used as measures of nutrition for prediction of survival and treatment outcomes. They are more accurate than methods like BMI and weight measurement and this makes them preferable for nutritional status measurement (43). Among HIV-infected patients, loss of FFM and FM has been found to be strong predictors of mortality in the ART era. Increases in FFM and FM have been seen with the use of ART (47) indicating improved nutrition in patients with the use of ART.

1.3.5 Summary of literature review

Literature is consistent on the association between HIV and nutritional status. HIV has been seen to be associated with depletion of body composition measures and wasting weight, FFM, FM and phase angle (9). The relationship between TB and nutritional status has also been established to be bidirectional with some researchers contending that TB is the primary driver of malnutrition in patients with HIV/TB co-infection (24, 23, 29). Decreases in body composition measures like FM, FFM and phase angle are associated with poor prognosis in those infected (31). There are however gaps that exist in the knowledge base. Most studies evaluating the relationship between HIV/AIDS, TB and nutritional status have been cross sectional in nature (3, 6, 10, 11, 13). Some studies for example, although they showed that the nutritional status of HIV/TB co infected patients was significantly worse than HIV alone (28, 29) but this study was cross sectional. There was no consideration of confounders that may also have an impact on nutritional status and temporality. Cross sectional studies do not deal with the issue of temporality that may be in question where conditions like HIV/AIDS,

TB and nutrition are concerned. These studies used t-tests and nonparametric Mann-Whitney U-tests for comparisons between groups (3, 28, 29). Most studies conducted also looked at impact of TB and impact of HIV/AIDS on nutrition separately without investigating the co infection (10,11,26). It is important to investigate the co infection because of the burden of HIV/AIDS and TB in our setting. A longitudinal study where there are repeated measures will help solidify the knowledge base. Few studies have investigated all the factors using rigorous longitudinal data analysis methods. Use of more rigorous statistical methods that allow for adjustment for confounders and analysis of repeated measures will yield more reliable results (36). Although studies done prior to the era of ART provide an evidence base (6, 13, 31) studies that also investigate the effect of ART are important. ART on its own has been seen to have an impact on body composition measures (32, 37, 64)

Setting may play an important role in this relationship however, with factors like endemic malnutrition in population, smoking and alcohol status, ART as well as gender playing important roles in these associations (40). Studies conducted in the era of ART have shown differences in responses of these measures to ART and more research is needed on this aspect (32, 37, 64). There is not enough information on the effect of TB on both body composition changes and body composition amongst HIV-infected individuals. This study investigated this effect.

1.4 Research Question

Does presence of TB at ART initiation (defined as TB one month before or after ART initiation) in HIV-infected individuals' have an effect on body composition?

1.5 Research Aim

To determine the effect of co-infection with TB on body composition among HIV-infected patients on HIV treatment over 12 months.

1.6 Objectives

1. To describe and compare baseline characteristics (including body composition measures) of HIV-infected adults with TB co-infection and those with HIV alone.
2. To assess the effect of TB on body composition changes over a period of 12 months in HIV-infected adults on ART.
3. To evaluate the relationship between TB and body composition measures among HIV-infected adults on ART over 12 months

CHAPTER 2: METHODOLOGY

2.0 Introduction

This chapter describes the methods used in the study as well as procedures undertaken to ensure validity of the results. It details the primary study, study design, a description of the study population and the variables that were collected and their measurement. It also details the data management and analysis processes.

2.1 Study design

This is a secondary data analysis of prospectively collected data of HIV-infected adults initiating ART at the Themba Lethu Clinic in South Africa.

2.2 Study Setting

The primary study was conducted at the Themba Lethu HIV Clinic at Helen Joseph Hospital, public-sector hospital in Johannesburg, South Africa.The clinic is the largest ART site in South Africaand provides HIV testing services and care before ART initiation and after (48). It offers TB services operating a TB focal point where TB can be diagnosed and treatment initiated (18).

2.3 Primary study

The Low Cost Monitoring (LCM) of HIV in Resource-limited Settings study enrolled 353 adult HIV-infected patients from January 2013 to May 2014 at the Helen Joseph Hospital. At enrolment, a baseline history, including capturing of information on demographic characteristics and any medications patients were currently taking was taken. Measurement of CD4 count, viral load, BIA and BMI was done at baseline and then every 6 months during the study. Total body FM, FFM, phase angle and other body composition measures were calculated from resistance and reactance using standard formulae. Body mass index was

calculated as weight in kg/ (height in meters) ^{2}. Active TB was confirmed by sputum smear microscopy and culture.

2.4 Study Population and Sampling

This study analysed the records of the 353 adult, HIV-infected, ART-naïve patients. Participants were recruited on the day they initiated standard first-line ART under the 2010South African National Department of Health ART treatment guidelines (49). All participants from the primary study who met the eligibility criteria described below were used in the main study. The sample size for the longitudinal data analysis was 128 patients and was obtained from participants with outcome data on all three visits (see Fig 3.1).

2.4.1 Inclusion Criteria

- HIV-infected adults (aged 18 years and older)
- Initiating ART on day of enrolment

2.4.2 Exclusion Criteria

- Participants who were pregnant at enrolment or who fell pregnant during the course of the study
- Participants who were already on ART or were ART-experienced at the start of the study

2.5 Measurement and data sources

Data for HIV-infected patients from the LCM study was analysed. Information collected upon initiation included patient demographics such as sex, age, employment and education as well clinical data such as TB co-infection status, CD4 count, BMI and viral load. Height, weight and body composition measures were collected for each patient at baseline, 6 months and 12 months. FM, FFM, TBW, ICW, ECW, phase angle α, basal metabolic rate (BMR) and

daily energy expenditure (DEE) were estimated using the Rudolph J. Liedtke (RJL) Body Composition 2.1 software from the resistance, reactance and patient details that were recorded (18).

The bioelectrical impedance of the body has been used as an indirect measure for body composition, because it is a reflection of both its structural and functional characteristics (41, 52). Kyle et al. recommend BIA as the preferred method for clinical assessment of body composition measures, especially FM and FFM (43). When you pass an electric current through the body the rate at which it passes depends on body composition. The body is composed of components through which the electric current can flow (water with ions) and those that provide resistance to the flow (body fat). There is a relationship between reactance and the resistance of the solution (22). BIA determines the electrical impedance/resistance of body tissue which provides an estimate of total body water (TBW). Other body composition measures can then be calculated from these measures.

2.5.1 Study measures

2.5.1.1 Outcome variable

Body Composition was used as a measure of nutrition in this study. There is generally no composite measure of the individual body composition measures and most researchers investigate the measures separately (6, 14, 50). We therefore analysed FM, FFM, TBW, ECW, ICW, DEE, BMR, phase angle, LDM and BMI as separate outcomes as some of the measures are correlated. The study therefore aimed to investigate the association between TB and each of the body composition measures separately.

To determine the effect of TB on percentage change of each measure, percentage change was calculated by taking the difference of body composition measures at different time points and dividing by the initial measure. We also used height normalised indices of FM and FFM which are fat free mass index (FFMI) and fat mass index (FMI) as these have an advantage of

compensating for differences in height. We defined wasting using FFM by cut off points of 16.7kg/$m^2$for men and 14.6kg/m^2 for women. We also defined it using FMI as <1.8kg/$m^2$for men and <3.9kg/$m^2$for women. These definitions were reported by Kyle et al. in his article on interpretation of body composition (51).

2.5.1.2 Exposure Variables

1. **Main exposure**
    - TB (positive smear and culture) at ART initiation, defined as TB diagnosed between one month before and one month after initiation. There was no information on TB treatment status.
2. **Socio-demographic characteristics**
    - Age in years at the time of ART initiation
    - Sex
    - Level of education: (< grade 8 and ≥ grade 8)
    - Current employment status (employed and unemployed)
    - Nationality (South African and other)
3. **Clinical characteristics**
    - Baseline viral load (copies/ml)
    - Baseline CD4 count (cells/mm^3)
    - ART drugs initiated
        - TDF/3TC/EFV
        - TDF/3TC/NVP
        - d4T/3TC/EFV

2.6 Data management and analysis

2.6.1 Data management

The data from the primary study was stored in an excel spread sheet. Data on some routine information like baseline characteristics for participants was stored in an electronic patient management system called Therapy Edge-HIV™. This data was exported from Therapy Edge-HIV™ and imported into SAS. Data was then imported into STATA 12™ for analysis. Data from Therapy Edge-HIV™ was merged with data from the LCM study using the patient's medical record ID.

The data was cleaned and checked for duplicates and missing data. For convenience in analysis some variables were renamed and recoded. All variables not needed for our study were dropped. For the purposes of this analysis, age at initiation of ART into 10 year age groups (18-29.9, 30-39.9, 40-49.9, and ≥50). BMI was categorised into underweight (<18.5kg/m^2), normal (18.5-24.9kg/m^2), overweight (25-29.9kg/m^2) and obese (≥30 kg/m^2). For the purposes of this analysis obese and morbidly obese were analysed within the same category. This was done because clinically the two groups are similar and statistically this allowed sufficient numbers in the groups for statistical analysis. CD4 count was analysed both as a continuous variable and a categorical variable. The categories for CD4 count were <100, 100-249, 250-349 and ≥350. Viral loads were log transformed and also categorised into detectable (≥400 copies /ml) and undetectable (<400 copies/ml) for analysis. Government prescribed HIV drug regimens were used as standard drug regimens with those on Tenofovir (TDF) based regimen in one group and those on Stavudine (d4t) based groups in another. Nationality was categorised as South African or foreign and education level of patients was categorised into either completed grade 8 or higher or education up to grade 8.For the purposes of multiple imputation, data was required in wide format in order to use data from other visits as predictors for imputing. Generalized Estimating Equations (GEE) however

required being in long format. Data was converted between the two formats as analysis required.

2.6.2 Exploratory Analysis

Exploratory analysis to summarize the main characteristics of the data and to identify outliers, trends and data patterns was done using box plots and histograms. Normal probability plots, quantile–quantile plots and histograms were used to determine if continuous variables were normally distributed. The Shapiro-Wilk test was also used to check for normality of data. Non-parametric statistical tests were then performed on all of the skewed variables. Exploratory analysis of data was done using the user written commands mstable and misschk as well as summarise in STATA to view the patterns of missingness across the visits.

2.6.3 Descriptive Analysis

As part of our descriptive analysis we looked at the missingness of data over the different visits. We determined the number of participants who had data for all three visits for the purposes of a complete case analysis. All participants enrolled into the LCM study who met the inclusion criteria for our analysis were used in baseline descriptive analyses regardless of availability of follow-up measures. Participants in the analysis were categorized as either co-infected with TB or without TB. Baseline characteristics for each group were summarized as medians with interquartile ranges (IQR), as all the continuous data was skewed, and proportions with confidence intervals determined using binomial exact methods for categorical data. Continuous variables were compared using Wilcoxon rank-sum tests between the two groups. Stratified analysis was performed by gender to assess for effect measure modification because of the significant effects of gender on normal body composition. Box plots of body composition parameters for men, women and those with TB and without were plotted. Two way comparisons of frequencies of categorical data were

done using Pearson's chi squared-tests where the expected value is greater than or equal to 5 and Fischer's exact where the expected cell value is less than 5. A p-value of <0.05 was considered as significant in all analysis.

Profile plots were used to graph the medians of the body composition parameters for the two groups HIV+/TB- and HIV+/TB+ over the three time points. The profiles of the groups were then compared to one another.

2.6.4 Inferential Analysis

2.6.4.1 Changes in body composition models by TB status (Objective 2)

For selected body composition measurements, the change from baseline to each follow-up visit by TB status was calculated by taking the difference .To calculate percentage change the difference was divided by the baseline value and this was multiplied by 100. Comparisons of median percentage change in body composition parameters between the two groups were done using the Wilcoxon rank-sum test. Changes were calculated from baseline to six months and baseline to 12 months. All statistical tests were two sided and a p–value of less than 0.05 was regarded as statistically significant.

2.6.4.2 Generalised Estimating Equations (GEE)(objective 3)

A subset of the study population which included only participants with body composition measures for all study visits was used for the models. Univariate GEE analysis was used to determine variables to adjust for in the multiple regression models. All variables with a significance level below 0.30 were included in multiple regression GEE model. Priori variables like age, gender and CD4 count were included in the multiple regression models despite lack of significance. GEE analysis was used to estimate the marginal effects of TB on body composition measures over time. Models were adjusted for sex, smoking status,

employment, level of education, baseline CD4count and viral load as determined from univariate analysis.

GEE were used as these gave more robust models even when the distribution of the response variable is not fully identified. Investigation of the robustness of the normality assumption for GEE showed that it performs well with moderately skewed data (53) therefore models were performed under the Gaussian distribution.

For model selection, quasi-likelihood under the independence model criterion (QIC) was used to select both the best working correlation and the best predictors to include in the model. In GEE models QICu may be taken as an approximation of QIC and is used for variable selection. Four types of working correlation structures were considered in model building. That is independent, exchangeable, autoregressive and unstructured. The QIC and QICu, which is an approximation to QIC, were used to select the most parsimonious model. The model with the lowest values of QIC and QICu was chosen.

2.6.4.3 Sensitivity Analysis using MI-GEE (Objective 3)

As part of our sensitivity analysis, we reanalysed the data using imputed values for all missing information including both predictor and response variables. We made the assumption that the missingness of the data was arbitrary and was missing completely at random (MAR). Multiple imputations provide complete sets of data by filling all missing data. Data was converted to wide format before imputation to ensure that values from the visits with observed data could be used to impute missing values. Transformations before imputation were done to provide distributions within which the imputations were to be done. Multiple imputations by the use of Multiple Imputation by Chained Equations (MICE) which uses the ICE command in STATA were conducted. Data was imputed based on the available

data and information from other time points was used as predictors of missing values. To ensure reproducibility of results a seed was set.

A total of 5 completed datasets were generated, analysed separately and then had the results combined using 'Rubin's Rules' by using mim in STATA. After imputation, the transformed data was back transformed to return all values to their original scale. The distribution of the imputed values was compared with the corresponding distribution of the observed variables. Data was summarised and values generated by each imputation were looked at separately. Box plots and two way plots of imputed variables were plotted to see if the distributions after imputation made sense. Multiple imputation-generalised estimating equations were then used for analysis.

For all models created using GEE, associations between body composition and TB were assessed and adjustments were made for smoking status, baseline CD4, viral load, sex and age as per the criteria described earlier.

2.7 Ethical considerations

The original study was reviewed and approved by the Wits Human Research Ethics Committee and informed consent for participation in the primary study was obtained from all participants. For the secondary data analysis ethical approval was also sought from the Wits Human Research Ethics Committee. Approval for the use of the data was sought from the Health Economics and Epidemiology Research Office. All exchange of data with the Health Economics and Epidemiology Research Office was by the use of password protected datasets. Data was kept in a secure computer and access was restricted to the supervisors and researcher only. All the data used did not have any identifying information. There was no information to link the data to the individuals who participated in the study.

CHAPTER 3: RESULTS

3.1 Total number of participants

A total of 353 participants enrolled into the LCM study were made available for this analysis. Of the 353 participants, 2 (0.57%) were excluded from analysis as they were pregnant, 2 (0.57%) were excluded for not being on ART and 4 (1.15%) did not have information on the exposure of interest, TB, resulting in 345 patients being included in the baseline analysis. A total of 5 (1.41%) died during the course of the study, 39 (11.04%) were considered lost to follow up at either 6 months or 12 months and 20 (5.86%) were transferred out before the end of the study. The baseline characteristics were divided into demographic, clinical and body composition measures. Comparisons were made between those with TB and those without TB.

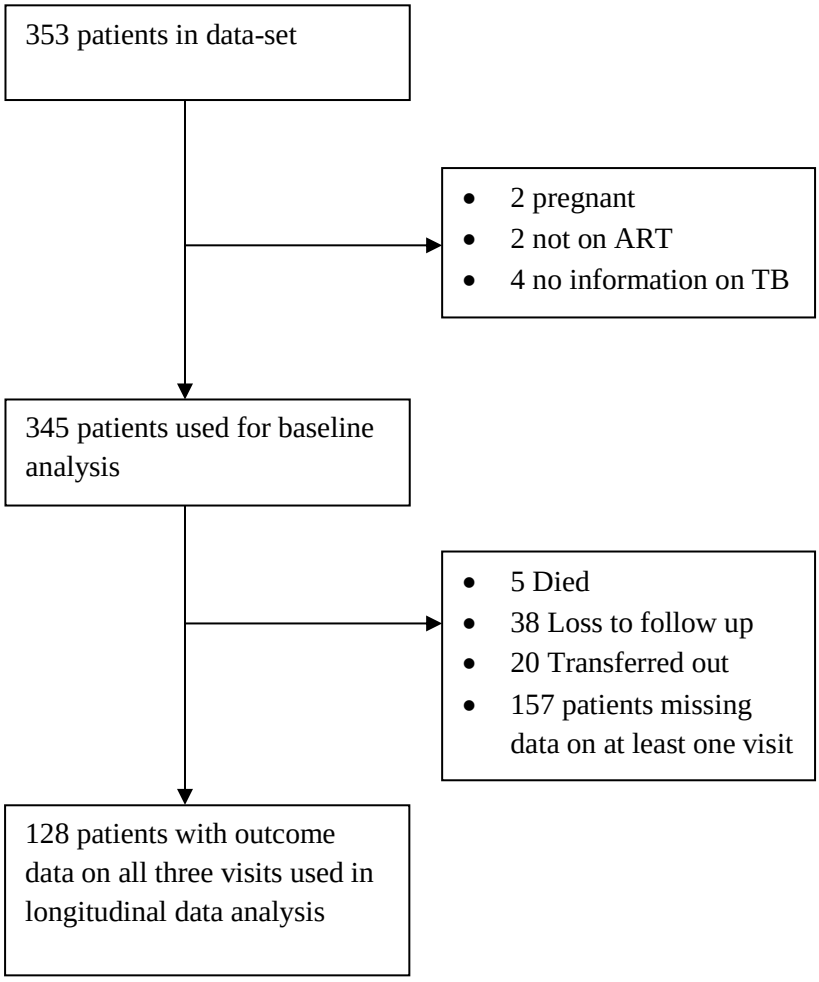

Fig 3.1: Flow chart of participants to illustrate arrival at complete case analysis cohort for longitudinal data analysis. *Diagram shows number of participants with missing outcome data at follow up visits and the patients excluded for not meeting the study inclusion criteria.*

3.2 Baseline Characteristics of the population

A total of 345 patients were analysed at baseline, of whom 36 (10.43%; 95%CI: 7.4%-14.15%) participants were co-infected with TB at baseline. The median age at ART initiation was 37.59years (IQR: 31.65-43.60). More than half (63.66%; 95%CI: 58.15%-68.57%) of patients were female and 84.62% (95%CI: 80.59%-88.47%) of the population was South African, with the remainder being from other Sub-Saharan African countries. Most participants were employed (58.55%; 95%CI: 53.15%-68.80%) either formally or informally. Only 8.14% (95%CI: 5.46%-11.51 %) of the participants smoked and 91.56% (95%CI: 88.04% -94.32%) of participants had completed at least grade 8.

Only a small proportion of the participants were underweight (6.66%; 95%CI: 4.27%-9.83%) and more than 50% (95%CI: 51.11%-61.82%) had a BMI between 18.5kg/m^2 and 24.9kg/m^2respectively with 14.41% (95%CI: 10.95%-18.65%) of the participants having BMI above 30kg/m^2. Only 13.04% (9.67%-17.06%) had a CD4 count above 350cells/mm^3.

3.3. Baseline demographic characteristics by TB status

Comparing patients with and without TB, most of the socio-demographic characteristics were similar between the two groups. However, those with TB were more likely to be younger than those without TB (p-value=0.006). Table 3.1 shows baseline demographic characteristics of the 345 patients stratified by TB status.

Table 3.1: Baseline demographic characteristics of 345 HIV-infected ART-naïve patients stratified by TB status

Characteristic	TB-ve (N=309)	TB +ve (N=36)	p-value
Gender			
Male	112(36.36)	13(36.11)	0.980
Female	196(63.64)	23(63.89)	
Employed			
No	124(40.13)	19(52.78)	0.150
Yes	185(59.87)	17(47.22)	
Nationality			
Other	47(15.26)	5(14.29)	0.883
South African	261(84.74)	30(85.71)	
Smoking			
No	283(91.88)	33(91.67)	0.750†
Yes	25(8.12)	3(8.44)	
Education			
< grade 8	26(8.72)	2(5.88)	0.750
≥ grade 8	272(91.28)	32(94.12)	
Age at initiation in years	37.35(31.75-44.20)	35.00(31.45-38.35)	0.060ł
Age at Initiation			
18-24.9	55(17.86)	4(11.11)	0.006†*
25-34.9	138(44.81)	27(75.00)	
35-44.9	49(15.91)	3(8.33)	
≥45	66(21.43)	2(5.56)	

† Fischer's exact p-value for sparse binary variables.
ł Wilcoxon rank sum test for skewed variables
* Variable significant at the 5% level of significance

3.4. Baseline clinical and body composition measures by TB status

Table 3.2 describes the baseline clinical and body composition measures by TB status. Fig 3.2 shows the baseline comparison of FM and BMI in patients with or without TB stratified by gender. Fig 3.3 is the graphical representation of median baseline body composition measures by TB status. Differences between TB+ve and TB-ve patients are not apparent except for BMI and FM. The box plots for FM and BMI stratified by gender show that these differences are more apparent in females than in males. The median BMI for TB-ve patients was 23.49 (IQR: 20.83-27.68) and that for TB+ve patients was 22.39 (IQR: 20.49-25.90) with a p-value of 0.050.

Table 3.2: Baseline clinical and body composition measures of patients stratified by TB status

Characteristic	TB -ve (n=309) N (%) or Median(IQR)	TB +ve (n=36) N (%) or Median(IQR)	p-value
BMI group, kg/m^2			
<18.5	19 (6.15)	4 (11.11)	0.091†
18.5-24.9	172 (55.66)	23 (63.89)	
25-29.9	69 (22.33)	8 (22.22)	
≥30	49 (15.86)	1 (2.78)	
BMI, kg/m^2	23.49 (20.83 - 27.68)	22.39 (20.49 - 25.90)	0.050*
CD4 cells\mm^3			
<100	70 (22.95)	13 (37.14)	0.027†
100-249	136 (44.59)	19 (54.29)	
250-349	70 (22.95)	2 (5.71)	
≥350	29 (9.41)	1 (2.86)	
CD4 cells/mm^3	207 (116.00 - 279.00)	113 (63.00 - 199.00)	<0.001*
Log viral load	5.00 (4.60 - 5.40)	5.20 (4.87 - 6.00)	0.010*
Height, meters	1.63 (1.58 - 1.69)	1.63 (1.57 - 1.70)	0.910
Weight, kilograms	64.00 (57.20 - 73.00)	63.80 (55.90 - 67.70)	0.160
Body Composition			
Phase α	7.20(6.30-8.10)	7.00 (5.30-8.70)	0.280
FM, kg	19.40 (11.95 - 27.35)	11.9(7.20 - 21.70)	0.003*
FFM, kg	44.70 (40.60 - 49.90)	45.6 (40.40 - 52.30)	0.700
LDM, kg	13.25 (11.70 - 14.70)	12.5 (11.30 - 14.50)	0.275
TBW, kg	32 (28.25 - 35.80)	33.20 (28.50 - 39.80)	0.459
ICW, kg	17.2 (15.25 - 20.75)	17.7 (15.00 - 23.00)	0.678
ECW, kg	14.40 (12.70 - 15.90)	14.9 (13.10 - 16.70)	0.434
BMR	1445 (1357 - 1573)	1433 (1357 - 1520)	0.506
DEE	1940 (1782 - 2153)	1899 (1769 - 2067)	0.670

*****Variable significant at the 5% level of significance. † Fischer's exact p-value for sparse binary variables.

While this shows borderline statistical significance, both group medians lie in the range of normal BMI and therefore the difference is not clinically significant. TB+ve participants had a statistically significantly lower median baseline CD4 count (113cells/mm^{3}IQR: 63-199) than TB-ve patients (207 cells/mm^{3}IQR: 116.0-279.0) (p-value<0.001) and those with TB also had a significantly higher median baseline log viral load than those without (p-value of 0.006). All body composition measures at baseline were not significantly different between the two groups at baseline except for FM. Patients with TB had a significantly lower FM

(median: 11.9; IQR: 7.20- 21.70) than patients without TB (median: 19.40; IQR 11.95-27.35) (p-value=0.003).

Table 3.3 shows a comparison of body composition measures by TB status stratified by gender. The differences between TB+ve patients and TB-ve patients are statistically significant and are more apparent in females compared to males. Comparison between TB+ve and TB-ve females showed statistically significant differences in BMI, CD4 count, log viral load and FM. These differences with the exception of log viral load were not observed in males.

Table 3.3: Comparison of clinical and body composition measures of 345 HIV-infected patients with TB and without TB by gender

		Female sex			Male-sex	
Characteristic	TB-ve N=196	TB +ve N=24	p-value	TB-ve N=113	TB+ve N=13	p-value
BMI, kg/m^2	25.30 (22.76 - 29.43)	23.32 (20.10 - 27.47)	0.027*	21.28 (19.77 - 23.70)	21.63 (20.29 - 22.86)	0.965
CD4 cells /mm^3	219 (126 - 286)	71 (40 - 214)	<0.001*	182 (89 - 248)	140 (103 - 173)	0.177
Log viral load	4.90 (4.49 - 5.35)	5.16 (4.79 - 5.91)	0.036*	5.12 (4.72 - 5.45)	6.00 (5.09 - 6.42)	0.045*
Height, meters	1.59 (1.55 - 1.63)	1.60 (1.55 - 1.63)	0.850	1.710 (1.67 - 1.75)	1.70 (1.69 - 1.76)	0.898
Weight, kg	65.40 (57.15-75.10)	62.00 (51.30 - 69.00)	0.063	63.00 (56.70 - 69.50)	64.20 (59.90 - 66.40)	0.840
Body Composition						
FMI, kg/m^2						
<1.8 /3.9†	15 (7.81)	7 (31.82)	<0.001*	49 (45.79)	9 (69.23)	0.096
≥1.8/3.9†	177 (92.19)	15 (78.95)		59 (54.21)	4 (30.77)	
Phase, α	6.90 (6.10 - 7.80)	6.10 (5.20 - 7.50)	0.097	7.65 (6.80 - 8.70)	7.20 (6.60 - 8.90)	0.680
FM, kg	23.60 (16.40 - 30.91)	16.8 (7.93 - 28.34)	0.024*	11.95 (7.52 - 19.35)	9.5 (4.95 - 13.98)	0.080
FFM, kg	42.50 (38.70 - 45.80)	41.20 (37.63 - 45.6)	0.407	50.40 (46.60 - 56.0)	52.30 (50.40 - 56.10)	0.224
LDM, kg	12.60 (11.40-13.60)	11.40 (11.05 - 13.34)	0.081	14.70 (13.50 - 16.05)	14.40 (12.60 - 15.70)	0.549
TBW, kg	30.00 (27.10 - 32.80)	29.8 (25.92 - 33.20)	0.704	36.15 (32.95 - 40.50)	40.90 (36.01 - 41.30)	0.080
ICW, kg	15.80 (14.40 - 17.30)	15.5 (12.9 - 17.70)	0.450	21.70 (19.40 - 24.05)	23.90 (22.57 - 24.73)	0.079
ECW, kg	14.20 (12.50-15.72)	14.70 (12.10 - 15.41)	0.739	14.60 (13.20 - 16.60)	16.70 (14.90 - 17.12)	0.089
BMR	1396 (1316 - 1487)	1361 (1294 - 1433)	0.197	1556 (1440 - 1648)	1520(1461 - 1590)	0.615
DEE	1851 (17.35 - 2063)	1821 (1723 - 1950)	0.195	2081 (1925 - 2268)	2061 (1939 - 2421)	0.959

*Variable significant at the 5% level of significance. †</≥ 1.8 for males </≥ for females.

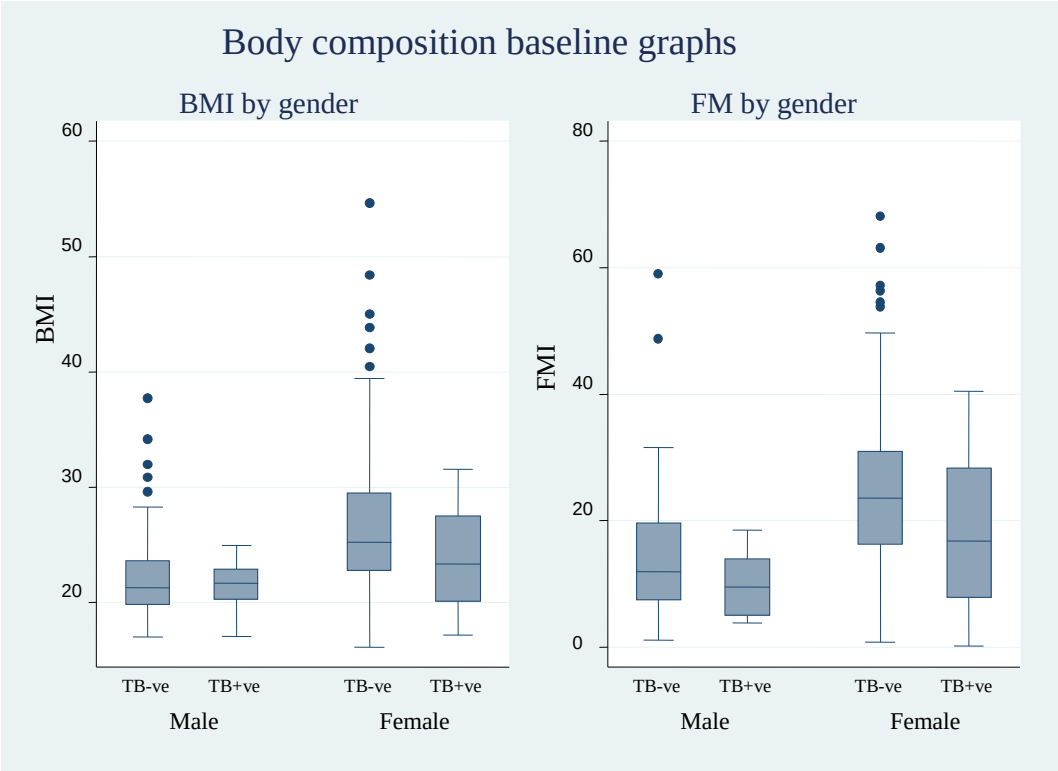

Fig 3.2: ***Baseline comparison of FM and BMI in patients with or without TB stratified by gender.*** *There are statistically significant differences between those with TB and those without TB in the female gender. The same differences are not significant in the males.*

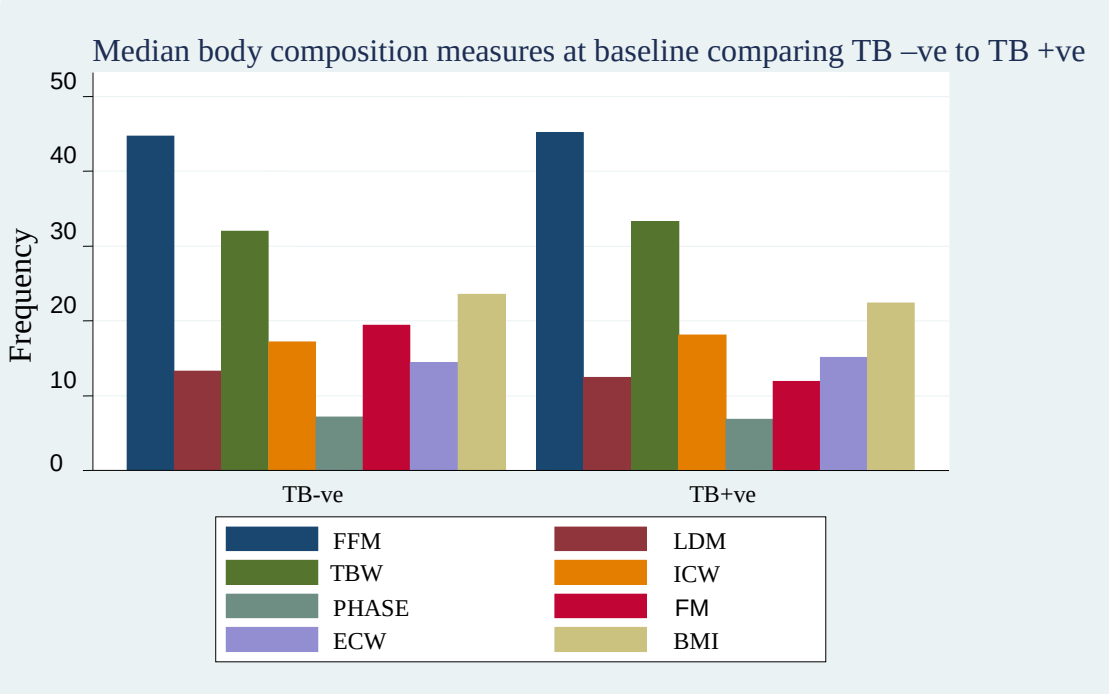

Fig 3.3: Median Baseline body composition measures at baseline by TB status. *Comparison of body composition measures between those with TB and those without at baseline shows that TB negative patients have a higher FM than those with TB. The differences with the other measures are not as apparent.*

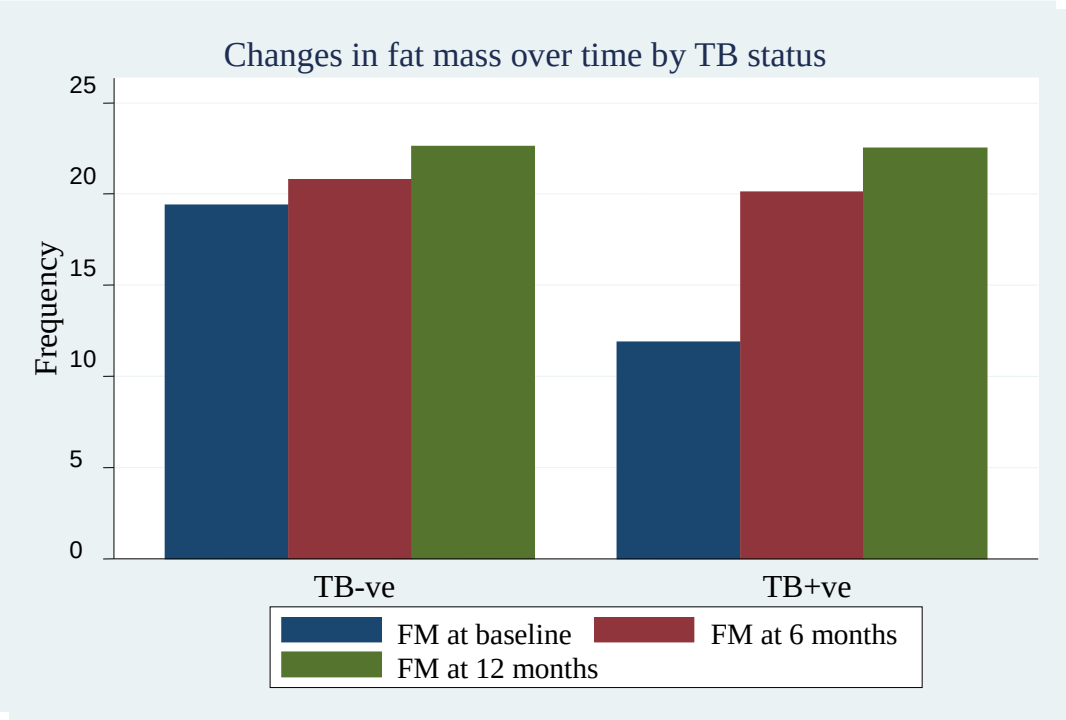

Fig 3.4: *Comparison of median FM mass over the three visits between patients with TB and patients without TB. There is an increase in FM on visit one through to visit three in both the TB-positive and the TB-negative patients.*

3.5. Complete Case analysis data

Only patients with outcome data for all three visits were used for complete case analysis. A total of 128 participants were available for the longitudinal data analysis (complete case analysis). Fig 3.1 shows the loss of follow up data over the one year period. We acknowledge that a lot of information is lost by considering only participants with outcome data for all three visits. A comparison of participants with missing outcome data and complete data over all the visits showed that there is a significant difference in gender between the two groups. This difference is important because gender is an important determinant of body composition. Table 3.4 shows a comparison of baseline characteristics between those included in complete case analysis (with all three visits) and those excluded (with at least one missing visit). In the complete case analysis group, 70.31% of participants were female whereas 59.24% of participants were female in the group with missing values (p-value 0.04). The percentage of

patients with TB is approximately 10% in both the included and excluded patients. The baseline demographic characteristics of those used in complete case analysis were not significantly different by TB co-infection as shown in Table 3.5. Baseline comparison of clinical and body composition measures (table 3.5) shows that TB+ve patients have significantly lower CD4 count (median: 85 cells/mm^3; IQR: 61 – 15) compared to TB-ve patients (median: 224 cells/mm^3; IQR: 129 – 306). TB+ve patients also had higher median baseline log viral load than TB-ve patients (p-value=0.043).

Table 3.4: **Baseline comparison of participants with all three visits (included patients) and participants with missing values on at least one of the visits (excluded patients)**

Baseline Characteristic	Included (N=128) N (%) or Median (IQR)	Excluded (N=211) N (%) or Median (IQR)	p-value
Gender			
Male	38 (29.69)	86 (40.76)	0.04*
Female	90 (70.31)	125 (59.24)	
Employed			
No	52 (40.63)	89 (41.98)	0.806
Yes	76 (59.38)	123 (58.02)	
Nationality			
Other	19 (14.84)	33 (15.71)	0.830
South African	109 (85.16)	177 (84.29)	
Smoking			
No	109 (90.83)	165 (90.66)	0.959
Yes	11(9.17)	17(9.34)	
Education			
< grade 8	11(8.80)	15(7.43)	0.655
≥ grade 8	114 (91.20)	187 (92.57)	
Age at initiation in years	36.25 (32.20 - 42.8)	37.30 (31.30 - 44.10)	0.613
Age at initiation			
18-24.9	18 (14.06)	40 (18.96)	0.283
25-34.9	70 (54.69)	94 (44.55)	
35-44.9.9	19 (14.84)	32 (15.17)	
≥45	21 (16.41)	45 (21.33)	
Drugs			
**TDF containing	111 (86.72)	170 (89.01)	0.537
**d4T containing	17 (13.28)	21 (10.99)	
BMI kg/m^2	23.59(21.00 - 27.65)	23.14(20.49 - 27.15)	0.396
BMI kg/m^2			
<18.5	7 (5.47)	15 (7.08)	0.881
18.5-24.9	72 (56.25)	121 (57.08)	
25-29.9	31 (24.22)	45 (21.23)	
≥ 30	18 (14.06)	31(14.62)	
CD4 count (cells/mm^3)			
<100	29 (22.66)	54 (25.47)	0.358
100-249	54 (42.19)	101 (47.64)	
250-349	30 (23.44)	42 (58.33)	
>350	15 (11.72)	15 (7.08)	
Tuberculosis			
TB –ve	114 (89.06)	191(90.09)	0.762
TB +ve	14 (10.94)	21 (9.91)	

TDF containing regimen: TDF/3TC/EFV or TDF/3TC/NVPd4T containing regimen: d4T/3TC/EFV

*Significant at the 5% significance level

Table 3.5: Baseline demographic characteristics of 128 HIV-infected ART-naïve patients stratified by TB status.

Characteristic	TB-ve (N=114)	TB +ve (N=14)	p-value
Gender			
Male	34 (29.82)	4 (28.57)	0.923†
Female	80 (78.18)	10 (71.43)	
Employed			
No	45 (39.47)	7 (50.00)	0.449
Yes	69 (60.53)	7 (50.00)	
Nationality			
Other	18 (15.79)	1 (7.14)	0.692†
South African	96 (84.21)	13 (92.86)	
Smoking			
No	97 (90.65)	12 (92.31)	0.661†
Yes	10 (9.35)	1 (7.69)	
Education			
< grade 8	10 (8.93)	1 (92.31)	0.680†
≥ grade 8	102 (91.07)	12 (92.31)	
Age at initiation in years	36.45 (31.90 - 43.50)	35.40 (33.20 – 37.60)	0.559

† Fischer's exact p-value for sparse binary variables.

Table 3.6: Baseline clinical and body composition measures of 128 patients stratified by TB status

Characteristic	TB -ve (n=114) N (%) or Median(IQR)	TB +ve (n=14) N (%) or Median(IQR)	p-value
BMI, kg/m^2	23.63 (21.04 - 27.68)	23.38 (20.95 - 27.62)	0.896
CD4 cells/mm^3	224.00 (129.00 – 306.00)	85 (61.00 – 151.00)	0.001*
log viral load	5.01 (4.61 – 5.445)	5.42 (5.09 – 5.97))	0.043*
Weight, kg	64.65 (58.20 – 71.80)	65.65 (60.00 – 69.00)	0.864
Body Composition			
Phase α	7.10 (6.20 – 8.10)	6.3 (5.2 – 8.7)	0.289
FM, kg	19.5 (12.30 – 28.40)	19.4 (9.90 -29.60)	0.979
FFM, kg	44.70 (40.10 – 49.20)	41.80 (39.10 – 48.5)	0.343
LDM, kg	13.15 (11.70 – 14.50)	11.55 (11.30 – 14.50)	0.299
TBW, kg	32.00 (28.00 – 35.40)	30.10 (26.80 – 33.30)	0.427
ICW, kg	17.3 (15.00 – 21.00)	15.7 (12.2 – 18.5)	0.187
ECW, kg	14.20 (12.60 – 15.70)	14.45 (13.00 – 15.70)	0.956
BMR	1445 (1361- 1563)	1431 (1341 - 1503)	0.556
DEE	1952 (1800 - 2110)	1901 (1744 - 1990)	0.412

* Significant at the 5% level of significance

3.5.1 Percentage change in body composition measures

Changes in both clinical and body composition measures from ART initiation at baseline visit until 6 months as well as from initiation until 12 months were calculated and compared between those with TB and those without. Although some results showed particular trends, most were non-significant. We note that this may be as a result of the small sample size resulting from attrition. The small sample size decreased the precision of the estimates resulting in large confidence intervals.

3.5.1.1 6 month changes (Objective 2)

Table 3.5.1.1 shows a comparison of changes in body composition measures over 6 months. Although not statistically significant, at 6 months patients with TB demonstrated a greater increase in BMI (1.60% vs. 2.10%; p-value=0.950) and FM (5.10% vs. 13.80%; p-value=0.610) than patients without TB. The difference in change in CD4 count between the two groups were statistically significant and patients without TB saw a smaller gain in overall CD4 count compared to those with TB (66.20% vs. 153.00%; p-value=0.002). Although not statistically significant, there was a larger increase in weight in patients with TB compared to those without TB (1.60% vs. 3.50%; p-value=0.879). The differences between all the changes in body composition measures between TB+ve patients and TB-ve patients were not significant. There is however a trend in the increase that suggests that TB+ve patients have larger increases compared to TB-ve patients.

3.5.1.2 12 month changes (Objective 2)

Table 3.5.1.2 shows a comparison of changes from baseline to 12 months. At 12 months, patients with TB demonstrated greater increase in FM than patients without TB (42.51% vs. 19.14%; p-value = 0.302), although this was not statistically significant. There was also a greater increase in BMI amongst those with TB compared to those without TB (1.6% vs.

2.2%; p-value = 0.506). There is a consistent trend of greater increase in the TB+ve patients compared to TB-ve patients as seen at 6 months

Table 3.5.1.1: Comparison of changes in clinical and body composition measures between patients with TB and patients without TB from baseline to six months using complete data

	TB -ve (n=114)			TB+ve (n=14)			
Characteristic	Baseline	6-months	% Change**	Baseline	6-months	%Change**	p-value
CD4, cells /mm^3	224 (129.0 - 224.0)	337(235.5 - 447.0)	66.2 (25.9 - 116.6)	85.5 (61.0 - 151.0)	226.0 (121.0 - 353.0)	153.0 (79.1- 303.3)	0.016*
BMI, kg/m^2	23.6 (21.0 - 27.7)	23.9 (21.7 - 28.0)	1.6 (-2.0 - 5.57)	23.4 (21.0 - 27.6)	24.5 (22.2 - 24.5)	2.1 (-3.3 - 7.2)	0.950
Weight, kg	64.7 (58.2 - 71.9)	66.0 (58.8 - 73.2)	1.6 (-1.9 - 5.7)	65.7 (60.0 - 69.0)	67.2 (59.5 -72.8)	3.5 (-3.3 - 7.2)	0.946
FM, kg	19.5 (12.3 - 28.4)	19.4 (9.9 - 29.6)	5.1 (-8.0 - 25.6)	20.2 (12.5 - 28.7)	21.8 (14.4 - 29.9)	13.8 (-15.8 - 62.7)	0.610
FFM, kg	44.7 (40.1 - 49.2)	44.8 (39.4 - 49.8)	-1.0 (-6.0 - 4.3)	41.8 (39.1 - 48.5)	44.3 (41.6 - 46.2)	0.3 (-10.3 - 7.8)	0.950
LDM, kg	13.2 (11.7 - 14.5)	12.7 (11.6-13.8)	-2.94 (-10.4 - 3.5)	12.5 (11.2 - 12.8)	12.5 (11.2 - 12.8)	-2.6 (-13.9 - 6.8)	0.980
TBW, kg	32 (28.0 - 35.4)	32.1 (28.1 - 35.5)	-0.6 (-5.1 - 7.3)	30.1 (26.8 - 33.3)	32.5 (29.0 - 34.2)	1.8 (-4.0 - 8.9)	0.590
ICW, kg	17.3 (15.0 - 21.0)	17.0 (14.6 - 20.0)	-2.24 (-9.3 -5.5)	15.7 (12.9 - 18.5)	16.8 (15.3 - 17.6)	1.7 (-7.0-10.1)	0.438
ECW, kg	14.2 (12.6 - 15.7)	14.6 (13.1 - 16.4)	0 (-4.0 - 8.0)	14.5 (13.0 - 15.7)	14.9 (13.8 -15.6)	5.8 (-3.2 - 8.5)	0.570
Phase Angle, α	7.1 (6.2 - 8.2)	6.7 (5.9 - 7.3)	-4.0 (-16.2 - 7.04)	6.3 (5.2 - 8.7)	6.3 (5.6 - 7.3)	-2.0 (-21.8 - 8.5)	0.492
BMR	1145(1361 - 1563)	1460 (1364 - 1579)	0.75 (-1.0 - 2.7)	1431 (1341 - 1503)	1466 (1339 - 1536)	1.0 (-1.4 - 2.4)	0.950
DEE	1952 (1800 - 2110)	1921(1774 - 2094)	0 .0(-2.1 - 2.4)	1901 (1744 - 1990)	1936 (1777-2050)	1.0 (-1.4 - 2.4)	0.409

**percent change is the ratio of the absolute increase over period divided by the baseline value ×100. The percentage change was compared between the two groups, HIV+ve/TB+ve and HIV+ve/TB-ve.

* Significant at 5% level of significance

Table 3.5.1.2: Comparison of changes in clinical and body composition measures between patients with TB and patients without TB from baseline to 12 months using complete data

	TB -ve (n=114)			TB +ve (n=14)			
Characteristic	Baseline	12-months	% Change**	Baseline	12-months	% Change**	p-value
BMI(kg/m^2	23.6 (21.0 - 27.6)	24.3 (21.9 - 29.1)	1.6 (2.0 - 5.5)	23.4 (21.0 - 27.4)	23.4 (22.4 - 30.7)	2.2 (-3.3 - 7.2)	0.506
FM, kg	19.5 (12.30 - 28.4)	22.9 (15.8 - 32.7)	19.14 (3.8 - 45.3)	20.2 (12.5 - 28.7)	24.6 (16.2 - 36.9)	42.5(-3.2 - 109.9)	0.302
FFM, kg	44.7 (40.1 - 49.2)	44.75 (39.4 - 49.8)	-4.1 (-7.8 - 1.5)	41.8 (39.1 - 48.5)	43.0 (37.0 - 44.5)	-1.8 (-12.52 - 6.9)	0.760
LDM, kg	13.2 (11.7 - 14.5)	12.8 (11.4 - 13.9)	-4.8 (-9.6 - 2.8)	12.5 (11.2 - 12.8)	12.1 (11.6 - 13.0)	-0.0 (-0.19 - 0.1)	0.822
TBW, kg	32.0 (28.0-35.4)	30.5 (26.7 -35.0)	-4.2 (-9.9-2.8)	30.1 (26.8 - 33.3)	30.6 (26.9 - 32.9)	-0.43 (-9.8 - 4.7)	0.480
ICW, kg	17.3 (15.0 - 21.0)	16.2 (13.7 - 19.9)	-6.8 (-13.9 - 0.45)	15.7 (12.9 - 18.5)	15.35 (13.4 - 17)	-1.7 (-10.74 - 2.53)	0.370
ECW, kg	14.2 (12.6 - 15.7)	14.0 (12.8 - 15.5)	3.2 (-3.6 - 8.2)	14.5 (13.0 - 15.7)	14.65 (13.0 - 15.9)	1.7 (-4.19 - 5.38)	0.312
Phase Angle, α	7.1 (6.2 - 8.2)	6.5 (5.7 - 7.1)	-8.96 (-20.3 - 4.14)	6.3 (5.2 - 8.7)	6.3 (5.8 - 6.6)	-0.04 (-37.6 - 16.92)	0.625
BMR	1145.5 (1361 - 1563)	1486 (1360 - 1606)	1.38 (-0.97 - 4.3)	1431 (1341 - 1503)	1484 (1339 - 1584)	3.02 (-0.97 - 6.56)	0.288
DEE	1952.5 (1800 - 2110)	1987 (1819 - 2.116)	0.84 (-2.94 - 4.88)	1901 (1744 - 1990)	1929.5 (1741 - 2084)	4.14 (-0.27 - 8.12)	0.124

**percent change is the ratio of the absolute increase over period divided by the baseline value ×100. The percentage change was compared between the two groups, HIV+ve/TB+ve and HIV+ve/TB-ve.

3.5.2 Profile plots and GEE models (Objective 3)

3.5.2.1 Phase angle

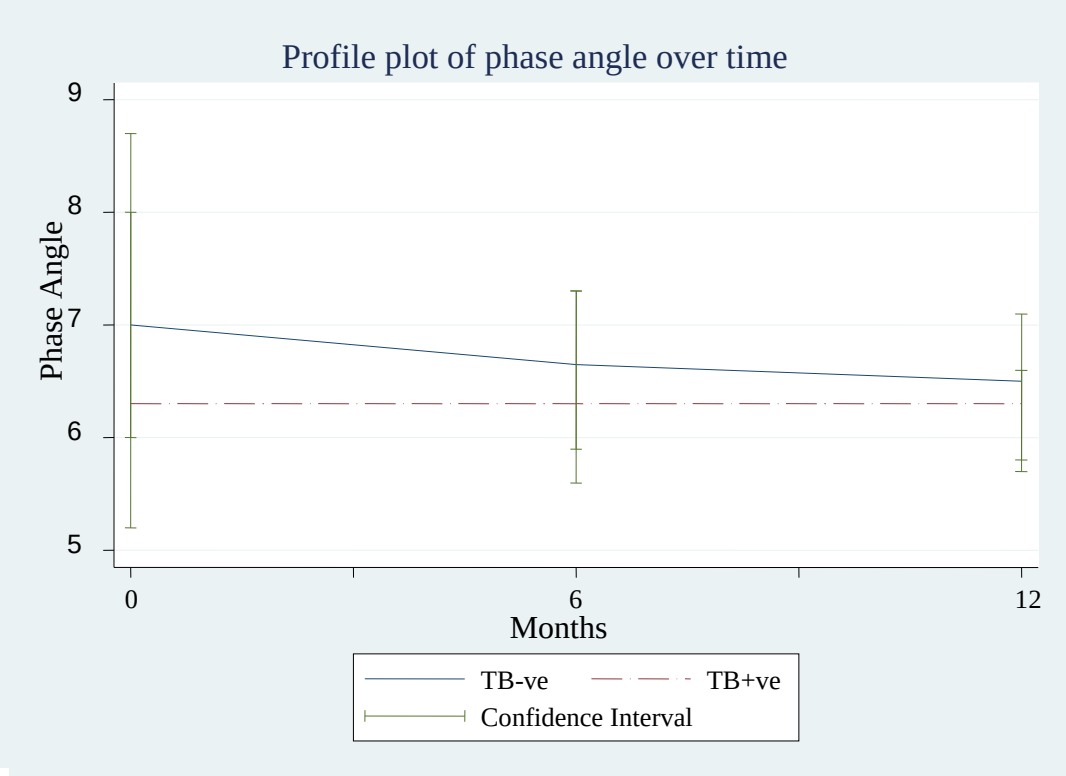

Fig 3.5.2.1*: The above graph shows the profile plot of median phase angle from baseline visit to 12 month visit.*

Fig 3.5.2.1 shows that at baseline those with TB had a lower median phase angle than those without TB. Over the period under analysis, patients without TB showed a decrease in phase angle and those with TB seemed to maintain the same phase angle. Table 3.5.2.1 shows GEE analysis of phase angle. In multiple regression analysis, there is an overall decrease in average phase angle over follow up. Each additional visit was associated with a 0.711 decrease in average phase angle (p-value=0.019). Being female was also associated with a decrease in average phase angle of 1.378 compared to being male (p-value=0.0390). The main exposure of interest, TB, was not associated with phase angle (p-value=0.695) at multivariable level but at univariate level those with TB had lower average phase angle (-0.872; p-value=0.040). CD4 count greater than 350cells/$mm^3$was associated with an increased average phase angle of 1.820 (p-value=0.048) compared to CD4 count less than 100 cells/mm^3in multivariable analysis.

Table3.5.2.1: GEE model for predictors of phase angle mass among 128 HIV-infected patients, 14 TB positive and 114 TB negative.

	Unadjusted			**Adjusted**		
Characteristic	Coefficient	Confidence Interval	p-value	Coefficient	Confidence Interval	p-value
Time	-0.852	-1.503 - -0.213	0.009	-0.711	-1.306 - -0.117	0.019*
Tuberculosis						
No	Reference					
Yes	-0.872	-1.703 - -0.041	0.040	-0.583	-1.683 - 0.522	0.659
Log viral load	-0.23	-0.977 - 0.516	0.543	-0.039	-0.735 - 0.655	0.911
Regimen						
TDF Containing	Reference					
d4T Containing	-0.0866	-0.814 - 0.6411	0.815			
Age at initiation						
18-24.9	Reference					
25-34.9	0.164	-1.532 - 1.86	0.849	0.030	-1.655 - 1.594	0.971
35-44.9	-0.25	-2.045 - 1.545	0.785	-0.304	-1.660 - 2.270	0.761
≥45	-0.698	-2.435 - 1.038	0.431	-0.759	-2.114 - 0.596	0.272
Gender						
Male	Reference					
Female	-0.771	-1.753 - 0.212	0.124	-1.378	-2.684 - -0729	0.039*
Smoking						
No	Reference					
Yes	1.087	-1.372 - 3.547	0.386			
Education						
<Grade 8	Reference					
≥Grade 8	-0.3955	2.968 - 2.177	0.763			
Employment						
Unemployed	Reference					
Employed	0.118	-0.774 - 1.01	0.796			
CD4 count						
<100 cells/mm^3	Reference					
100-249 cells/mm^3	0.283	0.497 - 1.063	0.477	0.161	-0.71 - 1.032	0.717
250-349 cells/mm^3	0.38	-0.479 - 1.258	0.380	0.565	-0.424 - 1.554	0.263
≥350 cells/mm^3	0.067	0.128 - 3.707	0.067	1.820	0.017 - 3.623	0.048

TDF containing: TDF/3TC/EFV or TDF/3TC/NVP
d4T containing: d4T/3TC/EFV
*Significant at the 5% confidence interval

3.5.2.2 Fat mass

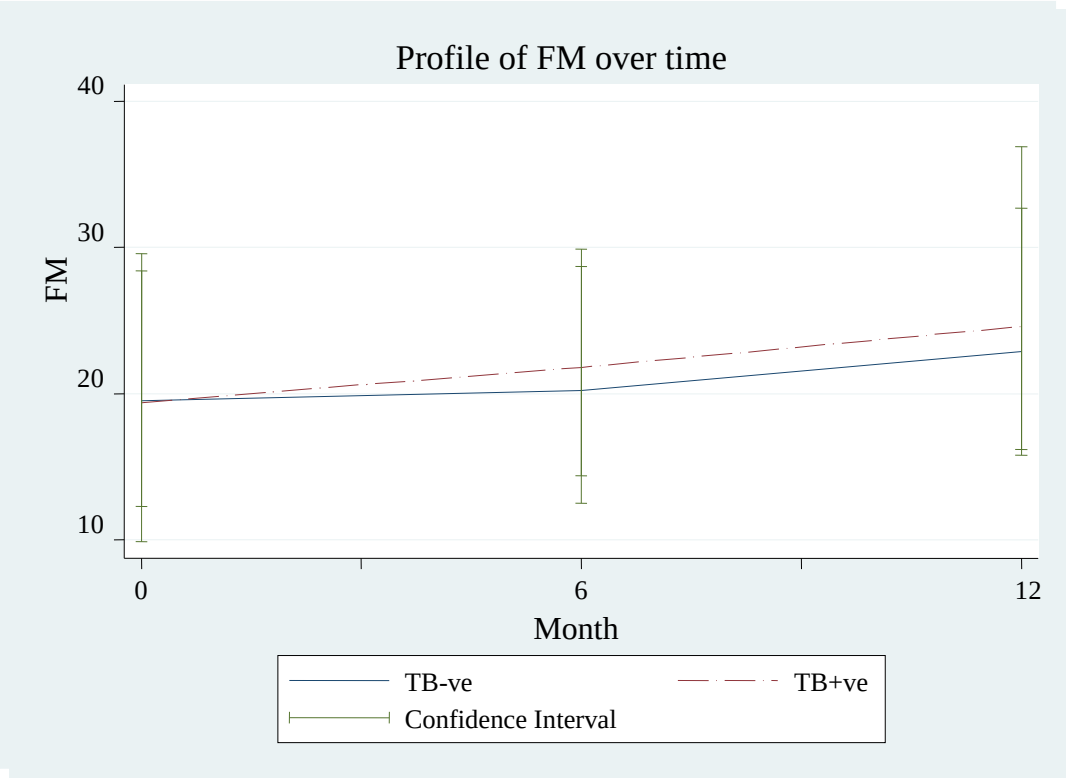

Fig 3.5.2.2 *The above graph shows the profile plot of median FM baseline visit to 12 month*

TB+ve patients showed similar FM to TB-ve patients at baseline in the profile plot for the complete case analysis. The rate of increase of FM appears higher in TB+ve patients compared to TB-ve patients by 12 month visit patients with TB+ve having higher average Mathis plot is suggestive of a faster gain in FM in patients with TB compared to patients without TB (Fig 3.5.2.2). Table 3.5.2.2 shows GEE analysis for predictors of FM. Univariate analysis by GEE shows that for each subsequent visit there is an increase in average FM of 2.248kg (p-value of <0.001) and at multiple regression level the increase in average FM is 2.231 kg (p-value = 0.001) for each subsequent visit. There is also an association between gender and FM. Being female was associated with increased average FM (10.989kg; p-value = 0.002) compared to males. Having an education beyond grade eight was associated with increased FM (7.675 kg; p-value= 0.001) compared to a less than grade eight education. There was no significant association between FM and TB at univariate level as well as multiple regression level (3.229 kg; p-value=0.889).

Table 3.5.2.2: GEE model for predictors FM among 128 HIV-infected patients, 14 TB positive and 114 TB negative.

	Unadjusted			**Adjusted**		
Characteristics	Coefficient	Confidence Interval	p-value	Coefficient	Confidence Interval	p-value
Visit	2.248	1.450 - 3.046	<0.001	2.231	1.382 - 3.080	<0.001*
Tuberculosis						
No	Reference					
Yes	-0.524	-6.356 - 5.308	0.86	3.229	-4.245 - 10.703	0.889
Regimen						
TDF containing	Reference					
d4T containing	-0.776	-7.913 - 6.36	0.831			
Log viral load	-2.704	-5.882 - 0.475	0.096	-1.234	-3.782 - 1.181	0.980
Age at initiation						
18 - 24.9	Reference					
25 – 34.9	0.058	-5.679 - 5.797	0.984	0768	-4.340 - 5.874	0.873
35 – 44.9	0.918	-6.046 - 7.882	0.439	3.573	-2.895 - 10.042	0.159
≥45	4.614	-4.250 - 13.479	0.578	4.016	-1.959 - 9.992	0.275
Gender						
Males	Reference					
Females	12.037	8.806 - 15.268	<0.001	10.989	6.722 - 15.256	0.002*
Smoking Status						
No	Reference					
Yes	-4.896	-11.674 - 1.882	0.157	-0.981	-8.266 - 6.303	0.792
Education						
< grade 8	Reference					
≥ grade 8	4.719	-0.384 - 9.822	0.070	7.675	3.033 - 12.317	0.001*
Employment						
Unemployed	Reference					
Employed	-1.864	-6.028 - 2.300	0.380			
CD4 count						
< 100 cells/mm^3	Reference					
100 - 249 cells/m^3	-1.543	5.563 - 2.476	0.452	0.794	-4.770 - 3.182	0.695
250 - 349/mm^3	8.582	2.307 - 14.856	0.007	3.887	-2.05 - 9.827	0.200
≥ 350/mm^3	5.315	-0.924 - 11.556	0.095	5.828	-0.423 - 12.07	0.068

TDF containing regimes: TDF/3TC/EFV or TDF/3TC/NVP
d4T containing regimen: d4T/3TC/EFV
* Significant at the 5% significance level

3.5.2.3 Body mass Index

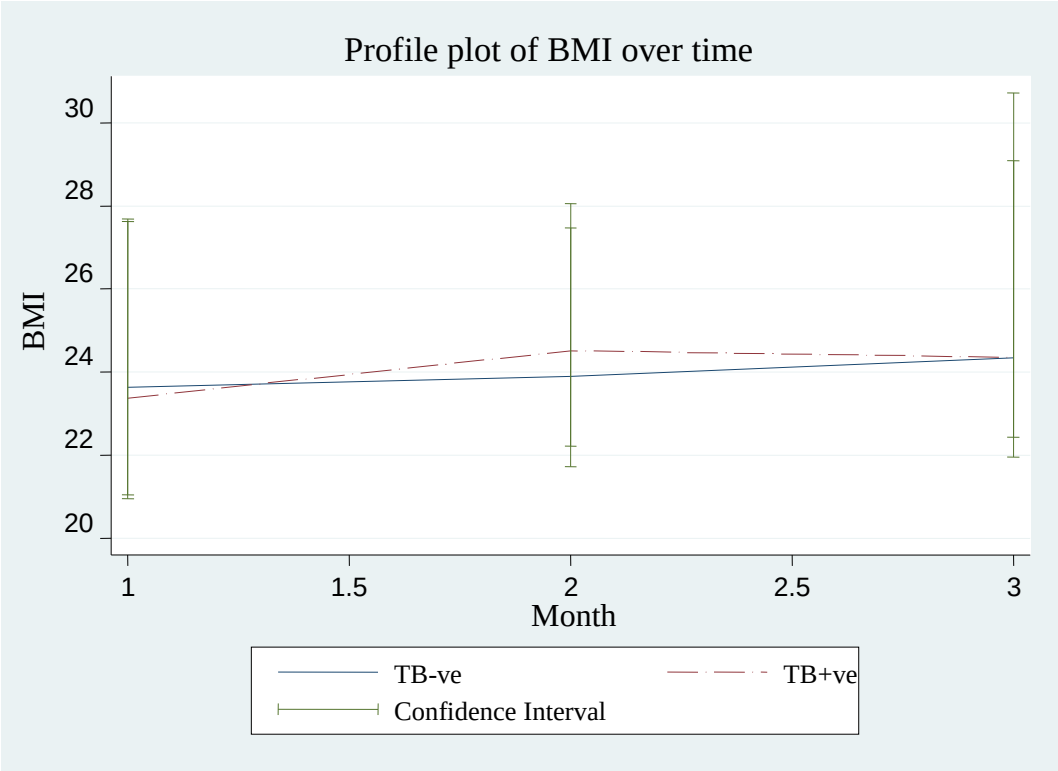

Fig 3.5.2.3: *The above graph shows the profile plot of median BMI from baseline visit to 12 month visit*

The median BMI between TB+ve and TB-ve patients is similar at baseline analysis although TB-ve patients have a slightly higher BMI. By 6 months, there is a faster increase in median BMI amongst TB+ve compared to TB-ve patients. This is however followed by a slight decrease in BMI amongst those with TB between 6 and 12 months, leaving both groups at very similar median BMI's at 12 months. Table 3.5.2.3 shows GEE analysis results for BMI. Those employed had 3.713kg/m^2 increased average BMI than those not employed (p-value=0.014). In multiple regression analysis there is an overall increase in average BMI over the study period. For each 6 month time period, there is an increase of 0.546kg/m^2 in average BMI within the population (p-value<0.001). There was no statistically significant difference between those with CD4 count less than 100cells/mm^3 and those in the 100-249 cells/mm^3 range however patients with CD4 count between 250-350cells/mm^3 had 3.658kg/m^2higher marginal BMI than those with CD4 count less than 100 cells/mm^3 (p-value=0.020) and those with CD4 count ≥350 cells/mm^3 also had a higher average marginal BMI than those in the

reference CD4 count category (p-value=0.029). There was no association between TB and BMI after adjusting for possible confounders (1.194; p-value=0.450)**Table 3.5.2.3: GEE model for predictors of BMI mass among 128 HIV-infected patients, 14 TB positive and 114 TB negative**

	Unadjusted			**Adjusted**		
Characteristic	Estimate	Confidence Interval	p-Value	Estimate	Confidence Interval	p-Value
Visit	0.547	0.367 - 0.729	<0.001	0.546	0.355 - 0.728	<0.001*
Tuberculosis						
Yes	Reference					
No	-0.403	-2.9 - 2.094	0.752	1.194	-1.909 - 4.297	0.451
Log viral load	-1.072	-2.857 - 0.712	0.239	-0.537	-2.243 - 1.170	0.538
Regime						
TDF containing	Reference					
d4T containing	0.311	-2.912 - 3.534	0.85			
Age at initiation						
18 -24.9	Reference					
25-34.9	0.591	-2.282 - 3.466	0.687	0.075	-1.822 - 3.172	0.596
35-44.9	2.264	-1.967 - 6.495	0.294	3.018	-0.851 - 6.888	0.126
≥45	2.897	-1.637 - 7.431	0.210	2.440	-1.278 - 6.158	0.198
Gender						
Males	Reference					
Females	5.142	3.609 - 6.675	<0.001	4.154	2.606 - 5.701	<0.001*
Nationality						
Other						
South African	1.322	-1.156 - 3.801	0.296	0.446	-3.247 - 6.672	0.687
Smoking Status						
No	Reference					
Yes	-2.124	-5.28 - 1.034	0.87			
Education						
< Grade 8	Reference					
≥ Grade 8	1.637	-0.847 - 4.121	0.397			
Employment						
Unemployed	Reference					
Employed	-0.775	-2.901 - 1.352	0.185	3.713	0.739 - 6.687	0.014*
CD4 count						
<100 cells/mm^3	Reference					
100-249 cells/mm^3	-0.465	-2.401 - 1.47	0.638	0.017	-1.956-1.991	0.986
250-349 cells/mm^3	4.378	1.300 -7.455	0.005	3.658	0.579-6.737	0.020*
≥ 350 cells/mm^3	3.811	-0.048-5.669	0.054	3.776	0.333-6.020	0.029*

TDF based: TDF/3TC/EFV or TDF/3TC/NVP

d4T based: d4T/3TC/EFV

*Significant at the 5% significance level

3.5.2.4 Fat free mass

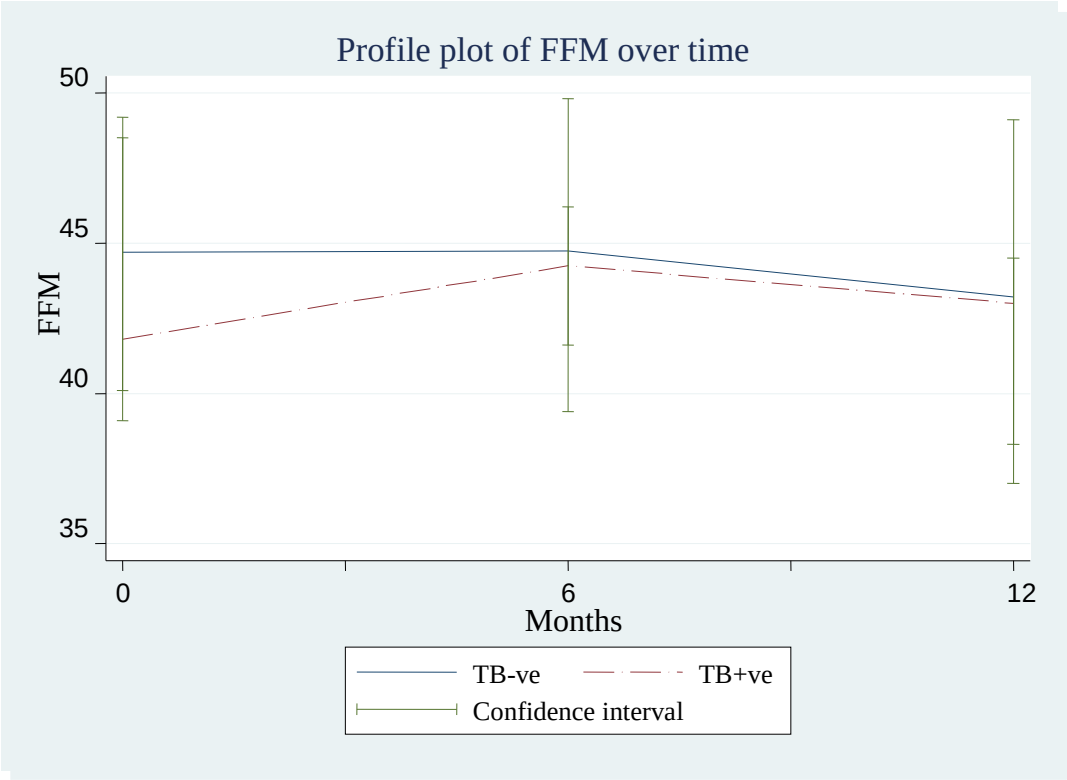

Fig 3.5.2.4*: The above graph shows the profile plot of median FFM from baseline visit to 12 month visit.*

The profile plot depicted in figure 3.5.2.4 shows that patients with TB have a lower FFM than those without TB at baseline. There is however an apparent increase amongst those with TB which is followed by a decrease in both groups. After adjusting for other variables in the multiple regression model, there is a decrease in FFM of 0.728 (p-value <0.001) for each increase in visit number from baseline till 12 months as shown in table 3.5.2.4. Each unit increase in log viral load was associated with a decrease in FFM of 1.647 (p-value= 0.016). There was an association between age and FFM. Those between the ages of 25 – 34.9 have a higher FFM than those in reference age group (4.141; p-value=0.004). Those over the age of 45 have 5.50 higher FFM than reference age group (p-value=0.003). Females have a lower FFM, (8.611; p-value = 0.01) than males. TB showed no significant association with FFM (-1.587; p-value=0.267).

Table 3.5.2.4: GEE model for predictors of FFM among 128 HIV-infected patients, 14 TB positive

	Unadjusted			**Adjusted**		
Characteristics	Coefficient	Confidence Interval	p-Value	Coefficient	Confidence Interval	p-Value
Visit	-0.786	-1.253 - 0.319	0.001	-0.728	-1.221 - -0.237	0.004*
Tuberculosis						
No	Reference					
Yes	-1.808	-5.245 - 1.629	0.302	-1.587	-4.386 - 1.213	0.267
Logviral load	-1.291	-2.883 - 0.302	0.112	-1.647	-2.983 - -0.309	0.016*
Regimen						
TDF containing	Reference					
d4T containing	1.041	-2.605-4.688	0.576			
Age at initiation						
18 – 24.9	Reference					
25-34.9	4.499	1.035 - 7.963	0.011	4.141	1..337 - 6.945	0.003*
35-44.9	4.967	1.132 - 8.802	0.011	5.989	2.279 - 9.701	0.002*
≥45	6.611	-1.692 - 14.915	0.119	5.501	1.842 - 9.168	0.003*
Gender						
Male	Reference					
Female	-8.269	-10.46 - -6.078	<0.001	-8.611	-10.683 - -6.540	<0.001*
Smoking						
No	Reference					
Yes	6.341	2.490 - 10.190	0.001	-0.458	-4.157 - 3.241	0.808
Education						
< grade 8	Reference					
≥grade 8	3.341	-0.204 - 6.886	0.065	3.2632	-0.499 - 7.025	0.089
Employment						
Unemployed	Reference					
Employed	0.663	-1.756 - 0.083	0.591			
CD4group						
<100 cells/m^3	Reference					
100-249 cells/m^3	-0.211	-3.000-2.580	0.882	-0.270	-2.56 - 2.019	0.817
250-349 cells/mm^3	0.883	2.478-4.244	0.607	1.333	-1.479 -1.14	0.353
≥350cells/mm^3	2.603	-1.919-7.125	0.259	3.161	-0.411-6.733	0.830

TDF based: TDF/3TC/EFV or TDF/3TC/NVP
d4T based:d4T/3TC/EFV
*Significant at 5% significance level

3.5.2.5 Total body water

Fig 3.5.2.5: *The above graph shows the profile plot of median phase angle from baseline visit to visit 3*

The profile change in fig 3.5.2.5 shows no significant improvement or changes in TBW over the study period. In multiple regression analysis, viral load, age at initiation and gender were associated with total body water. There was an overall decrease in TBW over the entire study period. For each unit increase in visit time, there a decrease in average TBW (0.506; p-value 0.018). An increase in log viral load was seen to be associated with a decrease in TBW (-1.3965; p-value =0.017).There was a trend in the association between age at initiation and TBW as shown in table 3.5.2.5. TBW increased as the age of initiation increased. Those in age group have a higher TBW than those in reference age group (3.538, p-value=0.002). The trend continues and those in age group over 45 years of age have the highest TBW (7.474; p-value=0.009). There was no association found between the main exposure of interest TB and TBW.

Table 3.5.2.5: GEE model for predictors of Total Body Water among 128 HIV-infected patients, 14 TB positive and 114 TB negative

	Unadjusted			Adjusted		
Characteristic	Coefficient	Confidence Interval	p-value	Coefficient	Confidence Interval	p-value
Time	-0.442	-0.586 - -0.028	0.036	-0.506	-0.925 - 0.087	0.018*
Tuberculosis						
No	Reference					
Yes	-1.039	-3.732 - 1.654	0.449	-0.993	-3.394 - 1.408	0.225
Log viral load	-1.013	-2.287 - 0.261	0.119	-1.396	-2.546 - -0.246	0.017*
Regimen						
TDF containing	Reference					
d4T containing	0.9106	-1.873 - 3.694	0.521			
Age at initiation						
18-24.9	Reference					
25-34.9	3.984	1.374 - 6.594	0.003	3.538	1.345 - 5.731	0.002*
35-44.9	4.604	1.733 - 7.476	0.002	4.653	2.309 - 6.997	<0..001*
≤45	5.861	-0.580 - 12.303	0.075	7.474	1.858 - 13.091	0.009*
Gender						
Male	Reference					
Female	-6.016	-7.764 - -4.269	<0.001	-6.061	-8.032 - -4.091	<0.001*
Nationality						
Other	Reference					
South African	-1.274	-3.724 - 1.176	0.308			
Smoking						
No	Reference					
Yes	4.591	1.414 - 7.768	0.005	-0.581	-3.519 - 2.357	0.698
Education						
<Grade 8	Reference					
≥Grade 8	2.36	-0.539 - 5.26	0.111	2.551	-0.712 - 5.814	0.125
Employed						
No	Reference					
Yes	0.314	-1.593 - 2.221	0.747			
CD4count						
<100 cells/mm^3	Reference					
100-249 cells/mm^3	-0.211	-3.000 -2.580	0.882	0.256	-1.589-2.095	0.788
250-34cells/mm^3	0.607	-2.478-4.244	0.607	0.303	-1.156 - 3.722	0.303
≥ 350 cells/mm^3	0.259	-1.919-7.125	0.259	1.768	-1.259- 4.795	0.252

*TDF based: TDF/3TC/EFV or TDF/3TC/NVP

*d4T based:d4T/3TC/EFV

* Significant at 5% significance level

3.6 Sensitivity Analysis

Due to substantial amounts of missing data over the follow up period, we conducted a complete case analysis of participants with outcome data for all three visits. We however also reanalysed our data after using multiple imputations to fill missing values for both predictors and outcome variables as part of our sensitivity analysis. Multiple imputations provide complete sets of data by filling all missing data. Below are the results our sensitivity analysis.

3.6.1 Changes in body composition

3.6.1.1 6 months

Table 3.6.1.1 shows comparison in measures after multiple imputations. Change in both clinical and body composition measures from ART initiation until 6 months were calculated and compared between those with TB and those without. At 6 months, patients with TB demonstrated a greater increase in BMI (2.21% vs. 4.20%; p-value=0.044), FM (6.50% vs. 23.0%; p-value <0.001), and CD4 count (70% vs. 113%; p-value <0.001) compared to those without TB. Patients without TB, however, had an increase in FFM (0.85%) vs. a decrease of 4.6% (p=0.007) for patients with TB. The same trend was observed with TBW as those without TB showed no change whilst those with TB showed a decrease of 1.95% (p=0.002).

3.6.1.2 12 months

At 12 months patients with TB demonstrated greater increase in FM (53.84% vs. 23.58%; p=0.000), BMR (1.68% vs. 3.98%; p-value <0.001 and DEE (1.54% vs. 3.98%; p=0.048) compared to those without TB. ECW in those with TB did not change whilst in those without TB there was a decrease – of 1.53% (p-value=0.047) (Table 3.6.1.2)

3.6.2 Generalized estimating equations

Table 3.6.2.1 shows GEE analysis of FM, FFM and BMI at multiple regression level. For each subsequent visit there is an increase in FM of 2.414kg (p-value of <0.001).There is also an association between gender and FM. Being female was associated with increased average FM (7.130kg; p value <0.001) compared to males. Having an education beyond grade eight was associated with increased FM (6.632 kg; p-value= 0.003) compared to a less than grade eight education. There was an association between age at initiation and FM. As age at initiation increases, there is an increase in FM. There was an association between TB and FM. Those with TB had a decreased FM compared to those without TB (-5.967; p-value = 0.041).

GEE analysis of BMI shows that those employed had 3.256kg/m^2 increased average BMI than those not employed (p-value=0.001). There was an overall increase in average BMI over the study period. For each 6 month time period, there is an increase of 0.543kg/m^2 in average BMI within the population (p-value=0.010). Gender was associated with BMI and as age at initiation increases, there is an increase in BMI. Analysis of FFM shows a decrease of FFM over the 12 months follow up period. It also shows an increase in average FFM as age at initiation increases. Females have a lower average FFM than males. Table 3.6.2.2 shows there was a decrease in average TBW over time and an increase in TBW as age at initiation increases. Being female is associated with an increased average TBW.

There are some differences in results obtained from the complete case analysis and the sensitivity analysis. The main difference is that FM was found to be significantly less in TB+ve individuals compared to TB-ve in GEE analysis (-5.967kg;p-value=0.041). For the

sensitivity analysis, age at initiation becomes a significant factor associated with body composition measures in multiple regression analysis. Gender and education are significantly associated with body composition measures in both analyses.

Table 3.6.1.1: Comparison of changes in clinical and body composition measures between patients with TB and patients without TB from baseline to six months

	TB Negative			TB Positive			
Characteristic	Baseline	6 months	%change**	Baseline	6 months	% change**	p-value
BMI, kg/m^2	23.5 (20.8 -27.7)	24(21.9 -28.0)	2.2 (-2.80-8.07)	22.50(20.2 -25.9)	23.8 (21.5 - 27.3)	4.2 (-2.8 - 10.7)	0.044*
FM, kg	19.1(11.5-27.1)	21.1(13.6-29.47)	6.50(-12.0 -40.0)	14.0(7.90-21.80)	19.6(13.0 -26.6)	23.0(10.8-118.7)	0.000*
CD4, cells/mm^3	207(116.0-279.0)	318(203-453)	70(22.9- 145.7)	113 (63-199)	227(133.8 – 353.0)	113(57.0 - 246.2)	0.000*
Weight, kg	64.00 (57.2 -73.00)	65.9 (59.7 -74.10)	1.90(2.7 -8.0)	63.80(55.9-67.7)	64.7(59.0 -70.9)	4.00(-3.3 -10.7)	0.892
Phase angle, α	7.19(6.3 -8.09)	6.79(5.7 -7.9)	-3.50 (-2.5 -15.4)	6.99 (5.5 -8.2)	6.49(5.3 -8.2)	0.17(-23.5-24.5)	0.114
FFM, kg	44.7 (40.8 -50.0)	44.8 (40.1 -49.6)	0.8 (-8.6-8.7)	45.6 (40.38 – 49.8)	44.1 (39.9 -47.9)	-4.60 (-14.2-7.70)	0.007*
LDM, kg	13.33(1.7-14.7)	12.8(11.30-14.70)	-1.16(-14.4-10.94)	12.5(11.15-14.40)	12.50(11.0-14.90)	-0.88(-14.60-15.76)	0.471
TBW, kg	32.0 (28.3 -35.9)	31.9 (28.3 -36.0)	0(-9.4 -11.5)	33.2(29.1 -39.8)	31.3(28.6 -34.2)	-1.95(-15.7-7.70)	0.002*
ICW, kg	17.20 (15.3-20.9)	17.17(14.9-19.9)	-1.7(-12.5-9.2)	16.80(15.00-22.80)	16.84(1530-19.48)	-1.70(-12.50-9.20)	0.168
ECW, kg	14.4 (12.7 -15.8)	14.7 (12.9 -16.3)	0.8 (-6.4-10.9)	14.9 (13.3 -16.7)	14.6 (12.9 -15.6)	0.6 (-12.3 -9.1)	0.205
BMR	1443(1357-1572)	1466(1352-1613)	0.81(2.1 -4.6)	1431(1341-1506)	1452(1339-1582)	2.3(-1.5 -6.2)	0.062
DEE	1938(1782-2151)	1943(1755-2183)	0.27(-6.30-6.00)	1886(1764-2061)	1886(1764-2061)	1.89(-2.2 -6.10)	0.120

**percent change is the ratio of the absolute increase over period divided by the baseline value ×100. The percentage change was compared between the two groups, HIV+ve/TB+ve and HIV+ve/TB-ve.

*Significant at 5% confidence level

Table 3.6.1.2: Comparison of changes in clinical and body composition measures between patients with TB and patients without TB from baseline to 12 months

	TB Negative			TB Positive			
Characteristic	Baseline	12 months	%change	Baseline	12 months	% change	p-value
BMI, kg/m^2	23.6 (20.8 - 27.6)	24.5 (21.9 – 29.1)	4.37(-1.1 -9.7)	22.4 (20.3 – 25.4)	24.9 (22.4 – 30.2)	9.4 (1.5 – 18.5)	0.139
FM, kg	19.1 (11.5 - 27.1)	23.60 (15.80 - 33.38)	20.3 (1.0 - 64.2)	14.0 (7.9 - 21.8)	23.8 (16.2 - 34.7)	53.8 (9.8 - 156.9)	0.000*
Phase, α	7.2 (6.3 - 8.1)	6.5 (5.5 - 7.6)	-9.5 (-25.8 - 10.9)	7.0(5.5 - 8.2)	6.4 (5.6 - 7.3)	-7.5 (-32.9 - 16.9)	0.776
FFM, kg	44.7 (40.8 -50.0)	44.9 (40.0 - 50.0)	-4.5 (-8.6 -0.8)	45.6 (40.4 - 49.8)	42.8 (38.7-44.5)	-3.2 (-12.5 -3.13)	0.326
LDM, kg	13.33(1.7-14.7)	12.8(11.40-14.10)	-4.4 (-9.15-3.05)	12.5(11.2 -14.4)	12.1(11.6-12.8)	2.65(-15.9 -8.8)	0.480
TBW, kg	32.0 (28.3 -35.90	30.8(26.7 -35.20	-4.7 (-10.2 -2.6)	33.2 (29.1 -39.8)	30.0(26.9-32.3)	-1.20(-9.8 -2.3)	0.112
ICW, kg	17.2(15.3-20.9)	16.4(13.8-19.9)	-6.9 (-14.28-0.00)	16.8 (15.0 -22.8)	15.1(13.4-17.0)	-6.2 (-10.7 -2.4)	0.140
ECW, kg	14.4 (12.7 - 15.8)	14.20(12.80-15.70)	-1.53(-7.5 -3.9)	14.9 (13.3 -16.7)	13.7 (13.0 -15.5)	0.0 (8.2 -9.9)	0.047*
BMR	1443(1357-1572)	1479(1356-1606)	1.7 (-1.02-4.26)	1431(1341-1506)	1486(1339-1565)	4.0 (-1.0 -8.2)	0.000*
DEE	1938(1782-2151)	1988(1809-2147)	1.5(-2.16-5.57)	1886(1764-2061)	1932(1741-2034)	3.98(-0.98-8.12)	0.048*

**percent change is the ratio of the absolute increase over period divided by the baseline value ×100. The percentage change was compared between the two groups, HIV+ve/TB+ve and HIV+ve/TB-ve.

*Significant at 5% significance level

Table 3.6.2.1: Predictors of FM, body mass index and FFM among 340 HIV-infected patients, 34 TB infected patients and 306 TB uninfected patients.

	Regression coefficients with their corresponding 95% confidence intervals and P-values for								
	FM(kg)			**BMI (kg/m^2)**			**FFM(kg)**		
	Coefficient	Confidence interval	P-value	Coefficient	Confidence interval	P-value	Coefficient	Confidence interval	P-value
Visit	2.414	1.527 - 3.300	0.000*	0.543	0.156 -0.929	0.010*	-0.250	-1.459 - -0.460	<0.001*
logviral	-0.936	-2.850 - 0.977	0.334	-0.272	-1.146 - 0.603	0.542	0.422	-1.682 - -0.024	0.045*
Age at initiation									
18-24.9	Reference								
25-34.9	2.887	-0.123 - 5.897	0.060	1.403	-0.280 -3.086	0.100	0.875	0.472 - 3.950	0.013*
35-44.9	5.625	2.128 - 9.121	0.002*	3.143	1.183 - 5.102	0.002*	1.009	1.106 - 5.125	0.003*
≥45	7.342	1.563 - 13.121	0.013*	3.789	0.988 - 6.590	0.009*	1.659	0.155 - 6.699	0.040*
Gender									
Male	Reference								
Female	7.130	3.618 - 10.643	<0.001*	4.335	2.775 - 5.892	0.000*	-0.743	-7.840 - -4.880	<0.001*
Education									
<grade 8	Reference								
≥ grade 8	6.632	2.320 - 10.944	0.003*						
Employment									
Unemployed	Reference								
Employed	-0.221	-2.652 - 2.210	0.858	3.256	1.388 - 5.123	0.001	0.638	-1.196 - 1.330	0.917
Tuberculosis									
No	Reference								
Yes	-5.967	-11.673 - -0.261	0.041*	-0.232	-1.934 - 1.468	0.787	0.984	-2.311 - 1.675	0.748
CD4									
<100 cells/mm^3	Reference								
100-250 cells/mm^3	0.516	-2.375 - 3.407	0.725	0.391	-1.008 - 1.789	0.582	0.625	-1.039 - 2.290	0.45
250-349 cells/mm^3	2.351	-1.575 - 6.277	0.240	1.082	-0.718 - 2.883	0.239	0.983	-0.250 - 3.633	0.087
≥350 cells/mm^3	0.337	-0.595 - 4.473	0.883	0.742	-1.585 - 3.069	0.531	1.291	-0.594 - 4.473	0.133

Table 3.6.2.2: Predictors of Phase angle and total body water for 340 HIV-infected patients, 34 TB infected and 306 TB uninfected patients

	GEE coefficients with their corresponding 95% confidence intervals and P-values for					
	Phase angle, α			**Total body water, kg**		
	Coefficient	Confidence interval	P-value	Coefficient	Confidence interval	P-value
time	-0.317	-1.166 - 0.533	0.429	-0.652	-1.019 - 0.284	0.001*
Logviral load	-0.085	-0.728 - 0.559	0.793	-0.669	-1.387 -0 .049	0.068
Age at initiation						
18-24.9	Reference					
25-34.9	0.056	-1.855 - 1.967	0.953	1.685	0.343 - 3.369	0.017*
35-44..9	-0.333	-2.251 - 1.585	0.720	2.629	1.196 - 4.668	0.001*
≥45	-0.557	-2.688 -1.573	0.593	2.718	0.462 - 5.909	0.022*
Gender						
Male	Reference					
Female	-0.199	-1.386 - 0.988	0.731	-4.762	-5.896 - 3.627	<0.001*
Nationality				-0.443	-1.625 - 0.739	0.462
Other	Reference					
South African				-0.471	-1.674 - 0.733644	0.443
Tuberculosis						
No	Reference					
Yes	-0.168	-1.585 - 1.249	0.805	-0.182	-1.711 - 1.348	0.812
CD4						
<100 cells/mm^2	Reference					
100-250 cells/mm^2	0.031	-0.825 - 0.886	0.943	0.841	-0.518 - 2.199	0.217
250-349 cells/mm^2	0.167	-1.163 - 1.497	0.791	1.366	-0.380 - 3.111	0.122
≥350 cells/mm^2	1.444	-2.212 - 5.099	0.420	1.112	-1.345 - 3.569	0.370

*Significance at the 5% level of significance

3.7. Comparison of Complete case analysis and imputed results

Although there are some similarities in the results found using complete case analysis and imputed values, there are some differences as well. For this reason we discuss the results obtained from the complete case analysis.

Fat Mass (FM)

Both GEE models for fat mass show that for there was a significant increase in average FM over the study period. They also show that females have a higher average FM than males and those with an education beyond grade eight had higher average FM than their less educated counterparts. Results from multiple imputation show that those with TB had significantly lower FM than patients without TB whilst there is no significant association between TB and FM. The only other difference is that age at initiation is significantly associated with FM in GEE models that use imputed data.

Body mass index (BMI)

In both complete case analysis and imputed data analysis, time, gender and employment are significantly associated with BMI. The coefficients and the significance levels are similar in both analyses. The one difference in results is that age at initiation is significantly associated with BMI when analysis is done using imputed data.

Fat free mass (FFM)

The analyses using complete case analysis and multiple imputation yielded similar results. There was a significant increase in FFM over the study period in both analyses. Log viral load and gender were both significantly associated with FFM. Age at initiation was found to be associated with FM in imputed data analysis but not in complete case analysis.

Phase angle

There were significant differences in models using complete case analysis and using multiple imputation. None of the variables were significant in the multiple imputation variables. The complete case analysis showed that there was a decrease in phase angle over time and being female was associated with a reduced phase angle.

CHAPTER 4: DISCUSSION

4.0 Introduction

This chapter is an overview of the study's findings for each outcome. We go on to discuss all the factors associated with each outcome, starting with the main exposure of the study which is TB. Relationships between the findings in our study compared to findings from other studies are explored and possible reasons for our findings are also discussed. The chapter also gives an overview of the relevance of the findings with regards to HIV, TB and nutrition and also highlights the limitations of the study. We also discuss potential areas of study to be pursued beyond this study.

4.1 Summary of results

At baseline patients with TB/HIV co-infection appeared with lower indicators of nutrition. They presented with lower FM, BMI, CD4 count and higher viral load than TB –ve patients. After stratification by gender the differences in measures between TB+ve and TB-ve patients were found to be significant in females but not in males as females with TB showed significantly lower FM, FMI and BMI. They also had lower CD4 counts and higher viral load than TB-ve females. There were no significant differences between TB-ve males and TB+ve males. Results of complete case analysis showed that TB+ve patients had greater increases in CD4 count, FM, weight, BMI and FFM although not statistically significant. The rest could have a clinical bearing so were noted. These results were consistent throughout study period.

GEE analysis showed no significant associations between TB and body composition measure. The analysis however showed significant associations between selected body composition measures and gender, education, employment, CD4 count and viral load. All patients enrolled in the study had been initiated on a first line ART regimen. The regimen of ART the participants were on showed no association with any of the body composition measures. A

study by Shlay et al on Long term body composition changes in ART showed Changes in total and regional body but the changes did not differ by ART regimen (38). The fact that all the patients in our study (Both HIV only and HIV/TB co-infected) were on ART and that only first line regime effect was investigated could influence our results. It could have resulted in our failure to see a significant difference in body composition as ART has its own effects which are to be further investigated.

Sensitivity analysis conducted multiple imputation showed similar associations as complete case analysis with the exception of age which showed associations with the different measures in the sensitivity analysis. The results also showed greater increases in FM and BMI in TB+ve than in TB-ve patient. Contrary to results from complete case analysis, FFM changes were significantly greater in TB-ve patients compared to TB+ve patients. The results of complete case analysis take precedence over results from imputed data. GEE analysis with imputed data showed a significant association between TB and FM, TB+ve patients have lower average FM than TB-ve patients with HIV.

4.2 Body composition and clinical measures

Our analysis showed that at baseline patients with HIV/TB co-infection had lower CD4 count compared to patients with HIV alone. Several other studies have concluded the same (54, 55). The results highlight the relationship between immunological failure and TB. Patients with TB have a compromised immunological status and patients with a compromised immune system are more likely to get TB. At baseline no temporality can be established. HIV/AIDS and TB combined have a negative impact on immunological status as measured by CD4 count. Females had a higher baseline CD4 count at baseline compared to males. This is in line with studies that have shown that male gender is associated with late presentation for treatment in HIV (56). This information is important when making interventions for early HIV counselling and testing.

In this analysis, we compared the change in CD4 count and body composition measures between patients with HIV alone and patients with HIV/TB co-infection. There was a greater increase of these measures in patients with HIV/TB co-infection from baseline to 6 months. This was consistent with results from a study that compared CD4 count recovery in HIV-infected/TB+ve and HIV-infected/TB-ve. Wanchu et al showed patients with co-infection had significantly greater increase in CD4 count compared to those with HIV alone (57). Patients with HIV/TB co-infection start out with significantly lower CD4 count but treatment with ART as well as anti TB treatment has a positive impact on CD4 count and the change is marked in these patients.

Our baseline analysis of body composition measures by TB status showed that TB+ve patients had a lower FM and BMI compared to individuals without TB when presenting for treatment. This is consistent with studies that have shown that HIV/TB co-infection results in lower body composition measures (3, 6, 13). The effect of TB in HIV-infected individuals is to further reduce nutritional status. Our results are also consistent with results from the study by Paton and Ng (29) which showed that TB is associated to lower FFM and FM than patients without TB. The authors measured body composition between in 18 patients with TB and 22 without TB. This study was however not conducted amongst HIV-infected patients only. In another study however they looked at the impact of TB on body composition of HIV-infected men in Brazil in a cross-sectional study. They found that nutritional status of those with co-infection was generally worse than those with HIV alone. They found that HIV/TB co-infected patients had a significantly lower mean BCM. Another finding was that FM and FFM showed small but insignificant reductions. The authors concluded that FM and FFM underestimated the nutritional deficit (6). A study among 944 patients with HIV, TB or HIV/TB co-infection in Uganda, it was shown that patients with TB co-infection had a lower

FM than their counter-parts with HIV-infection only (58). This was also in another study that showed that reduced FM and FFM was seen when patients present with TB (19).

It has been shown that TB may result in greater FM in females but lower FFM in males (36). We stratified our baseline analysis by gender and our analysis showed an association between TB and FM was in females but not in males. Females with HIV/TB co-infection showed significantly lower FM than females with HIV alone. This difference was not evident in males. The lack of difference in both FM and FFM amongst TB+ve and TB-ve males could be because patients in this group are more homogenous with regards to HIV-infection as measured by CD4 count. Males at baseline were generally worse off than females, with lower CD4 count and higher viral load but the differences in these measures between TB-infected and uninfected patients were smaller in males than females. This may result in factors other than TB status being responsible for similar reductions in FM and FFM in the groups. Viral load and CD4 count have been shown to be independent predictors of body composition measures (47)

The effect of gender on body composition measures is well recognised. Studies have shown that females have higher amounts of FM and lower amounts of FFM than males (40). In our analysis, we found that males had lower FM by 9kg compared to females and their FFM was 4.85kg higher in multiple regression GEE analysis. This is consistent with other studies among HIV-infected adults that have reached the same conclusion. A study by McDermott et al compared body composition, as measured by dual-energy X-ray absorptiometry, in 203 HIV-infected males and 62 HIV-infected females on ART. It found that amount and distribution of FM was higher among HIV-infected females than males receiving ART (21). The study suggested that females on ART tend to gain more FM than their male counterparts because of hormonal differences between the two genders. In their paper on body composition among TB-infected patients with and without HIV, Mupere et al found that gender and not HIV status was

associated with body composition changes in patients with TB (36). Females had significantly higher FM whilst males had higher FFM. This is consistent with our results where females had higher FM than males throughout the study period and males has a higher FFM

In the current study, among HIV-infected patients initiating ART and followed up for 12 months, sex, age, education, smoking, and baseline viral load and CD4 count were associated with different body composition measures. TB however showed no association with any of the body composition measures over the period. Our results show a large influence of gender on the wasting process in HIV-infected individuals more than TB.

4.2.1 Phase angle

We found no statistically significant association between TB and phase angle, although the decrease appeared more pronounced in patients without TB in the profile plot. This is consistent with our comparison of percentage changes. Females had lower average phase angle than males after adjusting for other possible confounders. There were no significant differences in phase angle between those with TB and those without TB, even after stratification by gender.

In our study the mean phase angle in the population was higher than the mean reported by Shah et al in their study of 163 HIV-infected females and 199 HIV-uninfected males. They reported a mean (standard deviation) of 5.42 (+/-1.05) and 5.76 (+/-0.93), respectively (15) while we found a mean (standard deviation) of 6.6 (+/-2.28) for males and 6.0 (+/-2.86) in females. In Uganda, the same author found that co-infection with pulmonary TB and HIV is associated with lower phase angle (15). Among HIV-infected individuals, there was an inverse relationship between phase angle and risk of death (2, 59). Schwenk et al in their study on Phase angle as a predictor of clinical progression and survival found phase angle was independently associated with these factors (2). A study conducted to investigate the

prognostic relevance of phase angle measured by BIA among 75 HIV-infected patients found that body composition as reflected by phase angle is a determinant of long time survival. Sanchez et al, in their study investigating changes in body composition among 24 participants with TB found that phase angle increased significantly during TB therapy (59). This is contrary to results from our study that showed a decrease in phase angle over the follow up period.

Between baseline and 6 months the phase angle decreased by 4% (-16.2%-7.04%) in TB-ve and 2% (-21.8%-8.5%) TB+ve.

4.2.2 Fat mass

A comparison of fat mass between those with TB and those without TB showed that HIV-TB co-infected had significantly lower fat mass than patients with HIV only. This is line with studies that show that HIV/TB co-infection is associated with loss in FM (58). When stratified by gender, the difference in FM between those with TB and those without TB was significant in females. This difference was however not seen in males. This is consistent with results from a study that showed that weight loss in HIV was associated with loss of FM in female patients and with loss of FFM in male patients (16). Infection with TB is likely to exacerbate FM loss and the difference is more pronounced in females. This might be the reason FM differences by TB-infection are more apparent in females and not in males.

HIV/TB co-infected patients have a lower FM than patients infected with HIV alone and HIV-uninfected patients (15, 47). This could mean that at baseline the effect of TB is to lower body fat.

A cross sectional study looking at predictors of body composition among TB patients in Mwanza found that TB was associated with a lower FM among HIV-uninfected patients but not among HIV-infected patients (40). The authors suggested that the association was not

seen in HIV-infected patients because other factors like opportunistic infections could explain the deficits in fat mass amongst HIV-infected patients. These results contradict the findings of our baseline analysis which suggest that TB may have an enhanced effect on FM of patients already infected with HIV. The effect of TB on FM persists in HIV-infected individuals in our study. We however observed a difference in HIV-infected females and not in males. Our suggestion is that females had generally higher CD4 counts and lower viral loads when compared to males. Females were immunologically better off than their female counterparts. Other factors i.e. other opportunistic infections or advanced HIV disease may be responsible for body composition measures in males and therefore no difference between TB-infected and those without TB in this group.

Although not statistically significant, TB co-infected patients had higher percentage increase in FM than TB-uninfected patients. A possible reason is the patients are worse off and taking drugs for both conditions; this may lead to a more drastic improvement than their healthier counterparts.

There was an increase in population FM over the three visits. The profile plot shows an upwards trajectory and at univariate analysis in GEE there was a 2.248kg increase in FM for each visit and this was maintained in multiple regression analysis. An increase in FM over the follow up period is consistent with results that patients on ART and on ATT treatment should experience an increase in FM. This represents an increase in nutritional status associated with improved outcomes.

Over 12 months, using GEE we determined that TB has no statistically significant effect on average FM. Gender however showed an association with FM. Females had significantly more FM than males and those with a higher education had significantly more FM than their less educated counterparts

4.2.3 Body mass index

Our results showed that there was an association between BMI and TB-infection in HIV-infected individuals. Although the difference in BMI between those with HIV/TB co-infection and HIV alone was statistically significant, it was not clinically significant. Both groups had a median BMI within normal range. Studies on HIV-infection and BMI have shown that HIV without treatment results in body wasting (45, 60).

Recent studies in Sub-Saharan African countries have shown that levels of obesity seem to be increasing (61, 62).The proportion of people with BMI <18.5kg/m^2 at baseline in the present study is less than 7%. This proportion was not statistically significantly different between those with TB and those without TB, although those with TB had a higher proportion of underweight participants. The median BMI was 23.5kg/m^2 and 22.39kg/m^2 for those without TB and those with TB, respectively, indicating that the study population was primarily comprised of people of normal weight according to WHO guidelines for BMI classification. A substantial number within our population had BMI above 25kg/m^2 which WHO classifies as overweight. Most studies amongst HIV-infected individuals have found the populations to comprise of larger numbers below 18.5kg/m^2.Swaminathan et al compared nutritional status in HIV-infected patients with TB and without TB. They found approximately 33% of the HIV-infected/TB-uninfected and 50% of HIV-infected/TB-infected had BMI<18.5 (16). This was clear evidence of the negative impact of TB and HIV on nutrition. This was not evident in our population. We did not find such large differences between those with TB and those without.

The GEE model showed that there was an increase in BMI over the follow up period. This was an expected change as all the participants were initiated on ART. A study of patients on ART in Nigeria found that patients gained weight during follow-up. There were no significant effects of age, sex, viral load or educational status on BMI change in this

population (63). The present study showed employment and gender were significantly associated with BMI. We suggest that patients who are employed may have a better socio-economic status and therefore be able to afford better nutrition. There was no association between TB and BMI over the follow up period.

4.2.4 Fat free mass

Although FFM appears lower in TB infected individuals, this difference was not statistically significant. There were also no significant differences in FFM after stratification by gender. Shah et al in their study compared nutritional status between HIV-infected and HIV-uninfected individuals with TB and found similar results to ours. They found that among 261 HIV-infected patients and 278 HIV-uninfected patients in Sub-Saharan Africa, HIV/TB co-infection was not associated with lower FFM but with smaller body cell mass.

Using the GEE model after adjusting for other variables, there was a decrease in FFM over the follow-up period. This could be seen as worrying as FFM has been seen to be associated with poor outcomes in HIV-infected individuals. In their study on the effect of ART on body composition measures, McDermott et al reported that patients on ART may experience increases in FFM (47) but a study by Silvia et al showed increases in BMI and not FFM with use of ART and weight gain in that study was due to changes in FM and not FFM (64). A study by Paton et al looked into impact of TB on body composition in HIV-infected males and found a mean FFM of 47.7 +/- 5.2 and 45.5 +/- 5.3 in HIV-infected patients without TB and with TB respectively. Although those with TB had lower FFM in their study, the difference was not statistically significant (6). This is similar to our result where those with TB have lower FFM but the difference is not statistically significant. In our analysis of factors associated with FFM, we identified sex, age and viral load as predictors of lower FFM. No association was found between TB and FFM after adjusting for possible confounders in our model.

4.2.5 Total body water

Although not statistically significant, baseline TBW was higher for TB+ve patients (33.20kg) compared to TB-ve patients (32.00kg) p-value=0.459. There is a decrease in TBW between baseline, 6 months and 12 months for both TB+ve and the TB-ve groups and no statistical significance in these differences. After adjusting for confounders in GEE analysis there was an overall decrease in average TBW (-0.728, p-value=0.004). After one year the median TBW was similar in the TB+ve and the TB-ve patients. GEE analysis showed that for every increase in log viral load and females had a lower viral load than males. (-8.611; p-value <0.001). These results are similar to results from a study by Ott et al showed an increase in TBW with a decrease in FFM in the early stages of HIV infection (46). As the extent of malnutrition increases, so does the proportion of TBW as well as depletion of FFM. The increase in water results in Oedema in those affected. A general decrease in TBW our patients could be indicative of increasing nutritional status due to ART and anti TB treatment. It is synonymous with the increase in FM and FFM.

4.3 Limitations of study

The primary study was conducted at one out-patient HIV treatment facility based within a public-sector hospital in South Africa so it is possible that experience differs at other primary healthcare facilities and the results may not be generalizable to other facilities.

Sample size was calculated for a different outcome measure and the study was not powered to assess the association between TB co-infection and body composition measures. There was a substantial amount of missing data. Over the follow up periods some patients have missing outcome data.. GEE on the complete case analysis assumed missing data in the main study was missing completely at random. This assumption is hardly ever met. Inferences made from complete case analysis may be wrong for the entire population if missingness

is not completely at random (MNAR). An attempt to resolve this problem was through multiple imputations of the missing data. The data missing was however on the outcome and literature on imputation of outcomes varies (65-67). Some authors have argued that multiple imputation can be suited for sensitivity analysis although complete case analysis should be the analysis of choice more often (66, 67). There has also been some argument that imputation might yield biased or incorrect estimates (65). Imputation is only useful if the imputed model is correct. The coefficients obtained from the missing data were different for some of the variables to those from those obtained from complete case analysis although there were some with similar results. More work is needed to confirm which of the coefficients obtained are correct. We present complete case results as the main results in this paper.

GEE modelling provides population averaged estimates and these are useful to an extent but cannot be used for subject specific inferences. Generalised linear mixed models could be used as the next step in analysis to make inferences that are not for the whole population. Information on severity of TB and treatment of TB could be useful in finding the relationship between TB and nutritional status. We did not have information on severity of TB or linkage to care for those with TB in our analysis. These could be confounders in the relationship between TB and nutritional status.

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As the sample size was small in the complete case analysis, it was difficult to assess interactions between sex and TB. Data on smoking did not take into consideration the amount smoked per day. As such, the information is an over generalisation of smoking as it was not collected for the purposes of this study.

Another limitation of the small sample size in our study is that it would have reduced our chances of detecting the true effect and the precision of the estimates. This may be the reason

that we have some clinically significant differences and results in our analysis that failed to reach statistical significance.

There is indication that there were changes in ART regimens amongst some participants in the study. This study did not access the reasons for switching regimens and so we cannot determine whether the patient switched due to virologic failure, toxicity, side effects or some other reason. This may be the explanation for the decreased nutritional status with regards to some of the body composition measures like FFM and phase angle. Patients may not have been responding to treatment as anticipated. There is need to investigate treatment failure and nutritional status. There is also a need to investigate effects of different ART strategies, both Non-Nucleoside Reverse Transcriptase inhibitors (NNRTI) –based and Protease Inhibitor (PI) –based on body composition against those who have not initiated ART. This might give a better understanding of the role ART plays in the dynamics of HIV/AIDS, TB and nutrition in the era of ART. Further analysis looking at severity of TB is also needed.

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CHAPTER5: CONCLUSIONS&RECOMMENDATIONS

5.0 Introduction

The conclusions and recommendations in this chapter are drawn from the discussion in the preceding chapter. The chapter gives recommendations for future research in this area of study.

5.1 Conclusions

Currently, efforts towards integration of HIV, TB and nutrition care are underway. Understanding determinants of body composition measures at baseline and during treatment with ART as well as TB treatment may help in the design of interventions that are targeted and can improve treatment outcomes. Such determinants include CD4 count, viral load, and socio-economic status as well as lifestyle factors like smoking.

In the current study, patients with HIV/TB co-infection had lower body composition measures than participants with HIV only at baseline. They also presented with a lower median CD4 count at baseline indicating increased severity of HIV-infection amongst patients with TB. This highlights the importance of contact tracing of TB cases to find other TB-infected people at early stages of infection. It is important that patients infected with TB and HIV be identified so that their health and nutritional status can be monitored.

Differences in body composition at baseline stratified by gender showed the effect of TB on both nutritional measures and immunological measures. The differences in females are more apparent as the group of patients is more heterogeneous with regards to severity of HIV-infection. Males generally had more severe HIV-infection as determined by CD4 count and viral load at baseline whether they had TB or not. These results show that males generally present at baseline when they are sicker than females and therefore have generally poorer

nutritional status at presentation. Efforts need to be made to identify HIV-infected men early for better treatment outcomes.

Although not statistically significant, results on changes from baseline to 6 months and from baseline to 12 months show that TB+ve have larger increases in both nutritional and immunological measures than TB-ve patients. This shows that patients respond really well to treatment and improvement after treatment commencement is marked. Treatment of these conditions is therefore of great importance as there seems to be substantial improvements in this group once treatment is started.

In all analyses using GEE, TB was not significantly associated with any of the body composition measures modelled. This could be suggestive that over the course of treatment, identifying people in need of nutritional supplementation should be based on a host of factors and body composition measures, not just TB. TB may not be the main and only driver of nutritional indicators in HIV-infected individuals.

More than 50% of participants had normal BMI and less than 7% had BMI <18.5kg/m^2. This indicates that this group of patients was not severely malnourished. As much as 14.41% of the participants had BMI above 30kg/m^2.The relationship between obesity, malnutrition and HIV need to be investigated. The face of HIV is changing with regards to nutrition. The marker for infection is no longer severely malnourished patients. Patients indicated for treatment with CD4 count below 350cells/mm^3 may actually be obese.

It is of great importance to study medication adherence, medical complications and drug regimens for both HIV and TB to further understand how these have an effect on body composition. Assessment of nutrition by BIA in HIV-infected individuals needs to be a part of diagnosis and therapy regardless of TB status.

5.2 Recommendations

Nutritional supplements

Measurement of nutritional status is very important amongst HIV-infected individuals regardless of TB status. At baseline, differences in body composition by TB status were more apparent in females but males presented with lower body composition measures regardless of TB status. Targeted nutritional interventions according to TB might only make sense for females. Males however should all receive nutritional intervention regardless of TB status. On average males present with poor nutritional and immunological indicators. Our recommendation is that the decision to give and maintain nutritional supplementation be made per individual after careful consideration of all other factors that could be associated with body composition and assessment of their nutritional status.

Identification of HIV-infected individuals and TB preventative therapy

Comparison of the two groups at baseline showed that HIV-infected patients with TB present with lower body composition measures compared to HIV-infected individuals without TB. This is evidence that TB in HIV exacerbates the malnutrition from HIV on its own. Identification of HIV-infected individuals early and getting them on treatment to reduce risk of acquiring TB is important. Use of TB preventative therapy for HIV-infected individuals could also be helpful.

Monitoring of nutritional status over treatment period

The study found that although changes of BMI and FFM over time were consistent with improved nutrition, phase angle, which has been shown to be a predictor of survival in chronic disease management, decreased with time. FFM also showed reduction over the study period. Over the period of 12 months the group did not show definite increases in

nutritional status. Thus, consistent monitoring after ART initiation of nutritional status is important in all HIV-infected individuals.

Predictors of body composition measures

Baseline CD4 and viral load were found to be important predictors of BMI, FFM and TBW. Education and employment were also found to be associated with measures of nutrition. Early diagnosis and treatment is therefore of importance in the nutritional status of HIV-infected individuals. Socio-economic status is an important predictor of nutritional status and could be used to determine those most in need of interventions.

Adherence and treatment failure

Although over time our study showed an increase in BMI and FM it also showed a decrease in phase angle and FFM on the entire group from baseline throughout the study. These are important predictors of treatment success in patients. It is important to investigate the response to treatment for different body composition measures. Patients on ART and TB treatment must be closely monitored to see if these decreases are a result of non-adherence, treatment failure due to medication resistance, or side effects of ART.

Longitudinal data data

Missing data is a major problem when it comes to analysis of missing data. It is important to ensure that there is minimal loss to follow up during longitudinal data data collection. Analysis using incomplete data is difficult and might not yield results that can be generalised to the entire population. Multiple imputation is one way of dealing with the problem of missing data. However it has its own pitfalls and may not yield accurate estimates (66). As part of our analysis we did a sensitivity analysis using multiple analyses to compare with the results obtained from our complete case analysis. It is

important to use explore different statistical methods to analyse datasets with missing data.

5.3 Potential areas for research

It is important to note that the information obtained in this study may not be generalizable to different populations. The amount of missing data makes it difficult to generalise. A more rigorous look at the effect of TB taking into account the severity, type and treatment of TB is important. This study lays the groundwork for future longitudinal data studies in different populations to study trends in nutritional status.

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NAME: (Principal Investigator)	Ms Caroline Govathson
DEPARTMENT:	School of Public Health University of the Witwatersrand Epidemiology and Biostatistics
PROJECT TITLE:	The Impact of Tuberculosis Infection on the Body Composition of HIV Positive Adult Patients on HAART in Johannesburg South Africa
DATE CONSIDERED:	29/11/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Charles Chasela
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/12/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

Principal Investigator Signature

Date 11 DEC 2013

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Health Economics and Epidemiology Research Office (HE²RO)

Themba Lethu Clinic, Helen Joseph Hospital
Perth Road, Westdene 2092, South Africa
Tel +27 (11) 276-8850, Fax +27 (11) 276-8885

A collaboration between the Wits Health Consortium of the University of the Witwatersrand
and the Center for Global Health & Development of Boston University

04 November 2013

INVESTIGATOR(S): Kate Shearer - Health Economics and Epidemiology Research Office
Charles Chasela – School of Public Health, University of the Witwatersrand
Caroline Govathson – School of Public Health, University of the Witwatersrand

TITLE OF RESEARCH PROJECT: **The impact of Tuberculosis infection on the body composition of HIV positive adult patients on HAART in Johannesburg South Africa.**

PERMISSION TO USE STUDY DATABASE

This letter serves to confirm that the abovementioned investigators have been granted permission to use the study database of the **Low cost monitoring of HIV in resource-limited settings** project. This study has approval from the University of the Witwatersrand HREC-Medical (M10418).

Permission has been granted for the period 01 November 2013 – 31 October 2014 on condition that the investigators sign

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

(ii) RTC confidentiality agreement

The investigators also agree to acknowledge the study and funding in any work that emanates from using this data (NIH/CFAR Creative and Novel Ideas in HIV Research CNIHR: Low cost monitoring of HIV in resource-limited settings UAB CFAR grant number P30AI027767).

Yours sincerely,

Dr Denise Evans
Senior Researcher
Health Economics and Epidemiology Research Office (Wits Health Consortium)
Mobile: +27 (0)82 713 8857
Fax: +27 (0)86 516 0727

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Website: http://www.bu.edu/cghd/

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Denise Evans

CLEARANCE CERTIFICATE	**M10418**
PROJECT	Low-Cost Monitoring of HIV in Research-Limited Settings
INVESTIGATORS	Dr Denise Evans.
DEPARTMENT	Department of Medicine/Clinical HIV Res Unit
DATE CONSIDERED	30/04/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 11/06/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr D Evans

DECLARATION OF INVESTIGATOR(S)

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

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...