OPTIMIZING THE DIAGNOSIS AND MANAGEMENT OF TUBERCULOSIS AND ASSESSING THE BURDEN OF HYPERTENSION AMONG HIV PATIENTS IN A HIGH TB AND HIV BURDEN SETTING

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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DECLARATION

This thesis has not been submitted in any other application for a degree and is the result of my own work and composition.

In some circumstances, work (such as Laboratory services and part of data collection) was conducted in partnership with other co-workers and institutions. The details of the contribution of the collaborators to this project, is as outlined below, and they are also mentioned in the acknowledgments section.

Activities	Responsibility
Continued recruitment of HIV-positive patients when I had to return to the UK before completing data collection for Chapter 2.	Dr Kufre - HIV unit, UATH
Sputum sample collection (Chapter 2)	Joy Haruna - Laboratory Scientist, UATH
Sputum samples' transport to the Zankli Medical Research Laboratory (Chapter 2)	Mr Madu (the Project Vehicle Driver)
Sputum smear microscopy and culture (Chapter 2)	Zankli Medical Research Laboratory, Bingham University, Karu, Nasarawa state.
Retrieval of HIV patients case folders (Chapter 5)	UATH's ART clinic's records staff
Data abstraction from HIV care records and TB treatment registers/cards (Chapters 4 and 5)	Three research assistants (Tope, Seun, and Deborah) and some nursing students.
HIV testing for recruitment of HIV-negative controls. (Chapter 5)	Staff of the HIV Counselling and Testing (HCT) unit and Microbiology department of University of Abuja Teaching Hospital
Provision or donation of sputum collection cups	TB and Leprosy Control Program of Federal Capital Territory (FCT-TBLCP)

*Co-workers were directly under my daily supervision and I regularly visited the TB Laboratory to ensure that things were done as they ought to be done

DEDICATION

This thesis is dedicated to God the Almighty, who has been my help, sustenance and sufficiency.

ABSTRACT

Background: TB is the commonest cause of death among people living with HIV (PLHIV) in Nigeria. It is difficult to diagnose TB among PLHIV and diagnosed patients often experience poor treatment outcome. Although, PLHIV are living longer because of increased access to antiretroviral therapy (ART), hypertension is emerging as a major co-morbidity in this population. This study explored better means of diagnosing TB among individuals with and without HIV, described TB treatment outcomes and their determinants, and assessed the burden of hypertension among PLHIV.

Methods: This thesis comprises four studies conducted in University of Abuja Teaching Hospital (UATH): one cross-sectional study of PLHIV to assess the performance of the WHO TB symptom screening algorithm; a prospective study to evaluate whether C-reactive protein (CRP) and Interferon gamma-inducible protein 10 (IP-10) could be used to screen individuals for TB; a retrospective study to describe the TB treatment outcome of PLHIV and HIV-negative patients and the risk factors for poor treatment outcome and a retrospective study to describe the prevalence and incidence of hypertension and its determinants among PLHIV registered over a period of 3 years.

Results: 202 PLHIV were screened for TB and 72.3% had symptoms of TB. However, only 3% and 6.5% had culture or culture plus smear confirmed TB, respectively. The WHO algorithm had 83.3% sensitivity, 29.1% specificity and 98.2% negative predictive value. CRP and IP-10 were measured in 408 patients with TB symptoms, of which, 21% had culture-confirmed TB. CRP had 91.4% and 33.2% sensitivity/specificity among all participants, 95.3% and 42.6% among HIV-negative patients, and 84.8% and 22.1% sensitivity/specificity among PLHIV, respectively. IP-10 had 87.3% and 40.9% sensitivity/specificity among all participants and 87.5% and 50.3% among HIV-negative and 79.4% and 47.2% among PLHIV, respectively. 998 patients were treated for TB by the hospital in the last 5 years. Of these, 62% had treatment success, 8.4% died and 18% were lost-to-follow up (LTFU). TB/HIV-coinfection rate was 44.3%. Treatment success was 52.3% among PLHIV and 70% among HIV-negative patients ($p = 0.001$) with higher deaths (15.5% versus 2.9%, $p = 0.001$) and LTFU (22.5% versus 14.3%, $p = 0.001$) among PLHIV. Poor treatment outcome and LTFU were more frequent among older individuals, PLHIV, those without sputum smear results and low body weight. Low weight and not receiving Co-trimoxazole were associated with poor outcome and LTFU among PLHIV. 12.8% of 883 PLHIV had hypertension at enrolment and 11.2% developed hypertension 12 months after enrolment. Hypertension was associated with older age, higher BMI, hepatitis B and higher CD4 counts. Patients with incident hypertension had higher systolic and diastolic blood pressure on enrolment.

Conclusion: The WHO TB screening algorithm performed as reported by WHO. However, most patients had symptoms of TB and required further tests, which limits its efficiency in high burden settings. CRP performed better than IP-10 and both markers performed better in HIV-negative than among PLHIV. TB treatment outcome was poor and was worse among PLHIV. Hypertension is a common problem among PLHIV. There is a need for better screening tools for TB among HIV-positive patients and to develop interventions to improve the outcome of patients with TB, especially among the elderly, PLHIV and those presenting with underweight. The high burden of hypertension among HIV patients signals the need to integrate care of hypertension and other noncommunicable diseases into HIV care programs.

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List of abbreviations

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine Transaminase
AOR	Adjusted Odds Ratio
ART	Antiretroviral Therapy
ARV	Antiretroviral
BMI	Body Mass Index
BP	Blood Pressure
CD4 Cells	Cluster of differentiation 4 cells
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
cIMT	Carotid intima-media thickness
CRP	C-Reactive Protein
CPT	Co-trimoxazole Prophylaxis Therapy
CSC	Commonwealth Scholarship Commission
CVD	Cardiovascular Diseases
DM	Diabetes Mellitus
DOTS	Directly Observed Therapy Short course
FACA	FCT's Agency for the Control of AIDS
FBC	Full Blood Count
FCDA	Federal Capital Development Administration
FDC	Fixed Dose Combination
FCT	The Nigeria's Federal Capital Territory (FCT), Abuja
FIND	Foundation for Innovative New Diagnostics
FMOH	Federal Ministry of Health
Hb	Haemoglobin concentration
HbA1C	Glycated Haemoglobin
HBV	Hepatitis B Virus
HCT	HIV Counselling and Testing
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
INH	Isoniazid
IP-10	Interferon Gamma-inducible Protein 10
IPT	Isoniazid Preventive Therapy
IQR	Interquartile Range
LAM	Lipoarabinomannan
LED-FM	Light Emitting Diode Florescent Microscopy
LFT	Liver Function Tests
LFTU	Loss/Lost-to-Follow up
LSTM	Liverpool School of Tropical Medicine
LTBI	Latent TB Infection
MDR-TB	Multidrug Resistant Tuberculosis
M&E	Monitoring and Evaluation
MTB	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
MTB/RIF	Mycobacterium Tuberculosis/Rifampicin Resistance
NALC	N-Acetyl-L-Cysteine

NaOH	Sodium hydroxide
NASCAP	National AIDS and STIs Control Program
NEPLWHAN	Network of People Living with HIV/AIDS in Nigeria
NK	Not Known
NPV	Negative Predictive Value
NTBLCP	National Tuberculosis and Leprosy Control Program
NTM	Non-Tuberculous Mycobacteria
OI	Opportunistic Infection
OR	Odds Ratio
OSP	Oral Swab PCR
P	Probability value
PCR	Polymerase Chain Reaction
PEP	Post-Exposure Prophylaxis
PLHIV	People Living with HIV/AIDS
PMTCT	Prevention of Mother-to-Child Transmission
POC	Point-of-Care
PPV	Positive Predictive Value
PWV	Pulse Wave Velocity
RFT	Renal Function Tests
RIF	Rifampicin
ROC	Receiver Operating Characteristic
RR	Rifampicin Resistance
SD	Standard Deviation
SDGs	Sustainable Development Goals
SPSS	Statistical Package for the Social Sciences
T2DM	Type 2 Diabetes Mellitus
TB	Tuberculosis
TBLCP	Tuberculosis and Leprosy Control Program
TPPs	Target Product Profiles
UATH	University of Abuja Teaching Hospital
UNAIDS	Joint United Nations Programme on HIV/AIDS
USA	United States of America
VOCs	Volatile Organic Compounds
WBC	White Blood Cells
WHO	World Health Organization
ZMC	Zankli Medical Centre
95%CI	95% Confidence Interval

Chapter 1

General Introduction

1.1 Introduction

Acquired Immune Deficiency Syndrome (AIDS) and Tuberculosis (TB) are the top two leading causes of death due to an infectious agent in the world [1]. Nigeria has about 3.2 million individuals infected with the Human Immunodeficiency Virus (HIV) [2] and the World Health Organization (WHO) classifies the country as fourth in the list of high TB burden countries [3], with an estimated 407,000 new cases in 2016 [4]. These two pathogens interact, and TB is the most important opportunistic infection and major cause of death among the HIV positive patients.

In spite of its high TB burden, the case detection rate in Nigeria is very low (16%) [5]. Diagnosis of TB in HIV patients is difficult because a high proportion of co-infected patients are sputum smear negative or have extrapulmonary forms of the disease [6-9]. A large proportion of TB cases are diagnosed late or remain undiagnosed, and autopsy studies from Sub-Saharan Africa, have reported that undiagnosed TB is a major cause of death among people living with HIV (PLHIV) [10-12] and cases diagnosed late have poor treatment outcome.

HIV prevalence varies across the states of Nigeria, as shown in Figure 1. The prevalence ranged from <1% in 5 states in the southern (Ogun, Ekiti, Edo, Delta and Ebonyi states) and 4 states in the northern (Kebbi, Zamfara, Katsina and Bauchi states) regions, to > 9% in a state in the southern (Rivers) and two states in the northern (Kaduna and Taraba) regions of the country. This study took place in the Nigeria's Federal Capital Territory (FCT), located at the centre of the country, and HIV prevalence there is in the range of 7 to 9%.

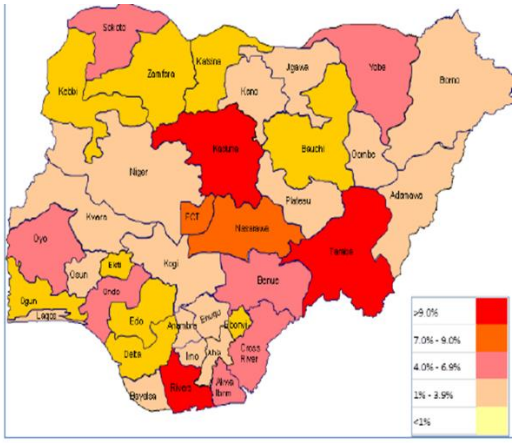


Figure 1.1. Map of Nigeria describing HIV prevalence by state. Source: Nigeria's National Reproductive and Health Survey (NARHS), 2012.

Sputum smear microscopy is still the most commonly used diagnostic test for TB diagnosis in Nigeria and globally. Though, it is cheap, fast and can be used at all levels of health care, its sensitivity is low, especially among HIV patients [13,14]. Sputum culture has high sensitivity but it takes up to 17 days for liquid and up to two months for solid culture to produce test results.

Given the difficulty and consequences of making a TB diagnosis among HIV-infected individuals, the WHO developed an algorithm for screening for (and excluding) TB among HIV patients. Patients without symptoms suggestive of TB (absence of cough > 2 weeks, fever, chronic weight loss or excessive night sweating) are considered to be unlikely to have TB and are provided with Isoniazid Preventive Therapy (IPT). Patients with any of the symptoms should undergo further testing for TB diagnosis and, if confirmed, commence anti-TB medication [15,16]. Several studies evaluating the 2007 WHO algorithm reported sensitivity and specificity ranging from 20% to 72% and 22% to 72.9%, respectively [15,17,18]. Surveys in high TB burden countries also reported that more than half of the patients with bacteriologically confirmed TB did not meet the WHO criteria and that many of patients presented without symptoms [13,19,20]. Consequently, the algorithm was modified in 2011 to

include any of the symptoms of fever, cough, weight loss and night sweats of any duration. The performance of the revised algorithm has not been evaluated in Nigeria or West Africa.

In 2010, the WHO approved the use of Xpert MTB/RIF for TB diagnosis [21,22]. Xpert MTB/RIF has high sensitivity (92% and 75% for sputum smear-positive and negative, respectively) and specificity of 99% [23], and in 2014, the WHO recommended that, when available, Xpert- MTB/RIF should be used as the first diagnostic test in HIV-infected individuals with signs and symptoms suggestive of TB[13,24]. To improve TB case detection among HIV-infected patients, WHO therefore recommends utilizing the standardized algorithms and to use Xpert MTB/RIF as the first diagnostic test. Although Xpert MTB/RIF availability is increasing in high burden settings, the test requires stable power supply and laboratory facilities, which make it difficult to be used at the lowest level of healthcare. Consequently, its implementation in some countries has not reduced the time to TB treatment initiation [25] or improved treatment outcome [26], and may not be cost-effective [27]. These challenges highlight the need for better diagnostics for TB.

The WHO conveyed a meeting in April 2014 to develop a consensus of four high-priority target product profiles (TPPs) for new TB diagnostics [28]. These include; a non-sputum-based point-of-care (POC) that can detect all forms of TB through identification of biomarkers or bio-signatures, a POC triage test that is simple, not costly, and can be used to rule out TB at the lowest level of healthcare, a sputum-based POC test that could replace smear microscopy, and a rapid Drug Susceptibility Testing (DST) at the microscopy level [28,29]. Of these, the most urgently needed diagnostic is the non-sputum-based POC that can accurately detect all forms of TB with a sensitivity higher than smear microscopy. As there are no products that are close to market, in this thesis, we evaluated the performance of C-reactive Protein (CRP) and Interferon Gamma Inducible Protein 10 (IP-10) as biomarkers to rule out active TB.

TB still carries a high risk of death in Nigeria. In addition to deaths due to undiagnosed TB, thousands of people die, are lost-to-follow up (LTFU) or fail to improve clinically during TB treatment, every year. In 2016, one million (17% of the global notified TB cases) either died, were LTFU, failed treatment or were not evaluated [30]. A review of 44516 TB patients registered between 2011and 2015 in Lagos, Nigeria, reported that 1424 (3.2%), 4717 (10.6%) and 937 (2.1%) of the patients died, LTFU or failed treatment, respectively [31]. A better understanding of TB treatment outcome and its determinants is needed to reduce poor outcomes. However, there are few studies from Abuja, Nigeria, comparing TB treatment outcome and its associated factors, between HIV-positive and HIV-negative patients.

In addition to TB, cardiovascular diseases are emerging as the leading cause of death among patients with HIV [32]. Although, increasing access to ART has improved their life expectancy and quality of life, these populations are now at risk of age-related non-communicable diseases, such as hypertension and diabetes [33,34 35]. HIV-infected patients in Nigeria may have metabolic complications of ART [36,37], which are coupled with a high prevalence of other traditional risk factors [38,39]. As hypertension is the most important risk factor for CVD, we conducted a survey to explore whether this is also a major health problem among patients with HIV in Nigeria.

1.2 Aim and scope of the study

This study assessed the performance and effectiveness of the current WHO symptom screen algorithm for TB diagnosis among HIV patients, evaluated the performance of two non-sputum-based biomarkers for TB screening, and determined TB treatment outcome and burden of hypertension among PLHIV, with a view to improving diagnosis and management of TB and hypertension among HIV patients, thereby preventing avoidable deaths in this population.

1.3 Main Objectives

(1) To assess the predictive value of the WHO symptom screening algorithm to exclude TB among people living with HIV (PLHIV)
(2) To determine the performance of CRP and IP-10 as screening tools for active TB
(3) To retrospectively determine the treatment outcome of patients with TB with and without HIV.
(4) To retrospectively determine the prevalence of hypertension among new patients with HIV at the time of enrolment.
(5) To retrospectively determine the incidence of hypertension among the same patients one year after initiation of ART.

1.4 Thesis structure

Each chapter focuses on one main objective and each chapter has a brief introduction, a literature review, methodology, results and discussion sections.

Chapter one gives an overall introduction to the thesis and presents the aim and objectives.

Chapter two evaluates the WHO algorithm for TB symptom screening among HIV patients.

Chapter three, evaluates the performance of CRP and IP-10 as screening tools for active TB.

Chapter four presents a retrospective study on TB treatment outcome and its determinants for treatment success, and lost to follow up among HIV-negative and HIV-positive patients.

Chapter five presents a retrospective study on the prevalence and incidence of hypertension among HIV patients.

Chapter six presents the general discussion/conclusion, which integrates all the components of this thesis

Chapter 2

Performance of the WHO symptom screening algorithm for TB diagnosis among HIV patients

2.1 Introduction

TB is preventable and curable; however, it is still the commonest cause of death among HIV patients. To reduce the catastrophic impact of TB/HIV-coinfection in high burden countries, the WHO TB symptom screening algorithm was developed to ensure early case detection and prompt treatment and prevention of TB among HIV patients. No published study has however evaluated the algorithm in Nigeria or West Africa.

TB case detection is low in Nigeria, according to the 2015 global TB report, Nigeria contributed 15% of the 3 million cases of TB that were not reported to WHO in 2013 [40] and close to half of the unreported TB cases in 2015, were in either Indonesia or Nigeria [41]. Since the symptom screening aims to improve TB case detection and prevent active TB, there is a need to evaluate the screening algorithm in the Nigeria.

This cross-sectional study consecutively recruited newly diagnosed HIV-positive patients, screened them for TB symptoms, and performed sputum smear microscopy and culture on all the patients, irrespective of the symptom screening outcome, with a view to assessing the performance of the algorithm.

2.2 Literature review

HIV is the strongest risk factor for the development of TB [41,42]. The risk of developing active TB among PLHIV is 17 – 22 times more than that of individuals not infected with HIV [43]. About one-third of the world population is infected with latent TB infection, but with competent host immunity, only very few of these will develop to active TB in their life time [44-46]. The risk of developing TB disease after exposure to MTB is about 10% for life-time for people without HIV and half of the risk will occur within the first two years after being infected [44,47]. Conversely, in PLHIV, this risk is increased to 10% per annum in the early and to 30% in the late stages of HIV infection [47]. HIV co-infection increases the risk of reactivation of latent TB, susceptibility to primary infection and re-infection after exposure to MTB [48-51]. Unlike other opportunistic infections, TB can occur at any stage of HIV, though its risk increases with the level of immunosuppression [47]. HIV also accelerates the rate of progression of TB disease [47]. Therefore, HIV patients are at higher risk of developing and dying from the disease compared to individuals who are not infected with HIV. Furthermore, TB and HIV co-infection is a great threat against the survival of PLHIV, being the most important opportunistic infection and cause of death among them [12,43,52-54]. It is estimated that 1.2 million PLHIV developed TB in 2015 worldwide [41,55,56]. In the same year, about one-third (390,000) of 1.2 million deaths occurring among HIV patients across the globe, were associated with TB and 75% of these occurred in sub-Saharan Africa [41,43]. Ironically, Africa, which has between 12% and 15% of the world population [57], bears the majority of the global TB/HIV burden [40]. According to the 2016 global TB report, the region has 22 of the 30 high TB/HIV burden countries of the world and the highest average TB/HIV co-infection rates [41]. Studies across sub-Saharan Africa showed that the prevalence of TB among HIV patients ranges from 8% in Botswana to 35% in South Africa [58].

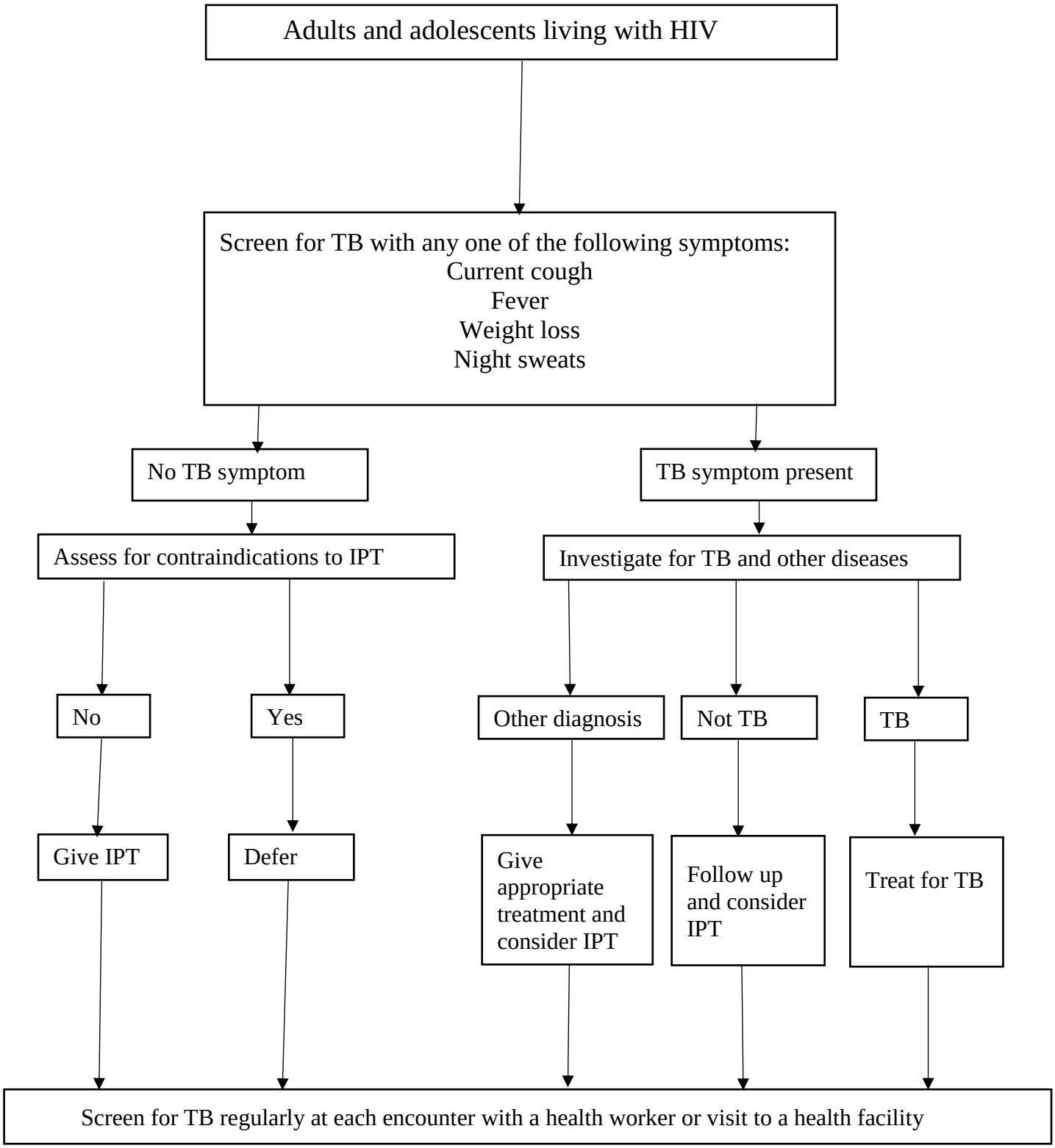
Nigeria, the most populous country in Africa, has the second largest number of PLHIV in the world and ranks 4th among the 30 high burden TB countries [3,40,59] and an average of 22% of TB patients are co-infected with HIV [60]. There are no national data on the prevalence of TB among HIV patients. However, four studies using smear microscopy reported that HIV/TB-coinfection ranged from 10.5% to 56.9% [61-64]. The huge variation in the prevalence may be due to disparity in the studies' methods of data collection and their target populations. For instance, a study of 1320 patients in an HIV clinic in Kano, in the North-west zone of the country, reported a TB prevalence of 10.5% [62], while a further study of 940 HIV patients with TB symptoms conducted at two ART clinics: one in Jos and the other in Lagos (in the North-central and South-west zones of the country, respectively) had a TB prevalence of 56.9% [61]. A third study of 200 HIV-infected patients with TB symptoms in Ile-Ife (South-west zone) found TB in 34.5% [64], while a fourth study of 86 HIV patients with TB symptoms in Lafia, in the North-central zone of the country, found a TB prevalence of 13.8% [63].

The main obstacles against adequate control of TB among HIV patients globally, particularly in sub-Saharan Africa, are the lack of well-equipped laboratory facilities [65-69] and the unusual clinical presentation of TB [70] . The clinical presentation of TB is altered in HIV patients. For instance, HIV infected patients may present with non-specific symptoms, which overlap with other opportunistic infections or patients may not have symptom at all and their sputum is more likely to be smear-negative [6,15,68]. Also, Chest X-ray findings are non-specific or normal in most cases. Because of this, conventional TB diagnostics such as smear microscopy and radiography perform poorly among HIV patients and the sensitivity of sputum smear microscopy may be as low as 9%-23% [66,68,71,72]. Therefore, the difficulty of TB diagnosis in sub-Saharan Africa is associated with a high mortality and morbidity among HIV patients.

To reduce the burden of TB among PLHIV, WHO recommends four strategies, which include antiretroviral therapy (ART) and the 3'I's (Intensified case-finding (ICF), Isoniazid preventive therapy (IPT) and Infection control for TB) [73-75]. ICF involves the systematic screening of all HIV patients for symptoms of TB and is the entry point to IPT. Patients without symptoms are eligible for IPT and symptomatic individuals can be investigated further, diagnosed and treated early [76]. Initially, chronic cough (> 2weeks) was the only symptom for screening [14,16]. However, this led to poor sensitivity as more than half of bacteriologically-confirmed cases were missed [14,20,77,78]. A meta-analysis of 12 observational studies from resource-constrained countries [79] reported that presence of any one of four symptoms (current cough, weight loss, fever and night sweats) performed better in determining whether PLHIV were likely to have TB, while the absence of symptoms had a high negative predictive value. WHO included this algorithm in its 2011 **g**uidelines for ICF and IPT, and recommended its use at the time of initial presentation for HIV care [76] (figure 2.1).

Some changes have been made to the 2011 WHO algorithm. In 2013, it was recommended that Xpert MTB/RIF should be the primary TB diagnostic test, instead of sputum smear microscopy [80]. Patients with negative Xpert MTB/RIF should be given IPT and should continue to be screened for TB symptoms at every clinic visit. All patients with positive Xpert MTB/RIF but no Rifampicin resistance, should be treated for TB and those with RIF positive results should undergo further drug susceptibility testing (DST). In 2016, WHO also indicated a repeat Xpert MTB/RIF, chest X-rays and a workup for diagnosing extra-pulmonary TB, should be conducted among Xpert/MTB negative patients[81].

Figure 2.1. WHO TB symptom screening algorithm for HIV-positive adults and adolescents, 2011 [76]



Source: WHO. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource- constrained settings

The sensitivity and specificity of the 2011 WHO TB symptoms screening is said to be 79% and 50% respectively, and when combined with chest X-ray, the sensitivity increases to 91% but specificity reduces to 39%. Most importantly, the absence of symptoms has a high negative predictive value of 97.7%[79] in a population with a TB prevalence of 5%. High negative predictive values are needed as a screening test to rule out a disease. A test's negative predictive value is affected by disease prevalence. The higher the disease prevalence, the lower the negative predictive value of a test. For instance, if the symptom screening were used among HIV patients with 10%, 20% and 30% TB prevalence, the negative predictive values would be 95.5%, 90.5% and 84.7% respectively. Which means that 4.5%, 9.5% and 15.3% of patients without any of the TB symptoms, will have TB and could be missed. Because the prevalence of TB among HIV patients in most of the high TB burden countries is above 5% [41,58], the performance of the symptom screening may be different from what was estimated in the initial meta-analysis.

To assess the performance of the WHO algorithm, a study needs to meet the criteria used in the original meta-analysis [79]. The criteria are: availability of data on the four symptoms, collection of sputum samples irrespective of signs and symptoms, and culturing at least one sputum sample for TB diagnosis for each participant.

Nine studies that meet these criteria have been published after the meta-analysis, but only seven were purposely designed to assess the symptom screening's performance [82-90]. Of these , 6 were conducted in South Africa [83-87,89], one in Ethiopia [82], one in Kenya [90] and one was a multi-national study from South Africa, Botswana, Malawi and Zimbabwe [88]. Seven studies were conducted among patients in ART clinics (of which, 4 were on ART-naïve patients only [72,87,88,90] and 3 on both ART-naïve and on ART patients) [85,89,90], one took place among pregnant women in antenatal clinics [84], and one on newly admitted HIV-positive patients on medical wards [86]. The symptom screening positivity rate, TB prevalence and performance of

the studies varied, as shown in summary table 2.1. The proportion of participants with positive symptom ranged from 6.6% to 91.1%, with the largest proportion of patients with symptoms (91.1%) found among patients on the medical wards [86]. Three of the four studies that recruited ART-naïve patients reported that between 70.1% to 80.2% of the patients had TB symptoms [82,87,88] and one study found symptoms in 53.2% of participants [90]. TB symptoms were present in 25.6%, 58.1% and 61.8% of pregnant, non-pregnant women and men, respectively [90]. Two of the 3 studies that included patients with or without ART, found a higher proportion of symptoms among ART-naïve patients [83,85] but the third study found little differences (88% and 82.7%, respectively) [89]. A higher proportion of positive TB symptoms screening was found among those not on ART.

In summary, almost all acutely ill patients admitted to medical wards and more than two-thirds of ART-naïve patients attending HIV clinics had at least one symptom. However, the prevalence of symptoms was low among those on ART and pregnant women attending ANC and lowest among participants on ART and those previously screened or treated for TB.

The prevalence of PTB in the nine studies ranged from 2.5 % to 32.6%. The prevalence was 2.5%, 4.1%-5.9%, 8.4%-17.3% and 32.6% among pregnant women, patients on ART, patients not on ART and patients in the medical wards, respectively. In a study conducted among patients on ART, TB was found in 11.2% participants. However, when participants were stratified into pregnant, non-pregnant women, and men, the prevalence was 5.9%, 11.5% and 13.6%, respectively [90].

Table 2.1. Summary of findings from studies that assessed the 2011 WHO's TB symptom screening algorithm

Author/Date	Country	Sample		Positive symptom screening	TB prevalence	Sensitivity	Specificity	PPV	NPV
Ahmad Khan /2014 [85]	South Africa	737	215 (Pre ART)	71.2%	15.8%	91.2%	32.6%	20.3%	95.2%
			522 (On ART)	44.6%	5.9%	51.6%	55.8%	6.8%	94.8%
Balcha 2014 [82]	Ethiopia	784		80%	16.9%	92.6%	23%	20%	94%
Hoffmann 2013 [84]	South Africa	1368		16%	2.5%	28%	84%	4.4%	98%
Kufa 2012 [89]	South Africa	422		85.6%	6.4%	100%	15.4%	7.5%	100%
Lawn, 2011 [87]	South Africa	468		70.1%	17.3%	84%	22.5%	18.5%	87%
Lawn 2015 [86]	South Africa	427		91.1%	32.6%	96.4%	11.4%	33.7%	86.8%
Rangaka 2012 [83]	South Africa	1429	657(PreART)	23.7%	13%	47.6%	79.8%	25.8%	91.1%
			772 (OnART)	6.6%	5.4%	23.8%	94.4%	19.6%	95.6%
Swindells 2013 [88]	Botswana, Malawi, South Africa, Zimbabwe	445		80.2%	12%	91%	21%	14%	94%
Surbhi Modi 2016 [90]	Kenya	738	738 (All)	53.2%	11.2%	74.1%	49.5%	15.6%	93.8%
			134 (pregnant)	25.6%	5.9%	28.2%	74.6%	5.9%	94.8%
			353 (other women)	58.1%	11.5%	78.3%	44.5%	15.8%	93.9%
			251 (men)	61.8%	13.6%	77.2%	40.6%	16.8%	92.0%

The performance of the WHO symptom screening in ruling out PTB, varies across studies. Of four studies that included ART-naïve patients, two reported the screening performed well [82,88], one reported inadequate performance [87] and one that it performed well among non-pregnant women [90] (sensitivity (78.3%) and negative predictive value (NPV) 93.9%) and men (sensitivity (77.2%) and NPV (92.0%)), but had a poor performance among pregnant women (sensitivity (28.2%) and NPV (94.8%)) [90].

One of the 3 studies that enrolled patients without or with ART reported good performance without stratification [89], one study reported it performed well among ART-naïve, but poorly among patients on ART [85] and one that the screening's performance was poor irrespective of ART status and poorer among patients on ART [83] (See table 2.1). This latter study also reported 52.4% and 76.2% of culture-confirmed TB cases were missed by the screening [83]. Among HIV-positive pregnant women, the sensitivity of symptom screening was very poor (28%)[84] (See table 2.1), as symptoms were absent in 76% of culture confirmed TB cases [84]. Also, symptom screening was not useful among acutely ill patients admitted to medical wards [86].

Some of the studies have reported that the addition of CD4 count, Body mass index (BMI) and Haemoglobin (Hb) improved the performance of the screening and reduced the number of patients that needed further diagnostic work ups [82,83]. However, these findings are not consistent across studies[88].

2.3 Methodology

2.3.1 Study design

This was a cross-sectional study from July 2016 to June 2017, among newly diagnosed HIV-positive patients attending the ART clinic of University of Abuja Teaching Hospital (UATH) in Abuja, Nigeria.

2.3.2 Description of study location

UATH is a 500-bed tertiary health facility of the Federal Government of Nigeria. It is located in Gwagwalada Area Council of the Federal Capital Territory (FCT) and provides specialized services in all disciplines of medicine. The hospital has the largest ART site in the FCT, with more than 15,000 HIV patients enrolled.

This health facility offers HIV Counselling and Testing (HCT) services. Patients attend voluntarily or are referred by other services for testing. New patients diagnosed at the HCT unit or referred from other centres are referred to the ART site for HIV care. The ART site receives referred patients from government, faith-based and private hospitals within FCT and its environs. The services provided include chronic HIV Care; ART adherence preparation, initiation and monitoring; prophylaxis and treatment of opportunistic infections (OIs); TB/HIV co-management; Prevention of Mother-to-Child Transmission (PMTCT); patients' education on positive living; Home-based care, various forms of patients' support, and cervical cancer screening. The ART site has the following units: Monitoring and Evaluation (M&E), Home-based Care and patients' Support, Medical teams, ART Adherence Counselling, Pharmacy, Laboratory, Paediatrics, and Cervical Cancer Screening. Newly diagnosed HIV patients report to the M&E unit, which enrols new patients into the program. Patients are then sent to the Home-based care and support unit, for first-visit counselling, history taking and documentation. Afterwards, patients attend the laboratory for baseline investigations, which include CD4 count, full blood count (FBC), renal (RFT) and liver

function tests (LFT) and Hepatitis B Virus (HBV) screening. Each newly enrolled patient is allocated to one of the medical teams, which comprises a doctor, a nurse and an ART Adherence counsellor. New patients are initially seen by the doctor, who takes a medical history, physical examination, clinical HIV staging and treatment of OI. Patients with symptoms of TB undergo chest X-rays and are referred to the Directly Observed Therapy Short-course (DOTS) unit, for sputum smear microscopy. Seriously ill patients are admitted to the medical wards. Patients with OI are sent to the Adherence Counsellors for counselling on the drug(s), HIV/AIDS and positive living, and to the pharmacy to collect drugs. Drugs for OI are provided for one week and patients are asked to return to collect the baseline investigations' results and for assessment of ART eligibility. Patients co-infected with TB commence TB treatment and two weeks later, if there are no problems, start ART that does not interact with TB medications. Patients without TB symptoms eligible for ART, receive ART adherence preparation and commence Co-trimoxazole Prophylaxis Therapy (CPT). Patients are given a two-week appointment on CPT alone. At the end of the two weeks, patients are assessed regarding CPT adherence and their understanding of ART adherence. Misunderstandings and problems with adherence are addressed during this visit. If the patient's adherence preparation is satisfactory, ART is initiated, and CPT is continued. Patients not eligible for ART are followed using CD4 counts and clinical staging every three months.

2.3.3 Sample size calculations

This study is like a prevalence study, the formula for the minimum acceptable sample size

(N) = Z^2 pq/(d^2)

Where, p = prevalence of disease among study population

q = (1-p)

d = precision (0.05)

Z = Z score at 95% confidence interval (1.96)

There is no study from Nigeria or West Africa that has evaluated the WHO TB symptom screening algorithm for TB diagnosis among HIV patients. The prevalence of TB obtained from studies elsewhere among newly diagnosed/pre-ART HIV patients, ranged from 8.4% to 17.3%. Since 17.3% will give the largest sample size, we decided to use it for sample size calculations. Using the above formula, the minimum acceptable sample size (N) = 1.96^2 X 0.173 X 0.827/ $(0.05)^2$ = 220. To make room for attrition during data collection, 10% of the sample size (22) was added, making the sample size 242, which was rounded up to 250. This ideal sample size however was not achieved because of prolonged healthcare workers strikes during data collection, coupled with the decentralization of HIV care which reduced the number of patients at the study's site. We could only recruit 202 newly diagnosed HIV patients over a period of 12 months. All patients that met the study's selection criteria, gave consent to participate and could produce sputum (either on the spot or after given a cup to try to produce early morning sputum at home and bring it to the clinic) were recruited.

2.3.4 Inclusion criteria

(1) Confirmed HIV positive status

(2) Age of 18 years and above

(3) Newly diagnosed

(4) Consent to participate in the study

(5) Enrolled at the ART site

2.3.5 Exclusion criteria

(1) Patients on temporary enrolment (because, these patients were usually passer-by/visitors who fell sick during their visit to Abuja and were diagnosed to be HIV-positive).
(2) Patients already diagnosed to have TB.
(3) Patients unable to provide informed consent and did not have legally assigned representatives.
(4) Patients with a high risk of imminent death (these are patients with severe respiratory distress, loss of consciousness, un-recordable blood pressure or any other sign of life threatening conditions).

2.3.6 Participant selection

Participants were identified through the ART site's M&E unit. All newly diagnosed HIV patients > 18 years enrolled at the ART site between 21st July 2016 and 15th June 2017, were invited to participate in the study. After obtaining consent, patients were interviewed and screened for TB symptoms (presence and duration of cough, fever, weight loss and night sweats) using a questionnaire (see appendix 6, section 8.6.1). Thereafter, patients were classified as having/not having symptoms of TB. Newly enrolled patients were consecutively recruited. A daily register of all newly diagnosed HIV patients was kept, whether they were recruited or not into the study (to make adjustment during data analysis).

Participants were interviewed using a structured questionnaire to assess their medical history and reasons for attending, ask for the presence of TB symptoms and undertake physical examination. All routine HIV care baseline investigations (CD4 Count, Full Blood Count, and Renal function test) were competed by the site staff.

2.3.7 Sample collection

Patients were asked to attempt producing a sputum sample on the spot. Specimens were examined to ascertain their quality and quantity, and specimens with deficient quality (mainly salivary or containing food particles) or quantity (< 0.5ml) were retaken. As patients without TB symptoms are more likely to produce sputum in the morning, patients unable to produce sputum on the spot, were taught how to produce good quality sputum, given a sputum collection cup, and asked to attempt to produce further sample during consecutive morning and bring the specimens to the clinic.

2.3.8 Laboratory tests

Sputum samples were transported (using a cold chain) to Zankli Medical Centre (ZMC) Laboratory, which is an accredited research laboratory, where it underwent smear microscopy, using a Light-emitting diode fluorescence microscopy **(**LED-FM) and culture. To ensure specimens were cultured within twenty-four hours of collection, a dedicated driver transported specimens to the laboratory, as soon as he was informed. In some instances, sputum samples were kept up to 48 hours in a refrigerator to maintain a cold chain.

Smear microscopy was done by LED-FM with auramine staining at a minimum of 30 low-power fields. After sputum decontamination and digestion with Mycoprep reagent, containing Sodium hydroxide, N-Acetyl-L-Cysteine, and sodium citrate (NaOH-NALC-Citrate), culture was performed using two solid Lowenstein-Jensen (LJ) culture media slopes per patient and monitored for 2 months for evidence of growth. Thereafter, MTB identification was done on specimens with growth, using SD Bioline TB Ag MPT64 Rapid test (SD, Korea). The laboratory staff were blinded to the outcomes of TB symptom screening and other clinical parameters of the participants.

Classification of patients

Patients were classified as *culture-confirmed TB, bacteriologically-confirmed TB* and *TB-negative,* based on the results of smear microscopy and culture.

Culture-confirmed: patients with positive *M. tuberculosis* culture.

Bacteriologically-confirmed: patients with positive culture and/or positive smear microscopy.

TB-negative: patients with negative smear microscopy and negative culture.

Patients deemed TB-negative were provided IPT and ART. Patients with TB were provided TB treatment and initiated ART two weeks later, as per the Nigerian ART guidelines.

Patients were also classified according to the WHO HIV clinical staging (see table 2.2)

Table 2.2. WHO HIV clinical staging of HIV disease in adults and adolescents

Clinical stage 1
Asymptomatic
Persistent generalized lymphadenopathy
Clinical stage 2
Moderate unexplained weight loss (<10% of presumed or measured body weight)
Recurrent respiratory tract infections (sinusitis, tonsillitis, otitis media, pharyngitis)
Herpes zoster
Angular cheilitis
Recurrent oral ulceration
Papular pruritic eruption
Fungal nail infections
Seborrhoeic dermatitis
Clinical stage 3
Unexplained severe weight loss (>10% of presumed or measured body weight)
Unexplained chronic diarrhoea for longer than 1 month
Unexplained persistent fever (intermittent or constant for longer than 1 month)
Persistent oral candidiasis
Oral hairy leukoplakia
Pulmonary tuberculosis
Severe bacterial infections (such as pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia)
Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Unexplained anaemia (<8 g/dl), neutropaenia (<0.5 × 109/L) and/or chronic thrombocytopaenia (<50 × 109/L)
Clinical stage 4
HIV wasting syndrome
Pneumocystis (jirovecii) pneumonia
Recurrent severe bacterial pneumonia
Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month in duration or visceral at any site)
Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs)
Extrapulmonary tuberculosis
Kaposi sarcoma
Cytomegalovirus infection (retinitis or infection of other organs)
Central nervous system toxoplasmosis
HIV encephalopathy
Extrapulmonary cryptococcosis, including meningitis
Disseminated nontuberculous mycobacterial infection
Progressive multifocal leukoencephalopathy
Chronic cryptosporidiosis
Chronic isosporiasis
Disseminated mycosis (extrapulmonary histoplasmosis, coccidioidomycosis)
Lymphoma (cerebral or B-cell non-Hodgkin)
Symptomatic HIV-associated nephropathy or cardiomyopathy
Recurrent septicaemia (including nontyphoidal Salmonella)
Invasive cervical carcinoma
Atypical disseminated leishmaniasis

Source: WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection [91].

2.3.9 Ethical consideration

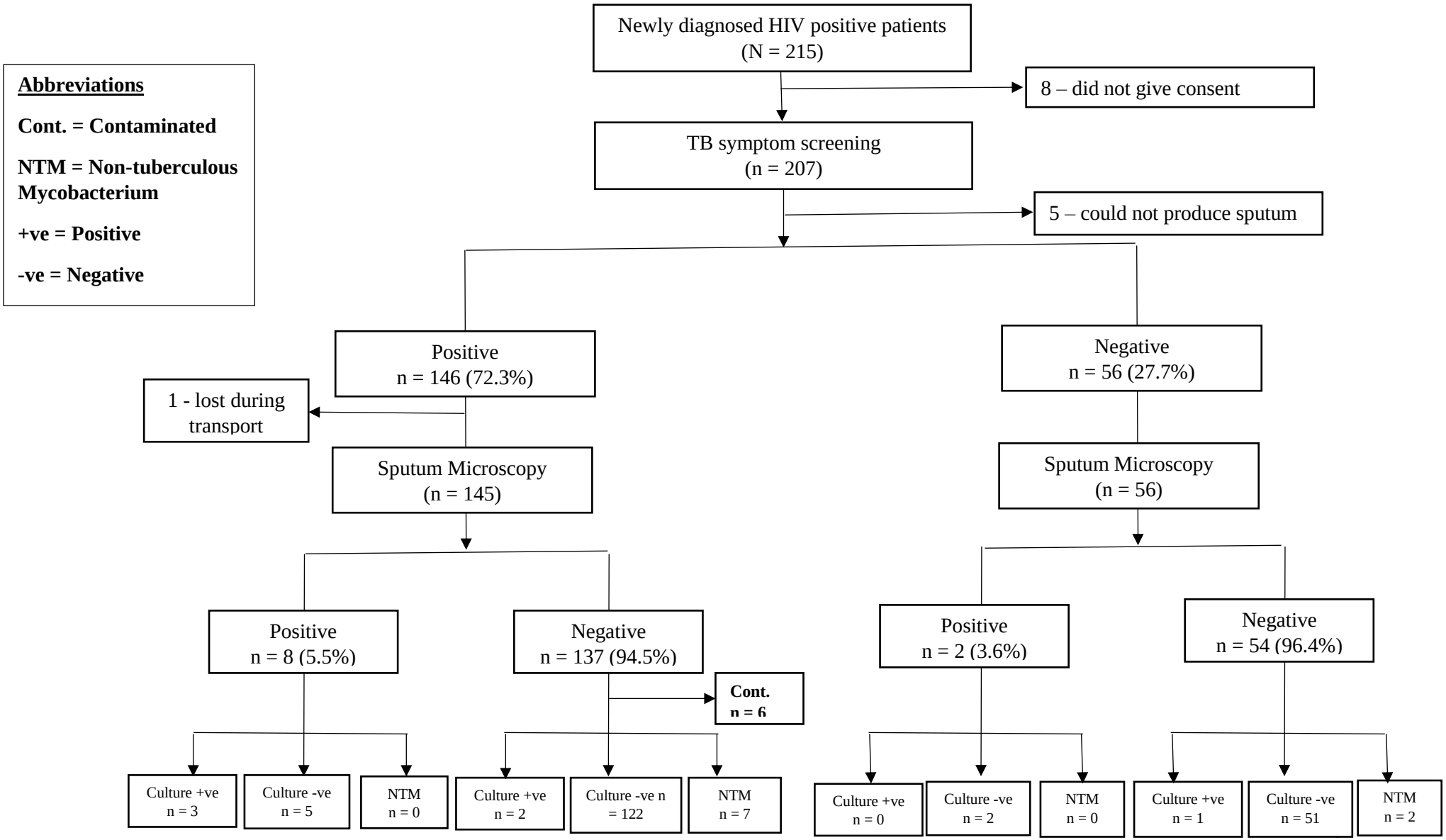
Ethical approval was obtained from the Research Ethics Committees of the Liverpool School of Tropical Medicine (LSTM), University of Abuja Teaching Hospital (UATH) and the Nigeria's Federal Capital Territory (FCT). Written and verbal informed consent were obtained from every participant. For participants that could not read or write, the study patient's information sheet was read in their local language in the presence of an approved representative, who served as a witness and signed the consent form on behalf of the patient. A stamp pad was made available for such patients, for thumb printing. Patients were made

aware their participation was voluntary and that refusing to participate would not affect the quality of treatment. All information collected was kept confidential.

2.3.10 Data analysis

Epi-Info 7 (US Centers for Disease Control and Prevention (CDC)) and the Statistical Package for the Social Science (SPSS) (IBM, version 24) were used for data analysis. Data entry, parts of descriptive statistics and bivariable analysis were conducted in Epi-Info. Data in Epi-Info were exported into SPSS for descriptive statistics, bi- and multivariable analyses. Descriptive statistics included counts, percentages, means and standard deviations (SD), medians and interquartile ranges. The characteristics of patients with and without TB symptoms were compared in bivariate analysis, using Chi-Square and Fisher's Exact tests and "Student t" tests. Mann-Whitney's U-tests were used for non-normally distributed continuous variables. Crude Odds ratios (COR) and 95% Confidence Interval (95% CI)) and mean differences with 95% CI, medians differences (95% CI) and P values were obtained. Variable with $p < 0.2$ were adjusted for age and gender in multivariable analysis, using binary logistic regression. As only 6 participants had positive culture, the performance of the WHO TB screening algorithm was determined in two scenarios: (1) using culture alone and (2) among bacteriologically confirmed TB cases (culture or smear-positive). The sensitivity, specificity, positive predictive value (PPV) and NPV were estimated.

Figure 2.2. Flow chart for the study on evaluation of the WHO algorithm for TB diagnosis among HIV patients



2.4 RESULTS

2.4.1 Demographic, clinical and laboratory characteristics of participants

Two hundred and two patients with a new diagnosis of HIV-infection were screened at the time they attended the clinics for the first time. Eighty (39.6%) participants were male and 122 (60.4%) female (ratio 2: 3). The age ranged from 17 to 75 years, with a mean (SD) of 35.5 (9.7) years and 182 (90.1%) were between 20 and 49 years. The mean (SD) age for males and females were 41 (7.9) and 31.9 (9.0) years, respectively (p = 0.001). Most patients were married/with partner (104, 51.5%) or single (61, 30.2%), as shown in table 2.3. Twenty three (12.1%) patients had no formal education, 78 (41.1%) and 55 (28.9%) had secondary and higher education, respectively. There was a wide range of occupations, with civil servants (33, 16.3%) and traders/business (80, 39.6%) being the most frequent occupations. There was also a large number of ethnic groups with Igbo (39, 19.3%) and Hausa (20, 9.9%) having the highest frequencies. One hundred forty-two (70.3%) participants were Christian and 60 (29.7%) Muslims.

Seventy-four (36.6%) patients had cough, 79 (39.1%) fever, 122 (60.7%) weight loss and 27 (13.4%) night sweats, resulting in 146 (72.3%) having at least one of the four symptoms. The median (IQR) duration of cough, fever, weight loss and night sweats were 3 (1 – 10), 2 (1 – 4), 8 (3.5 – 16) and 3 (1.5 – 6) weeks, respectively. Nine patients (4.5%) had a history of TB treatment and 11 (5.5%) did not know whether they had been treated for TB. The clinical staging of HIV infection classified 123 (61.5%), 33 (16.5%), 42 (21.0%) and 2 (1.0%) patients as stage 1, 2, 3, and 4, respectively.

The mean (SD) BMI, waist and hip circumference and Waist-to-hip ratios were 22.1 (3.8) kg/m^2, 82 (9.0) cms, 91.7 (8.9) cms and 0.90 (0.06), respectively. Hypertension (defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) was present in 23 (12.3%) patients. The median (IQR) CD4 cell count was 197 (101 – 350) cells/mm^3 with a

mean (SD) haemoglobin concentration of 9.7 (1.9) g/dl. Hepatitis B virus co-infection was present in 21 (13.4%) of 157 patients screened.

TB investigations were conducted for 201 participants. These, comprised 145 (99.3%) of the 146 symptomatic patients and all 56 patients without TB symptoms.

Sputum smear microscopy was positive in 10 (5.0%) and negative in 191 (94.6%) patients (one missing). Sputum culture was positive in 6 (3.0%), negative in 180 (89.1%), Non-TB Mycobacterium (NTM) in 9 (4.5%) and contaminated in 6 (3.0%). One culture was missing. If contaminated or missing, results are removed. The proportion of patients with culture-confirmed TB was 6/195 (3.1%). Smear microscopy was positive in seven patients with negative culture. Therefore, if smear or culture positive, results are considered bacteriologically confirmed, and 13/201 (6.5%) had bacteriologically confirmed TB, table 2.5.

Table 2.3. Characteristics of study participants

Characteristics	Category	N = 202*	Missing
Mean (SD) [Range] age (Years)	All	35.5 (9.7) [17.0 – 75.0]	
	Male	41.0 (7.9) [17.0 – 60.0]	
	Female	31.9 (9.0) [18.0 – 75.0]	
Age group	10 – 19	3 (1.5)	
	20 – 29	58 (28.7)	
	30 – 39	74 (36.6)	
	40 – 49	50 (24.8)	
	50 – 59	14 (6.9)	
	≥ 60	3 (1.5)	
Gender	Male	80 (39.6)	
	Female	122 (60.4)	
Marital status	Single	61 (30.2)	
	Married/with partner	104 (51.5)	
	Separated	11 (5.5)	
	Divorced	8 (4.0)	
	Widowed	18 (8.9)	
Educational Level	No formal education	23 (12.1)	12
	Primary	34 (17.9)	
	Secondary	78 (41.1)	
	Higher education	55 (28.9)	
Occupation	Students	11 (5.4)	1
	Unemployed	11 (5.4)	
	Civil Servants	33 (16.3)	
	Artisans	15 (7.4)	
	Business/Trading	80 (39.6)	
	Farming	14 (6.9)	
	House-wife	15 (7.4)	
	Others	22 (10.9)	
Ethnicity	Igbo	39 (19.3)	
	Hausa	20 (9.9)	
	Yoruba	14 (6.9)	
	Gbagyi	13 (6.4)	
	Tiv	12 (5.9)	
	Ebira	7 (3.5)	
	Igala	7 (3.5)	
	Fulani	6 (3.0)	
	Others	81 (40.1)	

***** Number and percentage unless specified

Table 2.4. Clinical characteristics and medical history of participants

Characteristics	Category	N = 202*	Missing	**Duration,** Median (IQR)	Missing
TB-related Symptoms	Cough	74 (36.6)		3 (1 – 10)	3
	Fever	79 (39.1)		2 (1 – 4)	7
	Weight loss	122 (60.7)	1	8 (3.5 – 16)	5
	Night sweats	27 (13.4)		3 (1.5 – 6)	
	Any of the above 4	146 (72.3)			
Duration of illness (weeks)				6 (2 – 16)	33
Functional Status	Working	168 (84.0)	2		
	Ambulatory	32 (16.0)			
	Bedridden	0 (0.0)			
WHO HIV clinical staging	I	123 (61.5)	2		
	II	33 (16.5)			
	III	42 (21.0)			
	IV	2 (1.0)			
Previous history of TB		9 (4.5)			
BMI (kg/m^2)	Mean (SD)	22.1 (3.8)	25		
Waist circumference (cm)	Mean (SD) [Range]	82.0 (9.0) [65.0 – 109.0]	50		
Hip circumference (cm)	Mean (SD) [Range]	91.7 (8.9) [70.0 – 118.0]	51		
Waist-to-Hip ratio	Mean (SD) [Range]	0.90 (0.06) [0.78 – 1.00]	51		
Systolic Blood Pressure (mmHg)	Mean (SD)	110.8 (15.6) [80 – 170]	15		
Diastolic Blood Pressure (mmHg)	Mean (SD)	71.5 (10.5) [40 – 110]	15		
Hypertension		23 (12.3)	15		
CD4 Cells count (Cells/mm^3)	Median (IQR)	197 (101 – 350)	1		
CD4 count ranges (Cells/mm^3)	< 100	50 (24.9)	1		
	100 – 199	52 (25.9)			
	200 – 350	49 (24.4)			
	> 350	50 (24.9)			
Haemoglobin (g/dl)	Mean (SD)	9.7 (1.9)	54		
White Blood Cells X 10^9/L	Median (IQR)	4.6 (4.1 – 6.1)	54		
Platelets count X 10^9/L	Median (IQR)	237.5 (180 – 277)	54		
Hepatitis B Virus screening	Positive	21 (13.4)	45		

***** Number and percentage unless specified**.**

Table 2.5. Participants' smear microscopy and culture results

Characteristics	Category	N = 202*	Missing
Smear microscopy	Negative	191 (94.6)	
	Scanty	9 (4.5)	
	+	0 (0.0)	
	++	0 (0.0)	
	+++	1 (0.5)	
	Not done	1 (0.5)	
Culture	Negative	180 (89.1)	
	NTM	9 (4.5)	
	MTB	6 (3.0)	
	Contaminated	6 (3.0)	
	Missing	1 (0.5)	
Smear or Culture positive		13 (6.5)	1

***** Number and percentage unless specified

2.4.2 Participants' characteristics by WHO symptom screening

Patients with symptoms (positive WHO screening) had similar age to patients without symptoms (36.2 (10.2) versus 33.7 (7.8), p = 0.063) and there were no differences in terms of gender, marital status, education, ethnicity, and religion (see table 2.6). Similarly, there were no differences in the median (IQR) CD4 cells counts and the proportion of participants with hepatitis B.

Patients with symptoms were more likely to have illnesses that hindered their daily activities (p = 0.001), to be at AIDS stage 2 (p = 0.025), 3 and 4 (p = 0.001), and to have lower blood pressure than patients without symptoms, table 2.7. The mean (SD) BMI for patients with symptom was lower than for patients without symptoms (21.8 (3.6) versus 23.3 (4.1), p = 0.021). Patients with symptoms had lower mean (SD) haemoglobin (9.4 (1.8) versus 10.3 (1.9), p = 0.005) and higher median (IQR) white blood cells (5.0 (4.1 – 6.7) versus 4.1 (3.8 – 4.9), p = 0.001) than patients without symptoms.

2.4.3 Multivariable analysis of the characteristics of participants with positive WHO TB symptom screening

Variables with p values < 0.2 in bivariable analysis were adjusted for age and gender. After the adjustment, the variables that remained statistically significant were the functional status, the WHO clinical staging, the systolic and diastolic blood pressures, BMI, haemoglobin concentration and white blood cell count (see table 2.8).

Patients with TB symptoms were less likely to carry out daily activities (p = 0.001), more likely to be in WHO clinical stages 2 (AOR (95 CI) = 2.8 (1.1 – 7.2)) or 3 and 4 (14.1 (3.2 – 62.4)) and to have lower systolic (0.97 (0.94 – 0.99)) and diastolic (0.93 (0.90 – 0.97)) blood pressure; BMI (0.88 (0.81 – 0.96)), and haemoglobin (0.7 (0.6 – 0.9)). Conversely, patients with TB symptoms had higher white blood cells count (1.5 (1.2 – 2.0), p = 0.003).

Table 2.6. Participants' characteristics stratified by WHO symptom screening outcome

Characteristics	Category	WHO symptom screening		OR (95% CI)	P value
		Positive N = 146 (%)	Negative N = 56 (%)		
Mean (SD) Age (years)	**A**ll	36.2 (10.2)	33.7 (7.8)	2.5 (1.3 – -0.1)*	0.063
	Male	41.7 (7.9)	38.8 (7.8)	2.9 (-1.1 – 7.0)*	0.152
	Female	32.3 (9.9)	30.8 (6.4)	1.5 (-2.0 – 5.0)*	0.402
Age Group	10 – 19	3 (2.1)	0 (0.0)		0.509
	20 – 29	38 (26.0)	20 (35.7)		
	30 – 39	52 (35.6)	22 (39.3)		
	40 – 49	38 (26.0)	12 (21.4)		
	50 – 59	12 (8.2)	2 (3.6)		
	≥ 60	3 (2.1)	0 (0.0)		
Gender	Male	60 (41.1)	20 (35.7)	1.3 (0.7 – 2.4)	0.484
	Female	86 (58.9)	36 (64.3)		
Marital status	Single	44 (30.1)	17 (34.0)	1	
	Married	71 (48.6)	33 (58.9)	0.8 (0.4 – 1.7)	0.603
	Separated	8 (5.5)	3 (5.4)	1.0 (0.2 – 4.3)	0.968
	Divorced	7 (4.8)	1 (1.8)	2.7 (0.3 – 23.7)	0.369
	Widowed	16 (11.0)	2 (3.6)	3.1 (0.6 – 14.9)	0.160
Educational Level	None	17 (12.4)	6 (11.3)	1	
	Primary	29 (21.2)	5 (9.4)	2.1 (0.5 – 7.7)	0.291
	Secondary	53 (38.7)	25 (47.2)	0.7 (0.3 – 2.1)	0.587
	Higher Ed.	38 (27.7)	17 (32.1)	0.8 (0.3 – 2.4)	0.671
Ethnicity	Hausa	16 (11.0)	4 (7.1)	1.9 (0.6 – 5.9)	0.291
	Igbo	32 (21.9)	7 (12.5)	2.1 (0.9 – 5.2)	0.099

	Yoruba	10 (6.8)	4 (7.1)	1.2 (0.3 – 3.9)	0.806
	Others	88 (60.3)	41 (73.2)	1	
Religion	Christianity	100 (68.5)	42 (75.0)	0.7 (0.4 – 1.5)	0.365
	Islam	46 (31.5)	14 (25.0)		

*Mean difference (95% CI).

Table 2.7. Clinical characteristics and medical history of participants, stratified by WHO symptom screening outcome

Characteristics	Category	WHO symptom screening		OR (95% CI)	P value
		Positive N = 146 (%)	Negative N = 56 (%)		
Functional Status	Working	112 (77.8)	56 (100.0)	Undefined	0.001
	Ambulatory	32 (22.2)	0 (0.0)		
WHO clinical staging	I	75 (52.1)	48 (85.7)		0.001
	II	27 (18.8)	6 (10.7)		
	III	40 (27.8)	2 (3.6)		
	IV	2 (1.4)	0 (0.0)		
WHO clinical staging	I	75 (52.1)	48 (85.7)	1	
	II	27 (18.8)	6 (10.7)	2.9 (1.1 – 7.5)	0.025
	III/iv	42 (29.2)	2 (3.6)	13.4 (3.1 – 58.1)	0.001
Previous TB	Yes	9 (6.2)	0 (0.0)		0.171
	No	129 (88.4)	53 (94.6)		
	Don't know	8 (5.5)	3 (5.4)		
Systolic BP (mmHg)	Mean (SD)	109.0 (15.3)	115.0 (15.7)	-5.9 (-10.9 – -1.0)*	0.019
Diastolic BP (mmHg)	Diastolic	70.2 (10.4)	75.0 (10.0)	-4.8 (-8.2 – -1.5)*	0.005
BMI (kg/m^2)	Mean (SD)	21.8 (3.6)	23.3 (4.1)	-1.5 (-2.8 – -0.2)*	0.021
BMI Range (kg/m^2)	< 18.0	14 (10.7)	1 (2.2)	4.9 (0.6 – 38.7)	0.131
	18.0 – 24.9	97 (74.0)	34 (73.9)	1	
	25.0 – 29.9	13 (9.9)	7 (15.2)	0.7 (0.2 – 1.8)	0.399
	≥ 30.0	7 (5.3)	4 (8.7)	0.6 (0.2 – 2.2)	0.457
Waist-to-Hip ratio	Mean (SD)	0.90 (0.054)	0.89 (0.047)	0.01 (-0.01 – 0.03)*	0.315
CD4 count (cells/mm^3)	Median (IQR)	193 (89 – 363)	207 (142 – 277)	-11.0 (-59.0 – 45.0)*	0.677
CD4 cell count range	< 100	41 (28.3)	9 (16.1)	1	
	100–199	34 (23.4)	18 (32.1)	0.4 (0.2 – 1.0)	0.061
	200 - 350	30 (20.7)	19 (33.9)	0.3 (0.1 – 0.9)	0.024
	> 350	40 (27.6)	10 (17.9)	0.9 (0.3 – 2.4)	0.799
Haemoglobin (g/dl)	Mean (SD)	9.4 (1.8)	10.3 (1.9)	-0.9 (-1.6 – -0.3)*	0.005
White Blood Cell X 10^9/L	Median (IQR)	5.0 (4.1 – 6.7)	4.1 (3.8 – 4.9)	0.9 (0.3 – 1.5)†	0.001
Platelets X 10^9/L	Median (IQR)	235 (182 – 296)	210 (167 – 259)	25.0 (-3.0 – 51.0)†	0.080
Hepatitis B Virus	Negative	97 (64.6)	39 (69.6)	1	
	Positive	16 (11.0)	5 (8.9)	1.3 (0.4 – 3.8)	0.645

	Unknown	33 (22.6)	12 (21.4)	1.1 (0.5 – 2.4)	0.795
Smear microscopy††	Negative	137 (94.5)	54 (96.4)	0.6 (0.1 – 3.1)	0.569
	Positive	8 (5.5)	2 (3.6)	1	
Sputum Culture (n = 195)**	Negative	127 (87.6)	53 (94.6)	1	
	NTM	7 (4.8)	2 (3.6)	1.5 (0.3 – 7.3)	0.642
	MTB	5 (3.4)	1 (1.8)	2.1 (0.2 – 18.3)	0.498
Smear or Culture positive	Yes	10 (6.9)	3 (5.4)	1.3 (0.3 – 4.9)	0.691
	No	135 (93.1)	53 (94.6)		

*Mean difference (95% CI). †Median difference (IQR). **Five cases with contaminated culture and one case with culture not done, were excluded.
WBC = White blood cell. ††A patient without sputum smear microscopy result was excluded.

Table 2.8. Multivariable analysis of the clinical characteristics and medical history of participants, stratified by WHO symptom screening outcome

Characteristics	Category	WHO symptom screening		OR (95% CI)	P value	AOR (95% CI)	P value
		Positive N = 146 (%)	Negative N = 56 (%)				
Functional Status	Working	112 (77.8)	56 (100.0)	Undefined	0.001	Undefined	0.001
	Ambulatory	32 (22.2)	0 (0.0)	1		1	
WHO clinical staging	I	75 (52.1)	48 (85.7)	1		1	
	II	27 (18.8)	6 (10.7)	2.9 (1.1 – 7.5)	0.025	2.8 (1.1 – 7.2)	0.033
	III/IV	42 (29.2)	2 (3.6)	13.4 (3.1 – 58.1)	0.001	14.1 (3.2 – 62.4)	0.001
Systolic BP (mmHg)	Mean (SD)	109.0 (15.3)	115.0 (15.7)	-5.9 (-10.9 – -1.0)*	0.019	0.97 (0.94 – 0.99)	0.002
Diastolic BP (mmHg)	Diastolic	70.2 (10.4)	75.0 (10.0)	-4.8 (-8.2 – -1.5)*	0.005	0.93 (0.90 – 0.97)	0.001
BMI (kg/m^2)	Mean (SD)	21.8 (3.6)	23.3 (4.1)	-1.5 (-2.8 – -0.2)*	0.021	0.88 (0.81 – 0.96)	0.003
BMI Range (kg/m^2)	< 18.0	14 (10.7)	1 (2.2)	4.9 (0.6 – 38.7)	0.131	4.7 (0.6 – 35.8)	0.136
	18.0 – 24.9	97 (74.0)	34 (73.9)	1		1	
	25.0 – 29.9	13 (9.9)	7 (15.2)	0.7 (0.2 – 1.8)	0.399	0.6 (0.2 – 1.6)	0.275
	≥ 30.0	7 (5.3)	4 (8.7)	0.6 (0.2 – 2.2)	0.457	0.5 (0.1 – 1.6)	0.242
CD4 cell count range	< 100	41 (28.3)	9 (16.1)	1		1	
	100–199	34 (23.4)	18 (32.1)	0.4 (0.2 – 1.0)	0.061	0.5 (0.2 – 1.2)	0.108
	200 - 350	30 (20.7)	19 (33.9)	0.3 (0.1 – 0.9)	0.024	0.4 (0.1 – 1.0)	0.051
	> 350	40 (27.6)	10 (17.9)	0.9 (0.3 – 2.4)	0.799	1.1 (0.4 – 3.2)	0.855
Haemoglobin (g/dl)	Mean (SD)	9.4 (1.8)	10.3 (1.9)	-0.9 (-1.6 – -0.3)*	0.005	0.7 (0.6 – 0.9)	0.005
White Blood Cell X 10^9/L	Median (IQR)	5.0 (4.1 – 6.7)	4.1 (3.8 – 4.9)	0.9 (0.3 – 1.5)†	0.001	1.5 (1.2 – 2.0)	0.003
Platelets X 10^9/L	Median (IQR)	235 (182 – 296)	210 (167 – 259)	25.0 (-3.0 – 51.0)†	0.080	1.0 (0.99 – 1.0)	0.125

*Mean difference (95% CI). †Median difference (IQR). **Five cases with contaminated culture and one case with culture not done, were excluded.
WBC = White blood cell. ††A patient without sputum smear microscopy result was excluded.

2.4.4 Performance of the WHO TB symptom screening algorithm

Table 2.9 shows the performance of the WHO screening algorithm, using culture as the reference standard. One hundred and thirty-nine (71.3%) of 195 patients with culture results had positive symptoms and 56 had no symptoms. Six patients had positive culture and 13 positive culture or smear microscopy. Among the 139 symptomatic participants, 5 (3.6%) had positive MTB culture, 8 (5.8%) had positive smear-microscopy and 10 (7.2%) positive culture or smear-microscopy. Among the 56 asymptomatic participants, 1 (1.8%) had positive culture, 2 (3.6%) had positive smear-microscopy and 3 (5.4%) positive culture or smear-microscopy (p = 0.675), as shown in table 2.9

Table 2.9. Performance of the WHO TB symptom screening algorithm

WHO symptom screening	**Sputum culture**			**Total**
	Positive	Negative		
Positive	5 (3.6%)	134 (96.4%)		139
Negative	1 (1.8%)	55 (98.2%)		56
			P = 0.675	
	Culture/smear microscopy			
	Positive	Negative		
Positive	10 (6.9%)	135 (93.1%)		145
Negative	3 (5.4%)	53 (94.6%)		56
			P = 0.691	

Taking culture as the reference standard, the sensitivity and specificity of the symptom screening score to identify patients with and without TB were 83.3% (95% CI = 35.9 - 99.6) and 29.1% (95% CI = 22.7 - 36.1), respectively. These resulted in a PPV and NPV of 3.6% (95% CI = 1.2 - 8.2) and 98.2% (95% CI = 90.5 – 100), respectively. The sensitivity and specificity were 100% (95% CI = 100- 100) and 26.3% (95% CI= 16.9 – 37.7) for males and 75% (95% CI = 19.4 – 99.4) and 31.0% (95% CI = 22.6 – 40.4) for females (p = 0.608) with PPV and NPV of 3.4% (95% CI = 0.4- 11.9) and 100% (95% CI = 100 – 100) for males and 2.8% (95% CI = 0.1- 14.5) and 96.3% (95% CI = 89.6 – 99.2) for females (p vales = 0.7), (see table 2.10)

Using bacteriologically confirmed TB as positive culture or smear-microscopy, the sensitivity and specificity of the symptom screening score was 76.9% (95% CI = 46.2 – 95.0) and 28.2% (95% CI = 21.9 – 35.2), resulting in a PPV and NPV of 6.9% (95%CI = 3.4 – 12.3) and 94.6% (95% CI = 85.1 – 98.9), respectively. The sensitivity and specificity were 71.4% (95% CI = 29.0 – 96.3) and 24.7% (95% CI = 15.3 – 36.1) for males and 83.3% (95% CI = 35.9 – 99.6) and 30.4% (95% CI = 22.2 – 39.7) for females (p = 0.626 and 0.716), with a PPV and NPV of 8.3% (95% CI = 2.8 – 18.4) and 90.0% (95% CI = 68.3 – 98.8) for males and 5.9% (95% CI = 1.9 – 13.2) and 97.2% (95% CI = 85.5 – 99.9) for females (see table 2.11).

Table 2.10. Performance of WHO symptom screening algorithm using sputum culture as the reference standard

Category	N	WHO symptom screening, n (%)		Culture + (%)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
		Positive	Negative					
All	195*	139 (71.3)	56 (28.7)	6 (3.1)	83.3 (35.9– 99.6)	29.1 (22.7 – 36.1)	3.6 (1.2 – 8.2)	98.2 (90.5 – 100.0)
Male	78	58 (74.4)	20 (25.6)	2 (2.6)	100 (100 – 100)	26.3 (16.9 – 37.7)	3.4 (0.4 – 11.9)	100 (100 – 100)
Female	117	81 (69.2)	36 (30.8)	4 (3.4)	75.0 (19.4 – 99.4)	31.0 (22.6 – 40.4)	2.8 (0.1 – 14.5)	96.3 (89.6 – 99.2)

*Six cases with contaminated culture and one case with missing culture were excluded

Table 2.11. Performance of WHO symptom screening algorithm using bacteriologically confirmed TB diagnosis (smear or culture-positive) as the reference standard

Category	N	WHO symptom screening, n (%)		Bact + (%)	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
		Positive	Negative					
All	201*	145 (72.1)	56 (27.9)	13 (6.5)	76.9 (46.2 – 95.0)	28.2 (21.9 – 35.2)	6.9 (3.4 – 12.3)	94.6 (85.1 – 98.9)
Male	80	60 (75.0)	20 (25.0)	7 (8.8)	71.4 (29.0 -96.3)	24.7 (15.3 – 36.1)	8.3 (2.8 – 18.3)	90.0 (68.3 – 98.8)
Female	121	85 (70.2)	36 (29.8)	6 (5.0)	83.3 (35.9 – 99.6)	30.4 (22.2 – 39.7)	5.9 (1.9 – 13.2)	97.2 (85.5 – 99.9)

*A patient without sputum smear microscopy result was excluded**.** Bact+ = culture or smear positive.

2.5 DISCUSSION

2.5.1 Summary of study findings

This is the first study from Nigeria evaluating the performance of the WHO TB symptom screening algorithm among PLHIV. The study evaluated the algorithm by collecting sputum samples from all newly diagnosed PLHIV, irrespective of TB symptoms, and screened them for TB using sputum smear microscopy and culture to determine the sensitivity, specificity, PPV and NPVs using culture then culture plus smear microscopy as the reference standards.

Seventy two percent of participants had at least one TB symptom. Factors independently associated with the presence of symptoms were a higher HIV/AIDS clinical staging, higher white blood cell counts and lower systolic and diastolic blood pressure, BMI and haemoglobin concentration. Only 3% of participants had culture-confirmed TB, and 6.5% were culture or smear-positive. Using culture, the algorithm had a sensitivity of 83.3%, specificity of 29.1%, with PPV and NPV of 3.6% and 98.2%. The sensitivity, specificity, PPV and NPV for males were 100%, 26.3%, 3.4% and 100% and 75%, 31%, 2.8% and 96.3% for females, respectively. Using culture plus smear microscopy, the algorithm had sensitivity of 76.9%, specificity of 28.2% and PPV and NPV of 6.9% and 94.6%. For males the sensitivity was 71.4%, specificity 24.7%, PPV and NPV of 8.3% and 90%, while females had 83.3% sensitivity, 30.4% specificity and PPV and NPV of 5.9% and 97.2%.

2.5.2 TB symptom screening

The finding that most participants had TB symptoms is consistent with studies from South Africa (70.1% to 88%) [85,87,89], Ethiopia (80%) [82], Nepal (77.3%) [92], Botswana, Malawi and Zimbabwe (80.2%) [88] . A further South African study screening newly diagnosed HIV patients admitted to medical wards reported 91% of patients had symptoms [86]. Some studies have reported lower prevalence of symptoms among HIV patients not on ART from South

Africa (23.7%) [83] and Kenya (53.2%) [90]. In Thailand and Vietnam, studies reported that 48.3% of patients had TB symptoms [93]. In South Africa, 6.6% of participants pre-ART [83] and 44.6% [85] on ART had TB symptoms. Two studies using the algorithm in pregnant HIV-positive patients in South Africa [84] and Kenya [90] and reported that 16% and 26% of the participants had symptoms.

The variation in the proportion of patients with symptoms is likely due to timing when patients present to the services. If patients mostly come when are acutely ill or had been admitted, it is likely hospital-based studies would enrol patients in advanced stages of HIV, when OIs can present with the same symptoms. In contrast, studies with a lower proportion of symptoms may be due to the effect of ART reducing the risk TB and OIs [94-96] [89].

2.5.3 Prevalence of TB among the study population

Only 3% of patients had culture-confirmed TB, which is lower than reported in previous studies, except in one study conducted among HIV-positive pregnant women in South Africa [84]. Four cross-sectional studies screening newly diagnosed/pre-ART HIV-positive patients in South Africa reported that 6.4% to 17.3% had TB [83,85,87,89]. In Ethiopia [82] and Kenya [90], 16.9% and 11.2% of participants were culture positive and in Botswana, Malawi, and Zimbabwe 12% had TB [88]. A systematic review and meta-analysis of studies up to 2011 found the pooled prevalence of TB to be 8.5% [79].

The possible reasons for the lower proportion of culture-confirmed cases in this study are that solid culture has a lower sensitivity [88]. Another possibility is the higher TB prevalence in Southern Africa. Thirdly, our sample size is smaller than previous studies, and this resulted in a low power and wide confidence intervals.

Some patients were smear-positive, but culture negative and the diagnostic yield increased to 6.5%. It is thus highly likely that some cultures may have been false negative. This could be

due to the need to transport sputum, the ZRL moving to new premises at the same time the study took place, and the need to keep sputum in the hospital over weekends. These issues may have compromised the integrity of the sputum, resulting in false negative samples.

2.5.4 Performance of the algorithm

Using culture, the sensitivity and NPV of the screening algorithm were 83.3% and 98.2%, respectively. This is similar to the 78.9% sensitivity and 97.7% NPV reported in the initial WHO systematic review and meta-analysis [79]. The good performance of the symptom screening among our patients may be because our population is similar to the populations included in the meta-analysis [79]. Findings from studies evaluating the performance of the algorithm have been inconsistent within and across different populations. Of the five South African studies among ART-naïve patients [83,85,87-89], three found that symptom screening performed well [85,88,89], but two showed it performed sub-optimally [83,87]. In one of the latter studies, 52.4% of culture-confirmed TB cases among ART-naïve patients did not have TB symptoms [83].

The study's findings have several implications. The algorithm's main target is patients who are yet to start ART. As 72.3% had symptoms and would need further TB diagnostic workups, this may lead to a large laboratory workload in most high TB and HIV burden settings. There is therefore a need for more specific screening methods to reduce the false positive rate among the population.

Second, the heterogeneity of the algorithm's performance, means that its applicability may not be extended across all populations of HIV patients. Its performance is better among patients who have not started ART and have not been screened for TB symptoms. However, its low specificity, makes a large proportion of patients undergo further TB diagnosis, delaying the initiation of IPT. However, as Xpert MTB/RIF is now the recommended primary

screening test, the delay may be reduced and its performance in this population should be evaluated. Alternatively, screening methods that are more sensitive, simple and cheap, will be needed to overcome the challenge posed by the large number of patients for further diagnostic workup.

When bacteriologically confirmed TB was used as the reference standard, the specificity of the screening algorithm dropped from 83.3% to 76.9%. Its NPV also decreased from 98.2% to 94.6%. This means that using this classification could potentially increase the proportion of false negatives and false positives, as LED smear microscopy has a lower specificity than culture and patients may have other Non-MTB mycobacteria.

2.5.5 Limitations and strength of the study

There are many limitations in this study. Firstly, the use of solid culture, which has lower sensitivity than liquid culture, may have missed cases. Though, liquid culture could have higher contamination rate [97-99]. Secondly, because of small number of cultured-confirmed TB cases, we could not determine factors associated with misclassifications. Thirdly, because the research laboratory was far from the hospital, some samples were not processed within 48 hours of collection, which might have affected the integrity of the specimens. This could be a reason for the high proportion of smear-positive but culture-negative TB cases found in this study. Lastly, we only diagnosed PTB, since non-sputum samples were not collected. Hence, we might have underestimated the TB burden among the studied population, since HIV-positive patients are more likely to have EPTB than the general population.

Nevertheless, this study has some strengths. This is the first study from West Africa, which evaluates the algorithm. In addition, the laboratory staff were blinded to the participants' symptom screening outcome and clinical parameters. Culture positive samples were

differentiated from MTBC and NTM (using SD Bioline TB Ag MPT64 rapid test), and 9 cases of NTM were isolated.

2.5.6 Conclusion

More than 70% of participants in this study had at least one TB symptom, using the screening algorithm, and therefore, most patients had to be tested for TB. However, only 3% and 6.5% of the participants had culture or bacteriologically-confirmed TB. The algorithm had sensitivity of 83.3% and NPV of 98.2%. The low specificity of the algorithm requires the majority of patients to undergo further TB diagnostic workup, reducing its benefits to the patient and the health system.

Chapter 3

C-reactive protein (CRP) and Interferon-gamma inducible protein 10 (IP-10) as screening tools for TB

3.1 Introduction

This chapter presents a prospective study that assessed the performance of two non-sputum-based biomarkers (CRP and IP-10) as potential screening tools for TB. Most of the current TB diagnostics are sputum-based. However, due to the unusual clinical presentation of TB among HIV patients, these diagnostics may miss many TB cases and new approaches are needed to improve TB case detection among HIV-infected and uninfected patients.

We recruited consecutive patients with TB symptoms, collected blood to measure the acute marker and tested sputum samples with Xpert MTB/RIF and culture to determine the serum levels of the biomarkers and the ideal cut-offs for ruling in/out TB.

3.2 Literature review

3.2.1 Background

The need for simple and inexpensive TB screening tools in high burden settings has stimulated research efforts towards immunodiagnosis, by looking for biomarkers that may be potential candidates to identify or rule out TB. Biological markers measured in blood or body fluids that accurately detect someone with or without TB, would greatly overcome the difficulty associated with TB diagnosis among HIV patients and be good for diagnosing EPTB or culture-negative TB [67].

Several biomarkers have been assessed as possible TB diagnostics or screening tools [100,101] and two, C-reactive protein (CRP) [102] and Interferon-gamma inducible protein 10 (IP-10) have potential as screening tests.

Most studies of these markers have focused on their performance for TB diagnosis and treatment monitoring [67]. We therefore reviewed the literature to describe their sensitivity and specificity within the context of TB diagnosis, with a view to prospectively evaluating their potential when used alone or in combination for screening purposes. This is a critical issue, because researchers reporting their performance for diagnosis would have selected a cut of point that is close to the upper left corner of a receiver operating curve (ROC). If we repositioned these markers for screening purposes, and positive tests were followed by confirmatory tests, it would be possible to select a marker with a higher sensitivity and lower specificity.

3.2.2 Performance of serum C-reactive protein for TB diagnosis

CRP is a non-specific acute phase protein that is a marker of inflammatory stages. Its level is higher in individuals with active TB than in latent TB infections (LTBI) or healthy controls. CRP could be used as a Point of Care (POC) screening tool with results available within 5 minutes [102,103]. Seven studies (3 from South Africa [104-106], two from South Korea [107,108], one from China [109], and one multi-country study in Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States and Zimbabwe [110] compared serum CRP levels between patients with TB, other respiratory diseases or healthy controls (table 3.1). Two studies in Africa, were conducted among HIV infected patients with symptoms of TB. [104,105] and one in HIV-negative and -positive participants [106].

A CRP cut-off of 8mg/L was a good marker to exclude patients without TB (NPV of 96%) [104] and a rapid CRP quantification method (NycoCard CRP test, Axis-Shield; Oslo, Norway) had similar sensitivity, specificity, PPV/NPV of 95%, 51%, 63% and 93% correspondingly [104]. A second study on PLHIV screened participants before ART initiation [105] and found that patients with TB had median CRP concentrations 9 times higher (57.8mg/L) than patients

without TB (6.4mg/L). A cut off < 1.5mg/L would rule out TB in all individuals without TB (NPV = 100%), while a cut off > 400mg/l would identify all participants with TB (PPV = 100%). However, only 14.3% and 2.0% of the patients screened were included by these two cut-off points and only 12.3% of TB cases would have been included [105]. The best performing cut-off in the study was a CRP < 5mg/L, which had sensitivity, specificity, PPV and NPV of 90%, 44%, 24% and 96% respectively [105].

The study from South Africa recruiting both HIV-negative and -positive patients, reported that a CRP ≤ 5mg/L had good value to rule out TB with sensitivity, specificity, PPV and NPV of 98%, 59%, 74% and 96%, respectively [106].

Two studies in South Korea among HIV-negative military personnel [107] and among participants with unknown HIV status [108] aimed to differentiate TB from non-TB respiratory disease (pneumonia) [107,108]. One study used several cut-off points (< 2.5, < 5.0, < 11.2 and < 15.0 mg/dl) and found that CRP concentrations < 11.2mg/dl had the best performing cut-off with sensitivity, specificity, PPV and NPV of 97%, 41%, 44% and 96%, respectively [107]. The second study aimed to exclude TB and a cut off < 12.5mg/dl had sensitivity, specificity, PPV and NPV of 90%, 59%, 54% and 92%, respectively [108].

In China however, serum CRP was higher in subjects with community-acquired pneumonia than in patients with TB, although the difference was not statistically significant [109]. A cut-off < 15mg/L distinguished patients with TB and those without TB, with a sensitivity and specificity of 82% and 60%, respectively [109]. Lastly, the multi-country study of PLHIV at the start of ART [110] reported that patients with TB were 2 – 3 times more likely to have serum CRP in the highest quartile. However, using a cut-off ≤ 12 mg/dl performed poorly (sensitivity, specificity, PPV and NPV of 44%, 81%, 41% and 83% respectively) to rule out TB [110]. Therefore, most (5/7) studies assessing the performance of serum CRP found that the

marker may be a promising tool to rule out TB. However, its performance was poor/sub-optimal in two studies [109,110].

3.2.3 Performance of IP-10 as screening tool for TB

IP-10 is also a non-specific biomarker and is secreted by monocytes, neutrophils, endothelial cells, and fibroblasts in response to inflammation [111]. Its biological functions include chemotaxis induction, cell growth inhibition, apoptosis, and mobilization of inflammatory cells such as activated T cells, macrophages, and Natural Killer cells to the sites of inflammation [112]. Alterations in IP-10 serum concentrations are high in viral, bacterial, autoimmune, vascular, and neurological diseases.

Although, IP-10 is non-specific, studies have shown that it could be a promising biomarker for diagnosing or excluding TB [113-115], as IP-10-positive cells are present in bronchoalveolar lavage [116] or lymph node aspirates [117]. IP-10 responses have been described in two forms: (a) naturally released in response to TB or LTBI and measured unstimulated in blood or (b) stimulated using TB-specific antigens. High levels of the two forms have been found in urine and blood of patients with TB [118,119]. Stimulated IP-10 requires cell incubation [120] and the process is not feasible for POC assays to rapidly rule out TB in high burden countries. Although stimulated responses are more likely to have higher specificity, this review focuses on the value of unstimulated IP-10.

Table 3.1. Characteristics of the CRP and IP-10 studies reviewed

Author, year	Country	HIV	Study subjects	Method used for TB diagnosis	Controls	Method for analysis
CRP						
Choi, 2007 [107]	South Korea	Neg	46 TB 67 non-TB.	Culture or SM	Non-TB patients: sick adults who were initially suspected of PTB, but who ended up had other disease.	Turbidimetry
Kang, 2009 [108]	South Korea	NK	30 TB 57 non-TB.	Culture or SM Symptoms, X-ray	Non-TB patients: sick adults who were initially suspected of PTB, but who ended up had other disease.	Immuno-turbidimetry
Wilson, 2011 [106]	South Africa	Neg/Pos	135 TB 115 non-TB	Culture or SM Symptoms, X-ray tissue biopsy	Non-TB patients: adults who were initially suspected of active TB, but who ended up had other disease.	Turbidimetry/ spectrophotometry
Lawn, 2013 [103]	South Africa	Pos	81 TB 415 non-TB.	Culture or SM	Non-TB patients: HIV Positive patients without TB.	ELISA
Niu, 2013 [109]	China	Neg	78 TB 113 non-TB	Culture or SM Symptoms, X-ray	Non-TB patients: sick adults who were initially suspected of PTB, but who ended up had other disease.	immune scatter turbidimetry
Drain, 2014 [104]	South Africa	Pos	45 TB 47 non-TB	SM Culture	Non-TB patients: sick adults who were initially suspected of PTB, but who ended up not having TB.	Immunometric semi-quantitative assay/ spectrophotometry
Tenforde, 2015 [110]	9 countries*	Pos	77 TB 255 non-TB	Expert panel culture Broncho alveolar lavage or lung tissue.	Non-TB patients: HIV Pos patients without TB.	ELISA
IP-10						
Hong, 2012 [121]	South Korea	Neg	46 TB 22 LTBI 32 healthy	Culture or SM	LTBI patients: household contacts with a TST Pos who had lived with a confirmed TB.	ELISA

					Health controls: heathy adults with a Neg TST who had not lived with a confirmed TB.	
Shiratori, 2014 [122]	Philippines	Neg	37 TB 30 healthy.	Culture or SM Symptoms, X-ray	Health controls: healthy volunteers with unknown history for other diseases	Luminex xMap
Yang, 2014 [120]	China	NK	123 TB 91 non-TB 33 LTBI 36 healthy	Culture or SM Symptoms, X-ray histology	Non-TB patients: sick adults who were initially suspected of active TB, but who ended up had but other disease. LTBI patients: household contacts with a TST Pos who had lived with a confirmed TB. Health controls: healthy adults	ELISA
Tenforde, 2015 [110]	9 countries*	Pos	77 TB 255 non-TB	Expert panel culture Broncho alveolar lavage or lung tissue.	Non-TB patients: HIV Pos patients without TB	ELISA
Petrone, 2015 [123]	Uganda	Neg/Pos	32 TB 79 non-TB	Culture or SM Symptoms, X-ray tissue	Non-TB patients: sick individuals who were initially suspected of PTB, but who ended up had other disease.	ELISA

* Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States and Zimbabwe.

Table 3.2. Performance of C-reactive protein and IP-10 as screening tools for TB. All studies were case control studies.

Author, Year	Cut-off point	Sensitivity	Specificity	PPV	NPPV
CRP					
Choi, 2007 [107]	11.2mg/dl	97%	41%	44%	96%
Kang, 2009 [108]	15mg/dl	100%	50%	52%	100%
Wilson, 2011 [106]	5mg/L	98%	59%	74%	96%
Lawn, 2013 [103]	5mg/L	90%	44%	24%	96%
Niu, 2013 [109]	15mg/l	82%	60%	59%	83%
Drain, 2014 [104]	8mg/L	97%	54%	74%	96%
	8mg/L	95%	51%	63%	92%
Tenforde, 2015* [110]	12mg/dl	44%	81%	41%	83%
IP-10					
Hong, 2012 [121]	119.5pg/ml	88%	91%	89%	89%
Shiratori, 2014 [122]	348pg/ml	95%	93%	95%	93%
Yang, 2014 [120]	1008pg/ml	88%	92%	97%	67%
Petrone, 2015 [123]	209.1pg/Ml	79%	94%	93%	82%
	209.1pg/Ml	79%	53%	34%	89%
	209.1pg/Ml	100%	17%	46%	100%
Tenforde, 2015* [110]	2922.43pg/ml	51%	71%	34%	83%

* Brazil, Haiti, India, Malawi, Peru, South Africa, Thailand, United States and Zimbabwe

Five studies (China [120], Philippines [122], South Korea [121], Uganda [123], and the same multi-country study described for CRP [110]) assessed the performance of unstimulated IP-10 as a screening tool for TB. All compared IP-10 serum levels between individuals with and without TB (table 3.1). In China, an IP-10 cut-off < 1008pg/ml had sensitivity and specificity of 88% and 92% to differentiate TB and LTBI [120]. In South Korea, patients with TB (HIV status not stated) had higher IP-10 concentration (174.9pg/ml) than patients with LTBI (102.7pg/ml) and those without infection (71.1pg/ml) [121]. A cut-off of 119.5pg/ml had sensitivity of 88% and specificity of 91% for differentiating between TB and LTBI [121].

In the Philippines [122], HIV-negative individuals with TB had a higher mean 1P-10 level (1290pg/ml) than healthy controls (242.6pg/ml). A cut-off of 342pg/ml had sensitivity and specificity of 95% and 93%, respectively [122].

In Uganda, IP-10 did not distinguish children with TB and non-TB respiratory disease [124]. HIV-negative and HIV-positive children with TB were compared to healthy adult donors. A cut-off of 209.1pg/ml had sensitivity, specificity, PPV and NPV of 79%, 93%, 94% and 81.6% respectively. Comparing HIV-negative children with TB and those with non-TB respiratory disease, had; sensitivity, specificity, PPV and NPV of 79%, 53%, 34% and 89% respectively. Comparing HIV-positive children with TB and children with non-TB respiratory diseases, had; sensitivity, specificity, PPV and NPV of 100%, 17%, 46% and 100% respectively.

The multi-country study recruited HIV-positive patients free of TB at the time they started ART and were followed to see if serum IP-10 predicted incident TB. A cut-off < 2922.43pg/dl had a sensitivity, specificity, PPV and NPV of 51%, 71%, 34% and 83% respectively [110]. However, there was no information on wether patients were receiving IPT or not. Higher baseline levels of IP-10 were associated with increased risk of incident TB, but the biomarker performed poorly in ruling out incident TB.

Although, different cut-off points were used throughout the studies, IP-10 levels had sub-optimal performance irrespective of HIV status.

In summary, CRP performed better than unstimulated IP-10 in ruling out TB or discriminating between TB and LTBI or other respiratory disease, though, different cut-off points were used across studies.

3.3 Methodology

3.3.1 Study design

This prospective study was conducted on patients with TB symptoms presenting at Asokoro General Hospital (AGH) and Nyanyan General Hospital (NGH), both in Abuja, Nigeria, between 17th August 2017 and 8th January 2018.

3.3.2 Description of study area/site

Abuja, the Federal Capital Territory (FCT) of Nigeria, is located in the North-central geopolitical zone of the country and is a rapidly growing city, with a population of about 3.6 million (3,564,100). The FCT has three tertiary, 14 secondary and 179 public health facilities, and 673 registered private health facilities across six area councils. HIV prevalence and TB/HIV-coinfection rate in the FCT are 7.5% and 32.3%, respectively.

This study was conducted at the DOTS clinics of the hospitals. AGH is a 160-bedded hospital and serves about 250,000 – 500,000 residents of Abuja. The hospital provides services in all major specialities, and comprehensive TB and HIV care. NGH is a 68-bedded hospital and serves people of lower socioeconomic status than AGH.

All patients attending the hospitals, report to the General Outpatients Department (GOP), where each of them will be seen by a Medical Officer, who will take the clinical history. Patients with symptom suggestive of TB are then referred to the DOTS clinic, for TB screening. At the DOTS clinic, patients receive investigation forms for smear microscopy or Xpert MTB/RIF and chest X-rays. Thereafter, patients go to the laboratory for investigations and collect the investigations' results at the DOTS clinic. Patients with positive TB test are registered for treatment and counselled. The staff of DOTS clinic ask about contacts and arrange for contact tracing.

Patients with drug susceptible TB receive a six-month-regimen, comprising a two-month intensive phase and a continuation phase of four months. The intensive phase consists of fixed dose combination (FDC) of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol. The continuation phase contains FDC of Rifampicin and Isoniazid. Patients with drug-resistant TB are referred for DST and to the drug-resistant TB treatment centres.

3.3.3 Patients' recruitment and data collection

Every consecutive patient with TB symptoms referred to the DOTS clinics was approached to participate in the study. After full informed consent, patients that agreed to participate were recruited by a trained community extension worker (CHEW), using a structured questionnaire (Appendix 6, section 8.6.2). Two sputum samples were collected from each participant to conduct culture and Xpert MTB/RIF. A rapid CRP test was conducted at the DOTS clinic, using the Actim CRP (Medix Biomedica). This is a semiquantitative immunochromatographic dipstick test, that is visually interpreted, and its result can be read within five minutes. The test was carried out by trained CHEWS, on whole blood collected from fingertips, and interpreted according to its manufacturer's instructions.

A 5ml blood sample was collected for serum IP-10 and CRP immunoassays. Specimens' were transported in a cold chain on a daily basis to Zankli Research Laboratory by a dedicated driver.

3.3.4 Inclusion criteria

(1) Age ≥ 18 years

(2) Cough of ≥ 2 weeks

(3) Consented to participate

(4) Agreed to provide the needed specimens' samples (blood and sputum)

3.3.5 Exclusion criteria

(1) Currently on TB treatment

(2) Already diagnosed of TB but has not commenced TB

(3) Patients with language barriers and no appropriate interpreter

(4) Patients willing to participate but could not read/write and no legally assigned representative to sign consent form and act as a witness.

3.3.6 Laboratory analysis of samples

The first sputum sample was tested with Xpert MTB/RIF. The second sputum sample was decontaminated and digested, using sodium hydroxide- N-acetyl-L-cysteine-Citrate solution (Mycoprep reagent), for culture using solid culture in LJ medium. Culture or Xpert-positive samples were classified as TB-positive, while those with both negative culture and Xpert results were classified as TB-negative.

The blood sample collected was analysed for serum IP-10 using an ELISA (R&D systems, Inc., Minneapolis, MN, USA) that uses a quantitative sandwich enzyme immunoassay.

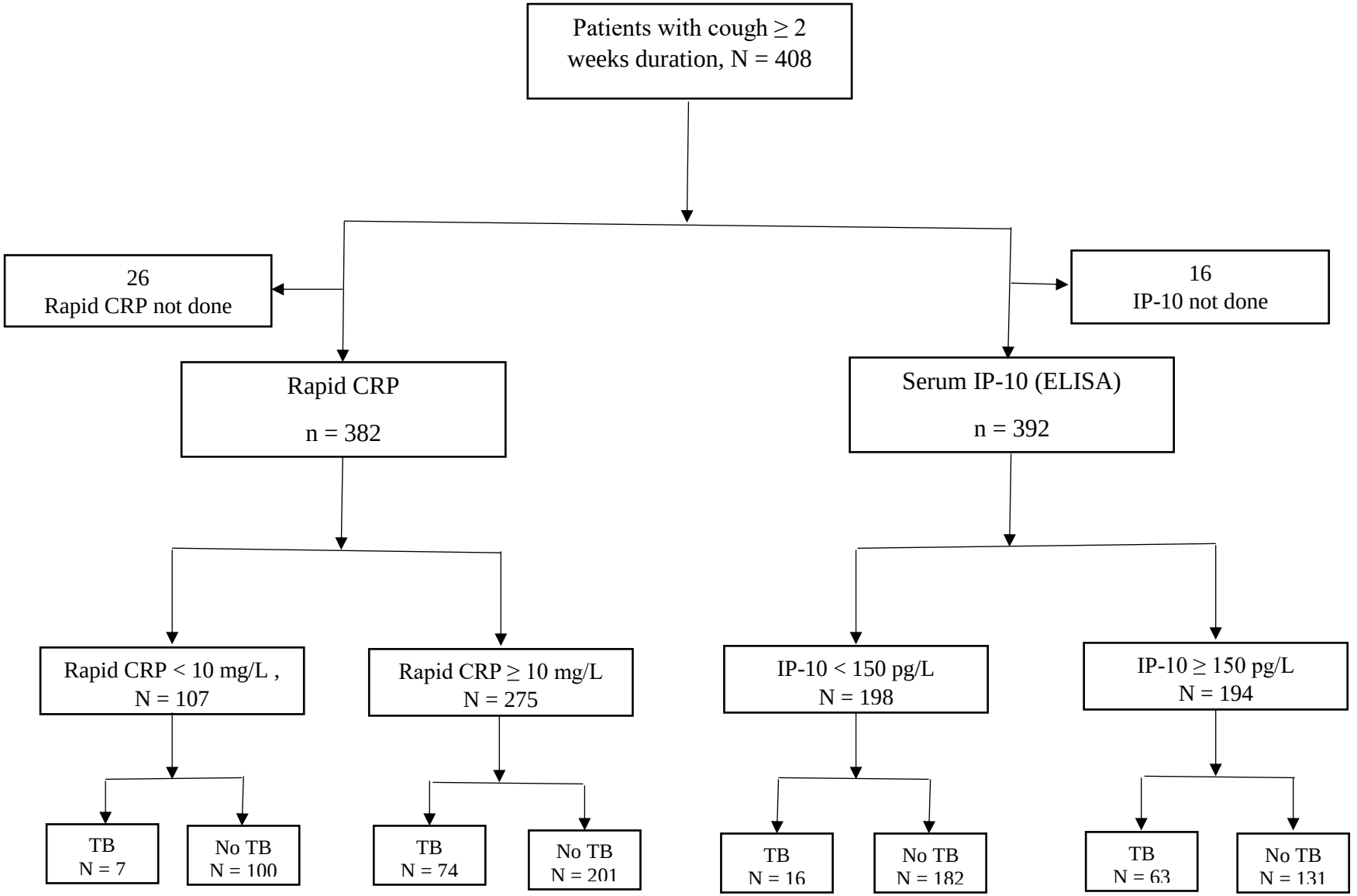
3.3.7 Ethical Considerations

Ethical approvals were obtained from the Research Ethics Committees of LSTM and the Nigeria's FCT. Written informed consent was obtained from all participants.

3.3.8 Data analysis

Data entry was conducted using Epi-Info 7 (CDC) and analysis of the data was performed in SPSS (IBM). Descriptive statistics were run to obtain counts, percentages, medians and interquartile ranges, as appropriate. Thereafter, crosstabulations and ROC curves were prepared to determine sensitivity and specificity of the CRP and IP-10 at various cut-off points and to ascertain the best cut-off point for the biomarkers.

Figure 3.1 The study flow chart



3.4 RESULTS

3.4.1 Participants characteristics and laboratory investigations

A total of 408 patients were recruited and had blood samples collected for CRP and IP-10. One hundred and ninety-four (47.5%) were male and 214 (52.5%) female. Their median (IQR) age was 36 (28.5 – 43.0) years.

All the patients had cough > 2 weeks, with a median (IQR) cough duration of 4 (2 - 6.5) weeks. Other frequent clinical symptoms were fever (271, 66.4%); weight loss (280, 68.6%); night sweats (210, 51.5%); chest pain (256, 62.7%); body weakness (233, 57.1%); loss of appetite (140, 34.3%) and haemoptysis (56, 13.7%). Almost all the patients (388, 95.1%) had HIV testing results and 182 (46.9%) were positive. Sixty (14.7%) participants had been previously treated for TB and were being re-investigated (table 3.3).

Nearly all (396, 97%) participants had sputum culture and 326 (82.3%) were culture-negative, with 62 (15.7%) being culture-positive and 8 (2%) contaminated. In addition, 407 (99.8%) patients had valid GeneXpert results. Of these, MTB was not detected in 329 (80.8%%), 77 (19%) had MTB and 1 (0.2%) an invalid result. Xpert MTB grades were very low in 10 (13%); low in 16 (20.8%); medium in 23 (29.9%) and high in 28 (36.3%). Rifampicin resistance was detected in 7 (9.1%) patients. TB was confirmed by either culture- or Xpert in 86 (21.1%) of the participants.

Rapid CRP test was available for 382 (93.6%) participants. Of these, 107 (28%), 126 (33%), 38 (9.9%) and 111 (29.1%) had serum CRP levels < 10000 ug/l, 10000 – 39999 ug/l, 40000 – 80000 ug/l, and > 80000 ug/l respectively. The median (IQR) IP-10 concentration was 148 (73 – 449) pg/ml (table 3.4).

Table 3.3 Characteristics of participants

Characteristics	Category	N = 408 n (%)	Missing
Age (Years)	Median (IQR)	36.0 (28.5 – 43.0)	
Gender	Male	194 (47.5)	
	Female	214 (52.5)	
Cough	No	0 (0)	
	Yes	408 (100)	
Cough duration (weeks)	Median (IQR)	4.0 (2.0 – 6.5)	
Nose bleeding	No	352 (86.3)	
	Yes	56 (13.7)	
Fever	No	137 (33.6)	
	Yes	271 (66.4)	
Weight loss	No	128 (31.4)	
	Yes	280 (68.6)	
Night sweats	No	198 (48.5)	
	Yes	210 (51.5)	
Chest pain	No	152 (37.3)	
	Yes	256 (62.7)	
Body weakness	No	175 (42.9)	
	Yes	233 (57.1)	
Loss of appetite	No	268 (65.7)	
	Yes	140 (34.3)	
Other illness	No	353 (86.5)	
	Yes	55 (13.5)	
HIV status	Negative	206 (53.1)	20
	Positive	182 (46.9)	
Previous TB treatment	None	348 (85.3)	
	Previous	58 (14.2)	
	Current	2 (0.5)	
If previously treated, treatment class	Relapse	32 (53.3)	
	Retreatment	2 (3.3)	
	Not known	26 (43.3)	
Months since last treatment	Median (IQR)	24.0 (7.0 – 58.0)	

***** Number and percentage unless specified, IQR = Interquartile Range,

Table 3.4. Laboratory results of participants

Characteristics	Category	N = 408 n (%)	Missing
Culture done	Yes	396 (97)	
Culture results	Negative	326 (82.3)	
	Positive	62 (15.7)	
	Contaminated	8 (2.0)	
Xpert	Xpert MTB/RIF	400 (98.0)	
	Ultra Xpert	7 (1.7)	
	Not done	1 (0.2)	
Xpert result	MTB not detected	329 (80.8)	
	MTB detected	77 (19.0)	
	Invalid	1 (0.2)	
Xpert grading	Very low	10 (13.0)	
	Low	16 (20.8)	
	Medium	23 (29.9)	
	High	28 (36.3)	
Xpert RIF resistance	Not detected	70 (90.9)	
	Detected	7 (9.1)	
Confirmed TB	No	322 (78.9)	
	Yes	86 (21.1)	
CRP rapid test done		382 (93.6)	
CRP rapid test result	< 10000 ug/l	107 (28.0)	26
	10000-40000 ug/l	126 (33.0)	
	40000-80000 ug/l	38 (9.9)	
	> 80000 ug/l	111 (29.1)	
IP 10 result (pg/ml)	Median (IQR)	148 (73 – 449)	16

***** Number and percentage unless specified. IQR = Interquartile Range

3.4.2 Performance of CRP and IP-10 as a screening marker for TB

Three cut-off points of CRP (≥ 10 mg/l, ≥ 40 mg/l and > 80 mg/l) were assessed, using sputum culture as the reference standard. CRP ≥ 10 mg/l performed fairly well among the study population but had a poorer performance among HIV-positive patients.

For all participants, a CRP ≥ 10 mg/l had 94.1% (95% CI = 83.0 – 95.6%) sensitivity with 33.2% (95% CI = 27.9 – 38.9%) specificity, PPV of 26.9% (95% CI = 21.8 – 32.6%) and NPV of 93.5% (95% CI = 87.0 – 97.3%).

Among HIV-negative patients, the sensitivity, specificity, PPV and NPV were; 95.3% (95% CI = 84.2 – 99.4%), 42.6% (95% CI = 34.7 – 50.8%), 31.5% (95% CI = 23.7 – 40.3%) and 97.1% (95% CI = 89.8 – 99.6%), respectively.

Among HIV-positive patients, the sensitivity, specificity, PPV and NPV were; 84.8% (95% CI = 68.1 – 94.9%), 22.1% (95% CI = 15.4 – 30.0%), 20.9% (95% CI = 14.4 – 28.8%), and 85.7% (95% CI = 69.7 – 95.2%), respectively.

A CRP cut-off ≥ 40 mg/l reduced the sensitivity but increased the specificity among all patients. The sensitivity and specificity were > 70% and NPV > 90% for the entire study population (sensitivity 72.8% (95% CI = 61.8 – 82.1%), specificity 70.1% (95% CI = 64.6 – 75.2%), PPV 39.6% (95% CI = 31.7 – 47.9%) and NPV 90.6% (95% CI = 86.1 – 94.0%)).

Among HIV-negative patients, cut-off ≥ 40 mg/l had a sensitivity of 76.7% (95% CI = 61.4 – 88.2%), specificity 75.5% (95% CI = 67.9 – 82.0%), PPV 46.5% (95% CI = 34.5 – 58.7%) and NPV 92.1% (95% CI = 86.0 – 96.2%). Among HIV-positive patients, the sensitivity was 66.7% (95% CI = 48.2 – 82.0%), specificity of 64.7% (95% CI = 56.1 – 72.7%), PPV of 31.4% (95% CI = 20.9 – 43.6%) and NPV of 88.9% (95% CI = 81.0 – 94.3%).

A cut-off point of CRP > 80mg/l, had lower sensitivity but higher specificity, as expected, as shown in table 3.5.

The performance of IP-10 at six different cut-off points (> 50 pg/ml, > 100 pg/ml, > 150 pg/ml, > 200 pg/ml, > 250 pg/ml and > 300 pg/ml) is shown in table 3.5.

For the whole study population, an IP-10 cut-off > 100 pg/ml had sensitivity of 87.3% (95% CI = 78.0 – 93.8%), specificity 40.9% (95% CI = 35.4 – 46.6%), PPV 27.2% (95% CI = 21.8 – 33.1%) and NPV 92.8% (95% CI = 87.1 – 96.5%).

The same cut-off point had sensitivity of 87.5% (95% CI = 73.2 – 95.8%), specificity of 50.3% (95% CI = 42.3 – 58.3%), PPV 30.7% (95% CI = 22.4 – 40.0%) and NPV 94.1% (95% CI = 86.8 – 98.1%) among HIV-negative patients.

HIV-positive patients had sensitivity of 85.3% (95% CI = 68.9 – 95.0%), specificity 32.4% (95% CI = 24.8 – 40.8%), PPV 23.2% (95% CI = 16.1 – 31.6%) and NPV of 90.2% (95% CI = 78.6 – 96.7%).

Increasing the IP-10 cut-off to > 150 pg/ml reduced the sensitivity but increased specificity. At this cut-off point, the sensitivity, specificity, PPV and NVP for all participants were 79.7% (95% CI = 69.2 – 88.0%), 58.1% (95% CI = 52.5 – 63.7%), 32.5% (95% CI = 25.9 – 39.6%) and 91.9% (95% CI = 87.2 – 95.3%), respectively.

For HIV-negative patients, IP-10 > 150 pg/ml had sensitivity of 80% (95% CI = 64.4 – 90.9%), specificity 69.2% (95% CI = 61.4 – 76.3%), PPV 39.5% (95% CI = 28.8 – 51.0%), and NPV 93.2% (95% CI = 87.1 – 97.0%).

Among HIV-positive patients, the cut-off had sensitivity of 79.4% (95% CI = 62.1 – 91.3%), specificity 47.2% (95% CI = 38.8 – 55.7%), PPV of 26.5% (95% CI = 18.2 – 36.1%) and NPV of 90.5% (95% CI = 81.5 – 96.1%). As shown in table 3.5, increasing the cut-off point, did not improve the performance of the biomarker.

Table 3.5 Performance of each of CRP and IP-10 as a screening tool for active TB

Population	Test	Cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All	CRP rapid	> 10 mg/L	91.4 (83.0 – 96.5)	33.2 (27.9 – 38.9)	26.9 (21.8 – 32.6)	93.5 (87.0 – 97.3)
		> 40 mg/L	**72.8 (61.8 – 82.1)**	**70.1 (64.6 – 75.2)**	**39.6 (31.7 – 47.9)**	**90.6 (86.1 – 94.0)**
		> 80 mg/L	61.7 (50.3 – 72.3)	79.7 (74.7 – 84.1)	45.0 (35.6 – 54.8)	88.6 (84.2 – 92.1)
	IP 10	> 50 pg/ml	94.9 (87.5 - 98.6)	17.6 (13.5 – 22.2)	22.5 (18.1 – 27.4)	93.2 (83.5 – 98.1)
		> 100 pg/ml	**87.3 (78.0 – 93.8)**	**40.9 (35.4 – 46.6)**	**27.2 (21.8 – 33.1)**	**92.8 (87.1 – 96.5)**
		> 150 pg/ml	79.7 (69.2 – 88.0)	58.1 (52.5 – 63.7)	32.5 (25.9 – 39.6)	91.9 (87.2 – 95.3)
		> 200 pg/ml	75.9 (65.0 – 84.9)	65.5 (59.9 – 70.8)	35.7 (28.5 – 43.5)	91.5 (87.1 – 94.8)
		> 250 pg/ml	69.6 (58.2 – 79.5)	70.6 (65.2 – 75.6)	37.4 (29.6 – 45.8)	90.2 (85.8 – 93.6)
		> 300 pg/ml	62.0 (50.4 – 72.7)	75.7 (70.6 – 80.4)	39.2 (30.6 – 48.3)	88.8 (84.3 – 92.3)
HIV -ve	CRP rapid	**> 10 mg/L**	**95.3 (84.2 – 99.4)**	**42.6 (34.7 – 50.8)**	**31.5 (23.7 – 40.3)**	**97.1 (89.8 – 99.6)**
		> 40 mg/L	76.7 (61.4 – 88.2)	75.5 (67.9 – 82.0)	46.5 (34.5 – 58.7)	92.1 (86.0 – 96.2)
		> 80 mg/L	62.8 (46.7 – 77.0)	81.9 (75.0 – 87.6)	49.1 (35.4 – 62.9)	88.8 (82.5 – 93.5)
	IP 10	> 50 pg/ml	95.0 (83.1 – 99.4)	23.9 (17.5 – 31.3)	23.9 (17.5 – 31.3)	95.0 (83.1 – 99.4)
		> 100 pg/ml	**87.5 (73.2 – 95.8)**	**50.3 (42.3 – 58.3)**	**30.7 (22.4 – 40.0)**	**94.1 (86.8 – 98.1)**
		> 150 pg/ml	80.0 (64.4 – 90.9)	69.2 (61.4 – 76.3)	39.5 (28.8 – 51.0)	93.2 (87.1 – 97.0)
		> 200 pg/ml	75.0 (58.8 – 87.3)	77.4 (70.1 – 83.6)	45.5 (33.1 – 58.2)	92.5 (86.6 – 96.3)
		> 250 pg/ml	67.5 (50.9 – 81.4)	81.1 (74.2 – 86.9)	47.4 (34.0 – 61.0)	90.8 (84.9 – 95.0)
		> 300 pg/ml	57.5 (40.9 – 73.0)	85.5 (79.1 – 90.6)	50.0 (34.9 – 65.1)	88.9 (82.8 – 93.4)
HIV +ve	CRP rapid	> 10 mg/L	84.8 (68.1 – 94.9)	22.1 (15.4 – 30.0)	20.9 (14.4 – 28.8)	85.7 (69.7 – 95.2)
		> 40 mg/L	**66.7 (48.2 – 82.0)**	**64.7 (56.1 – 72.7)**	**31.4 (20.9 – 43.6)**	**88.9 (81.0 – 94.3)**
		> 80 mg/L	57.6 (39.2 – 74.5)	78.7 (70.8 – 85.2)	39.6 (25.8 – 54.7)	88.4 (81.3 – 93.5)
	IP 10	> 50 pg/ml	94.1 (80.3 – 99.3)	11.3 (6.6 – 17.7)	20.3 (14.3 – 27.4)	88.9 (65.3 – 98.6)
		> 100 pg/ml	85.3 (68.9 – 95.0)	32.4 (24.8 – 40.8)	23.2 (16.1 – 31.6)	90.2 (78.6 – 96.7)
		> 150 pg/ml	**79.4 (62.1 – 91.3)**	**47.2 (38.8 – 55.7)**	**26.5 (18.2 – 36.1)**	**90.5 (81.5 – 96.1)**
		> 200 pg/ml	76.5 (58.8 – 89.3)	51.4 (42.9 – 59.9)	27.4 (18.7 – 37.5)	90.1 (81.5 – 95.6)
		> 250 pg/ml	70.6 (52.5 – 84.9)	58.5 (49.9 – 66.7)	28.9 (19.5 – 39.9)	89.2 (81.1 – 94.7)
		> 300 pg/ml	64.7 (46.5 – 80.3)	64.1 (55.6 – 72.0)	30.1 (19.9 – 42.0)	88.3 (80.5 – 93.8)

CRP = C-reactive protein, IP-10 = Interferon Gamma-inducible protein 10, PPV = Positive predictive value, NPV = Negative predictive value, 95% CI = 95% Confidence Interval, HIV -ve = HIV-negative, HIV +ve = HIV-positive

3.4.3 Performance of CRP and IP-10 combined

Table 3.6 shows the performance of the best combinations of CRP and IP-10. At a cut-off point of CRP ≥ 10 mg/l and an IP-10 > 500 pg/ml the sensitivity and specificity were 92.0% (95% CI = 83.4 – 97.0%) and 32.9% (95% CI = 27.5 – 38.6%), respectively.

Among HIV-negative participants, these combinations had a sensitivity of 97.5% (95%CI 86.8 – 99.9) and specificity of 43.0 (35.1 – 51.3), while among HIV-positive participants, sensitivity and specificity were 83.9 (66.3- 94.5%) and 21.1 (14.5 – 29.0), respectively.

A CRP cut-off point ≥ 40 mg/l combined with various IP-10 cut-off points are also shown in table 3.6. None of these combinations resulted in a higher yield of a better accuracy of the tests.

Table 3.6 Performance of CRP and IP-10 as a combined screening tool for active TB

Rapid CRP cut-off	IP-10 cut-off	Sensitivity (95% CI)			Specificity (95% CI)		
		All	HIV -ve	HIV +ve	All	HIV -ve	HIV +ve
> 10 mg/L	> 50 pg/ml	98.7 (92.8 - 100)	100 (91.2 - 100)	96.8 (83.3 – 99.9)	10.6 (7.3 – 14.7)	15.2 (9.9 – 22.0)	5.3 (2.1 – 10.5)
	> 100 pg/ml	97.3 (90.7 – 99.7)	100 (91.2 - 100)	93.5 (786 – 99.2)	20.2 (15.8 – 25.3)	26.5 (19.6 – 34.3)	13.5 (8.2 – 20.5)
	> 150 pg/ml	96.0 (88.8 – 99.2)	100 (91.2 - 100)	90.3 (74.2 – 98.0)	27.4 (22.4 – 32.9)	37.1 (29.4 – 45.3)	16.5 (10.7 – 24.0)
	> 200 pg/ml	93.3 (85.1 – 97.8)	97.5 (86.8 – 99.9)	87.1 (70.2 – 96.4)	27.7 (22.7 – 33.3)	37.7 (30.0 – 46.0)	16.5 (10.7 – 24.0)
	> 250 pg/ml	92.0 (83.4 – 97.0)	97.5 (86.8 – 99.9)	83.9 (66.3 – 94.5)	28.8 (23.6 – 34.3)	39.1 (31.2 – 47.3)	17.3 (11.3 – 24.8)
	> 300 pg/ml	92.0 (83.4 – 97.0)	97.5 (86.8 – 99.9)	83.9 (66.3 – 94.5)	29.8 (24.6 – 35.4)	40.4 (32.5 – 48.7)	18.0 (11.9 – 25.6)
	> 350 pg/ml	92.0 (83.4 – 97.0)	97.5 (86.8 – 99.9)	83.9 (66.3 – 94.5)	30.8 (25.6 – 36.5)	42.4 (34.4 – 50.7)	18.0 (11.9 – 25.6)
	> 400 pg/ml	92.0 (83.4 – 97.0)	97.5 (86.8 – 99.9)	83.9 (66.3 – 94.5)	31.2 (25.9 – 36.8)	42.4 (34.4 – 50.7)	18.0 (11.9 – 25.6)
	> 450 pg/ml	92.0 (83.4 – 97.0)	97.5 (86.8 – 99.9)	83.9 (66.3 – 94.5)	31.2 (25.9 – 36.8)	42.4 (34.4 – 50.7)	18.0 (11.9 – 25.6)
	> 500 pg/ml	**92.0 (83.4 – 97.0)**	**97.5 (86.8 – 99.9)**	**83.9 (66.3 – 94.5)**	**32.9 (27.5 – 38.6)**	**43.0 (35.0 – 51.3)**	**21.1 (14.5 – 29.0)**
> 40 mg/L	> 50 pg/ml	94.7 (86.9 – 98.5)	95.0 (83.1 – 99.4)	93.5 (78.6 – 99.2)	14.7 (10.9 – 19.3)	20.5 (14.4 – 99.4)	8.3 (4.2 – 99.2)
	> 100 pg/ml	90.7 (81.7 – 96.2)	92.5 (79.6 – 98.4)	87.1 (70.2 – 96.4)	32.2 (26.9 – 37.9)	40.4 (32.5 – 48.7)	24.1 (17.1 – 32.2)
	> 150 pg/ml	**88.0 (78.4 – 94.4)**	**92.5 (79.6 – 98.4)**	**80.6 (62.5 – 92.5)**	**46.9 (41.1 – 52.8)**	**56.3 (48.0 – 64.3)**	**37.6 (29.3 – 46.4)**
	> 200 pg/ml	85.3 (75.3 – 92.4)	90.0 (76.3 – 97.2)	77.4 (58.9 – 90.4)	51.0 (45.1 – 56.9)	62.3 (54.0 – 70.0)	39.1 (30.8 – 47.9)
	> 250 pg/ml	82.7 (72.2 – 90.4)	87.5 (73.2 – 95.8)	74.2 (55.4 – 88.1)	53.8 (47.9 – 59.6)	64.9 (56.7 – 72.5)	42.1 (33.6 – 51.0)
	> 300 pg/ml	81.3 (70.7 – 89.4)	85.0 (70.2 – 94.3)	74.2 (55.4 – 88.1)	56.2 (50.3 – 61.9)	66.9 (58.8 – 74.3)	44.4 (35.8 – 53.2)
	> 350 pg/ml	81.3 (70.7 – 89.4)	85.0 (70.2 – 94.3)	74.2 (55.4 – 88.1)	58.6 (52.7 – 64.3)	70.2 (62.2 – 77.4)	45.9 (37.2 – 54.7)
	> 400 pg/ml	81.3 (70.7 – 89.4)	85.0 (70.2 – 94.3)	74.2 (55.4 – 88.1)	59.6 (53.7 – 65.3)	71.5 (63.6 – 78.6)	45.9 (37.2 – 54.7)
	> 450 pg/ml	81.3 (70.7 – 89.4)	85.0 (70.2 – 94.3)	74.2 (55.4 – 88.1)	59.6 (53.7 – 65.3)	71.5 (63.6 – 78.6)	45.9 (37.2 – 54.7)
	> 500 pg/ml	76.0 (64.7- 85.1)	80.0 (64.4 – 90.9)	67.7 (48.6 – 83.3)	68.8 (63.2 – 74.1)	74.2 (66.4 – 80.9)	63.2 (54.4 – 71.4)
> 80 mg/L	> 50 pg/ml	94.7 (86.9 – 98.5)	95.0 (83.1 – 99.4)	93.5 (78.6 – 99.2)	16.4 (12.4 – 98.5)	21.9 (15.5 – 29.3)	10.5 (5.9 – 17.0)
	> 100 pg/ml	89.3 (80.1 – 95.3)	90.0 (76.3 – 97.2)	87.1 (70.2 – 96.4)	36.0 (30.5 – 41.8)	45.0 (36.9 – 53.3)	27.1 (19.7 – 35.5)
	> 150 pg/ml	**85.3 (75.3 – 92.4)**	**87.5 (73.2 – 95.8)**	**80.6 (62.5 – 92.5)**	**51.4 (45.5 – 57.2)**	**60.9 (52.7 – 68.8)**	**42.1 (33.6 – 51.0)**
	> 200 pg/ml	81.3 (70.7 – 89.4)	82.5 (67.2 – 92.7)	77.4 (58.9 – 90.4)	56.5 (50.6 – 62.3)	67.5 (59.5 – 74.9)	45.1 (36.5 – 54.0)
	> 250 pg/ml	77.3 (66.2 – 86.2)	77.5 (61.5 – 89.2)	74.2 (55.4 – 88.1)	60.6 (54.8 – 66.3)	70.9 (62.9 – 78.0)	50.4 (41.6 – 59.2)
	> 300 pg/ml	76.0 (64.7 – 85.1)	75.0 (58.8 – 87.3)	74.2 (55.4 – 88.1)	63.7 (57.9 – 69.2)	72.8 (65.0 – 79.8)	54.1 (45.3 – 62.8)
	> 350 pg/ml	76.0 (64.7 – 85.1)	75.0 (58.8 – 87.3)	74.2 (55.4 – 88.1)	66.1 (60.4 – 71.5)	76.2 (68.6 – 82.7)	55.6 (46.8 – 64.2)
	> 400 pg/ml	76.0 (64.7 – 85.1)	75.0 (58.8 – 87.3)	74.2 (55.4 – 88.1)	67.5 (61.8 – 72.8)	78.1 (70.7 – 84.5)	55.6 (46.8 – 64.2)
	> 450 pg/ml	76.0 (64.7 – 85.1)	75.0 (58.8 – 87.3)	74.2 (55.4 – 88.1)	67.5 (61.8 – 72.8)	78.1 (70.7 – 84.5)	55.6 (46.8 – 64.2)
	> 500 pg/ml	65.3 (53.5 – 76.0)	67.5 (50.9 – 81.4)	58.1 (39.1 – 75.5)	78.8 (73.6 – 83.3)	80.8 (73.6 – 86.7)	77.4 (69.4 – 84.2)

CRP = C-reactive protein, IP-10 = Interferon Gamma-inducible protein 10, PPV = Positive predictive value, NPV = Negative predictive value, 95% CI = 95% Confidence Interval, HIV -ve = HIV-negative, HIV +ve = HIV-positive

Table 3.7 is a hypothetical scenario of the number of cases that would be correctly diagnosed /excluded by the biomarkers at CRP and IP-10 cut offs, when applied to a population of 1000 participants and with a TB prevalence of 21%.

Among HIV-negative patients, a CRP ≥ 10 mg/l (sensitivity 95.3%, specificity 42.6%) would detect 200 out of 210 TB cases. In addition, 453 patients without TB would be CRP-positive and would undergo further TB diagnostic tests. Furthermore, CRP ≥ 10 mg/l and IP-10 > 500 pg/ml would improve the performance, increasing sensitivity from 95.3% to 97.5%, with a stable specificity (42.6% and 43.0%), thereby reducing the number of TB cases missed (from 10 to 5).

Other cut-off points of the biomarkers (either individually or in combination) would result in higher number of TB patients being missed. Also, the table shows that the biomarkers performed fairly well among HIV-negative patients, but poorly among HIV-positive patients.

Table 3.7. Number of patients that would be selected for further TB tests using CRP and IP-10 as TB screening tools with a TB prevalence of 21%.

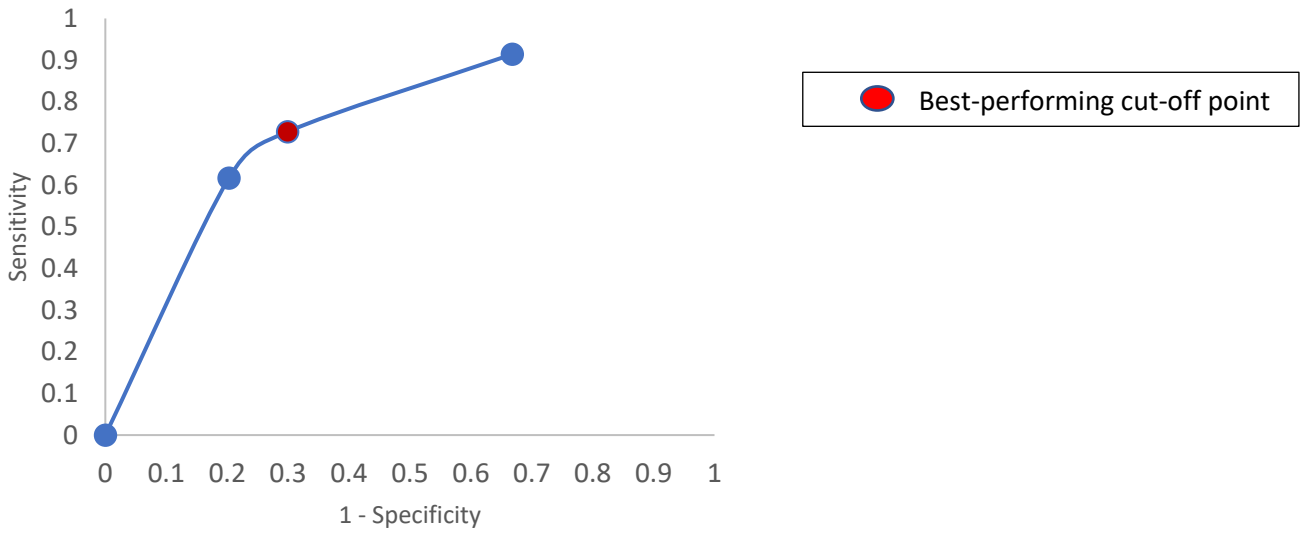
				Bact. Positive, n = 210		Bact. Negative, n = 790	
		Sensitivity	Specificity	Correctly selected	Missed	Correctly excluded	Included for further tests
All	CRP > 10mg/l	91.4%	33.2%	192	18	262	528
	> 40mg/l	72.8%	70.1%	153	57	554	336
	> 80mg/l	61.7%	79.7%	130	80	630	160
	IP-10 > 100pg/ml	87.3%	40.9%	183	27	323	467
	> 150pg/l	79.7%	58.1%	167	143	459	331
	CRP > 10mg/l & IP-10 > 500pg/ml	92.0%	32.9%	193	17	260	530
	CRP > 40mg/l & IP-10 > 150pg/ml	88.0%	46.9%	185	25	371	419
	CRP > 40mg/l & IP-10 > 450pg/ml	81.3%	59.6%	171	39	471	319
	CRP > 80mg/l & IP-10 > 100pg/ml	89.3%	36.0%	188	22	284	506
	CRP > 80mg/l & IP-10 > 150pg/ml	85.3%	51.4%	179	31	406	384
HIV-ve	CRP > 10mg/l	95.3%	42.6%	**200**	**10**	**337**	**453**
	> 40mg/l	76.7%	75.5%	161	49	596	194
	> 80mg/l	62.8%	81.9%	132	78	647	143
	IP-10 > 100pg/ml	87.5%	50.3%	184	26	397	393
	> 150pg/ml	80.0%	69.2%	168	42	547	243
	CRP > 10mg/l & IP-10 > 500pg/ml	97.5%	43.0%	**205**	**5**	**340**	**450**
	CRP > 40mg/l & IP-10 > 150pg/ml	92.5%	56.3%	194	16	445	345
	CRP > 40mg/l & IP-10 > 200pg/ml	90.0%	62.3%	189	21	492	298
	CRP > 40mg/l & IP-10 > 450pg/ml	85.0%	71.5%%	179	31	565	225
	CRP > 40mg/l & IP-10 > 500pg/ml	80.0%	74.2%	168	42	586	204
	CRP > 80mg/l & IP-10 > 100pg/ml	90.0%	45.0%	189	21	356	434
	CRP > 80mg/l & IP-10 > 150pg/ml	87.5%	60.9%	184	26	481	309
HIV +ve	CRP > 10mg/l	84.8%	22.1%	178	32	175	615
	> 40mg/l	66.7%	64.7%	140	70	511	279
	> 80mg/l	57.6%	78.7%	121	89	622	168
	IP-10 > 100pg/ml	85.3%	32.4%	179	31	256	534
	> 150pg/ml	79.4%	47.2%	167	43	373	417
	CRP > 10mg/l & IP-10 > 500pg/ml	83.9%	21,1%	176	34	167	623
	CRP > 40mg/l & IP-10 > 150pg/ml	80.6%	37.6%	169	41	297	493
	CRP > 80mg/l & IP-10 > 150pg/ml	80.6%	42.1%	169	41	333	457

3.4.4 ROC for CRP as a TB screening tool

Figure 3.2 shows a Receiver Operating Characteristic (ROC) curve for the performance CRP at ≥ 10 mg/l, ≥ 40 mg/l and > 80 mg/l for the whole study's population.

The highest point on the curves occurred at ≥ 10 mg/l, had a sensitivity of 91.4%, and specificity of 33.2%. CRP ≥ 40 mg/l had a lower sensitivity (72.8%), but a higher specificity (70.1%). The third point (CRP > 80 mg/l) had a sensitivity of 61.7% and specificity of 79.7%.

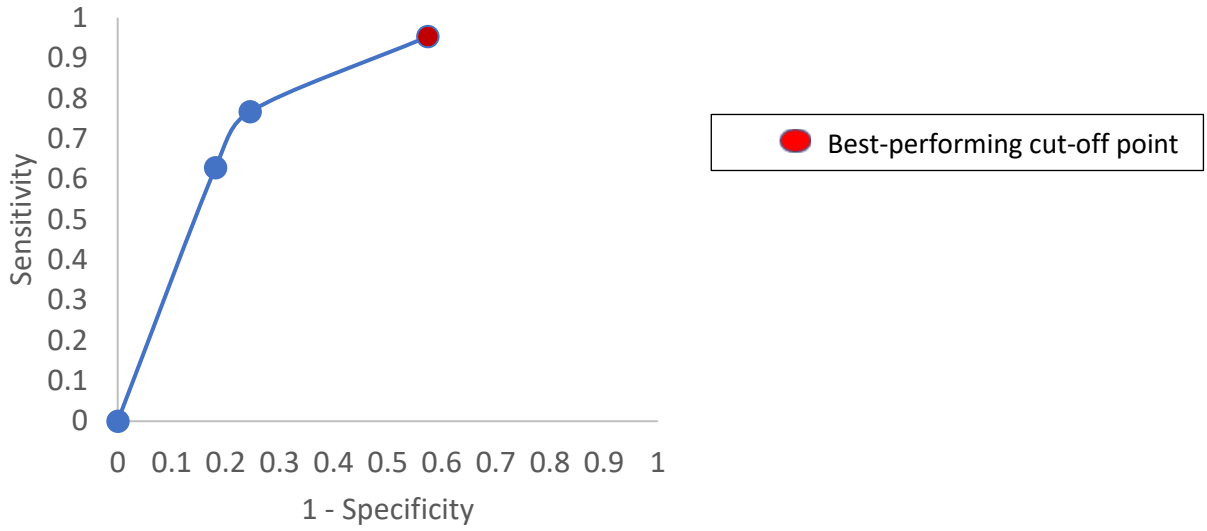
Figure 3.2 ROC for performance of CRP as a TB screening tool for all symptomatic patients



3.4.5 ROC curve for performance of CRP as a TB screening tool among HIV-negative patients

Figure 3.3 shows that CRP performed better among HIV-negative patients compared to the whole study population. The CRP's highest sensitivity among HIV-negative patients was 95.3% and its corresponding specificity was 42.6%. This occurred at CRP ≥ 10 mg/dl. The next point to the highest occurred at CRP ≥ 40 mg/l. Its sensitivity was 76.7% and its corresponding specificity was 75.5%.

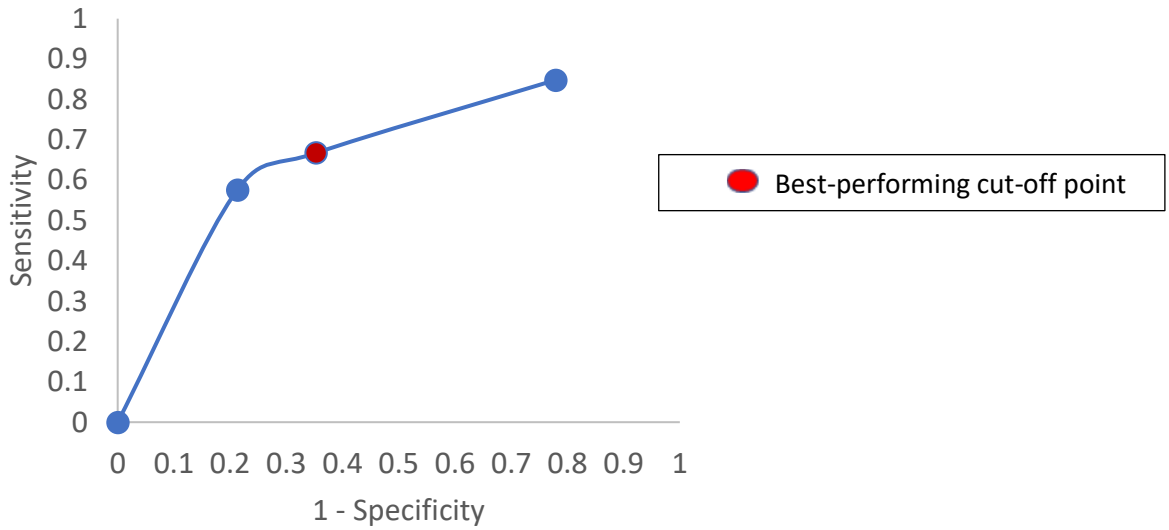
Figure 3.3 ROC curve for CRP among HIV-negative patients



3.4.6 ROC curve for CRP among HIV-positive patients

Figure 3.4 displays the ROC of CRP among HIV-positive patients. The highest point on the curve was at CRP ≥ 10 mg/l, and sensitivity at this point was 84.8%, with specificity of 22.1%. The point below this (CRP ≥ 40 mg/l) had a sensitivity of 66.7% and a specificity of 64.7%.

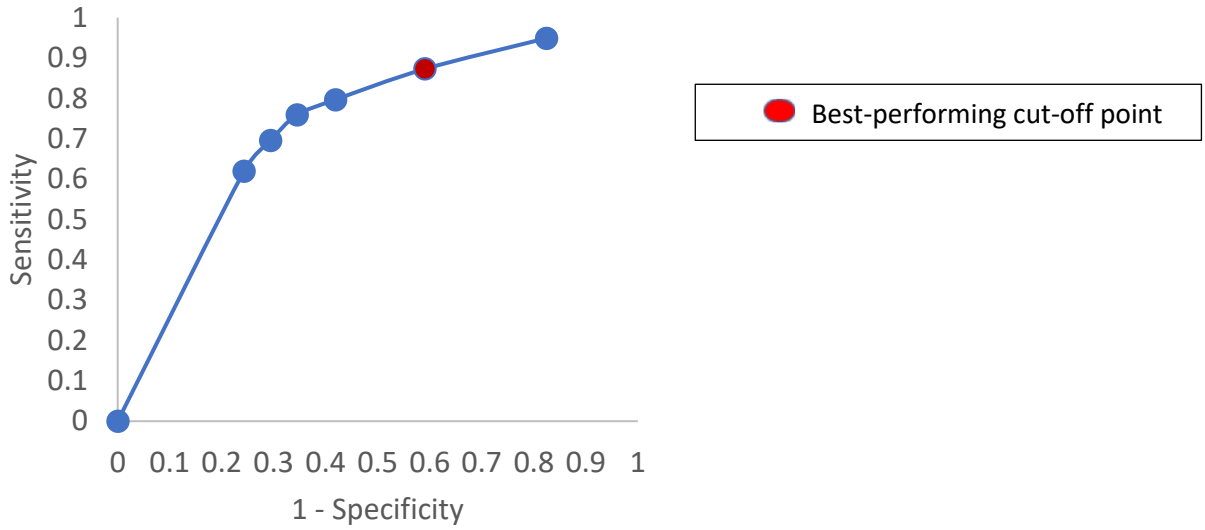
Figure 3.4 ROC curve for CRP among HIV-positive patients



3.4.7 ROC curve for IP-10 among all patients with TB symptoms

Figure 3.5 shows a ROC curve for IP-10 at different cut-off points (> 50 pg/ml, > 100 pg/ml, > 150 pg/ml, > 200 pg/ml, > 250 pg/ml and > 300 pg/ml). For the whole study population, the best-performing cut-off was > 100 pg/ml, with a sensitivity of 87.3% and a specificity of 40.9%.

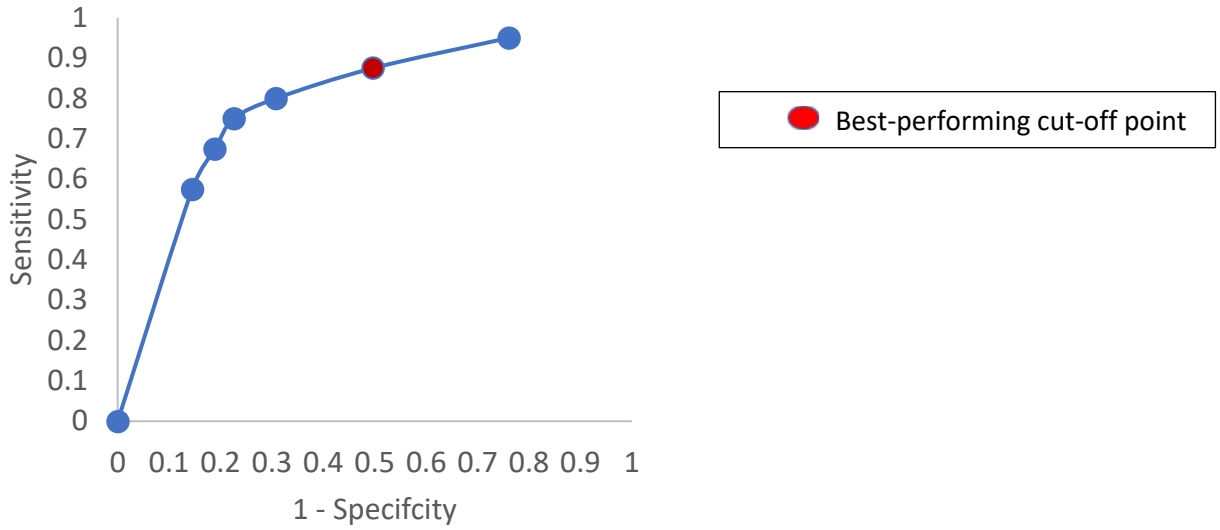
3.5 ROC curve for performance of IP-10 as a screening tool among patients with TB symptoms



3.4.8 ROC curve for IP-10 among HIV-negative patients

Among HIV-negative participants, the ROC curve (figure 3.6) best-performing IP-10 cut-off point had sensitivity of 87.5% and specificity of 50.3%. The sensitivity (87.5% versus 87.3%) and specificity (50.3% versus 40.9%) were slightly higher among HIV-negative patients.

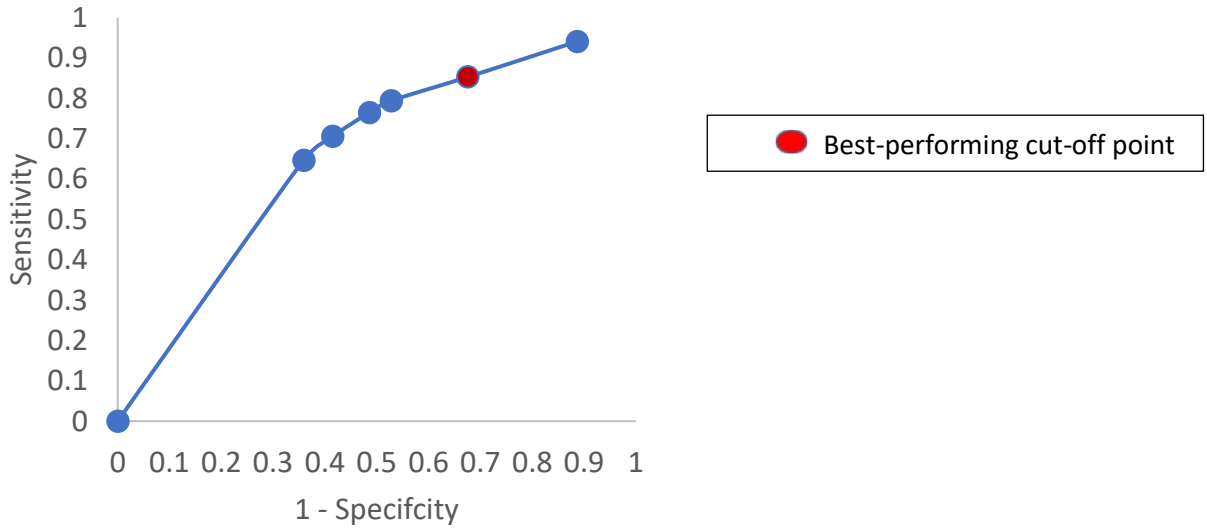
Figure 3.6 ROC curve of IP-10 among HIV-negative patients



3.4.9 ROC curve of IP-10 among HIV-positive patients.

As shown in figure 3.7, the highest point on the curve (IP-10 > 50 pg/ml) had a sensitivity of 94.1% but a specificity of 11.3%. The next point below this, was at a cut-off > 100 pg/ml with sensitivity of 85.3% and specificity 32.4%. The third cut off (> 150 pg/ml) had sensitivity and specificity of 79.4% and 47.2%, respectively. Hence, performance of IP-10 as a TB screening tool was poorer among HIV-positive patients compared to HIV-negative patients.

Figure 3.7 ROC curve of IP-10 among HIV-positive patients



3.5 Discussion

3.5.1 Summary of the study and its findings

In this study, 408 patients with cough of ≥ 2 weeks were recruited. Of these, 86 (21%) had TB and 46.9% were HIV-positive. CRP at a cut-off point of ≥ 10 mg/L had the best performance and sensitivity of 91.4% but specificity of 33.2%. The performance was better among HIV-negative patients (sensitivity 95.3%, specificity 42.6%) and worse among HIV-positive patients (sensitivity 84.8%, specificity 22.1%). When CRP cut-offs were increased the sensitivity decreased and specificity increased slightly, as expected.

IP-10 at a cut-off > 100 pg/ml had the best performance. In the whole group the sensitivity was 87.3% and specificity 40.9%. Again, performance was better among HIV-negative patients (sensitivity and specificity 87.5% and 50.3%) than among HIV-positive participants (sensitivity 85.3%, specificity 32.4%). Increasing the cut-off point to IP-10 decreased the sensitivity but increased specificity.

The combination of CRP and IP-10 in the whole study population with a CRP ≥ 40 mg/L & IP-10 > 150 pg/ml had sensitivity of 88% and specificity of 46.9%. However, among HIV-negative patients, the optimal cut-off point was CRP ≥ 10 mg/L & IP-10 > 500 pg/ml, which resulted in sensitivity and specificity of 97.5% and 43.0%, respectively. For HIV-positive patients, the optimal cut-off point was CRP > 80 mg/L & IP-10 > 150 pg/ml, with sensitivity of 80.6% and specificity of 42.1%.

3.5.2 Performance of CRP among patients with TB symptoms

We did not find any study that evaluated performance of rapid CRP on a study population comprising HIV-positive and HIV-negative patients. However, a study has shown that the performance of rapid CRP is comparable to that of ELISA (or other Lab-based CRP), when used in parallel with each other, with sensitivity (95% versus 97%) and specificity (51%

versus 54%) [104]. Hence, we may compare this finding with findings from previous studies that assessed performance of lab-based CRP among populations comprising HIV-positive and HIV-negative patients. The performance of rapid CRP among this study's whole population, is worse than findings from a prospective study in South Africa, conducted on 250 patients with TB symptoms (in which 135 patients with confirmed TB were compared with 115 patients without TB), and showed that the test had sensitivity of 98% and specificity of 59% [106]. Though, a lower cut-off point (CRP ≥ 5mg/L) than ours (CRP ≥ 10mg/L) was used in the study. The TB cases in the South Africa study were smear-negative cases, which might have lower bacillary load and different level of CRP production. In this study, we used solid culture and Xpert MTB/RIF, while in the South Africa study used liquid culture and histology, a major difference is the study design. While our study used a cross sectional design, the South Africa study used a case control design. The latter is an inadequate design to assess diagnostics, because it often generates over optimistic results, and this is likely one of the main differences. Similarly, the high proportion of participants with TB (> 50%) is likely to result in significant differences when a major issue with the test is a low specificity.

Our findings also differ from a systematic review and meta-analysis of 9 studies (of which 8 were from high burden settings) with 1299 participants, which assessed the accuracy of CRP for TB diagnosis [125]. The meta-analysis showed that CRP performed well, with a pooled sensitivity and specificity of 92% (95% CI = 83 – 97%) and 68% (95% CI = 49 – 82%), for patients recruited from outpatients settings. These differences could be due to the sample sizes, methods of TB confirmation and CRP analysis, the cut-off point used, and the studies combined in the meta-analysis.

Among HIV-positive patients, CRP performed poorly , which is contrary to findings from a prospective study from South Africa on 93 HIV-positive patients with TB symptoms [104], which reported that a cut-off ≥ 8 mg/L had 97% sensitivity and 51% specificity [104]. Two

prospective studies from Uganda used rapid CRP among HIV-positive patients and found that the biomarker had 89% sensitivity, which was similar to the WHO TB symptom screening algorithm and better specificity, leading to more patients starting IPT and ART [126,127]. One of the two studies was conducted on 1177 pre-ART patients [126] and found that CRP ≥ 10 mg/L had sensitivity of 89% and specificity of 72%.

Furthermore, a systematic review and meta-analysis of studies that assessed the diagnostic accuracy of CRP for active TB among HIV-positive patients in outpatient settings, also found that the biomarker performed well, with a pooled sensitivity and specificity of 92% and 70%, respectively [125].

Possible reasons for the difference between our study's finding and findings from the previous studies, may be due to difference in the studies' participants' characteristics, methods of TB confirmation and sample size. Firstly, for instance, two of the studies mentioned above, recruited HIV patients irrespective of TB symptom [126,127], but we recruited patients with cough of ≥ 2 weeks. Hence, it could be that patients in our study had longer symptom duration and possibly, other opportunistic infections that could have affected the performance of the diagnostics. As a meta-analysis [125] and some studies have shown that CRP had poor specificity among patients with TB symptoms who needed admission [125] or self-reported the symptom (due to high prevalence of pyogenic infections) [126].

However, our study findings are similar to a multi-centre case-control study nested into a clinical trial, which recruited participants from nine countries and determined the ability of CRP to discriminate between patients who had incident TB at 96 weeks after initiating ART and those who did not have TB, and showed that at a cut-off of ≥ 12 mg/L, the sensitivity and specificity of CRP were 44% and 81% respectively [110]. Though, this study was done on

patients who had been on ART for close to two years, and this might have decreased their risk of TB and immune response to CRP production.

For HIV-negative participants, CRP had better performance. Its sensitivity was good (95.3%), but its specificity was still lower (42.6%) than the WHO recommended TB screening tool[28]. This finding is corroborated by findings from a prospective study from South Korea, which evaluated the performance of CRP among HIV-negative patients (sensitivity 97% and specificity 41%) [107]. However, findings from another prospective study, among HIV-negative patients in China showed lower sensitivity (82%) but higher specificity (60%), at a cut-off of CRP ≥ 15 mg/L [109].

3.5.3 Performance of IP-10 as a screening tool

The performance of unstimulated IP-10 was sub-optimal among all categories of patients. Overall, IP-10 had sensitivity of 87.3% and specificity of 40.9%. This is consistent with findings from previous studies. For example, in Uganda, 111 children (comprising 80 HIV-negative and 31 HIV-positive) with presumptive TB and 33 healthy adults (as controls), found that the IP-10 performed poorly in differentiating patients with TB and other respiratory diseases (sensitivity 79%, specificity 94%) [123]. In addition, a systematic review and meta-analysis of 14 studies with 2075 participants, assessed the diagnostic sensitivity of IP-10 was 72% and specificity 82% [128]. However, a prospective study in Norway, of 164 subjects, found that unstimulated IP-10 differentiate active and LTBI, irrespective of HIV status [113].

Similar to CRP, IP-10 performance was better in HIV-negative than HIV-positive patients. The poor performance of IP-10 among HIV-positive patients is supported by a prospective study from Uganda which showed that IP-10 performance was poor in differentiating TB and not TB in HIV-negative and HIV-positive groups [123]. On the other hand, two prospective

studies conducted among HIV-negative patients: one from South Korea [121] and the other from Philippines [122], found that unstimulated IP-10 performed well in differentiating between patients with active TB and LTBI, with sensitivity and specificity of 88% and 91% [121] and 95% and 93%[122], respectively.

To see if using both CRP and IP-10 (one after the other) for TB screening, will lead to better performance of the biomarkers as a screening tool for active TB, we assessed the performance of the combination of each cut-off point of CRP with each of the cut-off points of IP-10. Our finding showed that the combination only led to slight improvement in the performance of the biomarkers, among HIV-negative patients. It increased the sensitivity by 2.2% (from 95.3% to 97.5%) and the specificity by 0.4% (from 42.6% to 43%). We did not find any study that assessed the performance of combination of the two biomarkers as a screening tool for active TB.

3.5.4 Limitations and strength of the study

This study has some limitations. Firstly, the use of solid culture as the reference standard, might have missed some TB cases that could be detected by liquid culture, and this might affect this study's findings. However, contamination rate could be higher in liquid culture compared to solid culture [97-99]. Secondly, we could not compare the performance of the biomarkers to that of the WHO TB symptoms screening, because we did not recruit patients irrespective of TB symptoms (we recruited only patients with cough of ≥ 2weeks)). Lastly, because we diagnosed only PTB, some EPTB cases might have been missed and this could make us to underrate the burden of TB in our study population.

However, this study has some strength. This study might be the first study from Nigeria, that assessed the performance of CRP and IP-10 as screening tools for active TB. Also, this could be one of the few studies that compared the performance of the biomarkers between HIV-

positive and HIV-negative patients. We also did not find any published previous study that assessed the performance of the combination of CRP and IP-10 as a screening tool for active TB. Hence, this might be the first study on the topic.

3.5.5 Conclusion

This study showed that close to half of the study population are HIV-infected and 21% of them had TB. In addition, the performance of rapid CRP was poor among HIV-positive patients, fair in the whole study's population and better among HIV-negative patients. IP-10's performance was poor among all categories of patients. Diagnostic algorithms that consider testing for CRP as a triage step for TB diagnosis, need to include screening for HIV. It is likely that HIV-infected individuals will need to be tested directly by a molecular test such as Xpert, while HIV-negative patients could undergo CRP screening and patients with CRP > 10 mg/L could undergo further tests.

Chapter 4

TB Treatment Outcome among HIV-infected and -uninfected patients

4.1 Introduction

Tuberculosis treatment success is a key indicator for monitoring the implementation of the End TB Strategy, which aims to drastically reduce the public health impacts of TB by 2035 [129]. The treatment success targeted is ≥ 90%, for both drug-susceptible and drug-resistant TB cases in all countries by 2025 [41,129], however, only one country in Sub-Saharan Africa (Tanzania) has reached this target among drug-susceptible patients. In 2015, 5,893,106 new and relapsed TB cases were registered globally, with a treatment success of 83%. In the same year, treatment success across Africa, ranged from 71% in Congo to 90% in Tanzania, with treatment outcome being worse among HIV-positive patients, ranging from 20% in Congo to 83% in Tanzania[130].

Few studies have compared the TB treatment outcomes between HIV-positive and HIV-negative patients in Nigeria or systematically described all unfavourable TB treatment outcomes. There is therefore a need for quality studies to create the evidence of the impact of HIV infection on TB treatment outcome in Nigeria. This study therefore systematically reviewed the records of TB patients treated at a large DOTS clinic at the University of Abuja Teaching Hospital (UATH), between January 2012 and December 2016.

The specific objectives of the study were to:

- Determine treatment outcome among TB patients treated in UATH between January 2012 and December 2016
- Compare TB treatment outcome between HIV-positive and HIV negative patients
- Determine risk factors to poor treatment outcome (death/treatment failure)
- Identify factors associated with loss-to-follow up among the patients

4.2 Literature review

It is well established that TB treatment is less successful among HIV-positive than HIV-negative patients. According to the 2016 Global TB report [41], only 75% of HIV-positive patients had a successful TB treatment outcome in 2014, compared to 83% for HIV-negative patients and these differences were similar across all regions, as shown in the table 4.1 [41].

Table 4.1. TB treatment success of HIV-positive and HIV-negative patients by WHO region.

WHO Region	**TB treatment success**		
	HIV-negative	HIV-positive	Difference
Africa	83%	77%	6%
Americas	77%	56%	21%
Eastern Mediterranean	92%	53%	39%
Europe	80%	41%	39%
South-East Asia	79%	74%	5%
Western Pacific	93%	72%	21%

Source: Global Tuberculosis Report, 2016

For example, a review of TB treatment records of 61,138 patients across Europe, reported a successful treatment outcome of 56.9% and 78.7% among HIV-infected and HIV-uninfected patients [131]. A further study from India of 57,045 patients, showed that 74.3% and 79.9% of patients with and without HIV achieved treatment success, respectively [132] and many other studies outside Africa have reported that HIV is associated with worse TB treatment outcome [133-135].

In Africa, treatment success was reported to be lower for HIV-infected patients in seven studies [131,132,136-140], similar in one study [141] and higher in nine studies [142-150].

Although TB treatment outcome is less successful among HIV-infected patients, only the death rates are consistently higher, which are between two times higher in Africa and 7.5 times higher in the Western Pacific Region [41]. In sub-Saharan Africa the death rate is between 2 and 8 times higher among HIV-positive compared to HIV-negative patients.

TB treatment default rates of HIV infected individuals are more heterogeneous, with lower default rates in eight studies [132,136,138,140,141,145-147] and higher rates in eleven studies [131,137,139,142-144,148-152] (see table 4.2).

Finally, 9 studies showed that a higher proportion of TB/HIV-co-infected patients were transferred out [132,136-138,144,145,147,151,152], but one study had similar rates [139] and three studies indicated HIV patients, were less likely to be transferred out [142,146,152], which is likely to reflect the heterogeneity of the participants and the study settings .

In sub-Saharan Africa, HIV/TB co-infection also worsens TB treatment outcome. Most studies in Ethiopia [138,145,147,148,150,152-156], Benin [144,151,157], Cameroon [137], Gabon [146], Malawi [139], South Africa [140] and Uganda [149] have consistently reported TB treatment is less successful among HIV patients, as summarised in table 4.2. TB treatment success ranged from 34.0% to 97.0% among HIV-positive and from 53.0% to 96.4% for HIV-negative patients, but was consistently less successful among HIV-infected patients, with a difference ranging from 0.5% to 29.7%, which is close to the gaps reported from other regions.

The treatment success rate among HIV-positive and negative patients with drug susceptible TB in Nigeria was 79% and 88.6%, respectively [41]. TB treatment outcome of HIV-infected and uninfected patients in the country, was described in five studies and all reported a lower treatment success among HIV-infected patients, ranging from 48.8% to 73.4% for HIV-positive and 73.6% to 90.2% for HIV negative patients, with a difference from 6.3% to 29.7% [136,142,143,158,159].

Various factors have been attributed to the poor TB treatment outcome among HIV-positive patients. The first one is the interaction between TB and HIV, whereby one pathogen accelerates the natural progression of the other [131,160]. Secondly, it is often stated that the late diagnosis and delayed treatment of TB or ART initiation among TB/HIV-co-infected patients, lead to poor treatment outcome [41].

Factors that modify TB treatment outcome among patients with HIV co-infection

Factors known to affect TB treatment outcome include ART. Patients on ART have better TB treatment outcome than those not on ART [132,139,144,158,161-163]. However, some studies have reported that the treatment outcome for patients with and without ART can be similar [151,162,164] or better than patients without HIV [163,165]. Patients who start ART early have generally better TB treatment outcome than those who initiate ART late [166-169].

Another factor affecting treatment outcome is the level of immunosuppression or CD4 count at the time of TB diagnosis, with the outcome being poorer among patients with CD4 counts < 200 cells/m^3 [163,170].

Poor outcome is associated with age among HIV-positive patients, with worse outcome in patients at the extremes of life (particularly in infants and the elderly) [136,159,163,165,171].

Treatment outcome is also better among HIV-infected new TB cases compared to retreatment cases [172,173] and HIV-positive patients with smear-positive TB have better treatment outcome than smear-negative cases with HIV [144,170], which is likely due to their remaining ability to create cavitations, which reflect an immunological response to TB.

Table 4.2. Studies on TB treatment outcomes by HIV status

Author/Year	Country	Sample size	HIV status	Treatment outcome				
				Successful	Unsuccessful			
					Default	Failure	Transferred out	Died
Ade/2016 [157]	Benin	220	HIV-	93.4%	6.6%			
			HIV+	97.0%	3.0%			
Ade/2014 [151]	Benin	378	HIV-	88.2%	3.6%		1.3%	6.9%
			HIV+	77.8%	5.5%		1.4%	15.3%
Ade/2013 [144]	Benin	3070	HIV-	91.4%	1.8%	2.1%	0.4%	4.2%
			HIV+	78.4%	4.4%	2.4%	1.4%	13.4%
Pefura Yone/2012 [137]	Cameroon	1647	HIV-	74.3%	18.0%	0.4%	5.4%	1.8%
			HIV+	65.0%	19.3%	0.0%	5.8%	9.8%
Amante/2015 [155]	Ethiopia	639	HIV-	71.3%	28.7%			
			HIV+	58.6%	41.4%			
Asres/2016 [154]	Ethiopia	790	HIV-	90.1%	9.9%			
			HIV+	83.1%	16.9%			
Belayneh/2016 [147]	Ethiopia	403	HIV-	77.2%	2.4%	0.4%	13.3%	3.6%
			HIV+	76.7%	0.6%	0.6%	14.8%	7.1%
Ejeta/2015 [148]	Ethiopia	1143	HIV-	74.0%	6.2%	0.1%	13.8%	5.8%
			HIV+	55.7%	9.1%	8.7%	10.5%	16.0%
Endris/2014 [152]	Ethiopia	206	HIV-	92.3%	0.5%	0.0%	3.3%	3.8%
			HIV+	83.4%	4.2%	0.0%	8.3%	4.2%
Gebreegziabher/2016 [156]	Ethiopia	697	HIV-	96.4%	3.6%			
			HIV+	81.7%	18.3%			
Gebremariam/2016 [138]	Ethiopia	1562	HIV-	89.6%	1.6%	0.4%	6.2%	2.2%
			HIV+	73.0%	0.6%	0.0%	9.1%	16.7%
Mekonnen/2016 [150]	Ethiopia	931	HIV-	92.3%	2.4%	2.0%		3.3%
			HIV+	82.8%	3.5%	3.1%		10.6%
Tesfahuneygn/2015 [145]	Ethiopia	4275	HIV-	90.5%	2.2%	0.3%	5.0%	2.0%
			HIV+	84.5%	1.7%	0.5%	6.7%	6.6%
Zenebe/2016 [153]	Ethiopia	380	HIV-	85.3%	14.7%			
			HIV+	64.0%	36.0%			

Belard/2016 [146]	Gabon	175	HIV-	59.3%	19.4%	2.8%	18.5%	0%
			HIV+	44.8%	17.9%	3.0%	9.0%	25.4%
Tweya/2013 [139]	Malawi	2264	HIV-	88.0%	6.0%	1.0%	2.0%	3.0%
			HIV+	85.0%	7.0%	0.5%	2.0%	6.0%
Adejumo/2016 [136]	Nigeria	535	HIV-	79.7%	14.6%	0.6%	1.1%	4.0%
			HIV+	73.4%	14.0%	0.0%	1.4%	11.2%
Babatunde/2016 [142]	Nigeria	600	HIV-	73.6%	17.6%	5.5%	1.8%	1.5%
			HIV+	64.1%	18.5%	6.8%	0.9%	9.7%
Ofoegbu/2015 [143]	Nigeria	389	HIV-	78.5%	17.0%	4.0%		
			HIV+	48.8%	36.8%	10.8%		
Ogbudebe/2016 [158]	Nigeria	647	HIV-	90.2%	9.8%			
			HIV+	71.3%	29.7%			
Oshi/2014 [159]	Nigeria	1668	HIV-	78.6%	21.4%			
			HIV+	65.8%	34.2%			
Budgell/2016 [140]	South Africa	448	HIV-	83.0%	10.6%	3.2%	0.0%	3.2%
			HIV+	80.2%	9.6%	0.6%	0.0%	9.6%
Kirenga/2014 [149]	Uganda	96	HIV-	53.0%	13.0%	3.0%		31.0%
			HIV+	34.0%	16.0%	11.0%		39.0%
McGreevy/2012 [141]	Haiti	153	HIV-	81.0%	10.0%	4.0%		4.0%
			HIV+	73.0%	5.0%	4.0%		18.0%
Karo /2016 [131]	Europe	61,138	HIV-	78.7%	10.2%	2.4%		6.2%
			HIV+	56.9%	20.2%	1.5%		13.5%
Lucenko/2014 [134]	Latvia	2476	HIV-	89.0%	11.0%			
			HIV+	73.0%	23.0%			
Shastri/2013 [132]	India	57,045	HIV-	79.9%	8.8%	2.8%	1.4%	7.1%
			HIV+	74.3%	4.4%	0.5%	5.1%	15.7%

4.3 METHODOLOGY

4.3.1 Study design and site description

This was a retrospective descriptive study of TB patients treated at the DOTS clinic of the University of Abuja Teaching Hospital (UATH), between January 2012 and December 2016. The clinic receives patients referred from the wards and clinics within UATH and private, faith-based and government hospitals in Abuja and its environs. Most patients are referred from Gwagwalada town. The treatment provided to patients is supplied by the National Tuberculosis and Leprosy Control Programme (NTBLCP) and the drugs are free-of-charge to the patients. TB screening and follow up tests for TB and HIV testing are provided free of charge to the patients. For treatment purposes, TB patients are categorized into two, based on whether patients had never had TB before (new cases) or patients return for treatment after default or relapse from previous TB (retreatment cases). New TB cases are treated with category 1 anti-TB regimen, which comprises an intensive phase of two months (during which patient receives a daily fixed dose combination of Rifampicin, Isoniazid, Ethambutol and Pyrazinamide) and a continuation phase of four months (during which patient receives a daily fixed dose combination of Rifampicin and Isoniazid). While retreatment cases received category 2 regimen.[174]. The treatment consists of a three-month intensive phase (which comprises daily injectable Streptomycin and a fixed-dose combination of Rifampicin, Isoniazid, Ethambutol and Pyrazinamide for the first two months, thereafter, injectable Streptomycin is removed while patient continues the fixed-dose combination for the remaining one month) and a five-month continuation phase, during which patient will be on a daily fixed-dose combination of Rifampicin, Isoniazid and Ethambutol. However, this regimen is now an obsolete treatment, because WHO has recommended that it should no longer be prescribed for this category of TB patients [174]. TB treatment outcomes are described in table 4.3.

Table 4.3. TB treatment outcome definitions

Outcome	Definition
Cured	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.
Treatment completed	A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.
Treatment failed	A TB patient whose sputum smear or culture is positive at month 5 or later during treatment
Died	A TB patient who dies for any reason before starting or during the course of treatment
Lost to follow-up	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more
Not evaluated	A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit
Treatment success	The sum of cured and treatment completed

Source: WHO. Definitions and reporting framework for TB [175].

4.3.2 Method of data collection

With the permission of the UATH's TB programme coordinator, all TB treatment registers and cards of patients diagnosed to have TB and initiated treatment at the centre, from January 2012 to December 2016, were retrieved from the clinic record room. With the help of two research assistants, the records were reviewed and a proforma/data extraction form was used to collect information regarding the patients' HIV status (positive, negative or undetermined), whether the diagnosis had microbiological confirmation by smear microscopy, Xpert MTB/RIF or culture; clinical presentation (as pulmonary or extra-pulmonary), treatment regimen started and date treatment initiated, treatment outcome (cured, treatment completed, treatment success (cured + treatment completed), loss-to-follow up, treatment failure, transferred out or not evaluated), basic demographic characteristics (age, gender, educational

status, occupation) and factors that could be associated with treatment outcomes (weight, new or re-treatment, ART use or CPT for HIV-positive patients).

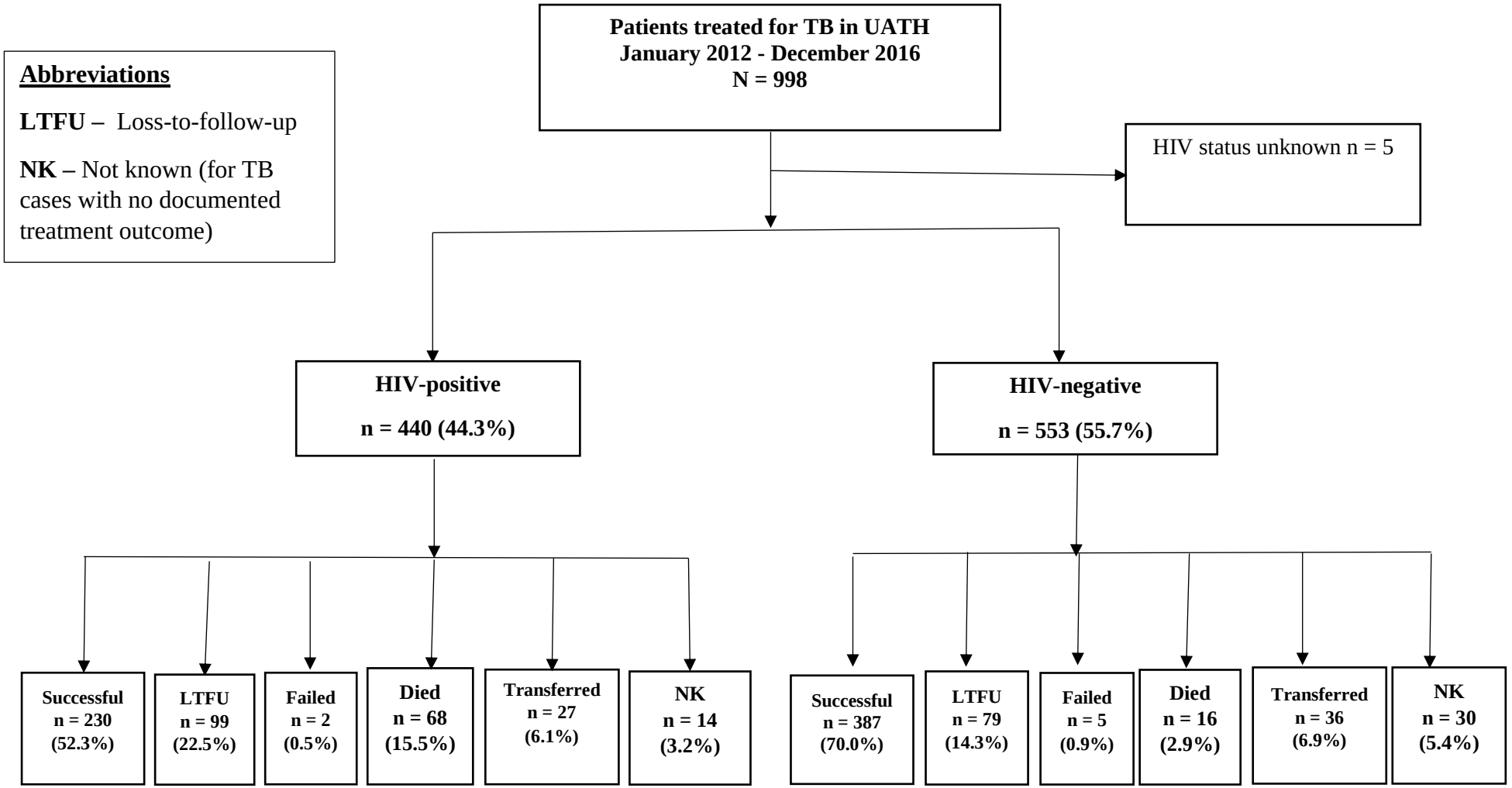
4.3.3 Data analysis

Data analysis was conducted using Epi-Info 7 (Centers for Disease Control and Prevention) and SPSS (version 24). After adequate data cleaning, descriptive statistics were done to obtain counts and percentages, means, standard deviations and charts for variables, as needed. Thereafter, bivariable analyses compared the characteristic of categorical variables between groups (e.g. HIV-positive and negative, treatment success versus treatment failure etc) using Chi-Square and Fisher's Exact tests, and OR with 95%CI. Comparisons of continuous variables were done using Student's t tests and the mean differences (95% CI) were determined and tested using paired T tests. Variables with p values < 0.2 were included in multivariable analyses using logistic regression adjusted for age, gender and HIV status to obtain Adjusted ORs (AOR)s to determine factors independently associated with treatment outcome. P values < 0.05 were considered statistically significant. Kaplan Meir survival analysis curves were drawn to compare death and LTFU trends by HIV status.

4.3.4 Ethical considerations

Ethical approvals were obtained from the Research Ethics committees of LSTM, UATH and FCT. Permission to have access to TB treatment records was sought from the UATH's TB coordinator and clinic staff. Informed consent from the patients was not obtained, as data had been collected from the patients and this requirement was waived by the ethics committees. All patient information was handled to maintain confidentiality.

Figure 4.1. TB treatment outcome study's flow chart



4.4 RESULTS

4.4.1 Baseline characteristics and treatment outcome

Nine hundred and ninety-eight patients were treated at the DOTS clinic of UATH from January 2012 to December 2016. Of these, 571 (57.2%) were male and 427 (42.8%) female (table 4.4). Their age ranged from 0 to 92 years with a mean (SD) of 34.5 (16.4) years for males and 29.7 (14.7) for females ($p = 0.001$). One hundred and twenty-four (12.4%) were children ≤ 14 years old. Of 895 patients with known marital status, 395 (44.1%) were single, 482 (53.9%) married, and 18 (2%) separated, divorced or widowed. Two hundred and ten (23.4%) were traders or business men/women and 159 (17.8%) students, with less frequent occupations including civil servants, artisans, farmers and others. Only 34 (3.8%) indicated they were unemployed. The majority (540, 62.5%) of patients belonged to minority ethnic groups such as Igala (50, 5.8%); Idoma (45, 5.2%); Tiv (35, 4.1%) and others. The three major Nigerian ethics groups (Igbo, Yoruba and Hausa) represented 19.6%, 9.4% and 8.6% of the participants, respectively.

Most (970, 97.2%) patients were classified as new and 28 (2.8%) as re-treatment TB cases. Nine hundred and forty-three (94.5%) cases were treated with category 1 and 55 (5.5%) with category 2 drugs. The majority (848, 85.0%) of cases had PTB and 150 (15%) EPTB. EPTB included 32 (21.3%) cases with enlarged lymph nodes, 22 (14.7%) abdominal TB and 21 (14%) TB of the spine. Forty (26.6%) patients had other forms of EPTB, with 35 not being documented. Of 848 PTB cases, 347 (40.9%) were smear-positive and 234 (27.6%) smear-negative. A further 196 (23.1%) were diagnosed with Xpert MTB/RIF and 71 (8.4%) with chest X-rays only, as shown in table 4.4. Two (1%) of the 196 cases tested with Xpert MTB/RIF were Rifampicin resistant and two (1%) had indeterminate results.

HIV status was documented for 993 (99.5%) participants and 440 (44.3%) were HIV-positive.

The mean (SD) weight at baseline, 2 and 5 months of treatment for males was 52.1 (15.9), 54.5 (16.3) and 58.6 (15.8) Kgs, with mean (SD) weight differences of 2.3 (4.4) and 5.2 (5.2) Kgs at 2 and 5 months, respectively. Similarly, the mean (SD) weight at baseline, 2 and 5 months for females, were 46.2 (16.6), 49.4 (14.7) and 52.4 (15.4) Kgs, with mean (SD) weight differences of 1.6 (4.6) and 3.7 (4.9) Kgs, respectively.

Treatment outcome was recorded as cured in 222 (22.2%) and completed in 397 (39.8%) patients, with successful treatment (cured + completed) in 619 (62%) patients. One hundred and eighty (18%) patients were LTFU, 7 (0.7%) had treatment failure, 63 (6.3%) were transferred out, 84 (8.4%) died and 45 (4.5%) were not evaluated. If patients not evaluated or transferred out are excluded, treatment success was 69.6%, with 9.4% of patients dying during the follow up.

Table 4.4. Baseline characteristics of patients treated for TB in UATH, 2012 – 2016.

Characteristics	Category	N = 998 (%)	Missing
Gender	Male	571 (57.2)	
	Female	427 (42.8)	
Mean (SD) [Range] age (years)	All	32.5 (15.8) [0 – 92]	
	Male	34.5 (16.4) [0 – 92]	
	Female	29.7 (14.7) [0 – 85]	
Age group (years)	< 5	58 (5.8)	
	5 – 14	66 (6.6)	
	15 – 24	152 (15.2)	
	25 – 34	282 (28.3)	
	35 – 44	243 (24.4)	
	45 – 54	114 (11.4)	
	55 – 64	46 (4.6)	
	≥ 65	37 (3.7)	
Marital status	Single	395 (44.1)	103
	Married	482 (53.9)	
	Separated	3 (0.3)	
	Divorced	2 (0.2)	
	Widowed	13 (1.5)	

Occupation	Student	159 (17.8)	102
	Unemployed	34 (3.8)	
	Civil servant	88 (9.8)	
	Artisan	70 (7.8)	
	Trading/Business	210 (23.4)	
	Farming	50 (5.6)	
	House-wife	86 (9.6)	
	Others	199 (22.2)	
Ethnicity	Igbo	169 (19.6)	134
	Yoruba	81 (9.4)	
	Hausa	74 (8.6)	
	Igala	50 (5.8)	
	Idoma	45 (5.2)	
	Tiv	35 (4.1)	
	Gbagyi	31 (3.6)	
	Ebira	30 (3.5)	
	Gwari	21 (2.4)	
	Fulani	17 (2.0)	
	Bassa	15 (1.7)	
	Ibiobio	13 (1.5)	
	Koro	12 (1.4)	
	Nupe	11 (1.3)	
	Jabba	10 (1.2)	
	Other	250 (28.9)	
Religion	Christian	643 (74.7)	137
	Islam	216 (25.1)	
	Others	2 (0.2)	
TB treatment category	New	970 (97.2)	
	Retreatment	28 (2.8)	
Anti-TB regimen category	Category 1	943 (94.5)	
	Category 2	55 (5.5)	
Anatomical site of TB	Pulmonary	848 (85.0)	
	Extra-pulmonary	150 (15.0)	
Type of PTB	SM-positive	347 (40.9)	
	SM-negative	234 (27.6)	
	SM-not done	267 (31.5)	
Forms of EPTB	Lymphadenitis	32 (21.3)	
	Abdominal	22 (14.7)	
	Spine	21 (14.0)	
	Breast	6 (4.0)	
	Prostatic	3 (2.0)	
	Meningitis	3 (2.0)	
	Pericarditis	3 (2.0)	
	Pleuritis	3 (2.0)	
	Endometrial	2 (1.3)	
	Other forms	20 (13.3)	
	Not documented	35 (23.3)	
[†]**Mode of diagnosis**	SM	349 (35.0)	
	X-rays	474 (47.5)	

	Xpert MTB/RIF	196 (19.6)	
	Clinical	88 (8.8)	
	Histology	56 (5.6)	
	Spine X-rays	21 (2.1)	
	Mantoux	2 (0.2)	
	MRI	1 (0.1)	
SM grade (n = 349)[a]	Scanty	35 (10.0)	
	+	91 (26.1)	
	++	125 (35.8)	
	+++	98 (28.1)	
Xpert MTB grade (n = 192)[b]	Very low	34 (17.7)	4
	Low	64 (33.3)	
	Medium	60 (31.3)	
	High	34 (17.7)	
Xpert RIF (n = 196)	Positive	2 (1.0)	
	Negative	192 (98.0)	
	Indeterminate	2 (1.0)	
HIV-infected		440 (44.3)	5
Baseline weight (kg)	Mean (SD) [Range]	49.6 (16.5) [3.5 – 105.5]	106
Weight gain at 2 m	Mean (SD) [Range]	2.0 (4.4) [-22.0 – 33.0]	454
Weight gain at 2 m	Mean (SD) [Range]	4.6 (5.2) [-12.0 – 32.0]	591
TB treatment outcome	Cured	222 (22.2)	
	Completed	397 (39.8)	
	Cured + completed	619 (62.0)	
	Loss-to-follow up	180 (18.0)	
	Failed	7 (0.7)	
	Transferred out	63 (6.3)	
	Died	84 (8.4)	
	Not evaluated	45 (4.5)	

[†] Some TB cases were diagnosed by more than one method
[a] In addition to the 347 smear-positive PTB cases, two EPT cases were smear-positive
[b]Four patients with positive Xpert MTB but had no grading for MTB detection, were excluded

4.4.2 Baseline characteristics of patients by HIV status

HIV-positive patients were less likely to be male (OR 95% CI = 0.6, 0.5 – 0.8, p = 0.001) and to be older (mean (SD) 33.7 (14.8) years) than HIV-negative patients (31.5 (16.7), p = 0.025), as shown in table 4.5. HIV-positive patients were older than HIV-negative patients of the same sex. HIV-positive patients were more likely to be married (p = 0.001), separated/divorced (p = 0.051) or widowed (p = 0.001) and less likely to be single.

HIV-positive patients were more likely to have PTB than HIV-negative patients (p = 0.001) and were more likely to have negative or missing smear microscopy (p = 0.001). The latter is

explained because patients with HIV are more likely to be screened with Xpert MTB/RIF as the first test for diagnosis (p = 0.001). Xpert MTB/RIF bacterial loads of HIV-positive patients were more likely to be very low than for HIV-negative patients (p < 0.05). However, there was no significant difference in Rifampicin resistance (p = 0.480).

HIV-positive patients had lower mean (SD) baseline body weight than HIV-negative patients (47.5 (16.6) Kgs versus 51.3 (16.2) Kgs, p = 0.001) and at 2 (50.1 (16.9) versus 53.7 (15.1), p = 0.001) and 5 months follow up (54.3 (17.0) versus 57.0 (15.2), p = 0.098). However, the mean (SD) weight difference at 2 and 5 months were not statistically significantly.

Table 4.5. Baseline characteristics of patients treated for TB in UATH by HIV status

Characteristics	Category	HIV-positive N = 440 (%)	HIV-negative N = 553 (%)	Odds ratio (95% CI)	P
Gender	Male	223 (50.7)	346 (62.6)	0.6 (0.5 – 0.8)	0.001
	Female	217 (49.3)	207 (37.4)		
Mean [SD] age (years)	All	33.7 (14.8)	31.5 (16.7)	2.2 (0.3 – 4.2) [a]	0.025
	Male	37.0 (15.4)	33.0 (16.8)	3.7 (1.2 – 6.7) [a]	0.005
	Female	30.4 (12.9)	29.0 (16.2)	1.4 (-1.4 – 4.2) [a]	0.318
Age group (years)	0 – 9	42 (9.5)	56 (10.1)		0.001
	10 – 19	26 (5.9)	51 (9.2)		
	20 – 29	64 (14.5)	163 (29.5)		
	30 – 39	149 (33.9)	133 (24.1)		
	40 – 49	112 (25.5)	71 (12.8)		
	50 – 59	30 (6.8)	36 (6.5)		
	60 – 69	12 (2.7)	23 (4.2)		
	≥ 70	5 (1.1)	20 (3.6)		
Marital Status	Single	134 (34.5)	261 (51.8)	1	
	Married	240 (61.9)	239 (47.4)	2.0 (1.5 – 2.6)	0.001
	Separated	4 (1.0)	1 (0.2)	7.8 (0.9 – 70.4)	0.051
	Widowed	10 (2.6)	3 (0.6)	6.5 (1.8 – 24.0)	0.001
Occupation	Student	35 (9.1)	124 (24.5)		0.001
	Unemployed	13 (3.4)	21 (4.1)		
	Civil servant	51 (13.2)	36 (7.1)		
	Artisan	32 (8.3)	38 (7.5)		
	Trading/Business	111 (28.8)	99 (19.5)		
	Farming	19 (4.9)	31 (6.1)		
	House-wife	37 (9.6)	48 (9.5)		
	Others	88 (22.8)	110 (21.7)		
Ethnicity	Hausa	23 (6.2)	51 (10.4)		0.112
	Igbo	74 (19.9)	94 (19.2)		
	Yoruba	31 (8.3)	49 (10.0)		
	Other	244 (65.6)	296 (60.4)		
Religion	Christianity	302 (80.7)	340 (70.2)		0.001

	Islam	71 (19.0)	143 (29.5)		
	Others	1 (0.3)	1 (0.2)		
TB treatment category	New	427 (97.0)	538 (97.3)	0.9 (0.4 – 1.9)	0.819
	Retreatment	13 (3.0)	15 (2.7)		
Treatment used	Category 1	420 (95.5)	518 (93.7)	1.4 (0.8 – 2.5)	0.222
	Category 2	20 (4.5)	35 (6.3)		
Anatomical site of TB	Pulmonary (PTB)	400 (90.9)	445 (80.5)	2.4 (1.6 – 3.6)	0.001
	Extra-pulmonary (EPTB)	40 (9.1)	108 (19.5)		
Type of PTB	Smear-positive	95 (23.8)	252 (56.6)	1	
	Smear-negative	144 (36.0)	88 (19.8)	4.3 (3.0 – 6.2)	0.001
	SM not done	161 (40.3)	105 (23.6)	4.1 (2.9 – 5.2)	0.001
Smear grade (n = 349)[b]	Scanty	13 (13.5)	22 (8.7)	1	
	+	25 (26.0)	66 (26.1)	0.6 (0.3 – 1.5)	0.291
	++	30 (31.3)	95 (37.5)	0.5 (0.2 – 1.2)	0.124
	+++	28 (29.2)	70 (27.7)	0.7 (0.3 – 1.5)	0.347
Xpert MTB (n = 192)[c]	Very low	24 (24.2)	10 (10.8)	1	
	Low	32 (32.3)	32 (34.4)	0.4 (0.2 – 1.0)	0.053
	Medium	29 (29.3)	31 (33.3)	0.4 (0.2 – 0.9)	0.039
	High	14 (14.1)	20 (21.5)	0.3 (0.1 – 0.8)	0.016
Xpert RIF (n = 196)	Positive	0 (0.0)	2 (2.1)		0.480
	Negative	100 (99.01)	92 (96.8)		
	Indeterminate	1 (0.9)	1 (1.1)		
Mean [SD] weight (kg)	At baseline	47.5 (16.6)	51.3 (16.2)	-3.8 (-5.9 – -1.6)[a]	0.001
	At 2 months	50.1 (16.9)	53.7 (15.1)	-3.6 (-6.4 – -0.9) [a]	0.009
	At 5 months	54.3 (17.0)	57.0 (15.2)	-2.7 (-5.9 – 0.5) [a]	0.098
Mean (SD) weight difference (kg)	At 2 months	1.7 (5.6)	2.2 (3.4)	-0.5 (-1.3 – 0.4) [a]	0.279
	At 5 months	4.7 (5.7)	4.5 (4.8)	0.2 (-0.9 – 1.3) [a]	0.704

[a]Mean difference (95% CI)
[b]In addition to the 347 smear-positive PTB, two EPTB were also smear-positive and were included
[c]Four patients with positive Xpert MTB but no grading for MTB detection, were excluded

4.4.3 TB treatment outcome by HIV status

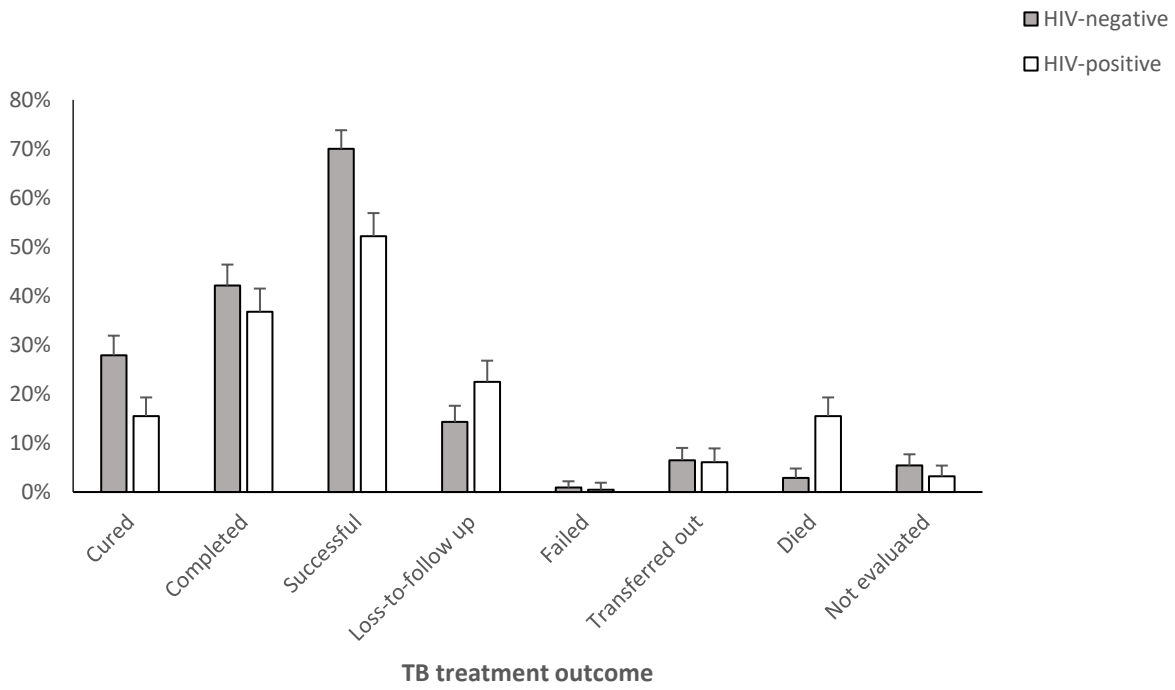
HIV-positive patients were less likely to be cured (68, 15.5%) or to complete treatment (162, 36.8%) than HIV-negative patients (154, (27.9%) and 233 (42.1%)) respectively), as shown in table 4.6 and Figure 4.2. Treatment success (cured + completed) was lower for HIV-positive patients (53.2% versus 70.0%, p = 0.001). HIV-positive patients had higher mortality (15.5% versus 2.9%, p = 0.001) and were more likely to be LTFU than patients without HIV (22.5% versus 14.3%, p = 0.001).

Table 4.6. Treatment outcome of TB patients between 2012 and 2016 by HIV status

Treatment Outcome	HIV-positive n = 440 (%)	HIV-negative n = 553 (%)	P value
Cured	68 (15.5)	154 (27.9)	0.001
Completed	162 (36.8)	233 (42.1)	0.089
Successful (cured + completed)	230 (52.3)	387 (70.0)	0.001
Loss-to-follow up	99 (22.5)	79 (14.3)	0.001
Failed	2 (0.5)	5 (0.9)	0.476
Transferred out	27 (6.1)	36 (6.5)	0.810
Died	68 (15.5)	16 (2.9)	0.001
Not evaluated	14 (3.2)	30 (5.4)	0.088

* HIV status of 5 patients was unknown.

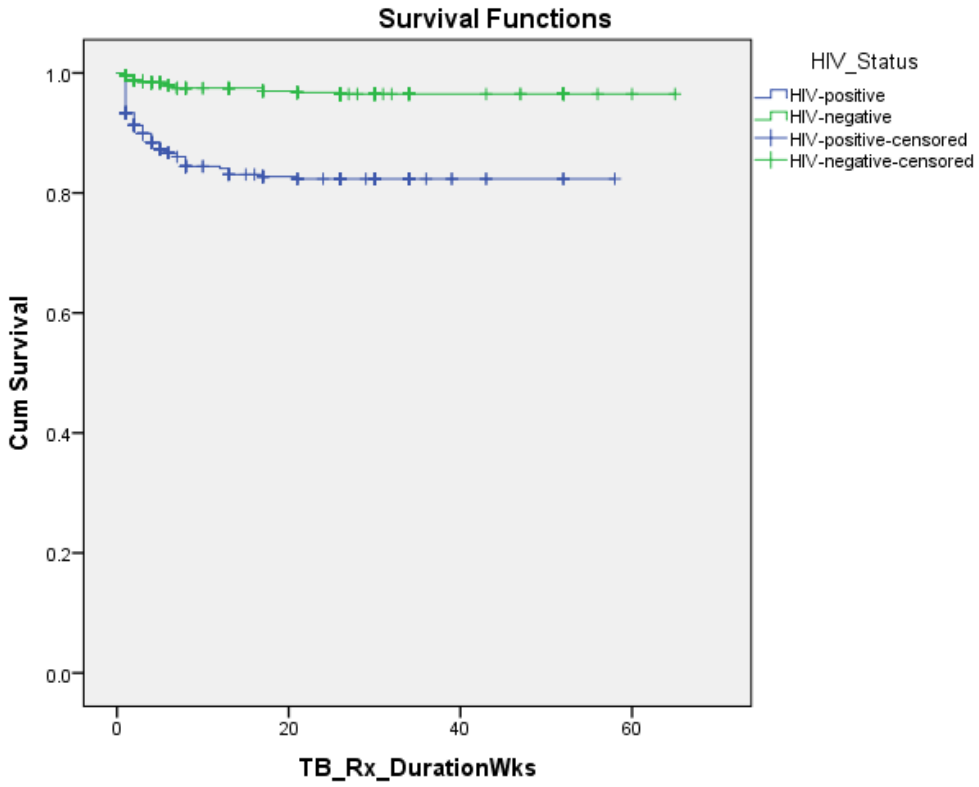
Figure 4.2. TB treatment outcome by HIV status at UATH, 2012 – 2016.



4.4.4 Kaplan-Meier Survival analysis for death by HIV status

Figure 4.3 describes the survival analysis for death by HIV status. There was a significant difference in the survival of HIV-negative and HIV-positive cases, throughout the course of treatment (Log Rank (p = 0.001)), with a higher death rate among HIV-positive patients. The majority of deaths occurred within the first 10 weeks of TB treatment.

Figure 4.3. Kaplan-Meier Survival analysis for death among TB patients by HIV status



Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	49.747	1	.000
Breslow (Generalized Wilcoxon)	51.295	1	.000
Tarone-Ware	50.742	1	.000

Test of equality of survival distributions for the different levels of HIV_Status.

4.4.5 Factors associated with treatment failure or death

Table 4.7 compares the characteristics of patients who died/failed treatment with those who had successful treatment. Patients who died/failed treatment were older (mean (SD) 37.8 (15.7) years) than patients with successful treatment (31.4 (15.3), p = 0.001). Patients who

died/failed treatment were more likely to be HIV-positive than patients with treatment success (OR, 95% CI: 5.6, 3.4 – 9.4, p = 0.001).

Patients who died/ failed treatment were more likely to be smear-negative (OR, 95% CI: 2.0, 1.1 – 3.8, p < 0.026), to be diagnosed by X-ray or Xpert MTB/RIF (p = 0.001) and to have lower weight gain at 2 and 5 months (-1.4 and 0.6 versus 2.1 and 4.6 Kgs, respectively, p = 0.008 and 0.057, respectively).

Table 4.7. Bivariable analysis of factors associated with death/failed TB treatment

Variable	**Category**	**Treatment outcome**		**Odds ratio (95% CI)**	**P**
		Death/ failure N = 91	**Success N = 619**		
Mean (SD) age (years)	All	37.8 (15.7)	31.4 (15.3)	6.4 (3.1 – 9.8)[a]	0.001
	Male	40.8 (15.9)	33.0 (16.2)	7.8 (3.1 – 12.5) [a]	0.001
	Female	33.7 (14.5)	29.2 (13.6)	4.5 (0.1 – 9.2) [a]	0.058
Age group*	0 – 9	6 (6.6)	60 (9.7)		0.001
	10 – 19	3 (3.3)	56 (9.1)		
	20 – 29	15 (16.5)	150 (24.2)		
	30 – 39	22 (24.2)	188 (30.4)		
	40 – 49	24 (26.4)	100 (16.2)		
	50 – 59	13 (14.3)	31 (5.0)		
	60 – 69	5 (5.5)	21 (3.4)		
	≥ 70	3 (3.3)	13 (2.1)		
Gender	Male	53 (58.2)	361 (58.3)	1.0 (0.6 – 1.6)	0.989
	Female	38 (41.8)	258 (41.7)		
Treatment category	New	91 (100.0)	600 (96.9)	Undefined	0.155
	Retreatment	0 (0.0)	19 (3.1)		
Anatomical site	Pulmonary	80 (87.9)	529 (85.5)	1.2 (0.6 – 2.4)	0.532
	EPTB	11 (12.1)	90 (14.5)		
Smear microscopy	Positive	21 (26.3)	262 (49.5)	1	
	Negative	24 (30.0)	148 (28.0)	2.0 (1.1 – 3.8)	0.026
	Not done	35 (43.8)	119 (22.5)	3.7 (2.0 – 6.6)	0.001
Treatment used	Category 1	87 (95.6)	589 (95.2)	1.1 (0.4 – 3.2)	0.851
	Category 2	4 (4.4)	30 (4.8)		
HIV status	Positive	70 (76.9)	230 (37.3)	5.6 (3.4 – 9.4)	0.001
	Negative	21 (23.1)	387 (62.7)		
Smear grade (n = 283)	Scanty	4 (19.0)	21 (8.0)	1	
	+	6 (28.6)	68 (26.0)	0.5 (0.1 – 1.7)	0.248
	++	4 (19.0)	96 (36.6)	0.2 (0.05 – 0.9)	0.042
	+++	7 (33.3)	77 (29.4)	0.5 (0.1 – 1.8)	0.272
Xpert grade (n = 142)	Very low	3 (13.6)	24 (20.0)	1	

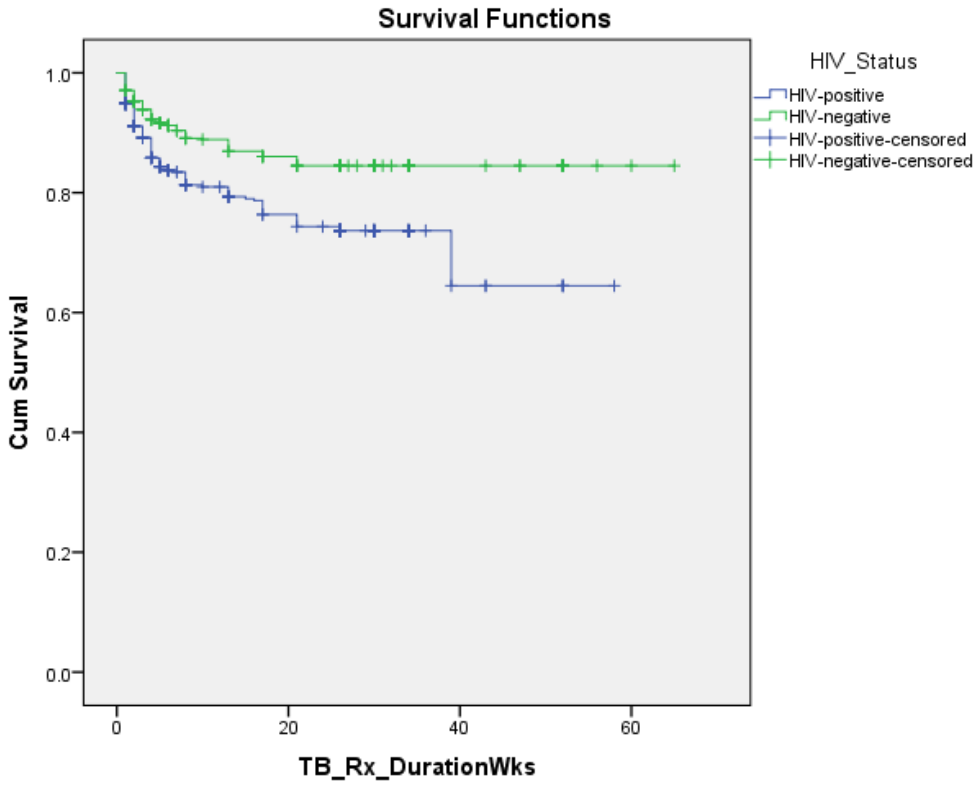
	Low	10 (45.5)	33 (27.5)	2.5 (0.6 – 10.1)	0.192
	Medium	8 (36.4)	38 (31.7)	2.0 (0.5 – 8.0)	0.341
	High	1 (4.5)	25 (20.8)	0.3 (0.0 – 3.4)	0.355
Mean (SD) weight	Baseline	49.8 (16.4)	50.3 (16.3)	-0.5 (-4.1 – 3.2) [a]	0.795
	2 months	55.8 (8.1)	52.6 (15.9)	3.2 (-6.2 – 12.6) [a]	0.505
	5 months	61.0 (6.0)	55.9 (16.1)	5.1 (-7.9 – 18.0) [a]	0.441
Weight difference	At 2 months	-1.4 (3.4)	2.1 (4.3)	-3.5 (-6.1 – -0.9) [a]	0.008
	At 5 months	0.6 (2.5)	4.6 (5.2)	-4.0 (-8.2 – 0.1) [a]	0.057

*n (%) unless otherwise stated. [a]Mean (95%CI). [b]Four patients without HIV status excluded; [c]Only sputum smear-positive included; [d]Three positive Xpert MTB patients had no MTB grading and were excluded

4.4.6 Kaplan-Meier Survival analysis for LTFU by HIV status

There was higher rate of LTFU among HIV-positive than HIV-negative patients throughout the course of TB treatment ($p < 0.001$) as shown in figure 4.4. In contrast to deaths, LTFU for HIV-positive patients continued to occur throughout the course of TB treatment.

Figure 4.4. Kaplan-Meier Survival curve analysis for LTFU among TB patients by HIV status



Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	15.899	1	.000
Breslow (Generalized Wilcoxon)	14.817	1	.000
Tarone-Ware	15.156	1	.000

Test of equality of survival distributions for the different levels of HIV Status.

4.4.7 Risk factors for LTFU

Patients LTFU were also more likely to be HIV-positive (p = 0.001) and to be older (mean (SD) age 34.8 (17.4)) than patients with successful treatment (31.4 (15.3), p = 0.017). Males LTFU were significantly older than males with successful treatment (38.3 (17.5) versus 33.0 (16.2), p = 0.005). However, women LTFU and with successful treatment had similar age (30.6 (16.5) versus 29.2 (13.6) years, respectively, p = 0.448). Patients LTFU were more likely to be smear-negative (p = 0.07), not to have smear microscopy and to be diagnosed by X-rays or GeneXpert (p = 0.001). The TB treatment category (new or retreatment), anatomical site and treatment used, were not associated with LTFU.

The mean (SD) weight at 2 months of treatment for LTFU patients was significantly lower (45.5 (16.6) Kgs) than for patients with successful treatment (52.6 (15.9), p = 0.03), although there was not significant weight change at 2 or 5 months, between the groups.

Table 4.8. Bivariable analysis of risk factors for LTFU

Variable	Category	Treatment outcome		Odds ratios (95% CI)	P
		Loss-to-follow up N= 180	Successful N= 619		
Mean (SD) age (years)	All	34.8 (17.4)	31.4 (15.3)	3.4 (0.6 – 6.3)[a]	0.017
	Male	38.3 (17.5)	33.0 (16.2)	5.3 (1.6 – 8.9) [a]	0.005
	Female	30.6 (16.5)	29.2 (13.6)	1.4 (-2.2 – 5.0) [a]	0.448
Age group*	0 – 9	19 (10.6)	60 (9.7)		0.022
	10 – 19	9 (5.0)	56 (9.1)		
	20 – 29	35 (19.4)	150 (24.2)		
	30 – 39	44 (24.4)	188 (30.4)		
	40 – 49	43 (23.9)	100 (16.2)		
	50 – 59	15 (8.3)	31 (5.0)		
	60 – 69	7 (3.9)	21 (3.4)		
	≥ 70	8 (4,4)	13 (2.1)		
Gender	Male	100 (55.6)	361 (58.3)	0.9 (0.6 – 1.2)	0.509
	Female	80 (44.4)	258 (41.7)		
Treatment category	New	174 (96.7)	600 (96.9)	0.9 (0.41 – 2.3)	0.858
	Retreatment	6 (3.3)	19 (3.1)		
Anatomical site	Pulmonary	155 (86.1)	529 (85.5)	1.1 (0.7 – 1.7)	0.827
	EPTB	25 (13.9)	90 (14.5)		
Type of PTB	SM-positive	43 (27.7)	262 (49.5)	1	
	SM-negative	38 (24.5)	148 (28.0)	1.6 (1.0 – 2.5)	0.068
	Not done	74 (47.7)	119 (22.5)	3.8 (2.5 – 5.8)	0.001
Treatment used	Category 1	169 (93.9)	589 (95.2)	0.8 (0.4 – 1.6)	0.499
	Category 2	11 (6.1)	30 (4.8)		
HIV status (n = 795)[b]	Positive	99 (55.6)	230 (37.3)	2.1 (1.5 – 3.0)	0.001
	Negative	79 (44.4)	387 (62.7)		
Smear grade (n=305)[c]	Scanty	7 (16.3)	21 (8.0)	2.6 (0.9 – 7.6)	0.087
	+	8 (18.6)	68 (26.0)	0.9 (0.3 – 2.4)	0.799
	++	18 (41.9)	96 (36.6)	1.4 (0.6 – 3.3)	0.385
	+++	10 (23.3)	77 (29.4)	1	
Xpert MTB/RIF (n = 149)[d]	Very low	4 (13.8)	24 (20.0)	1.0 (0.2 – 4.6)	0.957
	Low	11 (37.9)	33 (27.5)	2.1 (0.6 – 7.3)	0.252
	Medium	10 (34.5)	38 (31.7)	1.6 (0.5 – 5.8)	0.441
	High	4 (13.8)	25 (20.8)	1	
Mean (SD) weight (Kgs)	Baseline	48.1 (17.0)	50.3 (16.3)	-2.2 (-5.0 – 0.6) [a]	0.125
	After 2 months	45.5 (16.6)	52.6 (15.9)	-7.1 (-13.4 – -0.8) [a]	0.027
	After 5 months	54.0 (0.0)	55.9 (16.1)	-1.9 (-33.6 – 29.7) [a]	0.905
Weight difference	At 2 months	1.4 (5.5)	2.1 (4.3)	-0.7 (-2.5 – 1.1) [a]	0.429
	At 5 months	3.0 (0.0)	4.6 (5.2)	-1.6 (-11.8 – 8.6) [a]	0.753

*n (%) unless otherwise stated. [a]Mean (95%CI). [b]Four patients without HIV status excluded; [c]Only sputum smear-positive included; [d]Three positive Xpert MTB patients had no MTB grading and were excluded,
SM = Smear Microscopy

4.4.8 Factors associated with death or treatment failure among HIV-positive patients with TB

HIV was a key factor associated with death and treatment failure. Therefore, we conducted a further analysis of risk factors for death/treatment failure among this subgroup. HIV-positive patients who died/failed treatment were older (p = 0.016) and less likely to receive Co-trimoxazole preventive therapy (CPT) than HIV-positive patients with TB treatment success (p = 0.002). There was no significant association between death/treatment failure and gender, treatment category, anatomical site affected by TB, smear-positivity, smear grade, treatment used, Xpert MTB grade or ART ("Not on ART" versus "On ART", p = 0.794). The average body weight on enrolment, 2 or 5 months follow up, was not different between HIV-positive patients with death/treatment failure and successful treatment.

Table 4.9. Bivariable analysis of factors associated with death or treatment failure among HIV-positive TB patients

Variable	Category	Treatment outcome		Odds ratio (95% CI)	P
		Death/Failure N = 70 (%)	Success N= 230 (%)		
Mean (SD) age (years)	All	37.8 (15.6)	33.1 (13.8)	4.7 (0.9 – 8.5) [a]	0.016
	Male	40.6 (16.3)	36.3 (15.5)	4.3 (-1.4 – 10.0) [a]	0.136
	Female	33.9 (13.9)	30.0 (11.0)	3.9 (-0.9 – 8.9) [a]	0.107
Age group (years)	0 – 9	5 (7.1)	21 (9.1)		0.118
	10 – 19	3 (4.3)	14 (6.1)		
	20 – 29	7 (10.0)	34 (14.8)		
	30 – 39	20 (28.6)	87 (37.8)		
	40 – 49	20 (28.6)	55 (23.9)		
	50 – 59	9 (12.9)	12 (5.2)		
	60 – 69	4 (5.7)	5 (2.2)		
	≥ 70	2 (2.9)	2 (0.9)		
Gender	Male	41 (58.6)	114 (49.6)	1.4 (0.8 – 2.5)	0.187
	Female	29 (41.4)	116 (50.4)		
Treatment category	New	70 (100.0)	222 (96.5)	Undefined	0.205
	Retreatment	0 (0.0)	8 (3.5)		
Anatomical site of TB	Pulmonary	64 (91.4)	208 (90.4)	1.1 (0.3 – 2.9)	0.802
	Extrapulmonary	6 (8.6)	22 (9.6)		
Type of PTB	Smear-positive	15 (23.4)	57 (27.4)	**1**	
	Smear-negative	19 (29.7)	83 (39.9)	0.9 (0.4 – 1.9)	0.718
	Smear not done	30 (46.9)	68 (32.7)	1.7 (0.8 – 3.4)	0.155
Treatment used	Category 1	66 (94.3)	216 (93.9)	1.1 (0.3 – 3.4)	0.908
	Category 2	4 (5.7)	14 (6.1)		

Smear grade (n = 73)[b]	Scanty	3 (20.0)	5 (8.6)	1	
	+	5 (33.3)	15 (25.9)	0.6 (0.1 – 3.2)	0.511
	++	2 (13.3)	20 (34.5)	0.2 (0.02 – 1.3)	0.085
	+++	5 (33.3)	18 (31.0)	0.5 (0.1 – 2.6)	0.386
Xpert MTB/RIF (n = 71)[c]	Very low/Low	11 (57.9)	29 (55.8)	1.1 (0.4 – 3.2)	0.873
	Medium/High	8 (42.1)	23 (44.2)	1	
ART status	On ART	48 (68.6)	153 (66.5)	1	
	Not on ART	20 (28.6)	69 (30.0)	0-9 (0.5 – 1.7)	0-794
	Unknown	2 (2.9)	8 (3.5)	0.8 (0.2 – 3.9)	0.779
CPT status	On CPT	34 (48.6)	158 (68.7)	1	
	Not on CPT	30 (42.9)	56 (24.3)	2.5 (1.4 – 4.4)	0.002
	Unknown	6 (8.6)	16 (7.0)	1.7 (0.6 – 4.8)	0.281
Mean [SD] weight (kg)	At baseline	48.1 (16.2)	48.9 (16.5)	-0.711 (-5.2 – 3.8) [a]	0.757
	At 2 months	58.3 (10.4)	50.1 (16.7)	7.3 (-11.9 – 26.4) [a]	0.455
	At 5 months	64.0 (0.0)	54.2 (17.1)	17.1 (-24.1 – 43.7) [a]	0.569
Mean [SD] weight difference (kg)	At 2 months	0.0 (6.1)	1.8 (5.5)	-1.8 (-8.1 – 4.5) [a]	0.570
	At 5 months	-3.0 (0.0)	4.7 (5.7)	-7.7 (-19.1 – 3.6) [a]	0.180

[a]Mean difference and its 95% Confidence Interval. [b]Only sputum smear-positive patients were included.
[c]Two patients with positive Xpert MTB but no grading for MTB detection, were excluded.
CPT = Co-trimoxazole preventive therapy. Xpert = Xpert MTB/RIF

4.4.9 Factors associated with death/treatment failure among HIV-negative TB patients.

For completion, table 4.10 also shows the risk factors associated with death/treatment failure among HIV-negative patients. Males who died/failed treatment were older than males with treatment success (41.5 (15.2) versus 31.5 (16.4), p = 0.039) but this age difference was not observed in females. Patients who died/failed treatment were similar to patients with successful treatment with respect to gender, treatment category, anatomical site of TB, treatment used and proportion with Rifampicin resistance. The mean (SD) weight change at 2 months after commencement of TB treatment was lower for patients who died/failed treatment compared to patients with successful treatment (-1.9 (2.2) versus 2.2 (3.4), p = 0.001).

Table 4.10. Bivariable analysis of risk factors for death/treatment failure among HIV-negative TB patients

Variable	Category	Treatment outcome		Odds ratio (95% CI)	P
		Death/Failure N = 21 (%)	Success N= 387 (%)		
Mean (SD) age (years)	All	38.0 (16.3)	30.3 (16.0)	7.7 (0.6 – 14.7) [a]	0.033
	Male	41.5 (15.2)	31.5 (16.4)	10.0 (0.5 – 19.6) [a]	0.039
	Female	33.2 (17.3)	28.3 (15.1)	5.0 (-5.4 – 15.3) [a]	0.346
Age group (years)	0 – 9	1 (4.8)	39 (10.1)		0.039
	10 – 19	0 (0.0)	42 (10.9)		
	20 – 29	8 (38.1)	116 (30.0)		
	30 – 39	2 (9.5)	100 (25.8)		
	40 – 49	4 (19.0)	45 (11.6)		
	50 – 59	4 (19.0)	19 (4.9)		
	60 – 69	1 (4.8)	15 (3.9)		
	≥ 70	1 (4.8)	11 (2.8)		
Gender	Male	12 (57.1)	246 (63.6)	0.8 (0.3 – 1.9)	0.552
	Female	9 (42.9)	141 (36.4)		
Treatment category	New	21 (100.0)	376 (97.2)	Undefined	1.000
	Retreatment	0 (0.0)	11 (2.8)		
Anatomical site of TB	Pulmonary	16 (76.2)	320 (82.7)	0.7 (0.2 – 1.9)	0.447
	Extrapulmonary	5 (23.8)	67 (17.3)		
Type of PTB	Smear-positive	6 (37.5)	205 (64.1)	1	
	Smear-negative	5 (31.3)	64 (20.0)	2.7 (0.8 – 9.0)	0.115
	Smear not done	5 (31.3)	51 (15.9)	3.4 (0.98 – 11.4)	0.053
Treatment used	Category 1	21 (100.0)	371 (95.9)	Undefined	1.000
	Category 2	0 (0.0)	16 (4.1)		
Smear grade (n =212)[b]	Scanty	1 (16.7)	16 (7.8)	1	
	+	1 (16.7)	55 (26.7)	0.3 (0.02 – 4.9)	0.392
	++	2 (33.3)	76 (36.9)	0.4 (0.04 – 4.9)	0.491
	+++	2 (33.3)	59 (28.6)	0.5 (0.05 – 6.4)	0.626
Xpert MTB/RIF (n = 71)[c]	Very low/Low	2 (66.7)	28 (41.2)	2.9 (0.3 – 33.0)	0.570
	Medium/High	1 (33.3)	40 (58.8)		
Mean (SD) weight (kg)	At baseline	55.0 (16.5)	51.1 (16.1)	3.9 (-3.2 – 11.0) [a]	0.280
	At 2 months	54.9 (7.6)	53.5 (15.3)	1.4 (-9.3 – 12.0) [a]	0.804
	At 5 months	60.4 (6.5)	56.9 (15.4)	3.5 (-10.1 – 17.1) [a]	0.615
Mean (SD) weight difference (kg)	At 2 months	-1.9 (2.2)	2.2 (3.4)	-4.2 (-6.5 – -1.8) [a]	0.001
	At 5 months	1.3 (2.0)	4.6 (4.8)	-3.3 (-7.5 – 1.0) [a]	0.134

[a]Mean difference and its 95% Confidence Interval, [b]Sputum smear-positive PTB cases plus an EPTB case, which was also smear-positive were included, [c]A patient with positive Xpert MTB but no grading for MTB detection, was excluded

4.4.10 Risk factors for LTFU among HIV-positive TB patients treated at UATH

HIV-positive patients LTFU had similar age, gender, treatment category, anatomical site of TB, treatment used and Xpert MTB grade compared to patients with successful treatment, as

shown in table 4.11. HIV-positive patients LTFU were more likely to have missed CPT (p = 0.038) than patients with treatment success. The mean (SD) weight of patients LTFU was lower than for patients with treatment success on enrolment (44.1 (17.1) versus 48.9 (16.5), p = 0.023). Nevertheless, there was not significant weight change at 2 months, between the two groups.

Table 4.11. Bivariable analysis of risk factors for LTFU among HIV-positive TB patients

Variable		Treatment outcome		Odds ratio (95% CI)	P
		LTFU N = 99 (%)	Success N= 230 (%)		
Mean (SD) age (years)	All	32.9 (15.6)	33.1 (13.8)	-0.2 (-3.6 – 3.2) [a]	0.900
	Male	36.2 (16.0)	36.3 (15.5)	-0.1 (-5.4 – 5.2) [a]	0.973
	Female	29.8 (14.6)	30.0 (11.0)	-0.2 (-4.3 – 3.9) [a]	0.921
Age group (years)	0 – 9	14 (14.1)	21 (9.1)		0.692
	10 – 19	3 (3.0)	14 (6.1)		
	20 – 29	15 (15.2)	34 (14.8)		
	30 – 39	30 (30.3)	87 (37.8)		
	40 – 49	27 (27.3)	55 (23.9)		
	50 – 59	7 (7.1)	12 (5.2)		
	60 – 69	2 (2.0)	5 (2.2)		
	≥ 70	1 (1.0)	2 (0.9)		
Gender	Male	48 (48.5)	114 (49.6)	1.0 (0.6 – 1.5)	0.857
	Female	51 (51.5)	116 (50.4)		
Treatment category	New	94 (94.9)	222 (96.5)	0.7 (0.2 – 2.1)	0.502
	Retreatment	5 (5.1)	8 (3.5)		
Anatomical site of TB	Pulmonary	90 (90.9)	208 (90.4)	1.1 (0.5 – 2.4)	0.893
	Extrapulmonary	9 (9.1)	22 (9.6)		
Type of PTB	Smear-positive	15 (16.7)	57 (27.4)	1	
	Smear-negative	31 (34.4)	83 (39.9)	1.4 (0.7 – 2.9)	0.329
	Smear not done	44 (48.9)	68 (32.7)	2.5 (1.2 – 4.9)	0.010
Treatment used	Category 1	97 (98.0)	216 (93.9)	3.1 (0.7 – 14.1)	0.116
	Category 2	2 (2.0)	14 (6.1)		
Smear grade (n =73)[b]	Scanty	4 (26.7)	5 (8.6)	1	
	+	2 (13.3)	15 (25.9)	0.2 (0.0 – 1.2)	0.076
	++	5 (33.3)	20 (34.5)	0.3 (0.1 – 1.6)	0.164
	+++	4 (26.7)	18 (31.0)	0.3 (0.1 – 1.5)	0.141
Xpert MTB/RIF (n = 73)[c]	Very low	11 (52.3)	29 (55.8)	0.9 (0.3 – 2.4)	0.792
	Medium/High	10 (47.6)	23 (44.2)	1	
ART status	On ART	60 (60.6)	153 (66.5)	1	
	Not on ART	31 (31.3)	69 (30.0)	1.2 (0.7 – 1.9)	0.607
	Unknown	8 (8.1)	8 (3.5)	2.6 (0.9 – 7.1)	0.073
CPT status	On CPT	55 (55.6)	158 (68.7)	1	

	Not on CPT	34 (34.3)	56 (24.3)	1.7 (1.0 – 2.9)	0.038
	Unknown	10 (10.1)	16 (7.0)	1.8 (0.8 – 4.2)	0.176
Mean (SD) weight (kg)	At baseline	44.1 (17.1)	48.9 (16.5)	-4.7 (-8.8 – -0.7) [a]	0.023
	At 2 months	36.6 (15.6)	50.1 (16.7)	-14.5 (-23.4 – -5.7) [a]	0.001
	At 5 months	----------	54.2 (17.1)	----------	
Mean (SD) weight difference (kg)	At 2 months	0.8 (7.0)	1.8 (5.5)	-1.000 (-4.1 – 2.1) [a]	0.521
	At 5 months	----------	4.7 (5.7)		

[a]Mean difference and its 95% Confidence Interval. LTFU = Loss-to-follow up. [b]In addition to the 72 smear-positive PTB, an EPTB was also smear-positive and included. [c]Two patients with positive Xpert MTB but no grading for MTB detection, were excluded. ----- data not available. CPT = Co-trimoxazole preventive therapy

4.4.11 Risk factors for LTFU among HIV-negative TB patients treated at UATH

HIV-negative patients LTFU were older (mean (SD) age (37.8 (19.3) versus 30.3 (16.0), p = 0.001) and less likely to be on treatment category 1 than patients with successful treatment (p = 0.009). Patients LTFU had similar gender, treatment category, anatomical site of TB, and Xpert MTB grade to patients who completed treatment.

Table 12. Bivariable analysis of risk factors for LTFU among HIV-negative TB patients treated UATH

Variable	**Category**	**Treatment outcome**		**Odds ratio (95% CI)**	**P**
		LTFU N = 79 n (%)	**Success N = 387 n (%)**		
Mean (SD) age (years)	All	37.8 (19.3)	30.3 (16.0)	7.4 (3.4 – 11.4) [a]	0.001
	Male	40.4 (18.9)	31.5 (16.4)	9.0 (3.9 – 14.1) [a]	0.001
	Female	32.7 (19.5)	28.3 (15.1)	4.4 (-2.1 – 10.9) [a]	0.182
Age group (years)	0 – 9	5 (6.3)	39 (10.1)		0.009
	10 – 19	5 (6.3)	42 (10.9)		
	20 – 29	19 (24.1)	116 (30.0)		
	30 – 39	14 (17.7)	100 (25.8)		
	40 – 49	16 (20.3)	45 (11.6)		
	50 – 59	8 (10.1)	19 (4.9)		
	60 – 69	5 (6.3)	15 (3.9)		
	≥ 70	7 (8.9)	11 (2.8)		
Gender	Male	51(64.6)	246 (63.6)	1.0 (0.6 – 1.7)	0.867
	Female	28 (35.4)	141 (36.4)		
Treatment category	New cases	78 (98.7)	376 (97.2)	2.3 (0.3 – 17.9)	0.700
	Retreatment	1 (1.3)	11 (2.8)		
Anatomical site of TB	Pulmonary	63 (79.7)	320 (82.7)	0.8 (0.5 – 1.5)	0.534
	Extrapulmonary	16 (20.3)	67 (17.3)		
Type of PTB	Smear-positive	28 (44.4)	205 (64.1)	1	
	Smear-negative	6 (9.5)	64 (20.0)	0.7 (0.3 – 1.7)	0.425
	Smear not done	29 (46.0)	51 (15.9)	4.2 (2.3 – 7.6)	0.001

Treatment used	Category 1	70 (88.6)	371 (95.9)	0.3 (0.1 – 0.8)	0.009
	Category 2	9 (11.4)	16 (4.1)		
Smear grade (n = 234)[b]	Scanty	3 (10.7)	16 (7.8)	1	
	+	6 (21.4)	55 (26.7)	0.6 (0.1 – 2.6)	0.477
	++	13 (46.4)	76 (36.9)	0.9 (0.2 – 3.6)	0.895
	+++	6 (21.4)	59 (28.6)	0.5 (0.1 – 2.4)	0.422
Xpert MTB/RIF (n = 76)[c]	Very low/Low	4 (50.0)	28 (41.2)	1.4 (0.3 – 6.2)	0.714
	Medium/High	4 (50.0)	40 (58.8)		
Mean [SD] weight (kg)	Baseline	53.3 (15.4)	51.1 (16.1)	2.2 (-1.8 – 6.2) [a]	0.283
	At 2 months	58.0 (8.5)	53.5 (15.3)	4.5 (-5.1 – 14.1) [a]	0.359
	At 5 months	54.0 (0.0)	56.9 (15.4)	-2-9(-33.3 – 27.4) [a]	0.850
Mean (SD) weight difference (kg)	At 2 months	2.1 (2.1)	2.2 (3.4)	-0.1 (-2.2 – 2.0) [a]	0.913
	At 5 months	3.0 (0.0)	4.6 (4.8)	-1.6 (-11.1 – 8.0) [a]	0.748

[a]Mean difference and its 95% Confidence Interval. LTFU = Loss-to-follow up

[b] Sputum smear-positive PTB cases plus an EPTB case, which was also smear-positive were included

[c]A patients with positive Xpert MTB but no grading for MTB detection, were excluded

4.4.12 Multivariable analysis of risk factors for death/failed treatment

Variables with a P values ≤ 0.2 were included in the multivariable analysis and adjusted for age, gender and HIV status as shown in table 4.13. After adjustment, patients who died/failed treatment were older (AOR (95% CI) = 1.03 (1.01 – 1.04), p = 0.003), were 5 times more likely to be HIV-positive (AOR (95% CI) = 5.4 (3.2 – 9.1), p = 0.001) and to have a lower weight difference at 2 and 5 months follow up than patients with successful treatment (AOR (95% CI) = 0.8 (0.7 – 0.9), p = 0.005 and 0.8 (0.7 – 0.9), p = 0.001).

4.4.13 Multivariable analysis of risk factors for LTFU

After adjusting for age, gender and HIV status, patients LTFU were older (AOR (95% CI) = 1.02 (1.00 – 1.03), p = 0.015), more likely to be diagnosed by Xpert MTB/RIF or Chest X-rays (AOR (95% CI) = 3.2 (2.0 – 5.1), p = 0.001) and 2 times more likely to be HIV-positive (AOR (95% CI) = 2.1 (1.5 – 2.9), p = 0.001) and to have lower weight at baseline (AOR (95% CI) = 0.98 (0.97 – 0.99), p = 0.003) and 2 months (AOR (95% CI) = 0.96 (0.94 – 0.99), p = 0.003) than patients with treatment success.

Table 4.13. Multivariable analysis of factors associated with death/failed treatment among TB patients, UATH 2012 - 2016

Variable	Category	Treatment outcome		OR (95% CI)	P	AOR (95% CI)	P
		Death/failure N = 91 (%)	Success N = 619 (%)				
Age (years)	Mean (SD)	37.8 (15.7)	31.4 (15.3)	6.4 (3.1 – 9.8)[a]	0.001	1.03 (1.01 – 1.04)	**0.003**
Gender	Male	53 (58.2)	361 (58.3)	1.0 (0.6 – 1.6)	0.989	1.0 (0.6 – 1.7)	0.869
	Female	38 (41.8)	258 (41.7)	1			
Smear microscopy	Smear-positive	21 (26.3)	262 (49.5)	1		1	
	Smear-negative	24 (30.0)	148 (28.0)	2.0 (1.1 – 3.8)	0.026	1.1 (0.6 – 2.2)	0.707
	Not done	35 (43.8)	119 (22.5)	3.7 (2.0 – 6.6)	0.001	2.4 (1.3 – 4.6)	**0.006**
HIV status	Positive	70 (76.9)	230 (37.3)	5.6 (3.4 – 9.4)	0.001	5.4 (3.2 – 9.1)	**0.001**
	Negative	21 (23.1)	387 (62.7)	1			
Smear grade (n = 283)	Scanty	4 (19.0)	21 (8.0)	1		1	
	+	6 (28.6)	68 (26.0)	0.5 (0.1 – 1.7)	0.248	0.5 (0.1 – 1.9)	0.291
	++	4 (19.0)	96 (36.6)	0.2 (0.05 – 0.9)	0.042	0.3 (0.1 – 1.3)	0.094
	+++	7 (33.3)	77 (29.4)	0.5 (0.1 – 1.8)	0.272	0.5 (0.1 – 2.0)	0.300
Xpert grade (n = 142)	Very low	3 (13.6)	24 (20.0)	1		1	
	Low	10 (45.5)	33 (27.5)	2.5 (0.6 – 10.1)	0.192	3.1 (0.6 – 15.7)	0.165
	Medium	8 (36.4)	38 (31.7)	2.0 (0.5 – 8.0)	0.341	2.5 (0.5 – 12.2)	0.264
	High	1 (4.5)	25 (20.8)	0.3 (0.0 – 3.4)	0.355	0.5 (0.0 – 5.7)	0.556
Weight difference	At 2 months	-1.4 (3.4)	2.1 (4.3)	-3.5 (-6.1 – -0.9) [a]	0.008	0.8 (0.7 – 0.9)	**0.005**
	At 5 months	0.6 (2.5)	4.6 (5.2)	-4.0 (-8.2 – 0.1) [a]	0.057	0.8 (0.7 – 0.9)	**0.001**

*AOR = Adjusted odds ratio. n (%) unless otherwise stated. [a]Mean (95%CI). [b]Four patients without HIV status excluded; [c]Only sputum smear-positive included.
[d]Three positive Xpert MTB patients had no MTB grading and were excluded

4.4.14 Multivariable analysis of risk factors for death/treatment failure by HIV status

The type of PTB and use of CPT were predictors of death/treatment failure among HIV-positive patients, as shown in table 4.15. Patients with death/treatment failure were older (AOR (95% CI) = 1.02 (1.00 – 1.05), p = 0.057) and 2 times more likely to have Xpert MTB/RIF or a chest X-ray (AOR (95% CI) = 2.1 (1.0 – 4.2), p = 0.047) than patients with treatment success. Patients who died/failed treatment were 2.5 times less likely to have received CPT (AOR (95% CI) = 2.5 (1.4 – 4.4), p = 0.003) than patients with treatment success.

Age, the type of PTB and weight at 2 and 5 months were predictors of death/treatment failure among HIV-negative patients (table 4.16). Patients who died/failed treatment were older (AOR (95% CI) = 1.03 (1.01 – 1.05), p = 0.014), 2 times more likely to have smear-negative PTB (AOR (95% CI) = 2.4 (0.7 – 7.5), p = 0.146) or 3 times more likely to have PTB diagnosed by Xpert MTB/RIF or chest X-rays (AOR (95% CI) = 3.4 (1.0 – 11.6), p = 0.050), than patients with treatment success. Patients who died/failed treatment have lower body weight at 2 (AOR (95% CI) = 0.7 (0.6 – 0.8), p = 0.001) and 5 months (AOR (95% CI) = 0.9 (0.8 – 1.0), p = 0.004) than patients with treatment success.

4.4.15 Multivariable analysis of risk factors for LTFU by HIV status

After adjusting for age and gender, the variables that remained significant at predicting whether HIV-positive patient were LTFU or successfully treated were; the type of TB, use of CPT, baseline weight, and weight at 2 months. Patients LTFU were more likely to have smear-negative TB (AOR (95% CI) = 1.4 (0.7 – 2.9), p = 0.325) and the diagnosis being based on Xpert MTB/RIF or chest X-rays (AOR (95% CI) = 2.5 (1.3 – 5.1), p = 0.009) than patients with treatment success, as shown in table 4.17. Patients not on CPT (AOR (95% CI) = 1.7 (1.0 – 2.9), p = 0.037) and with a low weight at baseline (AOR (95% CI) = 0.97 (0.95 –

0.98), p = 0.001) and 2 months (AOR (95% CI) = 0.94 (0.88 – 0.99), p = 0.032) were more likely to be LTFU than patients with treatment success.

Adjustment was also made for age and gender for HIV-negative patients. After multivariable analysis age, type of TB and treatment used were statistically significant (table 4.18). Patients LTFU were older (AOR (95% CI) = 1.03 (1.01 – 1.04), p = 0.001) and more likely to have a diagnosis based on MTB/RIF or chest X-ray (AOR (95% CI) = 4.3 (2.3 – 7.9), p = 0.001), than successfully treated patients. However, patients with LTFU were less likely to be treated with category 1 anti-TB regimen (AOR (95% CI) = 0.4 (0.2 – 0.9), p = 0.036).

Table 4.14. Multivariable analysis of risk factors for LTFU patients treated at UATH between 2012 and 2016

Variable		Treatment outcome		OR (95% CI)	P	AOR (95% CI)	P
		LTFU N= 180 (%)	Successful N= 619 (%)				
Age (years)	Mean (SD)	34.8 (17.4)	31.4 (15.3)	3.4 (0.6 – 6.3)[a]	0.017	1.02 (1.00 – 1.03)	**0.015**
Gender	Male	100 (55.6)	361 (58.3)	0.9 (0.6 – 1.2)	0.509	0.9 (0.6 – 1.3)	0.637
	Female	80 (44.4)	258 (41.7)	1		1	
Type of PTB	Smear-positive	43 (27.7)	262 (49.5)	1		1	
	Smear-negative	38 (24.5)	148 (28.0)	1.6 (1.0 – 2.5)	0.068	1.2 (0.7 – 2.0)	0.507
	Not done	74 (47.7)	119 (22.5)	3.8 (2.5 – 5.8)	0.001	3.2 (2.0 – 5.1)	**0.001**
HIV status (n = 795)[b]	Positive	99 (55.6)	230 (37.3)	2.1 (1.5 – 3.0)	0.001	2.1 (1.5 – 2.9)	**0.001**
	Negative	79 (44.4)	387 (62.7)	1			
Smear grade (n = 305)[c]	Scanty	7 (16.3)	21 (8.0)	2.6 (0.9 – 7.6)	0.087	2.3 (0.8 – 6.8)	0.125
	+	8 (18.6)	68 (26.0)	0.9 (0.3 – 2.4)	0.799	0.8 (0.3 – 2.2)	0.692
	++	18 (41.9)	96 (36.6)	1.4 (0.6 – 3.3)	0.385	1.5 (0.7 – 3.6)	0.329
	+++	10 (23.3)	77 (29.4)	1		1	
Mean (SD) weight (Kgs)	Baseline	48.1 (17.0)	50.3 (16.3)	-2.2 (-5.0 – 0.6) [a]	0.125	0.98 (0.97 – 0.99)	**0.003**
	After 2 months	45.5 (16.6)	52.6 (15.9)	-7.1 (-13.4 – -0.8) [a]	0.027	0.96 (0.94 – 0.99)	**0.003**

*n (%) unless otherwise stated. [a]Mean (95%CI). [b]Four patients without HIV status excluded; [c]Only sputum smear-positive included; [d]Three positive Xpert MTB patients had no MTB grading and were excluded

Table 4.15. Multivariable analysis of factors associated with death or treatment failure among HIV-positive TB patients

Variable	Category	Treatment outcome		OR (95% CI)	P	AOR (95% CI)	P
		Death/Failure N = 70 (%)	Success N= 230 (%)				
Age (years)	Mean (SD)	37.8 (15.6)	33.1 (13.8)	4.7 (0.9 – 8.5) [a]	0.016	1.02 (1.00 – 1.05)	0.057
Gender	Male	41 (58.6)	114 (49.6)	1.4 (0.8 – 2.5)	0.187	1.2 (0.7 – 2.2)	0.479
	Female	29 (41.4)	116 (50.4)	1		1	
Type of PTB	Smear-positive	15 (23.4)	57 (27.4)	1		1	
	Smear-negative	19 (29.7)	83 (39.9)	0.9 (0.4 – 1.9)	0.718	0.9 (0.4 – 1.9)	0.727
	Smear not done	30 (46.9)	68 (32.7)	1.7 (0.8 – 3.4)	0.155	2.1 (1.0 – 4.2)	**0.047**
Smear grade (n = 73)[b]	Scanty	3 (20.0)	5 (8.6)	1		1	
	+	5 (33.3)	15 (25.9)	0.6 (0.1 – 3.2)	0.511	0.7 (0.1 – 3.9)	0.661
	++	2 (13.3)	20 (34.5)	0.2 (0.02 – 1.3)	0.085	0.2 (0.0 – 1.6)	0.135
	+++	5 (33.3)	18 (31.0)	0.5 (0.1 – 2.6)	0.386	0.4 (0.1 – 2.5)	0.303
Xpert MTB/RIF (n = 71)[c]	Very low/Low	11 (57.9)	29 (55.8)	1.1 (0.4 – 3.2)	0.873	1.1 (0.4 – 3.1)	0.919
	Medium/High	8 (42.1)	23 (44.2)	1		1	
CPT status	On CPT	34 (48.6)	158 (68.7)	1		1	
	Not on CPT	30 (42.9)	56 (24.3)	2.5 (1.4 – 4.4)	0.002	2.5 (1.4 – 4.4)	**0.003**
	Unknown	6 (8.6)	16 (7.0)	1.7 (0.6 – 4.8)	0.281	1.8 (0.7 – 5.1)	0.253

[a]Mean difference and its 95% Confidence Interval. [b]Only sputum smear-positive patients were included. [c]Two patients with positive Xpert MTB but no grading for MTB detection, were excluded. CPT = Co-trimoxazole preventive therapy. Xpert = Xpert MTB/RIF

Table 4.16. Multivariable analysis of factors associated with death or treatment failure among HIV-negative TB patients treated UATH between 2012 and 2016

Variable	Category	Treatment outcome		OR (95% CI)	P	AOR (95% CI)	P
		Death/Failure N = 21 (%)	Success N= 387 (%)				
Age (years)	Mean (SD)	38.0 (16.3)	30.3 (16.0)	7.7 (0.6 – 14.7) [a]	0.033	1.03(1.01 – 1.05)	**0.014**
Gender	Male	12 (57.1)	246 (63.6)	0.8 (0.3 – 1.9)	0.552	0.7 (0.3 – 1.7)	0.404
	Female	9 (42.9)	141 (36.4)	1		1	
Type of PTB	Smear-positive	6 (37.5)	205 (64.1)	1		1	
	Smear-negative	5 (31.3)	64 (20.0)	2.7 (0.8 – 9.0)	0.115	2.4 (0.7 – 7.5)	0.146
	Smear not done	5 (31.3)	51 (15.9)	3.4 (0.98 – 11.4)	0.053	3.4 (1.0 – 11.6)	**0.050**
Mean (SD) weight difference (kg)	At 2 months	-1.9 (2.2)	2.2 (3.4)	-4.2 (-6.5 – -1.8) [a]	0.001	0.7 (0.6 – 0.8)	**0.001**
	At 5 months	1.3 (2.0)	4.6 (4.8)	-3.3 (-7.5 – 1.0) [a]	0.134	0.9 (0.8 – 1.0)	**0.004**

[a]Mean difference and its 95% Confidence Interval [b]Sputum smear-positive PTB cases plus an EPTB case, which was also smear-positive were included
[c]A patient with positive Xpert MTB but no grading for MTB detection, was excluded

Table 4.17. Multivariable analysis of risk factors for LTFU among HIV-positive TB patients treated at UATH

Variable	Category	Treatment outcome		OR (95% CI)	P	AOR (95% CI)	P
		LTFU N = 99 (%)	Success N = 230 (%)				
Age (years)	Mean (SD)	32.9 (15.6)	33.1 (13.8)	-0.2 (-3.6 – 3.2) [a]	0.900	0.999 (0.982 – 1.017)	0.933
Gender	Male	48 (48.5)	114 (49.6)	1.0 (0.6 – 1.5)	0.857	1.0 (0.6 – 1.6)	0.877
	Female	51 (51.5)	116 (50.4)	1		1	
Type of PTB	Smear-positive	15 (16.7)	57 (27.4)	1		1	
	Smear-negative	31 (34.4)	83 (39.9)	1.4 (0.7 – 2.9)	0.329	1.4 (0.7 – 2.9)	0.325
	Smear not done	44 (48.9)	68 (32.7)	2.5 (1.2 – 4.9)	0.010	2.5 (1.3 – 5.1)	**0.009**
Treatment used	Category 1	97 (98.0)	216 (93.9)	3.1 (0.7 – 14.1)	0.116	3.1 (0.7 – 14.1)	0.135
	Category 2	2 (2.0)	14 (6.1)	1		1	
Smear grade (n =73)[b]	Scanty	4 (26.7)	5 (8.6)	1		1	
	+	2 (13.3)	15 (25.9)	0.2 (0.0 – 1.2)	0.076	0.2 (0.0 – 1.2)	0.080
	++	5 (33.3)	20 (34.5)	0.3 (0.1 – 1.6)	0.164	0.3 (0.1 – 1.7)	0.179
	+++	4 (26.7)	18 (31.0)	0.3 (0.1 – 1.5)	0.141	0.3 (0.0 -1.9)	0.197
Xpert MTB/RIF (n = 73)[c]	Very low	11 (52.3)	29 (55.8)	0.9 (0.3 – 2.4)	0.792	0.9 (0.3 – 2.4)	0.775
	Medium/High	10 (47.6)	23 (44.2)	1		1	
ART status	On ART	60 (60.6)	153 (66.5)	1		1	
	Not on ART	31 (31.3)	69 (30.0)	1.2 (0.7 – 1.9)	0.607	1.1 (0.7 – 1.9)	0.614
	Unknown	8 (8.1)	8 (3.5)	2.6 (0.9 – 7.1)	0.073	2.6 (0.9 – 7.2)	0.071
CPT status	On CPT	55 (55.6)	158 (68.7)	1		1	
	Not on CPT	34 (34.3)	56 (24.3)	1.7 (1.0 – 2.9)	0.038	1.7 (1.0 – 2.9)	**0.037**
	Unknown	10 (10.1)	16 (7.0)	1.8 (0.8 – 4.2)	0.176	1.8 (0.8 – 4.2)	0.178
Mean (SD) weight (kg)	At baseline	44.1 (17.1)	48.9 (16.5)	-4.7 (-8.8 – -0.7) [a]	0.023	0.97 (0.95 – 0.98)	**0.001**
	At 2 months	36.6 (15.6)	50.1 (16.7)	-14.5 (-23.4 – -5.7) [a]	0.001	0.94 (0.88 – 0.99)	**0.032**

[a]Mean difference and its 95% Confidence Interval. LTFU = Loss-to-follow up [b]In addition to the 72 smear-positive PTB, an EPTB was also smear-positive and included
[c]Two patients with positive Xpert MTB but no grading for MTB detection, were excluded ----- data not available. CPT = Co-trimoxazole preventive therapy

Table 18. Multivariable analysis of risk factors for LTFU among HIV-negative TB patients treated UATH

Variable	Category	Treatment outcome		OR (95% CI)	P	AOR (95% CI)	P
		LTFU N = 79 (%)	Success N = 387 (%)				
Age (years)	Mean (SD)	37.8 (19.3)	30.3 (16.0)	7.4 (3.4 – 11.4) [a]	0.001	1.03 (1.01 – 1.04)	**0.001**
Gender	Male	51(64.6)	246 (63.6)	1.0 (0.6 – 1.7)	0.867	0.9 (0.6 – 1.6)	0.808
	Female	28 (35.4)	141 (36.4)	**1**		1	
Type of PTB	Smear-positive	28 (44.4)	205 (64.1)	1		1	
	Smear-negative	6 (9.5)	64 (20.0)	0.7 (0.3 – 1.7)	0.425	0.6 (0.2 – 1.6)	0.303
	Smear not done	29 (46.0)	51 (15.9)	4.2 (2.3 – 7.6)	0.001	4.3 (2.3 – 7.9)	**0.001**
Treatment used	Category 1	70 (88.6)	371 (95.9)	0.3 (0.1 – 0.8)	0.009	0.4 (0.2 – 0.9)	**0.036**
	Category 2	9 (11.4)	16 (4.1)			1	

[a]Mean difference and its 95% Confidence Interval. LTFU = Loss-to-follow up

[b] Sputum smear-positive PTB cases plus an EPTB case, which was also smear-positive were included

[c]A patients with positive Xpert MTB but no grading for MTB detection, were excluded

4.5 Discussion

4.5.1 Summary of study's findings

This study describes the outcome of patients initiating TB treatment and identifies risk factors for death/treatment failure and LTFU. TB and HIV-co-infection was observed in 44.3% of patients. Overall, TB treatment success was low, with a high proportion of patients experiencing death or treatment failure and many being LTFU. HIV-positive patients were more likely to die and to be LTFU. Independent risk factors for death/treatment failure and for LTFU were older age, a diagnosis based on Xpert MTB/RIF or Chest X-rays, a positive HIV status and lower body weight on baseline.

The factors associated with poor outcomes varied among HIV-infected and uninfected patients. Among HIV-positive patients, risk factors for death/treatment failure included; an older age, a diagnosis not being based on smear microscopy, the lack of use of Co-trimoxazole and a low baseline body weight. Risk factors for LTFU were; not being diagnosed by smear microscopy, a lack of CPT use and a low body weight at baseline and at the 2-month follow up. For HIV-negative patients, older age and a diagnosis not based on smear microscopy were independent risk factors for both poor treatment outcome and LTFU. A low body weight differences at 2 and 5 months were risk factors for poor treatment outcome only and the use of category 2-treatment, was a risk factor for LTFU.

4.5.2 Prevalence of TB/HIV-coinfection among the study population

The proportion of patients co-infected with HIV (44.3%) is similar to reports from two other studies in Northern Nigeria: in Abuja, 42.7% of 1424 patients [143] and in Kano 45.5% of 389 patients were co-infected [176]. These two studies were also retrospective and conducted at the DOTs clinics of similar teaching/referral hospitals. The proportion of TB/HIV-coinfection in

our study was more than two times higher than in other retrospective studies from Southern Nigeria (16.9% [158], 19.7% [177], 20.6% [142] and 21.6% [178]).

HIV infection rates in Nigeria vary by State, from as high as 15.2% in Rivers state to as low as 0.2% % in Ekiti state [179], with a national of TB/HIV-co-infection rate of 16% [130] . Abuja has an HIV prevalence of 7.5% and this is reflected in the high TB and HIV co-infection rates among patients attending the hospitals.

Abuja has the fifth highest HIV prevalence in the country [179]. with a high proportion of migrant workers from all over Nigeria, who travel alone in search of employment. The UATH is also a teaching and a referral centre for patients not responding to treatment or difficult to diagnose. A further reason for the high TB/HIV co-infection rate, is the recent integration of the TB/HIV care and the implementation of the WHO algorithm for active TB case finding among HIV-positive patients, which may have increased TB case detection among HIV-infected patients.

The high TB/HIV-coinfection rate is also indicative of high transmission of TB in the community and underlines the importance of implementing Isoniazid Preventive Therapy [42], to prevent TB among newly diagnosed HIV infected patients.

4.5.3 Treatment outcome of patients with TB

The TB treatment success rate (62%) is lower than the WHO target of ≥ 90% [130]. This low rate is not unusual in Nigeria's referral hospitals. Ukwaja et al reported that at a Federal Medical Centre in Ebonyi State, Southern Nigeria, only 57.7% achieved treatment success [180], and two former studies in Abuja [143] and Kano [176], reported that only 65.8% and 52.3% of patients achieved treatment success. These three studies were five-year retrospective studies,

conducted at referral/teaching hospitals, which usually receive very sick or complicated patients.

Higher treatment success rates have been reported in other Nigerian studies, with treatment success ranging from 71.5% to 86.3% [136,142,158,177,178,181]. Although, these studies were also retrospective, four were conducted in primary health facilities and one in a secondary health facility, which are likely to treat mild and uncomplicated cases.

The low treatment success in UATH might also be due to the high proportion of patients co-infected with HIV and this hypothesis is supported by the low treatment success rate of all studies with high TB/HIV-co-infection rates in Nigeria [143,176,180].

The 8.4% case fatality is similar to two studies from southern Nigeria (8.2% [180] and 9.5% [181]). One was a five-year retrospective study, conducted at a DOTS clinic of a tertiary health facility [180], and the other, a four-year review of TB treatment records in primary health facilities [181]. This case fatality however is higher than reported by other four Nigerian studies, with mortality between 1% and 6% [136,142,143,158]. These latter studies comprised retrospective studies from southern [142,158,178] and northern Nigeria [143]. Two studies reviewed TB treatment records in teaching hospital clinics [142,143], one was a review of TB treatment records in rural areas [158] and one reviewed TB treatment records of a secondary health facility in Lagos [178].

Overall, deaths during TB treatment in high income countries with a low incidence of drug resistance range from 5% in Australia [182] to 9.9% in Israel [183]. In middle income countries, mortality ranges from 3.3% in India [184] to 15.9% in Taiwan [185]; and in low income countries. from 3.4% in Ethiopia [186] to 15.1% in Mozambique [162]. National rates however are not homogeneous at a lower scale, and the mortality is usually higher in settings treating advanced, referral cases or patients with social complications [170] and lower among immune-competent patients with early or mild infections.

Eighteen percent of UATH TB patients were LTFU. This is consistent with the 18.2% reported by a study from another Nigerian teaching hospital [142], but higher than in five studies conducted in primary or secondary health facilities (1% [181], 5.6% [158], 8.2% [177], 9.6% [178] and 15% [136]). These studies might have been located closer to where patients live. There are also studies reporting higher LTFU frequencies, ranging from 25.7% - 28.6% [142,176,180,187]. These studies were retrospective and conducted in teaching/referral hospitals, like our study, and may indicate that hospital receive referred patients from more remote locations or, given their retrospective design, that there was no effort to promote patients' adherence, as it could be expected in prospective studies.

Internationally, similar results were reported from Gabon (17.6%) [146] and Brazil (18.9%) [188], but most frequently, studies from other African (0.2% - 13.2%) [155,162,163,186,189-195], Asian (2.6% to 14.2%) [184,196-200] and European (5.0% to 10.7%) countries [131,201,202] reported lower LTFU, with very few studies reporting higher LTFU (Peru, 22.5%; [203] South Africa, 25.9% [204] and India, 30%) [205].

The high LTFU proportion in our study might be due to the study location in a referral centre. The centre may be far from where most of the patients live and result in financial and time commitments, which some patients might not be able to afford. Some patients referred to the hospital might have died during treatment, but their deaths were not recorded in the TB treatment registers. As many of the possible causes for the LTFU were not recorded, there is a need to put in place adequate tracking mechanisms (such as regular contact through phone call, visit by CHEWs, TB patients' support group, and community-based treatment) to elucidate the causes of the high LTFU with a view to addressing this problem.

Less than one percent of patients failed their TB treatment. This finding is similar to other studies from Nigeria (0.03% - 0.4%) [136,180,181,206], Ethiopia (0.3% [186] and 0.7% [190]) , South

Africa (0.4%) [195], India (0.3%) [205] and Pakistan (0.2%) [200]. However, higher treatment failure has been reported from Nigeria (2.3% [176], 2.5% [207] and 6.9% [143]) and other African countries (2.1 to 8.6%) [146,163,189,208], Pakistan (2.6% [198] and 5.0% [199]) and Uzbekistan (3.0%) [209]. Most studies with treatment failure ≥ 1% in Nigeria were conducted in teaching/referral hospitals, hence, a possible reason for the higher treatment failure rate, could be patient selection.

The low proportion of patients with treatment failure in our study, could be the result of a cohort effect due to the high mortality and LTFU resulting in the attrition of the cohort.

4.5.4 TB treatment outcome of HIV-positive and HIV-negative patients

Patients with TB with and without HIV had very different treatment outcomes. HIV-positive patients had lower treatment success rate. This finding is corroborated by many studies in Nigeria [136,142,143,158,159,178] and other African countries [138,148,149,155,156,191,204,210,211]. A few studies however have reported similar treatment success between HIV-positive and HIV-negative patients (e.g. Malawi [139] and South Africa [140]) and rarely a higher treatment success rate among HIV-positive patients in Benin [157].

The poor treatment success of HIV-positive patients is associated with the more aggressive and disseminated clinical presentations of TB, the difficulty of reaching a diagnosis, which results in many patients being diagnosed clinically (with a high likelihood of having other infections) and the well-established TB/HIV interaction, leading to accelerated progression of both HIV infection and TB [212]. Patients with advanced immunosuppression also have other opportunistic infections and malabsorption of TB drugs [213-215], drug-drug interactions (particularly Rifampicin and ARVs) and the pill burden from ART and TB medications [216,217].

HIV-positive patients were more likely to die during TB treatment. There is an established association between HIV-infection and increased risk of death during TB treatment [189,193,194,218-220], which may be due to a late TB diagnosis and the risk factors for low treatment success listed above. Most deaths among HIV-infected patients occurred within the first 12 weeks of TB treatment. This early mortality has been reported in other studies [176,221,222] and may be due to late TB diagnosis and the occurrence of IRIS, which usually occurs within the first few weeks after initiating ART [223]. Our data suggests that strategies to reduce deaths (such as, standard risk assessment (SRA) and enhance case management (ECM), targeted at patients who are HIV-positive, older or underweight) should work as early as possible, during TB treatment.

HIV-positive patients were more likely to be LTFU. There is a large number of studies reporting this finding in the medical literature (see for example [224-226]). HIV-TB co-infected patients are often frail and unemployed, and are often abandoned and stigmatised by their communities. These might result in their poor socioeconomic status and lack of support needed for them to keep TB treatment clinic appointment. Despite the enormous barriers that patients with TB-HIV co-infections face, not all studies report a high LTFU, with similar rates between HIV-positive and HIV-negative reported in several studies (see for example [201,203,227]).

The higher LTFU for TB/HIV-coinfected patients may reflect that many HIV-infected patients die at home but are not traced and their death is not captured in the registers. The negative socioeconomic impact of TB/HIV-coinfection also increases the risk of LTFU [224,228] and socioeconomic support has been shown to improve TB treatment outcome [229-231].

4.5.5 Risk factors for death/treatment failure

We defined poor treatment outcome as death/treatment failure, since the treatment outcome of patients transferred out or LTFU was unknown. Only 7 patients had treatment failure and we decided to combine deaths and treatment failure in the analysis. Older age and HIV status were independently associated with poor treatment outcome. Besides HIV, as previously discussed, poor outcome was associated with older age, as described by others [191,202] [193,194,218]. Older patients' comorbidities, such as DM [232], reduced immunity and increased TB treatment adverse effects [233,234] could increase their risk of poor treatment outcome.

Our study also showed a spurious association between death/treatment failure and the lack of a smear-based diagnosis. This is due to patients with HIV being more likely to be smear-negative and the implementation of the national policy to test HIV-positive individuals with presumptive TB with Xpert MTB/RIF as the first diagnostic test. Patients with presumptive TB and no smear-results, therefore are those with a difficult diagnosis, those too ill to produce sputum and those known to have HIV infections, who did not have the smear examinations.

A further marker associated with poor treatment outcome was the body weight difference at 2 and 5 months of follow up. Studies have shown an association between low body weight/underweight on admission and death [185,220,235-237]. Poor weight gain could be a marker of the same risk or reflect additional issues on follow up, such as poor medication adherence, poor treatment response or a wrong diagnosis in difficult to diagnose patients.

Some studies have reported associations between male gender and treatment outcome [162,183,189,218,227,238]. However, we did not find this association. Similarly, we did not find an association with retreatment, which could be due to > 97% of cases in the case series being newly diagnosed TB cases. Re-treatment cases often have drug-resistant TB and in Nigeria,

patients with Rifampicin resistance are considered to have MDR-TB. As MDR-TB cases are referred to specialist treatment centres, it is likely these are missing from the study cohort.

Older age, a lack of baseline smear microscopy and low body weight were also associated with poor treatment outcome for both HIV-positive and negative patients, and not receiving CPT was an additional risk factor for HIV-positive patients. The latter has been identified as a risk factor in some [220,239-243] but not all studies [191,194,237], and likely reflects the local context and the reasons why CPT was withheld during patient management.

Although, it is well established that not receiving ART during TB treatment is a risk factor for death [244], we did not find any association between ART and death/treatment failure. In a likely manner, some other studies have found similar associations to ours [162,191,220,221,237,245]. Some possible explanations for this finding could be that, because of the implementation of the WHO's recommendation in December 2009, that all patients with TB and HIV should be placed on ART, irrespective of their CD4 counts, all patients in the study were placed on ART during their TB treatment. However, many new patients would not have been on ART at the start of their TB treatment, as their TB diagnosis prompted the services to initiate HIV investigations. It is also likely the ART status was not updated in the TB treatment register/cards, which is a common problem in our setting. In addition, some HIV-positive patients might have died of IRIS during the early stages of TB treatment, and this could have masked the beneficial effect of ART on TB treatment outcome.

4.5.6 Risk factors for LTFU

Five patient's characteristics were associated with LTFU. Older age is a factor consistently reported in previous studies [159,187,198,226,246]. Older age is also associated with the risk of death and it could be that some of the patients classified as LTFU had died, but their deaths were not reported. Additionally, older patients might have retired from work and in the study

location context, retirement may lead to poverty, which is a risk factor for LTFU [196,198,224]. Comorbidities associated with ageing could also result in polypharmacy and an increased risk of drug adverse effects [198,247,248].

Similar to poor treatment outcome, patients with no smear microscopy were more likely to be LTFU. As explained above, this is likely a selection bias due to the way patients were diagnosed in the study setting.

Low body weights at baseline and 2 months later were associated with LFTU. It is likely the low body weight was a sign of advanced disease stages, poor treatment response and unreported/unnotified deaths misclassified as LTFU. Underweight could also make it impossible for patients to work and earn money, thereby resulting in poverty.

Among HIV-positive patients, were found four independent risk factors for LTFU. We could not find other studies identifying a lack of baseline smear results as a risk factor. This could be due to the same reasons discussed above. Other factors such as "not receiving CPT" or ART were discussed previously and one report reported a similar finding [242]. Low body weight at baseline and 2 months later, were risk factors for LTFU in this group of patients, as discussed above [249-251]. Our inability to find the association with ART could be due to improper documentation/updating of patients' status, and the implementation of the WHO's recommendations.

4.5.7 Limitations and strength of the study

This study has many limitations associated with the use of secondary data. The first one is that data on some important variables, such as socioeconomic status, height, CD4 count (for HIV-positive patients) and duration of symptoms, were not always captured in the registers and missing or incomplete data could not be recollected. There could also be misclassification of cases and whether patients received ART or CPT. Another limitation is

the location of the study. Teaching hospitals often receive complex and referred patients and its findings might not be generalisable to patients attending primary health care facilities.

However, this study has some strengths, which include a fairly large sample size, a comprehensive data analysis, the systematic screening and recording of HIV status and the addition of data on TB treatment outcome to the few existing studies in Nigeria. This is one of the very few studies that present data on risk factors for death and treatment failure and predictors of poor treatment outcome and LTFU.

4.5.8 Conclusion

This study describes a high TB/HIV-coinfection rate, poor treatment outcome and high LTFU. TB/HIV-infected patients had lower treatment success, higher risk of death and LTFU. Older age, a positive HIV status, a diagnosis not based on smear microscopy, and low body weight differences at 2 and 5 months, were independent predictors of death/treatment failure and LTFU. HIV-positive patients who died or had treatment failure, were more likely to be older, to have a diagnosis not based on smear microscopy and not receiving CPT. Risk factors for LTFU among this group were also a lack of smear results, not receiving CPT, and low weight at baseline and two months. Among HIV-negative patients, independent risk factors for death/treatment failure and LFTU were older age, a lack of smear results, a low body weight at baseline and the use of category 2 TB treatment.

To improve TB treatment outcomes among the study population, there is a need to scale up prevention of TB among HIV patients and HIV prevention in the communities, with a view to reducing TB/HIV-coinfection. Strategies and diagnostics for early detection of active TB among HIV patients in the community at large, should be put in place. There is also a need for better tracking of TB patients on treatment, to prevent LTFU. Standard risk assessment and enhanced case management should be considered for TB and TB/HIV-coinfected

patients, those with; older age, underweight, no baseline smear results and those on category 2 TB treatment. CPT should be given to all HIV-positive TB patients to reduce the risk of death among them.

Chapter 5

Prevalence and incidence of hypertension among HIV patients

5.1 Introduction

This chapter focuses on hypertension, a frequent comorbidity among HIV-positive patients, which may be due to increased risk of age-related diseases (as they are now living longer because of increased access to ART) and the metabolic complications of ART. Failure to detect and promptly treat hypertension among these patients, could worsen their HIV treatment outcome.

Currently, there are few studies on prevalence of hypertension among HIV-positive patients in Nigeria, and only one study from the country, has reported the incidence of hypertension among HIV-positive patients, in the last 5 years. Hence, there is a need for more studies, on the burden and risk factors for hypertension, and the steps needed for its proper control among HIV-positive patients. This chapter describes a retrospective review of medical records of HIV-positive patients enrolled in UATH, between January 2014 and December 2016 to describe the prevalence of hypertension at the time of enrolment and one year after enrolment to assess the disease's incidence in this cohort of patients.

Specific objectives

The objectives of this study were to determine:

i) The prevalence of hypertension among HIV-infected individuals at the time of enrolment at an HIV centre.
ii) The risk factors associated with hypertension among HIV-positive patients.
iii) The incidence of hypertension and risk factors for incident hypertension among HIV-positive patients

5.2 Literature review

5.2.1 Background

Hypertension or high blood pressure is a disease of public health importance globally, though, its burden is highest in low and middle-income countries. A systematic review and meta-analysis on the global prevalence of hypertension, pooling data from 1479 population-based studies with 19.1 million participating adults in 200 countries, reported that 1.13 billion persons have hypertension worldwide. The age-adjusted prevalence was 22.2% (24.1% for men and 20.1% women) [252]. The highest prevalence of hypertension occurred in low income countries, with 33% of women in African countries having hypertension. The WHO also reported a global prevalence of hypertension of 22% in 2014, which was highest (30%) in Africa [253].

The burden of hypertension among HIV-infected individuals varies from one part of the world to another, and a systematic review of studies in sub-Saharan Africa reported an association between hypertension and HIV prevalence [254]. According to a recent systematic review, the prevalence of hypertension among HIV-infected individuals ranges from 4.7% to 54.4% in high income countries and from 8.7% to 45.9% in low income countries [255]. In addition, a clinical trial (*Strategic Timing of AntiRetroviral Treatment* or *START*) conducted among HIV-patients recruited from all continents, found an overall prevalence of hypertension of 19.3% [256], but variations from 8.4% in Asia, 14.8% in South America, 19.3% in Europe/Australia/Israel, 21.2% in Africa, and 29.4% in North America [256].

Studies of hypertension among HIV patients in Sub-Saharan Africa have reported that the prevalence ranged from 4.8% in Kenya to 49.5% in Malawi [257-274] (table 5.1). In Nigeria, six studies reported a wide range of prevalence ranging from 9.5% to 46.0% of participants [275-280](See tables 5.1 and 5.2).

5.2.2 Incidence of hypertension among HIV-infected individuals

HIV-infected patients who are normotensive at the time of enrolment into care, may develop hypertension. Incident hypertension among these patients may be due to the established risk factors for hypertension among the general population (e.g. age, obesity) or result from HIV-related factors (HIV infection itself or the side effects of ART). In contrast to studies on the prevalence of hypertension however, there are very few studies documenting the incidence of hypertension among PLHIV. In Uganda, 13.1% of initially normotensive HIV patients developed hypertension (incidence 111.5/1000 persons/year) after a median follow up period of 394 days [281]. In the US studies, one study comparing the incidence of hypertension between HIV-infected and HIV-uninfected subjects, found that there was no different in the incidence among the groups (20.4% and 20.7%, respectively) [282]. A further two US studies conducted among HIV-infected subjects; one reported an incidence of 64.1/1000 person-years[283] and in the other study, 24.1% of participants had incident hypertension [284]. Although, these studies used different methods and reported findings differently, the incidence of hypertension seemed high among HIV patients, although it is unclear whether this incidence is higher than among HIV-uninfected counterparts.

5.2.3 Pathogenesis of hypertension among HIV patients

Three factors might increase the prevalence and incidence of hypertension among PLHIV. The increasing access to ART has improved the life expectancy of PLHIV [285-287]. As hypertension is associated with increasing age, ART is likely to be a confounder of an increasing lifespan. However, there is also evidence that HIV-infected persons experience premature ageing, by comparing their telomere length with HIV-uninfected counterparts [288].

It is also postulated that the chronic viremia and sustained immune activation of HIV infection, disrupts the endothelial nitric oxide synthase-nitric oxide (eNOS-NO) system,

which maintains vascular endothelial integrity. This disruption causes vascular endothelial damage, arterial stiffness and premature atherosclerosis, increasing the risk of hypertension. However, studies that measured arterial stiffness by pulse wave velocity and subclinical atherosclerosis (Carotid intima-media thickness) are inconsistent, with some studies reporting arterial stiffness and subclinical atherosclerosis [289-292], but others reporting similar findings to uninfected individuals [293-296]. A further potential factor increasing the risk of incident hypertension is the side effects of ART. Some ART regimens (Protease Inhibitors (PIs)) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)) cause dyslipidaemia, hyperglycaemia, lipodystrophy and insulin resistance[297,298]. Some studies have reported increased aortic stiffness [299] or Carotid intima-media thickness [300] among patients on ART compared to patients not on ART, but others studies have not found this association [264,301].

Table 5.1. Prevalence of hypertension among HIV patients

Author/Date	Country	Study population	Study design	Sample size	Prevalence of Hypertension
Menanga/2015 [257]	Cameroon	Adults	Cross-sectional	44	43.2%
Ekali/2013 [258]	Cameroon	Adults	Cross-sectional	143	14.7%
Abebe/2016 [259]	Ethiopia	Adults	Cross-sectional	262	17.1%
Mohammed/2015 [260]	Ethiopia	Adults	Cross-sectional	393	34.4%
Guehi/2016 [302]	Ivory Coast	Adults	Clinical Trial	733	4.9%
Bloomfield/2011 [262]	Kenya	Adults	Retrospective	12194	8.7%
Bloomfield/2014 [263]	Kenya	Adults	Retrospective	49475	4.8%
Muronya/2011 [264]	Malawi	Adults on ART	Cross-sectional	174	49.5%
Iwuala/2015 [275]	Nigeria	Adults	Cross-sectional	145	40.7%
Muhammad/2016 [277]	Nigeria	Adults	Cross-sectional	200	9.5%
Osegbe/2016 [280]	Nigeria	Adults	Cross-sectional	200	17.5%
Diouf/2012 [265]	Senegal	Adults on ART	Retrospective	242	28.1%
Nguyen/2016 [266]	South Africa	Adults	Retrospective	748	14.7%
Julius/2011 [267]	South Africa	Adults on ART	Cross-sectional	304	19.1%
Rabkin/2015 [268]	South Africa	Adults on ART	Cross-sectional	175	37.8%
Schoffelen/2015 [269]	South Africa	Adults	Cross-sectional	904	23.0%
Wensink/2015 [270]	South Africa	Adults	Cross-sectional	903	23.0%
Njelekela/2016 [303]	Tanzania	ART-naïve adults	Cross-sectional	34111	12.5%
Kagaruki/2014 [271]	Tanzania	Adults	Cross-sectional	671	26.2%
Mateen/2013 [272]	Uganda	Adults	Cross-sectional	5563	27.9%
Okello/2015 [281]	Uganda	Adults	Retrospective	4122	7.9%
Sander/2015 [301]	Uganda	Adults	Clinical trial	1006	11.9%
Muyanja/2016 [273]	Uganda	Adults on ART	Cross-sectional	250	5.2%
Magodoro/2016 [274]	Zimbabwe	Adults	Cross-sectional	1033	10.2%

5.2.4 HIV infection and hypertension

Studies comparing the prevalence of hypertension among HIV-infected and HIV-uninfected individuals in Africa and South America indicated that hypertension was lower among PLHIV (nine studies, [278,289,293,294,304-308]), similar between the two groups in one [279] and higher among PLHIV in one study [276]. Hypertension ranged from 11% to 46% among PLHIV and 10% to 44% among HIV-negative subjects (table 5.2). A recent systematic review reported a lower prevalence of hypertension among PLHIV [309], but a further systematic review among pregnant women, found no association between infected and uninfected mothers [310]. Therefore, findings from this review are heterogeneous, although, the majority of the studies indicated that HIV infection is associated with a lower prevalence of hypertension. Most of these studies also reported that older age, male gender and a higher body mass index (BMI) were associated with hypertension among PLHIV [278,279,304,305,308]. Hence, the high burden of hypertension in regions with high HIV burden [252,253] may be associated with an overall increase in the prevalence of traditional risk factors for hypertension.

5.2.5 ART and hypertension

A potential determinant of hypertension among PLHIV is the use of ART. This life-saving therapy however, is associated with metabolic complications, such as dyslipidaemia, hyperglycaemia, lipodystrophy and insulin resistance, which may increase the risk of hypertension. Studies comparing the prevalence of hypertension between PLHIV with and without ART from Africa (7 studies) and the United States (two studies) reported a higher prevalence of hypertension among individuals on ART than ART-naïve PLHIV (seven studies) [277,278,280,311-314], and a lower prevalence in two studies [279,315]. The prevalence of hypertension ranged from 12.3% to 38.0% among ART-exposed patients and 2.0% to 38% among ART-naïve patients, with difference ranging from 3% to 19% (table 5.3). Thus, most

studies indicate ART might be associated with a higher prevalence of hypertension. Other HIV-related factors such as CD4 count, level of immunosuppression, clinical stage and viral load are inconsistently associated with hypertension.

Table 5.2. Comparison of prevalence of hypertension between HIV-positive and HIV-negative individuals

Author/Date	Country	Study population	Study design		Sample Size	HIV status	Prevalence of Hypertension
Ngatchou/2013 [289]	Cameroon	Adults	Cross-sectional	304	96	HIV-	44.0%
					108	HIV+	41.0%
Amusa/2016 [276]	Nigeria	Adults	Cross-sectional	200	50	HIV-	10.0%
					150	HIV+	46.0%
Nduka/2016 [278]	Nigeria	Adults	Cross-sectional	512	106	HIV-	42.5%
					406	HIV+	14.5%
Ogunmola/2014 [279]	Nigeria	Adults	Cross-sectional	403	153	HIV-	13.7%
					250	HIV+	14.0%
Malaza/2012 [304]	South Africa	Adults	Cross-sectional	10,429	7915	HIV-	27.9%
					2514	HIV+	19.5%
Zhou/2012 [305]	South Africa	Adults	Cross-sectional	1420	892	HIV-	24.8%
					528	HIV+	20.3%
Sliwa/2012 [306]	Soweto	Adults	Retrospective	5328	4810	HIV-	44.0%
					518	HIV+	20.0%
Peck/2014 [308]	Tanzania	Adults	Cross-sectional	454	153	HIV-	16.3%
					301	HIV+	14.3%
Kwarisiima/2016 [307]	Uganda	Adults	Cross-sectional	65,000	61,455	HIV-	14.0%
					3545	HIV+	11.0%
Monteiro/2012 [293]	Brazil	Adults	Cross-sectional	343	82	HIV-	32.0%
					261	HIV+	20.0%
Pacheco/2016 [294]	Brazil	Adults	Cross-sectional	11558	11023	HIV-	36.3%
					535	HIV+	31.4%

Table 5.3. Comparison of prevalence of hypertension between ART-naïve and ART-exposed HIV patients

Author/Date	Country	Study population	Study design	Sample size		ART status	Prevalence of Hypertension
Dimala/2016 [311]	Cameroon	Adults	Cross-sectional	400	200	ART-naive	19.0%
					200	ART-exposed	38.0%
Nsagha/2015 [312]	Cameroon	Adults	Cohort	215	55	ART-naive	14.5%
					160	ART-exposed	29.4%
Muhammad/2013 [277]	Nigeria	Adults	Cross-sectional	200	100	ART-naive	2.0%
					100	ART-exposed	17.0%
Nduka/2016 [278]	Nigeria	Adults	Cross-sectional	406	100	ART-naive	9.0%
					306	ART-exposed	16.3%
Ogunmola/2014 [279]	Nigeria	Adults	Cross-sectional	250	120	ART-naive	19.0%
					130	ART-exposed	12.3%
Osegbe/2016 [280]	Nigeria	Adults	Cross-sectional	200	100	ART-naive	12.0%
					100	ART-exposed	23.0%
Botha/2014 [315]	South Africa	Adults	Cohort	137	71	ART-naive	38.0%
					66	ART-exposed	28.8%
Nduka/2016 [314]	USA	Adults	Systematic review	44903	9086	ART-naive	10.5%
					28908	ART-exposed	14.5%
Medina-Torne/2012 [313]	USA	Adults	Cross-sectional	707	198	ART-naive	29.0%
					509	ART-exposed	32.0%

5.3 METHODOLOGY

5.3.1 Study design

This was a retrospective review of the medical records of all patients newly diagnosed to have HIV enrolled at the UATH's ART site from January 2014 to December 2016.

5.3.2 Description of the study location

The ART site was described in chapter 2. Vital signs, including blood pressure, weight and height are routinely collected on all patients at the time of their first visit to the ART site and at every follow up appointment (except height). These measurements are filed in the registers and kept in the hospital central records office by the M&E unit. The records are then digitised into an ART-site database.

5.3.3 Data collection

At the time of data collection, it became apparent that the M&E database was not up to date. The investigator then requested permission to access the records and the records office staff were hired to retrieve the folders. Three research assistants and nursing students reviewed the records and extracted data on blood pressure, weight, height, body mass index, age, gender, level of education and occupation at the time of enrolment.

In addition, to compare the effect of HIV on hypertension, we had to include HIV-negative controls from the clinical records. However, HIV testing was not being done routinely at the hospital, and it was decided to recruit HIV-negative patients from the hospital's HCT. The same data as PLHIV of patients with HIV-negative results were collected prospectively from all consecutive patients who tested HIV-negative at the HCT unit, from July to September 2017 and consented to participate. Since the HCT unit is the major point of entry into the

ART clinic, these patients (controls) might be similar to the cases (expect for HIV infection). Hypertension was defined as a systolic BP ≥ 140 mmHg or a diastolic BP ≥ 90 mmHg.

To determine the incidence of hypertension one year after initiation of ART**,** the blood pressure of the PLHIV with a normal blood pressure on enrolment were reviewed to extract the blood pressure one year after enrolment. The blood pressure recorded for the study was the BP measurement recorded closer to the 12th month after initiation of ART +/- 2.4 months. If there were multiple BP measurements, the recording closer to the 12th month was taken. In addition, information extracted includes weight, Body mass index, ART regimen/s and date of initiation and co-morbidities at the time of follow up.

5.3.4 Ethical considerations

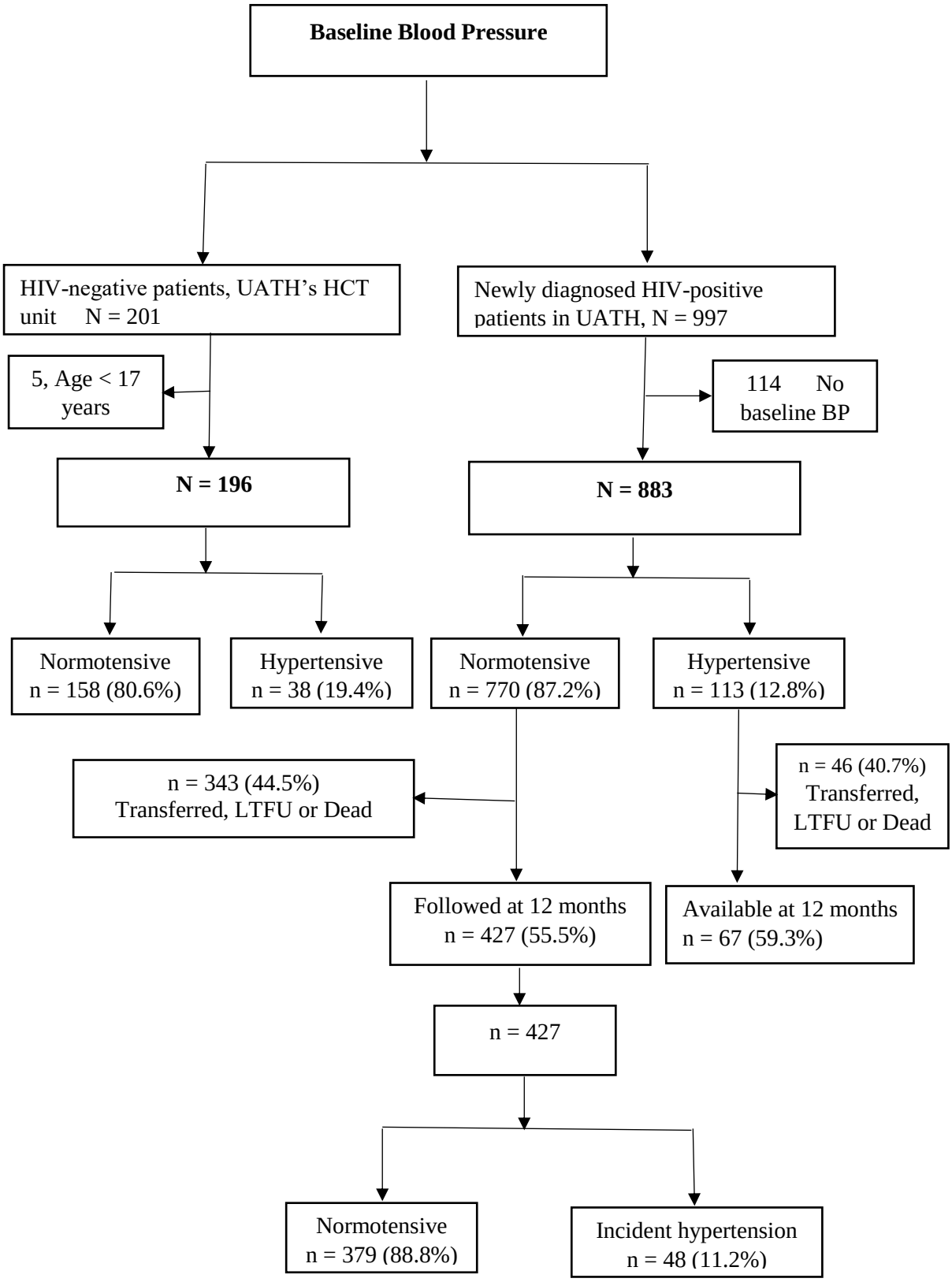
Ethical approvals were described in chapter 2. In addition, written permission was obtained from the head of the records unit to access the files. Records staff were informed of the study and their support was sought to retrieve the files. Patients informed consent was not obtained, since the study only used secondary data. The information extracted from the records was kept confidential and did not include patients' identifiers.

5.3.5 Data analysis

Statistical analysis was done using Epi-Info (US CDC) and SPSS (version 24). Descriptive statistics used included percentages, counts, means (SD) or medians and interquartile ranges. Comparison of the prevalence of hypertension and categorical variables between HIV-positive and HIV-negative patients was done, using Chi-Square and Fisher's exact tests, as appropriate. Continuous variables were compared between groups by Student's T tests and Mann-Whitney's U-test (for non-normally distributed variables). Known confounders for hypertension were adjusted in a multivariable analysis, using logistic regression. The characteristics of patients with hypertension and those without hypertension were compared

by bivariable analyses for HIV-negative and HIV-positive patients separately. The characteristics of HIV-positive patients with and without incident hypertension were compared by bivariable analyses and variables with $p < 0.2$ were adjusted for age and gender in multivariable analyses. Factors associated with prevalent or incident hypertension were determined through multivariable analyses using binary logistic regressions. Crude and adjusted odds ratios with 95% CIs, and P values were obtained for each of the included variables.

Figure 5.1. Flow chart for the prevalence and incidence of hypertension among HIV-positive patients



5.4 RESULTS

5.4.1 Baseline characteristics of participants

A total of 883 HIV-infected and 196 HIV-uninfected patients were included. Four hundred and ninety-four (55.9%) HIV-positive patients were reviewed one year later and of these, 474 (96.3%) had initiated ART. The characteristics of the participants are described in table 5.4. HIV-positive and -negative patients had similar age, although, HIV-positive males were older than HIV-negative males (p = 0.02) and HIV-positive females were younger than HIV-negative females (p = 0.23). HIV-positive patients were more likely to be female, married/widowed and less educated (P < 0.03 for all). Patients with HIV had lower mean (SD) BMI than HIV-uninfected patients (22.0 (4.4) and 25.2 (5.9), respectively, p = 0.001).

The mean (SD) diastolic and systolic blood pressures on enrolment were lower for HIV-positive than for HIV-negative patients (71.1 (10.7) versus 73.5 (11.7)) and 112.1 (15.5) versus 122.0 (18.5) mmm/Hg, respectively, p < 0.01). HIV-positive patients were less likely to have hypertension (p = 0.01).

Table 5.4. Baseline characteristics of participants by HIV status

Characteristics	Category	HIV-pos N = 883 (%)	HIV-neg N = 196 (%)	P	Missing
Age (years)	Mean (SD)	35.8 (9.6)	35.9 (11.9)	0.978	
	Male	39.9 (8.7)	36.8 (11.3)	0.020	
	Female	33.6 (9.3)	35.1 (12.3)	0.226	
Age group	10 – 19	8 (0.9)	7 (3.6)	0.013	
	20 – 29	230 (26.0)	56 (28.6)		
	30 – 39	358 (40.5)	68 (34.7)		
	40 – 49	203 (23.0)	38 (19.4)		
	50 - 59	62 (7.0)	18 (9.2)		
	≥ 60	22 (2.5)	9 (4.6)		
Gender	Male	309 (35.0)	85 (43.4)	0.028	
	Female	574 (65.0)	111 (56.6)		
Marital status	Single	228 (26.1)	83 (42.3)		10
	Married	596 (68.3)	108 (55.1)	0.001	
	Separated/Divorced	19 (2.1)	3 (1.5)	0.188	
	Widowed	30 (3.4)	2 (1.0)	0.022	
Education	No formal education	57 (8.7)	4 (2.2)		245
	Primary	138 (21.0)	15 (8.4)	0.454	
	Secondary	226 (34.5)	31 (17.4)	0.224	

	Higher education	235 (35.8)	128 (71.9)	0.001	
Occupation	Student	80 (9.2)	44 (24.4)	0.001	10
	Unemployed	38 (4.4)	9 (4.6)		
	Civil Servant	93 (10.7)	56 (28.6)		
	Artisan	80 (9.2)	4 (2.6)		
	Trading/business	251 (28.8)	39 (19.9)		
	Farming	42 (4.8)	2 (1.0)		
	Housewife	133 (15.2)	7 (3.6)		
	Others	156 (17.9)	34 (17.3)		
Ethnicity	Igbo	156 (18.0)	53 (27.0)	0.003	14
	Yoruba	72 (8.3)	24 (12.2)		
	Tiv	80 (9.2)	5 (2.6)		
	Igala	59 (6.8)	11 (5.6)		
	Hausa	54 (6.2)	7 (3.6)		
	Gbagyi	47 (5.4)	7 (5.6)		
	Ebira	40 (4.6)	8 (4.1)		
	Idoma	23 (2.6)	12 (6.1)		
	Fulani	16 (1.8)	2 (1.0)		
	Gwari	14 (1.6)	4 (2.0)		
	Ibiobio	15 (1.7)	1 (0.5)		
	Bassa	14 (1.6)	2 (1.0)		
	Nupe	10 (1.2)	5 (2.6)		
	Eggon	12 (1.4)	0 (0.0)		
	Tarok	6 (0.7)	4 (2.0)		
	Bassange	7 (0.8)	2 (1.0)		
	Kataf	5 (0.6)	1 (0.5)		
	Others	239 (27.5)	48 (24.5)		
Religion	Christianity	659 (75.8)	160 (81.6)	0.184	14
	Islam and other (n = 2)	300 (24.1)	36 (18.4)		
BMI	Mean (SD)	22.0 (4.4)	25.2 (5.9)	0.001	357
BMI Group	< 18.0	85 (15.7)	13 (7.2)	0.081	
	18.0 – 24.9	340 (62.7)	91 (50.6)	1	
	25.0 – 29.9	92 (17.0)	43 (23.9)	0.011	
	≥ 30.0	25 (4.6)	33 (18.3)	0.001	
Systolic BP	Mean (SD)	112.1 [15.5]	122.0 [18.5]	0.001	
Diastolic BP	Mean (SD)	71.1 [10.7]	73.5 [11.7]	0.009	
Hypertension		113 (12.8)	38 (19.4)	0.011	

BP = Blood pressure (mmHg). BMI = Body mass index ((kg/m^2) SD = Standard deviation

Further characteristics pertaining only to HIV-infected patients are shown in table 5.5. Seven hundred and ninety-four (97.3%) HIV-infected patients were working, 19 (2.3%) were ambulatory and 3 (0.3%) were bedridden. Five hundred and forty-three (66.8%), 145 (17.8%), 108 (13.3%) and 17 (2.1%) patients were in AIDS clinical stages 1, 2, 3, and 4, respectively. The median (IQR) CD4 cell count was 214 (91 – 381) cells/mm^3, with 206 (26.5%), 164 (21.1%), 187 (24.0%) and 221 (28.4%) having CD4 cell counts < 100, 100 –

199, 200 – 350 and > 350 cells/mm^3, respectively. Hepatitis B infection was reported in 45 (5.1%) of 516 patients with screening results.

Table 5.5. Baseline characteristics of HIV-positive patients

Characteristics	Category	N = 883 Frequency (%)	Missing values
Functional status	Working	794 (97.3)	67
	Ambulatory	19 (2.3)	
	Bedridden	3 (0.3)	
WHO stage	I	543 (66.8)	
	II	145 (17.8)	
	III	108 (13.3)	
	IV	17 (2.1)	
CD4 count (cells/mm^3)	Median (IQR)	214.0 (91.0 – 381.3)	105
CD4 count range	< 100	206 (26.5)	105
	100 – 199	164 (21.1)	
	200 – 350	187 (24.0)	
	> 350	221 (28.4)	
Haemoglobin (g/dl)	Mean (SD)	10.6 (1.9)	509
Platelets X 10^9/L	Median (IQR)	229.5 (185.3 – 298.0)	355
Creatinine (mMol/L)	Median (IQR)	57.0 (48.0 – 75.0)	698
WBC X 10^9/L	Median (IQR)	4.9 (4.0 – 6.4)	348
ALT (U/L)	Median (IQR)	35.0 (27.0 – 45.0)	710
Hepatitis B	Yes	45 (5.1)	367
	No	471 (94.9)	

WBC = White blood cell. ALT = Alanine Transaminase. SD = Standard deviation. IQR = Interquartile range.

5.4.2 Risk factors for hypertension among HIV-positive patients

Among HIV-infected individuals, patients with hypertension were older (mean (SD) age 40.0 (9.3) versus 35.2 (9.5) years for normotensive, p = 0.001) and were more likely to have overweight (p = 0.024) and obesity (p = 0.001). Hypertensive patients were more likely to have CD4 counts > 100, higher mean (SD) haemoglobin (11.2 (2.2) versus 10.5 (1.9)), p = 0.014) and to be co-infected with Hepatitis B (OR, 95% CI = 2.0 (0.9 – 4.2), p = 0.076) than normotensive patients.

Table 5.6. Risk factors for hypertension among HIV-positive patients

Characteristics	Category	Hypertensive N = 113 (%)	Normotensive N = 770 (%)	OR (95% CI)*	P
Age (years)	Mean (SD)	40.0 (9.3)	35.2 (9.5)	4.7 (2.9 – 6.6)*	0.001
	Male	43.1 (8.6)	39.3 (8.6)	3.8 (1.2 – 6.3)*	0.004
	Female	37.3 (9.1)	33.2 (9.2)	4.1 (1.7 – 6.6)*	0.001
Age group	10 - 19	0 (0.0)	8 (1.0)		0.001
	20 - 29	17 (15.0)	213 (27.7)		
	30 - 39	36 (31.9)	322 (41.8)		
	40 - 49	41 (36.3)	162 (21.0)		
	50 - 59	16 (14.2)	46 (6.0)		
	≥ 60	3 (2.7)	19 (2.5)		
Gender	Male	52 (46.0)	257 (33.4)	1.7 (1.1 – 2.5)	0.009
	Female	61 (54.0)	513 (66.6)		
Marital status	Single	21 (18.8)	207 (27.2)	1	
	Married	84 (75.0)	512 (67.3)	1.6 (0.97 – 2.7)	0.062
	Separated	1 (0.9)	18 (2.4)	0.5 (0.1 – 4.3)	0.567
	Widowed	6 (5.4)	24 (3.2)	2.5 (0.9 – 6.7)	0.077
Educational level	None	7 (7.7)	50 (8.8)	1	
	Primary	20 (22.0)	118 (20.9)	1.2 (0.5 – 3.0)	0.685
	Secondary	27 (29.7)	199 (35.2)	1.0 (0.4 – 2.4)	0.945
	Higher Ed.	37 (40.7)	198 (35.0)	1.3 (0.6 – 3.2)	0.513
Occupation	Student	7 (6.2)	73 (9.6)		0.130
	Unemployed	1 (0.9)	37 (4.9)		
	Civil Servant	18 (15.9)	75 (9.9)		
	Artisan	8 (7.1)	72 (9.5)		
	Trading/ business	38 (33.6)	213 (28.0)		
	Farming	3 (2.7)	39 (5.1)		
	House-wife	17 (15.0)	116 (15.3)		
	Others	21 (18.6)	135 (17.8)		
Ethnicity	Hausa	7 (6.4)	47 (6.2)		0.904
	Igbo	22 (20.0)	134 (17.7)		
	Yoruba	10 (9.1)	62 (8.2)		
	Others	71 (64.5)	516 (68.0)		
Religion	Christianity	87 (77.7)	572 (75.6)		0.790
	Islam	25 (22.3)	183 (24.2)		
	Others	0 (0.0)	2 (0.2)		
Baseline					
BMI (kg/m^2)	Mean (SD)	24.3 (5.4)	21.7 (4.1)	2.6 (1.3-3.9)*	0.001
BMI group	< 18.0	4 (5.6)	81 (17.2)	0.4 (0.1 – 1.1)	0.074
	18.0 – 24.9	39 (54.9)	301 (63.9)	1	
	25.0 – 29.9	19 (26.8)	73 (15.5)	2.0 (1.1 – 3.7)	0.024
	≥ 30	9 (12.7)	16 (3.4)	4.3 (1.8 – 10.5)	0.001
Systolic BP (mmHg)	Mean (SD)	137.9 (15.6)	108.3 (11.3)	29.6 (26.6-32.6)*	0.001
Diastolic BP	Mean (SD)	88.9 (8.3)	68.5 (8.3)	20.4 (18.8-22.1)*	0.001
Functional status	Working	100 (98.0)	694 (97.2)		1.000
	Ambulatory	2 (2.0)	18 (2.6)		
	Bedridden	0 (0.0)	2 (0.3)		
WHO stage	I	78 (77.2)	465 (65.3)		0.024
	II	17 (16.8)	128 (18.0)		
	III	6 (5.9)	102 (14.3)		
	IV	0 (0.0)	17 (2.4)		

CD4 count	Median (IQR)	220.0 (128-370)	211.5 (84.5–385.5)	20.0 (-16.0–57)†	0.269
CD4 count range	< 100	18 (17.3)	188 (27.9)	1	
	100 – 199	28 (26.9)	136 (20.2)	2.2 (1.1 – 4.0)	0.018
	200 – 350	28 (26.9)	159 (23.6)	1.8 (0.98 – 3.4)	0.057
	> 350	30 (28.8)	191 (28.3)	1.6 (0.9 – 3.0)	0.116
Haemoglobin (g/dl)	Mean (SD)	11.2 (2.2)	10.5 [1.9]	0.8 [0.2 – 1.4]	0.014
Platelets X 10^9/L	Median (IQR)	221.0 (180-290)	230.0 (186.5-300.5)	-5.0 (-27– 16)†	0.641
Creatinine	Median (IQR)	57.0 (48.5 – 74.5)	57.0 (48.0 – 75.0)	-1.0 (-9.0 – 6.0)†	0.791
WBC X 10^9/L	Median (IQR)	4.8 (3.9 – 6.1)	4.9 (4.0 – 6.4)	-0.2 (-0.6 – 0.3)†	0.464
ALT (U/L)	Median (IQR)	30.0 (23.5 – 45.5)	36.0 (27.3 – 45.0)	-3.0 (-8.0 – 4.0)†	0.400
Hepatitis B		10 (14.3)	35 (7.8)	2.0 (0.9 – 4.2)	0.076

* Abbreviations as in table 3.

5.4.3 Factors associated with hypertension on enrolment among HIV-negative patients

Among HIV-negative patients, participants with hypertension were older (44.6 (13.3) versus 33.8 (10.5) years for normotensive patients, p = 0.001). for both males and females.

Hypertensive patients had higher BMI (27.8 (6.3)) than normotensive patients (24.6 (5.6), p = 0.005), and had higher waist and hip circumference (90.9 (11.7) versus 82.3 (16.3) cms, p = 0.006 and 101.6 (12.5) versus 95.5 (14.4) cms, respectively, p = 0.035).

Table 5.7. Factors associated with hypertension among HIV-negative patients

Characteristics	Category	Hypertensive N = 38 (%)	Normotensive N = 158 (%)	OR (95% CI)*	P
Age (years)	Mean (SD)	44.6 (13.3)	33.8 (10.5)	10.8 (6.2 – 15.5)*	0.001
	Male	42.9 (14.0)	35.1 (9.9)	7.8 (0.7 – 14.9)*	0.032
	Female	46.3 (12.8)	32.8 (10.9)	13.4 (7.8 – 19.1)*	0.001
Age group	10 - 19	0 (0.0)	7 (4.4)		0.001
	20 - 29	4 (10.5)	52 (32.9)		
	30 - 39	13 (34.2)	55 (34.8)		
	40 - 49	7 (18.4)	31 (19.6)		
	50 - 59	9 (23.7)	9 (5.7)		
	≥ 60	5 (13.2)	4 (2.5)		
Gender	Male	19 (50.0)	66 (41.8)	1.4 (0.7 – 2.8)	0.358
	Female	19 (50.0)	92 (58.2)		
Marital status	Single	8 (21.1)	75 (47.5)		0.006
	Married	29 (76.3)	79 (50.0)		
	Separated/Divorced	0 (0.0)	3 (1.9)		
	Widowed	1 (2.6)	1 (0.6)		
Educational level	No formal education	2 (6.7)	2 (1.4)	1	
	Primary	5 (16.7)	10 (6.8)	0.5 (0.1 – 4.7)	0.543
	Secondary	4 (13.3)	27 (18.1)	0.1 (0.0 – 1.4)	0.092
	Higher education	19 (63.3)	109 (73.6)	0.2 (0.0 – 1.3)	0.090
Occupation	Student	0 (0.0)	44 (27.8)		0.001
	Unemployed	3 (7.9)	6 (3.8)		
	Civil Servant	11 (28.9)	45 (28.5)		
	Artisan	1 (2.6)	4 (2.5)		
	Trading/business	12 (31.6)	27 (17.1)		
	Farming	1 (2.6)	1 (0.6)		
	House-wife	2 (5.3)	5 (3.2)		
	Others	8 (21.1)	26 (16.5)		
Ethnicity	Hausa	2 (5.3)	5 (3.2)		0.930
	Igbo	10 (26.3)	43 (27.2)		
	Yoruba	5 (13.2)	19 (12.0)		
	Other	21 (55.3)	91 (57.6)		
Religion	Christian	32 (84.2)	128 (81.0)	1.3 (0.5 – 3.3)	0.648
	Islam	6 (15.8)	30 (19.0)		
BMI	Mean (SD)	27.8 [6.3]	24.6 [5.6]	3.1 (1.0 – 5.3)*	0.005
BMI group	< 18.0	1 (3.0)	12 (8.2)	0.5 (0.1 – 4.2)	0.522
	18.0 – 24.9	13 (39.4)	78 (53.1)	1	
	25.0 – 29.9	8 (24.2)	35 (23.8)	1.4 (0.5 – 3.6)	0.522
	≥ 30.0	11 (33.3)	22 (15.0)	3.0 (1.2 – 7.6)	0.021
Waist Circ.	Mean (SD), cm	90.9 (11.7)	82.3 (16.3)	8.7 (2.5 – 14.9)*	0.006
Hip Circ.	Mean (SD), cm	101.6 (12.5)	95.5 (14.4)	6.1 (0.4 – 11.7)*	0.035
Waist-to-Hip ratio	Mean (SD),	0.90 [0.08]	0.86 [0.02]	0.03 (-0.02 – 0.08)*	0.181
Systolic BP	**Mean (SD)** ,mmHg	150.1 (13.3)	115.3 [12.1]	34.8 (30.4 – 39.2)*	0.001
Diastolic BP	**Mean (SD)**,mmHg	87.9 (9.1)	70.0 (9.3)	17.9 (14.5 – 21.2)*	0.001

* Mean difference for means and its 95% Confidence Interval. BP = Blood pressure (mmHg). BMI = Body mass index ((kg/m^2). Waist Circ. = Waist circumference (cm). Hip Circ. = Hip circumference (cm).
OR = Odds Ratio. P = Probability value.

5.4.4 Incidence of hypertension and risk factors for incident hypertension among PLHIV.

A total of 389 (44.1%) of the 883 PLHIV included in the cross-sectional survey had been transferred out, died or LTFU one year later (table 5.8). Of the remaining 494 patients in the cohort, 67 (13.6%) had been hypertensive on enrolment, and 427 patients were available to assess the incidence of hypertension. Of these, 48 (11.2%, 95%CI = 8.5%-14.7%) patients had developed hypertension.

As many patients were lost, patients who remained in the cohort were compared to those transferred out/LTFU. Patients transferred out/LTFU had similar age, marital or educational status, occupation, BMI, baseline hypertension or Hepatitis B infection compared to those who remained in care. However, patients LTFU were more likely to be male (p = 0.007), to have lower haemoglobin (p = 0.005) and higher serum creatinine (p = 0.009) on admission (table 1 in appendix 2).

The patients' mean (SD) BMI one year later was 23.6 (4.4), with 89 (24.5%) and 23 (6.3%) of 494 patients having overweight or obesity, respectively. The median (IQR) CD4 cell count was 340 (212 – 528) cells/mm^3 and 474 (96.3%) were on ART. The mean (SD) duration on ART was 11.0 (2.4) months as described in table 5.5. Of 116 patients with viral load results, 74 (63.8%) had undetectable viral load (< 20 copies/ml), 19 (16.4%) had viral loads ranging from 20 to 999 copies/ml and 23 (19.8%) > 1000 copies/ml.

Table 5.8. Characteristics of HIV-positive patients participating one year later

Characteristics	Category	N = 883 (%)	Missing
Patient's status	Active in care	494 (55.9)	
	Transferred/LTFU/Died	389 (44.1)	
Baseline hypertension	Present	67 (13.6)	
	Absent	427 (86.4)	
BMI	Mean (SD)	23.6 (4.4)	131
BMI group	< 18.0	22 (6.1)	
	18.0 – 24.9	229 (63.1)	
	25.0 – 29.9	89 (24.5)	
	≥ 30.0	23 (6.3)	
Systolic BP at 1 year	Mean (SD) (mmHg)	113.8 (16.8)	
Diastolic BP at 1 year	Mean (SD) (mmHg)	72.7 (10.8)	
Incident hypertension (n = 427)	Yes	48 (11.2)	
	No	379 (88.8)	
Cell count at 1 year (cells/mm^3)	Median (IQR)	340.0 (212.0 – 528.0)	167
CD4 count range (cells/mm^3)	< 100	14 (4.3)	
	100 – 199	62 (19.0)	
	200 – 350	93 (28.4)	
	> 350	158 (48.3)	
Haemoglobin (g/dl)	Mean (SD)	11.5 (1.4)	341
Platelets count X 10^9/L	Median (IQR)	237.0 (197.0 – 289.0)	319
White Blood Cells X 10^9/L	Median	4.9 (4.2 – 6.1)	324
Viral load (copies/ml)	Median (IQR)	20.0 (20.0 – 49.0)	378
Viral load group (copies/ml)	< 20)	74 (63.8)	378
	20 – 999	19 (16.4)	
	≥ 1000	23 (19.8)	
ART	On ART	474 (96.3)	2
	Not on ART	18 (3.7)	
Duration on ART (months)	Mean (SD)	11.0 (2.4)	
ART regimen	TDF/FTC/EFV	403 (85.0)	
	TDF/FTC/NVP	4 (0.8)	
	ZDV/3TC/NVP	26 (5.5)	
	ZDV/3TC/EFV	8 (1.7)	
	Other	33(7.0)	
ART components	PI included	9 (2.0)	27
	PI not included	438 (98.0)	

BP = Blood pressure. BMI = Body mass index ((kg/m^2). ART = Antiretroviral therapy. PI = Protease Inhibitor. TDF/FTC/EFV = Tenofovir + Emtricitabine + Efavirenzes. TDF/FTC/NVP = Tenofovir + Emtricitabine + Nevirapine. ZDV/3TC/NVP = Zidovudine + Lamivudine + Nevirapine. ZDV/3TC/EFV = Zidovudine + Lamivudine + Efavirenzes

The 48 patients with incident hypertension were compared to the 379 patients without hypertension, to identify risk factors. Patients with incident hypertension were older (mean (SD) 39.3 (9.3) versus 34.7 (9.4) years for normotensive participants, p = 0.001) and more likely to be male (p = 0.024). Their baseline systolic and diastolic blood pressure was higher than for patients without incident hypertension (mean baseline systolic and diastolic BP (SD) 115.6 (12.7) versus 108.3 (10.5), p = 0.001) and 71.9 (8.2) versus 68.6 (8), p = 0.009), respectively). The baseline BMI of patients with incident hypertension was similar to the baseline BMI of normotensive patients. Patients with incident hypertension had higher median (IQR) baseline serum creatinine and Alanine transaminase (71.5 versus 51.5, p = 0.021 and 41.5 versus 35.5, p = 0.049), but similar frequencies of Hepatitis B infection.

The mean (SD) BMI at one year follow up was higher for patients with incident hypertension (25.4 (4) versus 23 (4.1), p = 0.001) and patients were more likely to be obese (p = 0.020) than for those who remained normotensive. The median white blood cell count was lower for patients with incident hypertension (4.4 and 5.0, respectively, p = 0.038), but there were no significant differences in the median CD4 count, the type and duration of ART.

Table 5.9. Factors associated with incident hypertension among HIV-positive patients

Characteristics	Category	Hypertensive N = 48 (%)	Normotensive N = 379 (%)	OR (95% CI)*	P
Age (years)	Mean [SD]	39.3 (9.3)	34.7 (9.4)	4.7 (1.8-7.5)*	0.001
	Male	41.7 (8.0)	38.1 (8.6)	3.6 (-0.4-7.6)*	0.078
	Female	37.4 (10.0)	33.3 (9.3)	4.2 (0.4-7.9)*	0.029
Age group	10 - 19	0 (0.0)	5 (1.3)		0.024
	20 - 29	6 (12.5)	118 (31.1)		
	30 - 39	19 (39.6)	153 (40.4)		
	40 - 49	17 (35.4)	73 (19.3)		
	50 - 59	5 (10.4)	20 (5.3)		
	≥ 60	1 (2.1)	10 (2.6)		
Gender	Male	21 (43.8)	107 (28.2)	2.0 (1.1-3.6)	0.027
	Female	27 (56.2)	272 (71.8)		
Marital status	Single	5 (10.4)	110 (29.3)		0.009
	Married	40 (83.3)	247 (65.7)		
	Separated	1 (2.1)	7 (1.9)		
	Divorced	2 (4.2)	3 (0.8)		
	Widowed	0 (0.0)	9 (2.4)		

Education	None	2 (6.1)	24 (8.4)		0.652
	Primary	4 (12.1)	59 (20.6)		
	Secondary	14 (42.4)	100 (35.0)		
	Higher Ed.	13 (39.4)	103 (36.0)		
Occupation	Student	1 (2.1)	41 (11.0)		0.249
	Unemployed	2 (4.3)	17 (4.6)		
	Civil Servant	9 (19.1)	32 (8.6)		
	Artisan	2 (4.3)	30 (8.1)		
	Trading/business	13 (27.7)	101 (27.2)		
	Farming	2 (4.3)	14 (3.8)		
	House-wife	9 (19.1)	68 (18.3)		
	Others	9 (19.1)	69 (18.5)		
Ethnicity	Hausa	5 (10.9)	20 (5.3)		0.478
	Igbo	8 (17.4)	67 (17.9)		
	Yoruba	2 (4.3)	24 (6.4)		
	Others	31 (67.4)	263 (70.3)		
Religion	Christianity	32 (66.7)	280 (74.7)		0.313
	Islam	16 (33.3)	94 (25.1)		
	Others	0 (0.0)	1 (0.3)		
Baseline BMI	Mean (SD)	22.9 (4.5)	21.8 (4.0)	1.1 (-0.2-2.4)*	0.098
BMI group	< 18.0	4 (9.5)	44 (16.1)	0.6 (0.2-1.8)	0.357
	18.0 – 24.9	27 (64.3)	177 (64.6)	1	
	25.0 – 29.9	7 (16.7)	43 (15.7)	1.1 (0.4-2.6)	0.887
	≥ 30	4 (9.5)	10 (3.6)	2.6 (0.8-9.0)	0.124
Systolic BP	Mean (SD)	115.6 (12.7)	108.3 (10.5)	7.3 (3.5-11.1)*	0.001
Diastolic BP	Mean (SD)	71.9 (8.2)	68.6 (8.0)	3.2 (0.8-5.6)*	0.009
Functional status	Working	47 (100.0)	366 (98.9)	Undefined	1.000
	Ambulatory	0 (0)	4 (1.1)		
WHO stage	I	34 (72.3)	254 (68.8)	1	
	II	8 (17)	72 (19.5)	1.7 (0.7-4.0)	0.213
	III	3 (6.4)	37 (10.0)	1.2 (0.5- 3.0)	0.670
	IV	2 (4.3)	6 (1.6)	0.6 (0.2-1.6)	0.286
CD4 count	Median (IQR)	170 (104- 293)	228 (108 – 401)	-40.0 (-97-12)†	0.128
CD4 count range	< 100	10 (22.2)	84 (23.7)	1	
	100 – 199	15 (33.3)	73 (20.6)	1.7 (0.7- 4.1)	0.213
	200 – 350	12 (26.7)	83 (23.4)	1.2 (0.5- 3)	0.670
	> 350	8 (17.8)	114 (32.2)	0.6 (0.2- 1.6)	0.286
Haemoglobin g/dl	Mean (SD)	10.6 (1.8)	10.7 (1.8)	-0.2 (-0.9-0.6)*	0.689
Platelets X 10^9/L	Median (IQR)	224 (197- 300)	222 (180- 296)	9.0 (-15- 34)†	0.455
Creatinine	Median (IQR)	71.5 (50- 108.5)	51.5 (46.8-71.3)	13.0 (1- 32)†	0.021
WBC X 10^9/L	Median (IQR)	5.6 (4.4- 6.6)	5.1 (4.0- 6.5)	0.3 (-0.3- 1)†	0.268
ALT (U/L)	Median (IQR)	41.5 (36.8- 46)	35.5 (27.3-45)	7.0 (0-13.0)†	0.049
Hepatitis B	Yes	3 (10.0)	19 (8.4)	1.2 (0.3- 4.4)	0.770
	No	27 (90)	207 (91.6)		
1 year later					
BMI (kg/m^2)	Mean (SD)	25.4 (4)	23.0 (4.1)	2.4 (1.0-3.8)*	0.001
BMI group	< 18.0	0 (0)	20 (7.3)	Undefined	0.229
	18.0 – 24.9	21 (53.8)	181 (66.1)	1	
	25.0 – 29.9	13 (33.3)	61 (22.3)	1.8 (0.9-3.9)	0.108
	≥ 30.0	5 (12.8)	12 (4.4)	3.4 (1.2-11.2)	0.020
Systolic BP	Mean (SD)	139.9 (17.1)	108.0 (10.1)	31.9 (26.8-36.9)*	0.001
Diastolic BP	Mean (SD)	90.2 (8.6)	69.1 (7.0)	21.1 (18.9-23.3)*	0.001
CD4 Cell count	Median (IQR)	261 (180-464)	254 (225-528)	-58.0 (-127-7)†	0.077
CD4 count range	< 100	4 (10.3)	9 (3.6)	1	

	100 – 199	8 (20.5)	44 (17.7)	0.4 (0.1--1.7)	0.210
	200 – 350	11 (28.2)	71 (28.6)	0.3 (0.1-1.3)	0.123
	> 350	16 (41.0)	124 (50.0)	0.3 (0.1- 1.1)	0.060
CD4 change	Median (IQR)	78 (0-211)	101 (7-180)	-2.0 (-54-53)†	0.937
Haemoglobin g/dl	Mean (SD)	11.9 (1.6)	11.4 (1.4)	0.5 (-0.1-1.2)*	0.120
Platelets X 10^9/L	Median (IQR)	220 (196-252)	245 (199 – 290)	-21 (-50-7)†	0.141
WBC X 10^9/L	Median (IQR)	4.4 (3.9-5.3)	5.0 (4.4 – 5.9)	-0.6 (-1.1-0)†	0.038
Viral load	copies/ml	20 (20-67)	20 (20 – 91)	0.0 [0-0)†	0.795
Viral Load group	< 20	8 (57.1)	52 (65.0)	1	
(copies/ml)	20 – 999	4 (28.6)	9 (11.3)	2.9 (0.7-11.6)	0.136
	≥ 1000	2 (14.3)	19 (23.8)	0.7 (0.1-3.5)	0.649
Receiving ART	yes	45 (97.8)	346 (95.3)	2.2 (0.3-17)	0.434
ART (months)	Median (IQR)	12.0 (10.5-12.0)	12.0 (10-12.0)	-0.1 (-0.8-0.7)†	0.873
ART regimen	TDF/FTC/EFV	45 (100.0)	352 (98.9)		0.732
	TDF/FTC/NVP	0 (0)	2 (0.6)		
	Others	0 (0)	2 (0.6)		
ART components	PI included	1 (2.2)	6 (1.7)	1.3 (0.2-10.6)	0.837
	PI not included	45 (97.8)	338 (98.3)		
Functional status	Working	44 (95.7)	351 (97.8)		0.394
	Ambulatory	2 (4.3)	6 (1.7)		
	Bedridden	0 (0.0)	2 (0.6)		
WHO stage	I	41 (89.1)	320 (88.2)		1.000
	II	4 (8.7)	33 (9.1)		
	III	1 (2.2)	8 (2.2)		
	IV	0 (0)	2 (0.6)		

* Mean difference and its 95% Confidence Interval. †Median difference and its Interquartile Range.
BP = Blood pressure (mmHg). BMI = Body mass index ((kg/m^2)

5.4.5 Multivariable analysis of risk factors for prevalent hypertension among HIV-positive patients

Variables entered into the multivariable analysis included age, gender, BMI, CD4 count range and Hepatitis B. Hypertensive patients were older (AOR (95% CI) = 1.06 (1.03 – 1.08), p = 0.001), more likely to be male (AOR (95% CI) = 1.6 (1.0 – 2.8), p = 0.074) and to have higher BMI (AOR (95% CI) = 1.1 (1.1 – 1.2), p = 0.001) than patients without hypertension. Correspondingly, hypertensive patients were less likely to be underweight (AOR (95% CI) = 0.4 (0.1 – 1.1), p = 0.068) and more likely to be overweight (AOR (95% CI) = 2.2 (1.2 – 4.2), p = 0.011) or obese (AOR (95% CI) = 5.4 (2.0 – 14.6), p = 0.001).

Hypertensive patients were more likely to have CD4 count ≥ 100 cells/mm^3 (100 – 199 cells/mm^3 AOR (95% CI) = 2.1 (1.1 – 4.0), p = 0.022), 200 – 350 cells/mm^3 AOR 1.9 (1.0 –

3.5), p = 0.059), > 350 cells/mm^3 (AOR= 1.9 (1.0 – 3.7), p = 0.042) and to be infected with Hepatitis B (AOR (95% CI) = 2.1 (1.0 – 4.5), p = 0.063).

5.4.6 Multivariable analysis of factors associated with baseline hypertension among HIV-negative patients

Patients with hypertension were older than non-hypertensive patients (AOR (95% CI) = 1.07 (1.03 – 1.11), p = 0.001). However, all other variables selected became non-statistically significant, as shown in table 5.11.

Table 5.10. Multivariable analysis of risk factors for hypertension among HIV-positive patients

Characteristics	Category	Hypertensive N = 113 (%)	Normotensive N = 770 (%)	OR (95% CI)*	P	Adjusted OR (95% CI)	P
Age (years)	Mean (SD)	40.0 (9.3)	35.2 (9.5)	4.7 (2.9 – 6.6)*	0.001	1.06 (1.03 – 1.08)	**0.001**
Gender	Male	52 (46.0)	257 (33.4)	1.7 (1.1 – 2.5)	0.009	1.6 (1.0 – 2.8)	0.074
	Female	61 (54.0)	513 (66.6)	1		1	
Marital status	Single	21 (18.8)	207 (27.2)	1		1	
	Married	84 (75.0)	512 (67.3)	1.6 (1.0 – 2.7)	0.062	1.1 (0.6 – 1.9)	0.732
	Separated/Divorced	1 (0.9)	18 (2.4)	0.5 (0.1 – 4.3)	0.567	0.5 (0.1 – 4.5)	0.568
	Widowed	6 (5.4)	24 (3.2)	2.5 (0.9 – 6.7)	0.077	1.7 (0.6 – 5.4)	0.352
Educational level	None	7 (7.7)	50 (8.8)	1			
	Primary	20 (22.0)	118 (20.9)	1.2 (0.5 – 3.0)	0.685		
	Secondary	27 (29.7)	199 (35.2)	1.0 (0.4 – 2.4)	0.945		
	Higher Ed.	37 (40.7)	198 (35.0)	1.3 (0.6 – 3.2)	0.513		
Occupation	Student	7 (6.2)	73 (9.6)	1		1	
	Unemployed	1 (0.9)	37 (4.9)	0.3 (0.0 – 2.4)	0.244	0.2 (0.0 – 1.7)	0.139
	Civil Servant	18 (15.9)	75 (9.9)	2.5 (1.0 – 6.3)	0.053	1.2 (0.5 – 3.0)	0.759
	Artisan	8 (7.1)	72 (9.5)	1.2 (0.4 – 3.4)	0.786	0.6 (0.2 – 1.8)	0.332
	Trading/ business	38 (33.6)	213 (28.0)	1.9 (0.8 – 4.3)	0.152	1.0 (0.4 – 2.5)	0.954
	Farming	3 (2.7)	39 (5.1)	0.8 (0.2 – 3.3)	0.759	0.3 (0.1 – 1.5)	0.154
	House-wife	17 (15.0)	116 (15.3)	1.5 (0.6 – 3.9)	0.370	1.1 (0.4 – 2.9)	0.886
	Others	21 (18.6)	135 (17.8)	1.6 (0.7 – 4.0)	0.293	0.7 (0.3 – 1.9)	0.506
Baseline							
BMI (kg/m^2)	Mean (SD)	24.3 (5.4)	21.7 (4.1)	2.6 (1.3-3.9)*	0.001	1.1 (1.1 – 1.2)	**0.001**
BMI group	< 18.0	4 (5.6)	81 (17.2)	0.4 (0.1 – 1.1)	0.074	0.4 (0.1 – 1.1)	0.068
	18.0 – 24.9	39 (54.9)	301 (63.9)	1		1	
	25.0 – 29.9	19 (26.8)	73 (15.5)	2.0 (1.1 – 3.7)	0.024	2.2 (1.2 – 4.2)	**0.011**
	≥ 30	9 (12.7)	16 (3.4)	4.3 (1.8 – 10.5)	0.001	5.4 (2.0 – 14.6)	**0.001**
Systolic BP (mmHg)	Mean (SD)	137.9 (15.6)	108.3 (11.3)	29.6 (26.6-32.6)*	0.001	1.3 (1.2 – 1.4)	**0.001**
Diastolic BP	Mean (SD)	88.9 (8.3)	68.5 (8.3)	20.4 (18.8-22.1)*	0.001	1.6 (1.3 – 1.9)	**0.001**

CD4 count range	< 100	18 (17.3)	188 (27.9)	1		1	
	100 – 199	28 (26.9)	136 (20.2)	2.2 (1.1 – 4.0)	0.018	2.1 (1.1 – 4.0)	**0.022**
	200 – 350	28 (26.9)	159 (23.6)	1.8 (0.98 – 3.4)	0.057	1.9 (1.0 – 3.5)	0.059
	> 350	30 (28.8)	191 (28.3)	1.6 (0.9 – 3.0)	0.116	1.9 (1.0 – 3.7)	**0.042**
Hepatitis B	Yes	10 (14.3)	35 (7.8)	2.0 (0.9 – 4.2)	0.076	2.1 (1.0 – 4.5)	0.063
	No	60 (85.7)	411 (92.2)	1		1	

* Mean difference and its 95% Confidence Interval. †Median difference and its Interquartile Range. BP = Blood pressure (mmHg). BMI = Body mass index (kg/m^2), ALT = Alanine Transaminase, WBC = White blood cells

Table 5.11. Multivariable analysis of risk factors associated with hypertension among HIV-negative patients

Characteristics	Category	Hypertensive N = 38 (%)	Normotensive N = 158 (%)	OR (95% CI)*	P	AOR (95% CI)	P
Age (years)	Mean (SD)	44.6 (13.3)	33.8 (10.5)	10.8 (6.2 – 15.5)*	0.001	1.07 (1.03 – 1.11)	**0.001**
Gender	Male	19 (50.0)	66 (41.8)	1.4 (0.7 – 2.8)	0.358	1.5 (0.6 – 3.8)	0.408
	Female	19 (50.0)	92 (58.2)	1		1	
Marital status	Never married	8 (21.1)	75 (47.5)	0.3 (0.1 – 0.7)	0.003	0.7 (0.3 – 1.9)	0.510
	Ever married	30 (78.9)	83 (52.5)	1		1	
Educational level	No formal education	2 (6.7)	2 (1.4)	1		1	
	Primary	5 (16.7)	10 (6.8)	0.5 (0.1 – 4.7)	0.543	0.6 (0.1 – 5.3)	0.669
	Secondary	4 (13.3)	27 (18.1)	0.1 (0.0 – 1.4)	0.092	0.5 (0.5 – 4.8)	0.527
	Higher education	19 (63.3)	109 (73.6)	0.2 (0.0 – 1.3)	0.090	0.9 (0.1 – 5.7)	0.722
BMI	Mean (SD), kg/m^2	27.8 [6.3]	24.6 [5.6]	3.1 (1.0 – 5.3)*	0.005	1.1 (1.0 – 1.1)	0.08
BMI group	< 18.0	1 (3.0)	12 (8.2)	0.5 (0.1 – 4.2)	0.522	0.5 (0.1 – 5.9)	0.617
	18.0 – 24.9	13 (39.4)	78 (53.1)	1		1	
	25.0 – 29.9	8 (24.2)	35 (23.8)	1.4 (0.5 – 3.6)	0.522	1.2 (0.5 – 3.4)	0.677
	≥ 30.0	11 (33.3)	22 (15.0)	3.0 (1.2 – 7.6)	0.021	2.0 (0.7 – 5.7)	0.172
Waist Circ.	Mean (SD)	90.9 (11.7)	82.3 (16.3)	8.7 (2.5 – 14.9)*	0.006	1.01 (0.97 – 1.06)	0.550
Hip Circ.	Mean (SD)	101.6 (12.5)	95.5 (14.4)	6.1 (0.4 – 11.7)*	0.035	1.01 (0.96 – 1.06)	0.618
Waist-to-Hip ratio	Mean (SD)	0.90 [0.08]	0.86 [0.02]	0.03 (-0.02 – 0.08)*	0.181	3.0 (0.2 – 51.7)	0.442
Systolic BP	Mean (SD)	150.1 (13.3)	115.3 [12.1]	34.8 (30.4 – 39.2)*	0.001	2.0 (1.2 – 3.1)	**0.005**
Diastolic BP	Mean (SD)	87.9 (9.1)	70.0 (9.3)	17.9 (14.5 – 21.2)*	0.001	1.3 (1.2 – 1.4)	**0.001**

* Mean difference for means and its 95% Confidence Interval. BP = Blood pressure (mmHg). BMI = Body mass index ((kg/m^2). Waist Circ. = Waist circumference (cm). Hip Circ. = Hip circumference (cm). OR = Odds Ratio. P = Probability value

5.4.7 Multivariable analysis of factors associated with incident hypertension among HIV-positive patients

After adjustment, variables that retained statistical significance for incident hypertension were age, marital status, baseline BMI, baseline systolic and diastolic blood pressure and BMI at 1 year. Newly hypertensive patients were older (AOR (95% CI) = 1.04 (1.02 – 1.07), p = 0.001), more likely to be male (AOR (95% CI) = 1.6 (0.9 – 3.1), p = 0.121) and to be married (AOR ratio (95% CI) = 2.9 (1.1 – 7.6), p = 0.033) than patients without hypertension. Newly hypertensive patients had higher baseline BMI (AOR (95% CI) = 1.1 (1.0 – 1.2), p = 0.065) and were more likely to be obese (AOR (95% CI) = 4.1 (1.2 – 14.3), p = 0.029). Patients with incident hypertension had higher baseline systolic (AOR (95% CI) = 1.06 (1.02 – 1.09), p = 0.001) and diastolic (AOR (95% CI) = 1.04 (1.00 – 1.08), p = 0.039) blood pressures, compared to patients without incident hypertension.

At one year after enrolment, hypertensive patients had a higher BMI (AOR (95% CI) = 1.1 (1.1 – 1.2), p = 0.001) and were more likely to be obese (AOR (95% CI) = 4.2 (1.2 – 14.3), p = 0.021), than patients without the hypertension.

Table 5.12. Multivariable analysis of factors associated with incident hypertension among HIV-positive patients

Characteristics	Category	Hypertensive N = 48 (%)	Normotensive N = 379 (%)	OR (95% CI)*	P	Adjusted OR (95% CI)	P
Age (years)	Mean [SD]	39.3 (9.3)	34.7 (9.4)	4.7 (1.8 – 7.5)*	0.001	1.04 (1.02 – 1.07)	**0.002**
Gender	Male	21 (43.8)	107 (28.2)	2.0 (1.1 – 3.6)	0.027	1.6 (0.9 – 3.1)	0.121
	Female	27 (56.2)	272 (71.8)	1		1	
Marital status	Never married	5 (10.4)	110 (29.3)	1		1	
	Ever married	43 (89.6)	266 (70.7)	3.6 (1.4 – 9.2)	0.006	2.9 (1.1 – 7.6)	**0.033**
Baseline BMI	Mean (SD)	22.9 (4.5)	21.8 (4.0)	1.1 (-0.2 – 2.4)*	0.098	1.1 (1.0 – 1.2)	0.065
BMI group	< 18.0	4 (9.5)	44 (16.1)	0.6 (0.2 – 1.8)	0.357	0.6 (0.2 – 2.0)	0.445
	18.0 – 24.9	27 (64.3)	177 (64.6)	1		1	
	25.0 – 29.9	7 (16.7)	43 (15.7)	1.1 (0.4 – 2.6)	0.887	1.3 (0.5 – 3.4)	0.529
	≥ 30	4 (9.5)	10 (3.6)	2.6 (0.8 – 9.0)	0.124	4.1 (1.2 – 14.3)	**0.029**
Systolic BP	Mean (SD)	115.6 (12.7)	108.3 (10.5)	7.3 (3.5 – 11.1)*	0.001	1.06 (1.02 – 1.09)	**0.001**
Diastolic BP	Mean (SD)	71.9 (8.2)	68.6 (8.0)	3.2 (0.8 – 5.6)*	0.009	1.04 (1.00 – 1.08)	**0.039**
CD4 count	Median (IQR)	170 (104 – 293)	228 (108 – 401)	-40.0 (-97.0 – 12.0)†	0.128	0.999 (0.997 – 1.001)	0.217
Creatinine	Median (IQR)	71.5 (50.0 – 108.5)	51.5 (46.8 – 71.3)	13.0 (1.0 – 32.0)†	0.021	1.003 (0.997 – 1.009)	0.299
ALT (U/L)	Median (IQR)	41.5 (36.8 – 46.0)	35.5 (27.3 – 45.0)	7.0 (0.0 – 13.0)†	0.049	1.031 (0.987 – 1.077)	0.170
Measurements at 1 year							
BMI (kg/m^2)	Mean (SD)	25.4 (4.0)	23.0 (4.1)	2.4 (1.0 – 3.8)*	0.001	1.1 (1.1 – 1.2)	**0.001**
BMI group	< 18.0	0 (0.0)	20 (7.3)	undefined	0.229	undefined	
	18.0 – 24.9	21 (53.8)	181 (66.1)	1		1	
	25.0 – 29.9	13 (33.3)	61 (22.3)	1.8 (0.9 – 3.9)	0.108	2.2 (1.0 – 5.0)	0.055
	≥ 30.0	5 (12.8)	12 (4.4)	3.4 (1.2 – 11.2)	0.020	4.2 (1.2 – 14.3)	**0.021**
Systolic BP	Mean (SD)	139.9 (17.1)	108.0 (10.1)	31.9 (26.8 – 36.9)*	0.001	1.3 (1.2 – 1.4)	**0.001**
Diastolic BP	Mean (SD)	90.2 (8.6)	69.1 (7.0)	21.1 (18.9 – 23.3)*	0.001	1.5 (1.2 – 1.9)	**0.001**
CD4 Cell count	Median (IQR)	261 (180 – 464)	254 (225 – 528)	-58.0 (-127.0 – 7.0)†	0.077	0.999 (0.997 – 1.001)	0.192
CD4 count range	< 100	4 (10.3)	9 (3.6)	1		1	
	100 – 199	8 (20.5)	44 (17.7)	0.4 (0.1 -1.7)	0.210	0.4 (0.1 – 1.9)	0.419
	200 – 350	11 (28.2)	71 (28.6)	0.3 (0.1 – 1.3)	0.123	0.4 (0.1 – 1.6)	0.189

	> 350	16 (41.0)	124 (50.0)	0.3 (0.1 – 1.1)	0.060	0.4 (0.1 – 1.5)	0.162
Haemoglobin g/dl	Mean (SD)	11.9 (1.6)	11.4 (1.4)	0.5 (-0.1 – 1.2)*	0.120	1.3 (0.9 – 1.9)	0.204
Platelets X 10^9/L	Median (IQR)	220 (196 – 252)	245 (199 – 290)	-21.0 (-50.0 – 7.0)†	0.141	0.996 (0.990 – 1.001)	0.138
WBC X 10^9/L	Median (IQR)	4.4 (3.9 – 5.3)	5.0 (4.4 – 5.9)	-0.6 (-1.1 – 0.0)†	0.038	0.7 (0.5 – 1.0)	0.066

* Mean difference and its 95% Confidence Interval. †Median difference and its Interquartile Range. BP = Blood pressure (mmHg). BMI = Body mass index (kg/m^2)

5.5 DISCUSSION

5.5.1 Summary of the study's findings

To determine if there is an association between HIV-infection and the prevalence of hypertension, we compared the prevalence of hypertension between HIV-positive patients at the time of enrolment into an HIV treatment site and HIV-negative controls. We explored risk factors for hypertension among HIV-positive and HIV-negative participants separately. Thereafter, we evaluated the incidence of hypertension among HIV-positive patients one year after enrolment and determined factors associated with incident hypertension.

We found that patients with HIV are less likely to have hypertension at the time of first attendance (12.8% versus 19.4% among controls). Among HIV-positive patients, hypertension was associated with an older age, male gender, higher BMI, higher CD4 count ($\geq$ 100 cells/mm^3) and Hepatitis B-coinfection. The only risk factor associated with hypertension among HIV-uninfected individuals was an older age. Furthermore, 11.2% of HIV-positive patients had incident hypertension after 12 months of follow up. Independent predictors of incident hypertension included; an older age, if they had been married, overweight and obesity a year earlier and higher baseline systolic and diastolic blood pressures, a higher BMI with overweight and obesity one year later. ART and other HIV-related factors were not associated with incident hypertension.

5.5.2 Effect of HIV infection on hypertension

Findings from studies comparing the prevalence of hypertension among HIV-infected and uninfected patients are inconsistent. Some studies have reported a lower prevalence of hypertension among HIV-positive patients, while others have not found this association, with a few reporting a higher prevalence of hypertension among HIV-positive patients.

Four Nigerian cross-sectional studies have compared the prevalence of hypertension among HIV-positive and -negative patients [276,278,279,316]. One of these found a lower prevalence [278], two studies found no difference [279,316] and one a higher prevalence of hypertension among HIV-positive patients [276]. Our findings are consistent with a systematic review of Sub-Saharan Africa studies, which concluded that HIV-infected individuals are more likely to have lower systolic and diastolic blood pressure [309]. Similar lower prevalence of hypertension among HIV-positive patients were reported in cross-sectional [304,307,317,318] and retrospective [306] studies from other African countries. However, two literature reviews [319,320] and a systematic review and meta-analysis [321], reviewing studies across the globe, found that HIV infection was associated with an increased risk of hypertension. In like manner, a very large study from USA ,which reviewed records of close to 30 million (29,060,418) elderly beneficiaries of Medicare and compared cardiovascular risk factors between HIV-positive and HIV-negative individuals, found a higher prevalence of hypertension among HIV-positive groups [322].

The inconsistency in these studies could be due to difference in the study populations, and most importantly, the participants characteristics. For instance, some studies compared ART-naïve HIV-positive patients with HIV-negative patients, while others compared combinations of ART-naïve and ART-exposed HIV-positive patients, with HIV-negative patients [279,316]. As patients with ART often gain weight, the association reported might be due to these confounders, as ART could alter the relationship. A further confounder is the accessibility to health services and ART. In Nigeria and in most of Sub-Saharan countries, patients had very limited access to ART in previous decades. Patients with HIV often had no access to ART and therefore were undernourished at the time of accessing the services, and mostly received palliative care. In other settings, groups at risk of HIV-infection are often professionals, lorry drivers etc, who had more access to health services and were tested at earlier disease stages.

These groups of patients often had lifestyles associated with obesity and hypertension, and thus, it is not surprising studies provide a wide range of results. Patients in Abuja have increasingly been able to access ART. However, many patients are still diagnosed late and patients tend to arrive in a poor overall health condition. It is thus likely this explains the lower prevalence of hypertension among these chronically infected patients.

5.5.3 Prevalence and risk factors for hypertension among HIV-negative patients

The 19.4% (95% CI = 13.8 – 25.0%) prevalence of hypertension found among HIV-negative patients is higher than reported in other cross-sectional studies from Nigeria [276,279,316]. One of the studies, from northern Nigeria, found a prevalence of 10% [276], and studies in southern Nigeria reported prevalence of 10.2% [316] and 13.7% [279]. However, one study in northern Nigeria, reported a very high prevalence of hypertension (42.5%) [278]. These differences could be due to the participants characteristics and study design. For instance, one of the studies had only 50 participants in its HIV-negative arm, while in another, HIV-negative participants were younger than (33.9 years) [276], compared to a mean of 35.9 years in this study. As we observed an increase of 7% in the prevalence of hypertension for every year of age, this difference may explain the variation of findings.

The prevalence of hypertension among HIV-negative patients in this study is similar to cross-sectional studies in Canada (20.3%) [323] and Italy (19.7%) [324].

The only risk factor for hypertension among HIV-negative patients, was older age, which is a traditional risk factor for hypertension among the general population. This has also been found in many studies conducted on HIV-negative patients [316,325].

5.5.4 Prevalence and risk factors for hypertension among PLHIV

Among HIV-positive patients, 12.8% were hypertensive at the time they were enrolled into HIV care. This is similar to a cross-sectional study from Lagos, Nigeria, which reported 12% of ART-naïve HIV-positive patients were hypertensive [280]. This prevalence is also consistent with a systematic review of 44,903 participants from 39 studies across the globe (with a view to determining the effect of ART on hypertension), which reported an aggregated prevalence of 10.5% among HIV-positive patients not on ART [314]. A further systematic review and meta-analysis of 63,554 participants from 49 studies worldwide, to determine the global prevalence of hypertension among HIV patients, also had similar findings, with a prevalence of 12.7% [326]. Additionally, a systematic narrative review (with a focus on the burden, risk factors, and drugs used in managing hypertension in this population) showed that the prevalence of hypertension among HIV-positive patients, ranged from 8.7 to 45.9% in low and middle-income countries and from 4.7 to 54.4% in high-income countries [255]. In like manner, a large cross-sectional study in Tanzania of 34,111 ART-naïve HIV-positive patients, showed that 12.5% had hypertension [303]. However, two studies from the north-east and South of Nigeria reported a higher prevalence of 19.3% [327] and 19% [279] among ART-naïve patients. Participants in the north-east study were slightly older with mean age of 37.4 years.

The risk factors for hypertension among HIV-positive are mostly the so-called traditional risk factors (older age, male gender and higher BMI) which are well established factors [311,327,328]. Additionally, previous studies have identified a higher CD4 count as an independent risk factor for hypertension [303,308,329], which is likely due to the fact that patients with high CD4 count might be in early stage of HIV infection, which could have made them to have higher body weight or BMI compared to patients at the late stage of the infection. In addition, an early stage of the infection could be associated with less vascular endothelial damage or

atherosclerosis that chronic infection might cause. Hepatitis B as a risk factor for hypertension has also been reported by others[282], which might reflect the effect of liver damage or additional chronic infection with its associated chronic inflammation on blood pressure. Some studies have shown an association between hepatitis C and hypertension [297,330,331], however hepatitis C screening is not routinely done in HIV care in Nigeria.

5.5.5 Incidence and risk factors for incident hypertension among PLHIV

The incidence of hypertension 12 months after participants' enrolment into the ART program was 11.2%. This is lower than the incidence of 31% reported by a retrospective study in Jos, north-east Nigeria [327].This could be partly due to patients in Jos being older than in Abuja. However, our incidence is similar to a longitudinal study of 4122 HIV-positive patients on ART in Uganda (13.1%) [281] and a prospective study in North America of 68,405 HIV-positive patients initiating ART (14%)[332]. In Tanzania, a prospective study showed that 9.6% of 955 HIV-positive patients had incident hypertension [333]. This lower incidence may be due to the lower mean follow up period of 144 days. Conversely, a higher incidence was found in a review of 6816 HIV-positive patients in the USA (20.4%) [334]. Follow up period varied across studies and a prospective cohort of 823 HIV-positive women in the USA reported a high incidence of 35% after a mean follow up of 9.6 years [335]. The inconsistency in reports of hypertension incidence could be similar to those discussed from prevalent hypertension, reflecting disease severity, adherence to ART, lifestyle changes and differences in the studies' follow up duration and participants' characteristics, such as age and BMI.

Independent risk factors for incident hypertension found in this study included; older age, ever been married, overweight/obesity at the time of enrolment, a high baseline systolic and diastolic blood pressure, BMI or overweight/obesity at 12 months. Previous studies have shown that age and higher BMI are independent predictors of incident hypertension [327,333,336].

We did not find other studies reporting an association with marriage. This finding in Nigeria's context may reflect the age of the patient and is likely to be a marker of the lifestyle and diet of the participants. Women tend to cook at home and a higher BMI is associated with affluence and health. To our knowledge, this is also the first study that reported an association between the pre-ART baseline blood pressure (systolic and diastolic) and the incidence of hypertension. This could mean that patients with incident hypertension might have been in pre-hypertensive stages before ART, and then developed hypertension with the increased weight associated with ART and viral suppression. It is likely that the traditional risk factors of hypertension might still be the main determinants among this group of patients. The implication of this finding could be that blood pressure of every HIV-positive patient should be thoroughly monitored, and special attention and preventive measures, should be directed at patients in pre-hypertension stages prior to ART initiation.

Despite similar studies reporting that ART [314] or its components [282] are risk factors for incident hypertension, this and other studies [283,327,333] did not find an association. This could be due to the fact that almost all (96.3%) participants in this study, were on ART, and that 98% of the patients on ART did not receive PI.

5.5.6 Limitations and strength of this study

Like other retrospective studies, this study has many limitations. The data used were collected without having this study or its objectives in mind and many relevant variables were not captured. Some data were incomplete, some variables were not standardized and there are likely recording and measuring errors that may or may not have occurred by random, introducing systematic biases. Patients may have been interviewed under stress with a newly diagnosed HIV infection and this may have resulted in recall bias or insincerity in their responses and a white coat effect that increased the likelihood of high blood pressure

readings. Though, this may be less likely to happen, due to the fact that patients with incident hypertension were found to have higher baseline systolic and diastolic blood pressures.

A large proportion of the patients available on enrolment were transferred out, LTFU or died in the interim period. These losses would certainly introduce biases, as patients who die or LTFU are more likely to have severe HIV infections, and may have very low BMIs and thus lower blood pressure. However, the comparison of patients LTFU and those remaining in the cohort did not identify significant differences. The prevalence and incidence of hypertension in this study could be lower than the true value, because of the short follow up period. Some patients with normal blood pressure on enrolment might have been on anti-hypertensive treatment, which was not disclosed or recorded by the health workers. We are also limited to make associations of causality, given the cross-sectional nature of the study. Lastly, it is not possible to rule out effects of unknown confounders on this study.

Nevertheless, this study has some strength. Firstly, it is one of the very few studies in Nigeria that determined incident hypertension among HIV-positive patients. Secondly, this may be the first study that documented that HIV-positive patients with incident hypertension had higher systolic and diastolic blood pressures at the time of enrolment. It will also contribute data on prevalence of hypertension among HIV-positive patients, to the few studies available in Nigeria.

5.5.7 Conclusion

This study found a lower prevalence of hypertension among HIV-positive patients compared to HIV-negative patients and established that patients who initiate ART have a high incidence of hypertension one year after initiation of therapy. Traditional risk factors of hypertension (older age, higher BMI, and male gender) were the main factors associated with both prevalence and incidence of hypertension in this population. Hepatitis B infection and a

relatively high CD4 count, were independent risk factors for prevalent hypertension, and higher blood pressures (systolic and diastolic) on enrolment, were independently associated with incident hypertension.

To control hypertension among HIV-positive patients, health practitioners of HIV/AIDS control programs should;

- Monitor blood pressure after initiation of ART
- Encourage lifestyle and dietary modifications (healthy eating habits), especially after ART initiation.
- Pay special attention on blood pressure monitoring among older patients
- Identify patients in pre-hypertensive stage at the time of enrolment and direct strategies for the prevention of hypertension towards them.
- Regularly screen HIV-positive patients for hepatitis B (and C), and institute appropriate treatment to reduce the risk of developing hypertension
- Frequently monitor blood pressures of patients with high CD4 count
- If possible, patients with hypertension should undergo renal function screening

Further prospective studies should be used in future research to avoid this study's limitations. Further studies are also needed, because there are very few studies reporting the incidence of hypertension among PLHIV, especially in the last decade, when the availability of ART has increased. Lastly, the effect of baseline blood pressures on incident hypertension should be assessed prospectively among HIV-positive patients.

Chapter 6

General Discussion and Conclusion

6.1 Introduction

This chapter integrates the findings of all the sub-studies, suggests future research and presents final conclusions.

Studies presented in this thesis are important because, a holistic approach is needed to sustain and improve the life expectancy brought by universal access to ART for PLHIV. Despite TB being preventable and treatable, it is the commonest cause of death among HIV-infected individuals. Hence, this thesis focuses on improving diagnosis and understanding the risk factors for poor treatment outcome of TB among HIV patients. In addition, we determined the burden of hypertension, an important risk factor for CVDs, now emerging as the leading cause of death among these patients.

To achieve the aim of this research, we conducted four studies comprising a cross-sectional study, a prospective study and two retrospective studies. In the first study, we evaluated the performance of the WHO TB symptom screening for early diagnosis and prevention of TB among HIV patients. The second study assessed the performance of CRP and IP-10 as screening tools for TB. This is a high-priority in TB diagnostic research, particularly for HIV patients and other vulnerable populations, such as children, who may not be able to produce sputum. The third study reviewed TB treatment records to determine the treatment outcome and its determinants and compare these between HIV-positive and HIV-negative patients. In the fourth study, medical records of HIV-positive patients were reviewed to determine the prevalence of hypertension on enrolment and the incidence of hypertension 12 months after enrolment.

6.2 Summary of findings from the studies

More than 70% of the newly diagnosed HIV patients had at least one of the symptoms of TB, but only 3% and 6.5% had culture-confirmed and bacteriologically-confirmed TB. The sensitivity and negative predictive value of the screening algorithm was 83.3% and 98.2% respectively. CRP sensitivity and specificity to identify individuals with TB was 91.4% and 33.2%, and was higher among HIV-negative individuals (95.3% and 42.6%) than in HIV-positive participants (84.8% and 22.1%). IP-10 sensitivity and specificity were lower than CRP and were 87.3% and 40.9% among all participants. The sensitivity and specificity also varied; HIV-negative participants (87.5% and 50.3%) and HIV-positive participants (85.3% and 32.4%). The combination of CRP and IP-10 increased the performance slightly among HIV-negative participants (sensitivity 97.5% and specificity 43%).

TB treatment outcome (of patients treated at the DOTS clinic of UATH) was very poor, which manifested as low treatment success (62%), and high death (8.4%) and LTFU (18%) rates. TB/HIV-coinfection rate was high (44.3%). HIV-positive patients were less likely to have treatment success, more likely to die and to be LTFU. However, there was no difference in treatment failure rate between the two groups. Risk factors to poor treatment and LTFU were older age, HIV infection, lack of baseline smear results, low body weight and underweight. Among HIV-positive patients, older age, low body weight, lack of baseline smear results, and not receiving CPT were predictors of poor treatment outcome and LTFU. ART use was not found to be associated with TB treatment outcome. For the HIV-negative patients' population, besides older age, low body weight and lack of baseline smear result, which were associated with poor treatment outcome and LTFU; being on category 2 anti-TB medication was associated with LFTU.

The prevalence of hypertension was higher among HIV-negative than HIV-positive patients (19.4% versus 12.8%). The incidence of hypertension among HIV-positive patients, one year after enrolment was 11.2%. Risk factors for hypertension were the traditional risk factors in the general population (older age, higher BMI). Additional risk factors among HIV-positive patients were higher CD4 counts and Hepatitis B infection. In addition to the traditional risk factors, incident hypertension was associated with higher systolic and diastolic blood pressure at enrolment.

6.3 Implications of the study findings

Findings from each of the studies presented in this thesis have many theoretical, service and policy implications. With respect to the evaluation of the WHO TB symptom screening algorithm, the fact that more than 70% of HIV-positive patients had a TB symptom, but only very few (3 – 6.5%) of them ended up having TB, means that the current limited laboratory services in high burden settings may not be able to cope with the work-overload resulting from testing three-quarters of all HIV-positive patients for TB. In addition, this will delay initiation of IPT and ART. Therefore, there is a need for better TB screening methods that are simple, rapid and not costly, for HIV patients, so that patients who need IPT will be able to start it and ART can also be commenced on time. Moreover, as previous studies have shown the algorithm does not work well in pregnant women, the screening algorithm may not be useful for all categories of HIV-positive patients. Hence, there may be a need to either have different TB screening algorithm for different categories of patients or look for one that could perform well in all groups of HIV patients. The poor performance of CRP and IP-10 among HIV-positive patients, implies that simple, rapid and effectives TB screening tools are still needed for early diagnosis and prompt and appropriate initiation of preventive therapy.

The high TB/HIV-coinfection rate found in the TB treatment outcome study, could mean that there are many undiagnosed HIV patients in the community. Since HIV is the strongest risk factor for TB and one-third of the population are infected with LTBI, it could also mean that many cases of HIV infection were not diagnosed early. There is a need for community-based HIV and TB screening, and institution of IPT for all HIV patients without TB.

The poor TB treatment outcomes among TB patients treated in UATH suggests a weak TB service, limited diagnostic and screening at lower health centres, which might have made many of patients to attend the hospital after poor response to treatment or difficulty with diagnosis at primary/secondary health facilities. Hence, there may be a need to strengthen TB diagnostic and screening capacities of TB treatment centres at the primary health care level, with a view to ensuring early diagnosis, prompt treatment and better outcome.

Regarding the high rate of LTFU, there may be a need to put appropriate and effective patients' tracking mechanisms in place. Also, factors associated with LTFU found in this study (such as, HIV-infection, older age, underweight/low body weight, not receiving CPT, being on category 2 TB treatment regimen, and lack of baseline smear results), should be put into consideration in strategy to address this issue, so that special attention can be directed towards patients with any of the risk factors.

The high burden of hypertension among HIV patients, reinforces the need to integrate hypertension (and other non-communicable diseases) management into the existing HIV care program, so that any HIV patient with the comorbidity, will receive treatment for it in addition to HIV care, at the same clinic and visit. Furthermore, an integrated management of these conditions may increase treatment adherence for ART, which is also low in this setting.

6.4 Future research

To address the limitations in the studies presented in this thesis, future researches should aim to meet the sample size required. Also, to avoid delay in sputum samples' processing and culture, there is a need to ensure that TB research laboratory and study's site(s) are not too far from each other.

Moreover, the fact that majority of patients with TB symptom (using the WHO symptom screening) ended up not having TB and were consequently delayed in initiating preventive therapy and ART, is an indication that more research for a rapid method to rule out TB is needed. Additionally, due to the unexpected and unavoidable delay in manufacturers' release of most of the new TB diagnostics (such as Ultra-sensitive GeneXpert, Genedrive, and electronic Nose (E-nose)) we had planned to evaluate in this study, the diagnostics were not available for this study. Hence, the evaluation of these diagnostics and others promising ones, whenever they are available, will be an important research towards overcoming the challenges with TB diagnosis among HIV patients.

Furthermore, future studies that recruit patients irrespective of TB symptoms, will be needed to re-evaluate the performance of CRP and IP-10 as a screening tool for active TB, in Nigeria/West Africa, to be able to compare the performance of the biomarkers to that of the WHO symptom screening algorithm. Because, we could not do this in our study, since only patients with TB symptom were recruited into the study.

In addition, there may be a need for more studies on factors associated with the combination of death and treatment failure, as poor TB treatment outcomes. Because, apart from our study, only one published previous study [208] was found to have assessed the factors. Future studies, should also determine factors associated with poor TB treatment outcomes among HIV-negative patients, since very few studies have done this in Sub-Saharan Africa.

Lastly, future studies should assess the effect of baseline blood pressure on incidence hypertension among HIV-positive patients, because we did not find any previous study to compare our finding on this factor with (association between higher baseline blood pressure (systolic and diastolic) and incident hypertension).

6.5 Conclusion

Four studies (comprising a cross-sectional, a prospective and two respective studies) were presented in this thesis. The main aims of the studies were to; improve TB diagnosis, understand determinants of TB treatment outcome and assess the burden of hypertension and its associated factors among HIV-patients, with a view to preventing premature deaths among the population. Findings from the studies showed that the WHO TB symptom screening algorithm performed as expected, however, the fact that the majority (> 70%) of patients with TB symptom, who underwent further diagnostic workup, ended up not having the disease, will make the implementation of the screening algorithm difficult in resource-limited settings, due to the under-equipped and insufficient laboratory services in these settings. In addition, the performance of CRP as a TB screening tool, was fair among HIV-negative patients, but poor among HIV-positive patients. IP-10 performed poorly in all categories of patients in the study. Also, treatment outcomes were poor among TB patients treated at the DOTS clinic of UATH. The treatment outcome was poorer among HIV-positive patients compared to HIV-negative patients. HIV-positive patients were less likely to have treatment success, but more likely to die and LTFU, compared to HIV-negative patients. The studies also showed that the burden of hypertension is high among HIV patients and its main determinants are the traditional risk factors, in addition to Hepatitis B infection.

To ensure early diagnosis, prompt treatment and prevention of TB among HIV-patients, there is therefore, a need to search for and find simple, rapid and inexpensive TB screening tool for

the patients. Additionally, TB treatment outcome can be improved by ensuring that special attention/care are directed towards HIV-positive, older and underweight patients, and all TB/HIV-coinfected patients are placed on CPT. Moreover, the high burden of hypertension among HIV patients, may be properly reduced by integrating hypertension screening into the current HIV control programs in resource-limited settings.

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8. Appendices

8.1 Appendix 1. Additional tables on TB treatment outcome study

Table 1. Bivariable analysis of factors associated with death during TB treatment

Variable	Category	Treatment outcome		Odds ratio (95% CI)	P
		Death	**Success**		
		N = 84	**N = 619**		
Mean (SD) age (years)	All	38.3 (16.1)	31.4 (15.3)	6.9 (3.4 – 10.4)	**0.001**
	Male	41.1 (16.3)	33.0 (16.2)	8.1 (3.3 – 12.9)	**0.001**
	Female	34.2 (15.0)	29.2 (13.6)	5.1 (0.123 – 10.0)	**0.045**
Age group*	0 – 9	6 (7.1)	60 (9.7)		**0.001**
	10 – 19	3 (3.6)	56 (9.1)		
	20 – 29	11 (13.1)	150 (24.2)		
	30 – 39	22 (26.2)	188 (30.4)		
	40 – 49	21 (25.0)	100 (16.2)		
	50 – 59	13 (15.5)	31 (5.0)		
	60 – 69	5 (6.0)	21 (3.4)		
	≥ 70	3 (3.6)	13 (2.1)		
Gender	Male	50 (59.5)	361 (58.3)	1.1 (0.7 – 1.7)	0.834
	Female	34 (40.5)	258 (41.7)	1	
Treatment category	New	84 (100%)	600 (96.9)	Undefined	0.151
	Retreatment	0 (0%)	19 (3.1)	1	
Anatomical site	Pulmonary	74 (88.1)	529 (85.5)	1.3 (0.6 – 2.5)	0.517
	EPTB	10 (11.9)	90 (14.5)	1	
Smear microscopy	Positive	15 (20.3)	262 (49.5)	1	
	Negative	24 (32.4)	148 (28.0)	2.8 (1.4 – 5.6)	**0.003**
	Not done	35 (47.3)	119 (22.5)	5.1 (2.7 – 9.7)	**0.001**
Treatment used	Category 1	80 (95.2)	589 (95.2)	1.0 (0.3 – 3.0)	0.973
	Category 2	4 (4.8)	30 (4.8)	1	
HIV status	Positive	68 (81.0)	230 (37.3)	7.2 (4.1 -12.6)	**0.001**
	Negative	16 (19.0)	387 (62.7)	1	
Smear grade (n = 277)	Scanty	3 (20.0)	21 (8.0)	1	
	+	3 (20.0)	68 (26.0)	0.3 (0.1 – 1.6)	0.158
	++	2 (13.3)	96 (36.6)	0.15 (0.02 – 0.9)	**0.041**
	+++	7 (46.7)	77 (29.4)	0.6 (0.2 – 2.7)	0.537
Xpert grade (n = 139)	Very low	2 (10.5)	24 (20.0)	1	
	Low	9 (47.4)	33 (27.5)	3.3 (0.6 – 16.6)	0.151
	Medium	7 (36.8)	38 (31.7)	2.2 (0.4 – 11.5)	0.347
	High	1 (5.3)	25 (20.8)	0.5 (0.04 – 5.6)	0.559
Mean (SD) weight	Baseline	49.0 (16.8)	50.3 (16.3)	-1.3 (-5.1 – 2.5)	0.517
	2 months	51.0 (2.2)	52.6 (15.9)	-1.5 (-4.2 – 1.2)	0.242
	5 months	----------	55.9 (16.1)	----------	
Weight difference	At 2 months	-2.3 (4.4)	2.1 (4.3)	-4.4 (-8.2 – -0.6)	**0.024**
	At 5 months	----------	4.6 (5.2)		

*n (%) unless otherwise stated. [a]Mean difference (95% CI).

Table 2. Multivariable analysis of factors associated with death among TB patients

Variable	Category	Treatment outcome		Odds ratio (95% CI)	P	AOR (95% CI)	P
		Death N = 84	Success N = 619				
Mean (SD) age	All	38.3 (16.1)	31.4 (15.3)	6.9 (3.4 – 10.4)[a]	**0.001**	1.03 (1.01 – 1.05)	**0.004**
Gender	Male	50 (59.5)	361 (58.3)	1.1 (0.7 – 1.7)	0.834	1.1 (0.7 – 1.9)	0.632
	Female	34 (40.5)	258 (41.7)	1		1	
Type of PTB	Smear-positive	15 (20.3)	262 (49.5)	1		1	1
	Smear-negative	24 (32.4)	148 (28.0)	2.8 (1.4 – 5.6)	**0.003**	1.6 (0.7 – 3.3)	0.247
	Smear not done	35 (47.3)	119 (22.5)	5.1 (2.7 – 9.7)	**0.001**	3.4 (1.7 – 6.9)	**0.001**
HIV status	Positive	68 (81.0)	230 (37.3)	7.2 (4.1 -12.6)	**0.001**	7.0 (3.9 – 12.4)	**0.001**
	Negative	16 (19.0)	387 (62.7)	1		1	
Smear grade	Scanty	3 (20.0)	21 (8.0)	1		1	
	+	3 (20.0)	68 (26.0)	0.3 (0.1 – 1.6)	0.158	0.4 (0.1 – 1.9)	0.236
	++	2 (13.3)	96 (36.6)	0.15 (0.02 – 0.9)	**0.041**	0.2 (0.03 – 1.3)	0.091
	+++	7 (46.7)	77 (29.4)	0.6 (0.2 – 2.7)	0.537	0.7 (0.1 – 3.6)	0.673
Xpert grade (n = 139)	Very low	2 (10.5)	24 (20.0)	1		1	
	Low	9 (47.4)	33 (27.5)	3.3 (0.6 – 16.6)	0.151	5.0 (0.8 – 32.1)	0.088
	Medium	7 (36.8)	38 (31.7)	2.2 (0.4 – 11.5)	0.347	3.7 (0.6 – 22.2)	0.152
	High	1 (5.3)	25 (20.8)	0.5 (0.04 – 5.6)	0.559	0.9 (0.1 – 12.2)	0.928
Weight difference	At 2 months	-2.3 (4.4)	2.1 (4.3)	-4.4 (-8.2 – -0.6)[a]	**0.024**	0.81 (0.66 – 0.99)	**0.036**

*n (%) unless otherwise stated. [a]Mean difference (95%CI)

8.2. Appendix 2. Additional table on prevalence and incidence of hypertension among HIV patients

Table 1. Characteristics of HIV-positive patients transferred/LTFU/dead and patients who remained in care 1 year after enrolment.

Characteristics	Category	Patients' status at 1 year		OR (95% CI)*	P
		LTFU/Transferred /Died, N = 389(%)	In care N = 494 (%)		
Age (years)	**Mean (SD)**	35.7 (9.5)	35.9 (9.6)	-0.2 (-1.4-1.1)*	0.794
	Male	40.1 (8.5)	39.8 (8.9)	0.4 (-1.6-2.3)*	0.719
	Female	32.8 (.1)	34.2 (9.4)	-1.3 (-2.9-0.2)*	0.094
Age group	10 - 19	3 (0.8)	5 (1.0)		0.741
	20 - 29	95 (24.4)	135 (27.3)		
	30 - 39	169 (43.4)	189 (38.3)		
	40 - 49	88 (22.6)	115 (23.3)		
	50 - 59	25 (6.4)	37 (7.5)		
	≥ 60	9 (2.3)	13 (2.6)		
Gender	Male	155 (39.8)	154 (31.2)	1.5 (1.1-1.9)	0.007
	Female	234 (60.2)	340 (68.8)		
Marital status	Single	103 (26.9)	125 (25.5)		0.202
	Married	258 (67.4)	338 (69.0)		
	Separated	5 (1.3)	14 (2.8)		
	Widowed	17 (4.4)	13 (2.7)		
Education	None	26 (9.2)	31 (8.3)		0.929
	Primary	60 (21.1)	78 (21.0)		
	Secondary	100 (35.2)	126 (33.9)		
	Higher Ed.	98 (34.5)	137 (36.8)		
Occupation	Student	36 (9.3)	44 (9.1)		0.104
	Unemployed	18 (4.7)	20 (4.1)		
	Civil Servant	45 (11.6)	48 (9.9)		
	Artisan	43 (11.1)	37 (7.6)		
	Trade/business	107 (27.6)	144 (29.6)		
	Farming	24 (6.2)	18 (3.7)		
	House-wife	46 (11.9)	87 (17.9)		
	Others	68 (17.6)	88 (18.1)		
Ethnicity	Hausa	26 (6.8)	28 (5.8)		0.191
	Igbo	68 (17.7)	88 (18.1)		
	Yoruba	40 (10.4)	32 (6.6)		
	Others	250 (65.1)	337 (69.5)		
Religion	Christianity	298 (78.4)	361 (73.8)		0.207
	Islam	81 (21.3)	127 (26.0)		
	Others	1 (0.3)	1 (0.2)		
BMI	Mean (SD)	21.6 (4.7)	22.2 (4.2)	-0.6 (-1.4-0.2)*	0.131
BMI group	< 18.0	36 (20.3)	49 (13.4)	1.6 (0.99-2.6)	0.052
	18.0 – 24.9	106 (59.9)	234 (64.1)	1	
	25.0 – 29.9	30 (16.9)	62 (17.0)	1.1 (0.7 – 1.7)	0.793
	≥ 30	5 (2.8)	20 (5.5)	0.6 (0.2 – 1.5)	0.247
Systolic BP	Mean (SD)	110.7 (15.3)	113.3 (15.5)	-2.6 (4.7– -0.5)*	0.013
Diastolic BP	Mean (SD)	70.4 [10.9]	71.7 [10.6]	-1.3 (-2.7-0.1)*	0.073
Hypertensive		46 (11.8)	67 (13.6)	0.9 (0.6-1.3)	0.443
Functional status	Working	319 (95.2)	475 (98.8)		0.004
	Ambulatory	14 (4.2)	6 (1.2)		
	Bedridden	2 (0.6)	0 (0.0)		
WHO stage	I	205 (61.6)	338 (70.4)	1	
	II	55 (16.5)	90 (18.8)	1.0 (0.7-1.5)	0.969
	III	64 (19.2)	44 (9.2)	2.4 (1.6-3.7)	0.001

	IV	9 (2.7)	8 (1.7)	1.9 (0.7-4.9)	0.211
CD4 Cell count	Median (IQR)	197.0 (69– 362)	223.0 (109-388)	-23.0 (-49–1.0)*	0.063
CD4 count range	< 100	100 (31.7)	106 (22.9)	1	
	100 – 199	61 (19.4)	103 (22.2)	0.6 (0.4-0.95)	0.029
	200 – 350	73 (23.2)	114 (24.6)	0.7 (0.5-1.0)	0.058
	> 350	81 (25.7)	140 (30.2)	0.6 (0.4-0.9)	0.013
Haemoglobin (g/dl)	Mean (SD)	10.2 (2.0)	10.8 (1.9)	-0.6 (-1.0-0.2)*	0.005
Platelets X 10^9/L	Median (IQR)	239.5 (188– 304)	224.0 (183-296)	10.0 (-5–25)†	0.179
Creatinine, mMol/L	Median (IQR)	63.0 (49.0 – 83.0)	53.0 (48-72)	7.0 (2–12)†	0.009
WBC (cells X 10^9/L)	Median (IQR)	4.8 (3.9 – 6.2)	5.1 (4.0- 6.5)	-0.2 (-0.5-0.1)†	0.253
ALT (U/L)	Median (IQR)	32.0 (26.0 – 46.0)	36.0 (28- 45)	-1.0 (-5.0-3.0)†	0.688
Hepatitis B	infected	17 (7.8)	28 (9.4)	0.8 (0.4-1.5)	0.525

* Mean difference and its 95% Confidence Interval. †Median difference and its Interquartile Range. BP = Blood pressure. BMI = Body mass index (kg/m^2), ALT = Alanine Transaminase, WBC = White blood cells

8.3. Appendix 3. Research Ethics application

FOR OFFICE USE ONLY	Application Number	Date considered	Reviewed by

Please complete this form in typescript. It is essential that this form is completed fully and the relevant enclosures are received if the study is to receive proper scrutiny by the Research Ethics Committee. If any documentation is missing proposals will not be submitted for review.

Applicant contact details

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Administration Charges:

An administration charge of £250 for awards of over £10,000 and £50 for those below will be made for ethical approval

Is the proposed work already funded?	**Yes** ☒	**No** ☐	**n/a** ☐
Total budget of proposal £ 45,000.000			
Name of Funding Organisations: 1. Commonwealth Scholarship Commission (gave a research support fund of £15,000.00) 2. Epistem, circa (provided £30,000.00 as funds for testing the Genedrive) 3. National Tuberculosis and Leprosy Control Programme of Nigeria(NTBLCP)- in kind contribution of cartridges for Xpert MTB/RIF testing			

Check List:

The following **Check List** must be completed. Please confirm that the following are enclosed.

	Yes	Not Applicable
1 Completed Internal School Transfer Form (ISF) for administration charges	☒	
8 Copies of the completed Application Form without staples	☒	
1 Copy of the Research Protocol	☒	☐
8 Copies of the Questionnaire/case record form without staples	☒	☐
8 Copies of the Consent Form without staples	☒	☐
8 Copies of the Patient Information Sheet without staples	☒	☐
8 Copies of the Translator Agreement without staples	☐	☒
8 Copies of the Draft interview/FGD or observation/Check list enclosed (if used) without staples	☐	☒
8 Copies of the Declaration page - last page of application (**1 signed and initialled** by the applicant plus 7 copies)	☒	
8 Collated Applications – please ensure you collate all documents into 8 separate applications and **NOT** 8 copies of each document	☒	

The completed forms should be sent to: Lois Thomas, Secretary, Research Ethics Committee, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA e-mail: lstmrec@liv.ac.uk

APPLICATION FORM FOR ETHICAL APPROVAL

(Revised December 2013)

Please refer closely to the Guidance Notes when completing this form.

All questions must be answered. Any form with sections left blank or answered with N/A will be returned. This form must contain all information necessary for the Research Ethics Committee to make a decision on Ethical Approval.

APPLICANT FULL NAME (w/ title)	Dr. Rotimi Samuel, OWOLABI
PROJECT TITLE	Optimizing the Diagnosis and Management of Tuberculosis (TB) and Diabetes Mellitus (DM) among HIV Patients in a high TB and HIV burden setting.

Ethical Approvals

Have you submitted this proposal to the LSTM Research Ethics Committee before?	**YES** ☐	**NO** ☒
If 'YES', please give date of previous review:		
To which other ethical committees has/will this protocol be submitted? (please list)? The ethical committee of University of Abuja Teaching Hospital		
APPROVALS – Please list any ethical review committees which have already approved the protocol.		
In-Country Ethical Approval - Please list the country(ies) where the research will be carried out and whether or not in-country ethical approval is required.		
Country 1: Nigeria	**YES** ☒	**NO** ☐
Country 2:	**YES** ☐	**NO** ☐
Country 3:	**YES** ☐	**NO** ☐
Country 4:	**YES** ☐	**NO** ☐
Add additional lines if your research is taking place in more than four countries.		
Please note if you have marked 'NO' for any country above <u>WRITTEN EVIDENCE</u> must be provided to confirm that in-country ethical approval is not required. This evidence should be attached as an annex to the application. Acceptable evidence includes: • a letter from the national Ministry of Health or other relevant regulatory authority • a letter from an authorised signatory at a local partner		

A letter from a co-investigator or other researcher at a local partner institution is NOT sufficient evidence. Your ethics application will not be considered until evidence is provided.

Research Team

List LSTM research team and all collaborators.

(Please include all overseas collaborators and give their affiliations, qualifications and role in the study).

NAME	ORGANISATION	QUALIFICATIONS	ROLE IN STUDY	GEOGRAPHIC LOCATION
Prof. Luis Cuevas	LSTM	MD, MTropMed, DTCH	First Supervisor	Liverpool
Mr. Russell Dacombe	LSTM	BSc, MSc	Second Supervisor	Liverpool
Dr. Emily Adams	LSTM	BSc, PhD	Advisor	Liverpool
Dr. Derek Sloan	LSTM	MBBS, PhD	Advisor	Liverpool
Prof. Lovett Lawson	Zankli Medical Centre	MBBS, MSc, DTM&H, PhD	Local Supervisor	Abuja, Nigeria
Emmanuel Agogo	National Agency for the Control of AIDS/HIV(NACA)	MBBS, MtropMed	Advisor and facilitator for the study in Nigeria. Procurement of Xpert cartridges.	Abuja, Nigeria
Saddiq Abdurrahman	Assistant director of the FCT health services. Head of Disease control programs (including the FCT-TBLCP)	MBBS, MtropMed	Advisor and facilitator for the study in Nigeria. Access to patients attending the DOTS clinics of the TBLCP of the FCT	Abuja, Nigeria

If proposal is for work relating to a MPhil/PhD, please state the name and Department of Supervisor/Tutor	
Supervisor	Prof. Luis Cuevas
Department	Clinical Sciences
Signature	

Sponsorship and Indemnity Cover Please see sponsorship and indemnity guidance notes and request form on the LSTM intranet http://pcwww.liv.ac.uk/lstmintranet/research_management/applyingforRG/intro.htm		
Have you submitted a sponsorship and indemnity request form to the Research Office?	YES ☒	NO
If no, do you intend to submit a form and if so, when?		
If you do not intend to submit the form please give reasons.		

GLOSSARY OF TERMS

Please provide a list of acronyms used in the application with their full name and any relevant explanation that would be helpful to committee members that may not be an expert in your area of work.		
AIDS	:	Acquired Immune Deficiency Syndrome
ART	:	Antiretroviral Therapy
ARV	:	Antiretroviral
CD4 Cells	:	Cluster of differentiation 4 cells
CI	:	Confidence Interval
CPT	:	Co-trimoxazole Prophylaxis Therapy
DM	:	Diabetes Mellitus
DOTS	:	Directly Observed Therapy Short course
FACA	:	FCT's Agency for the Control of AIDS
FBC	:	Full Blood Count
FCDA	:	Federal Capital Development Administration
FCT	:	The Nigeria's Federal Capital Territory (FCT), Abuja
FCT-TBLCP	:	Federal Capital Territory Tuberculosis and Leprosy Control Program
FMOH	:	Federal Ministry of Health
GeneXpert	:	Xpert **MTB**/RIF
HbA1C	:	Glycosylated Haemoglobin
HBV	:	Hepatitis B Virus
HCT	:	HIV Counselling and Testing
HIV	:	Human Immunodeficiency Virus
INH	:	Isoniazid
IPT	:	Isoniazid Preventive Therapy
LED-FM	:	Light Emitting Diode(LED) Florescent Microscopy
LFT	:	Liver Function Tests
MDR	:	Multidrug Resistant Tuberculosis
M&E	:	Monitoring and Evaluation unit
MTB/RIF	:	Mycobacterium Tuberculosis/Rifampicin Resistance
NASCAP	:	National AIDS and STIs Control Program

NEPLWHAN	:	Network of People Living with HIV/AIDS in Nigeria
NTBLCP	:	National Tuberculosis and Leprosy Control Program
OI	:	Opportunistic Infection
OR	:	Oral Swab
OSP	:	Oral Swab PCR
PCR	:	Polymerase Chain Reaction
PEP	:	Post-Exposure Prophylaxis against HIV infection
PLWHA	:	People Living with HIV/AIDS
PMTCT	:	Prevention of Mother-to-Child Transmission of HIV
POC	:	Point-of-Care
RFT	:	Renal Function Tests
RIF	:	Rifampicin
RR	:	Rifampicin Resistance
T2DM	:	Type 2 Diabetes Mellitus
TB	:	Tuberculosis
UATH	:	University of Abuja Teaching Hospital
VOCs	:	Volatile Organic Compounds
WHO	:	World Health Organization
Xpert	:	Xpert MTB/RIF
ZMC	:	Zankli Medical Centre
95%CI	:	95% Confidence Interval

SECTION A

STUDY OUTLINE

A.1	**LAY SUMMARY:** Please use simple language which is understandable to a non-scientific/non-academic audience. Sufficient detail of the protocol must be given to allow the Committee to make an informed decision without reference to other documents. Please spell out all acronyms. *(max 300 words)*

Acquired Immune Deficiency Syndrome (AIDS) and Tuberculosis (TB) are the leading causes of death due to single infectious agents and Nigeria has a high TB and Human Immunodeficiency Virus (HIV) burden, with 590,000 TB (1) and 3.4 million HIV cases (2). TB is the most important opportunistic infection causing death among HIV patients.

Patients with HIV should be screened for TB. The World Health Organization (WHO) recommends utilising a standardised algorithm consisting of symptoms of TB. Patients without symptoms are then given Isoniazid Preventive Therapy (IPT) and those with symptoms are investigated to initiate TB treatment. The algorithms have not been evaluated in Nigeria and asymptomatic individuals with TB or symptomatic patients with TB may be being missed.

It is also well established that it is difficult to make a diagnosis of TB among HIV patients due to the low bacilli load on presentation and the low sensitivity of the current TB diagnostics. We will evaluate several new diagnostics for TB. These diagnostics have not been evaluated in Nigeria and information on their performance in an HIV population is limited.

Diabetes Mellitus (DM) is a disease that increases the risk of TB and is more prevalence in patients with HIV. In 2013, an estimated 15% of the global adult TB cases were attributed to DM, and WHO recommends bidirectional screening of TB and DM. There is limited data on the prevalence of DM among patients with HIV with/without TB.

This study will evaluate the WHO-TB diagnostic algorithms among patients with HIV with and without symptoms of TB, and explore whether new diagnostics could increase the identification of TB cases. The study will explore better ways of diagnosing TB and DM among adult patients with HIV, with a view to characterising whether this is a problem among the study population.

A.2	**IMPORTANCE OF THE RESEARCH:** Please state the intended value of the research and explain how this research fits in with national/international research priorities. *(max 300 words)*

The prevalence of HIV is 3% in Nigeria and 7% in Abuja, the Federal Capital Territory (FCT). TB/HIV Co-infection rates are 22% and 32.3%, respectively. Despite the high TB burden in Nigeria, case detection is estimated to be very low (16%). Nigeria contributes 500,000(15%) to the 3.3 million cases of TB that are not notified to the WHO each year (3).

Optimizing TB diagnosis in Nigeria will greatly improve TB case detection, and thereby significantly reduce the global TB notification gap.

Most of the current TB diagnostics take a long time to confirm a diagnosis of TB and have reduced sensitivity among HIV patients. My study will explore better ways of diagnosing TB among HIV patients by assessing the effectiveness of the two current WHO algorithms for exclusion of TB and for improving TB diagnosis among new HIV patients; and the performance of selected new TB diagnostics in this population.

Exclusion of TB among newly diagnosed HIV patients is very important for initiation of IPT, as it reduces the risk of developing TB. The study will generate information on how to exclude TB among new patients with HIV and to improve the diagnosis of TB among HIV patients.

Type 2 Diabetes mellitus (T2DM) increases the risk of TB and WHO recommends screening for DM among TB patients. HIV increases the overall inflammatory processes of the body and patients with HIV have a higher risk of developing insulin resistance. In addition, several ARVs antagonise the effect of insulin and the risk of T2DM increases with the administration of ART.

The DM screening aspect of the study will document the burden of DM in HIV patients, highlighting the importance of early detection to reduce the risk of developing TB and the metabolic and chronic complications of DM.

A.3	**DUPLICATION OF RESEARCH:** Indicate what steps have been taken to ensure this work has not already been carried out. If this project or a similar one has been done before what is the value of repeating it? *(max 300 words)*

An extensive literature review of studies conducted in the topics of my dissertation was conducted.

The 2007 WHO guidelines on exclusion of TB among HIV patients recommends that all patients with HIV should be asked for symptoms of TB. Patients who do not have symptoms are given a medicine (called Isoniazid) to prevent them from developing TB in the future. This is called Isoniazid Preventive Therapy (IPT). This approach was evaluated in Ethiopia. Guidelines were then updated in 2011.The updated guidelines have not been evaluated in Africa.

Towards the end of 2010, WHO approved the use of a new test (Xpert MTB/RIF) for TB diagnosis. This automated molecular diagnostic test, can detect TB and drug (Rifampicin) resistance within two hours, and can be used in settings outside the reference Laboratories. The test has sensitivity of 92% and specificity of 99%, but it is less sensitive (75 %) among HIV patients. To improve diagnosis of TB, the manufacturers of Xpert MTB/RIF, developed Ultra-sensitive Xpert MTB/RIF (Xpert Ultra), which has higher sensitivity than the current Xpert. This new device is currently being evaluated for WHO endorsement in 4 countries. There are no studies reported. QuantuMDx Q-POC, xRapid-TB and Fluorobot are promising new TB diagnostics which performance need to be evaluated before they can be approved for general use. The e-nose included in the proposal is currently being developed. There is no data published on its performance. There is no published data on use of combination of C-reactive protein(CRP) and IP-10 (at higher sensitivity but lower specificity) as a screening test in TB diagnosis

The prevalence of DM among TB patients has been reported from many countries (e.g. Uganda and Tanzania), but not from Nigeria. There is no data on the prevalence of DM among HIV patients in the FCT.

A.4	**OBJECTIVES:** List the major objectives of the study. These must be clearly stated and achievable by the proposed design and methods

Objectives

(1) To assess the predictive value of the WHO algorithm to exclude TB among people living with HIV (PLHIV)
(2) To determine the performance of selected new diagnostics for TB among PLHIV
(3) To optimize the WHO algorithm to diagnose TB among symptomatic patients with HIV using alternative/additional diagnostic pathways and diagnostics.
(4) To assess the proportion of patients with HIV or TB who have type 2 Diabetes Mellitus (T2DM)
(5) To compare the performance of Fasting Blood Sugar (FBS) and Glycated Haemoglobin (HbA1C) for the diagnosis of T2DM among patients with HIV and or TB.
(6) To retrospectively determine the treatment outcome of patients with TB with and without HIV.
(7) To retrospectively determine the prevalence of hypertension and DM among new patients with HIV at the time of enrolment.
(8) To retrospectively determine the incidence of hypertension and DM among the same patients one year after initiation of ART.

A.5	**METHODOLOGY:** Please include the methodology for each objective (if different) and justify the rationale behind the use of the chosen methodology. Please use simple language which is understandable to a non-scientific/non-academic audience and spell out all acronyms.

Description of study location for patients' enrolment:

The following ART sites will be invited to participate in the study:

(1) University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja, Nigeria

(2) Wuse General Hospital, Wuse, Abuja, Nigeria

The University of Abuja Teaching Hospital (UATH) is a 500-bed tertiary health facility of the Federal Government of Nigeria. It is located in Gwagwalada Area Council of the FCT, and provides specialised services in all disciplines of medicine. The hospital has the largest ART site in the FCT, with more than 15,000 HIV patients enrolled. An average of 8 new HIV patients is enrolled per day, with 160 new HIV patients registered per month.

Wuse General Hospital is a district hospital of the Federal Capital Development Administration (FCDA) and is located in Wuse neighbourhood in Abuja Municipal Area Council. It provides specialized medical services, and is one of the largest health facilities in the FCT with ART services. The HIV site has more than 5000 HIV patients enrolled and 30-40 new patients diagnosed per month.

The two facilities offer HIV Counselling and Testing (HCT) services. Patients attend voluntarily or are referred by other services for testing. New patients diagnosed at the HCT unit or referred from other centres are referred to the ART sites for HIV care. ART sites receive referred patients from government, faith-based and private hospitals. The services provided include chronic HIV Care; ART adherence preparation, initiation and monitoring; prophylaxis and treatment of opportunistic infections (OIs); TB/HIV co-management; Prevention of Mother-to-Child Transmission (PMTCT); patients' education on positive living; Home-based care, various forms of patients' support, and cervical cancer screening. The ART sites have the following units: Monitoring and Evaluation (M&E), Home-based Care and patients' Support, Medical teams, ART Adherence Counselling, Pharmacy, Laboratory, Paediatrics, and Cervical Cancer Screening. Newly diagnosed HIV patients report to the M&E unit, which enrols new patients into the program. Patients are then sent to the Home-based care and support unit, for first-visit counselling, history taking and documentation. Afterwards, patients attend the laboratory for baseline investigations, which include CD4 count, full blood count (FBC), renal (RFT) and liver function tests (LFT) and Hepatitis B Virus (HBV) screening. The patient is allocated to one of the medical teams which comprises one doctor, one nurse and one ART Adherence counsellor. New patients are initially seen by the doctor, who takes a medical history, physical examination, clinical HIV staging and treatment of OI. Patients with symptoms of TB undertake chest X-rays and are referred to the Directly Observed Therapy Short-course (DOTS) unit for sputum smear microscopy. Seriously ill patients are admitted to the medical wards. Patients with OI are sent to the Adherence Counsellors for counselling on the drug(s), HIV/AIDS and positive living, and to the pharmacy to collect drugs. Drugs for OI are provided for one week and patients are asked to return to

collect the baseline investigations and assessment of ART eligibility. Patients co-infected with TB commence TB treatment and two weeks later, if there are no problems, start ART that does not interact with TB medications. Patients without TB symptoms eligible for ART receive ART adherence preparation and commence Co-trimoxazole Prophylaxis Therapy (CPT). Patients are given a two-week appointment on CPT alone. At the end of the two-weeks, patients are assessed regarding CPT adherence and their understanding of ART adherence. Misunderstandings and problems with adherence are addressed during this visit. If the patient's adherence preparation is satisfactory, ART is initiated and CPT is continued. Patients not eligible for ART are followed using CD4 counts and clinical staging every three months.

The study objectives will be achieved as follows;

(1) To assess the predictive value of the WHO algorithm to exclude TB among PLHIV

This will be a cross-sectional study from January 2016 to December 2016, among newly diagnosed HIV-positive patients attending the participating ART sites in Abuja, FCT.

Study's participants' selection

Participants will be identified through the ART sites' M&E units of UATH and Wuse district hospital. All newly diagnosed HIV patients >18 years enrolled at the ART sites, will be invited to participate. After obtaining consent, patients will be interviewed and screened for TB symptoms (presence and duration of cough, fever, weight loss and night sweats) using a questionnaire. Patients will be classified as having/not having symptoms of TB. It is expected that two-thirds of patients will not have symptoms, will be classified as 'WHO-algorithm negative' and will be eligible to participate in this study component, constituting the reference population. A randomly selected sample of participants will be recruited from this reference population. A daily register of all newly diagnosed HIV patients with their TB symptoms screening will be kept, whether they are recruited or not into the study (to make adjustment during data analysis).

Participants will be interviewed to describe medical history, reasons for attending, probe the lack of TB symptoms and undertake physical examination and all routine HIV investigations (CD4 Count, FBC, RFT and LFT and chest X-rays).

Sample collection

Patients will be asked to attempt producing three sputum samples, two samples on the spot, the second sample, one hour after the first, and one in the morning of the following day.

As patients without TB symptoms are more likely to produce sputum in the morning, patients unable to produce sputum on the spot will be asked to attempt to produce further samples during consecutive mornings and to bring the specimens to the clinic.

In addition, we will collect 50 ml of urine and 10ml of blood from each patient at the same time of sputum collection. Please see explanation for these samples in objective 2.

Laboratory tests

Sputum will be transported to Zankli Medical Centre (ZMC) Laboratory (using a cold chain), where it will undergo smear microscopy (using a Light-emitting diode (LED) fluorescence microscope **(**LED-FM)), ULTRA-Xpert MTB/RIF, conventional Xpert and culture. To ensure specimens are cultured within twenty four hours of collection, there will be daily transportation of specimens to the research laboratory. Please see objective 2 for a description of laboratory methods.

Classification of patients

Patients will be classified as *TB-positive*, *TB-unlikely* and *TB possible* based on the results of WHO endorsed diagnostics. It is assumed that ULTRA will have been endorsed at the time of the study. However, this can be revised if endorsement is not successful.

TB-positive: patients who have positive culture, Xpert/ULTRA-Xpert/MTB/RIF results.

TB-unlikely: patients who have negative culture and negative ULTRA/Xpert MTB/RIF results.

TB-possible: patients with positive smear microscopy (LED-FM) but contaminated culture and negative or inconclusive Xpert MTB/RIF results and abnormal X-ray. Patients in this group will be asked to provide new specimens for ULTRA.

Patients deemed not to have TB, will be provided IPT, as per WHO, and ART as per clinic guidelines. Patients with TB, will be provided TB treatment and initiate ART two weeks later, as per the Nigerian ART guidelines. Patients with TB-possible, will be re-assessed with the repeated investigations and if still unconfirmed, will be re-assessed three months later to evaluate their clinical condition (see follow up below).

Follow up:

All participants will be followed up according to the clinic routine appointments for individuals with and without TB. At the 3-month follow up, patients will be re-examined to confirm they have not developed symptoms of TB. Patients will also be asked for the presence of symptoms during each routine appointment. If a 'TB-negative' patient is discovered to develop symptoms in the course of follow-up, the patient will discontinue IPT and will undergo a new round of TB tests.

The Home-based Care and Support unit has a good patients' tracking system, and we will collaborate with the unit to track the participants who do not come for follow-up.

(2) To determine the performance of new diagnostics for TB among PLHIV

A cross sectional study design will also be used to evaluate the performance of selected new TB diagnostics among symptomatic patients. These will include the ULTRA Xpert MTB/RIF on sputum and urine samples, xRapid-TB, QuantuMDx-Q-POC on sputum and C-reactive protein and IP-10 in blood.

OuantuMDx Q-POC platform (QuantuMDx Group Ltd, Newcastle, UK), is a new portable, solar battery-operated and handheld DNA analyser that is being developed to detect Mycobacterium TB and drug resistance within 15 minutes, using sputum sample. However, the manufacturer has indicated the processed sputum that will undergo the molecular test could be screened to identify the bacilli, which could have an application as a smear microscopy device. I will evaluate an early prototype of this device in March 2016.

XRapid-TB is a mobile health device that uses the iPhone processor and its imaging to recognise the shape and colour of *Mycobacterium tuberculosis*, when attached to a microscope. According to its manufacturer (xRapid, London, UK), the device can withstand harsh weather and high temperature. It can be stored, taken and used anywhere with minimal training. LSTM will take the device to conduct pilot field evaluation by screening ZN stained smears using conventional light microscopy and the new device to assess the agreement of the readings and against culture (to assess sensitivity and specificity).

C-reactive protein (CRP) and Interferon gamma-induced protein 10 (IP-10): are acute phase proteins produced in response to inflammation. Although, CRP and IP-10 are non-specific biomarkers, a recent systematic review showed that elevated CRP and IP-10 levels have a median sensitivity of 92% and 90%, respectively and specificity of 62% and 76% respectively, when used for active TB diagnosis, irrespective of HIV status (unpublished). Combination of the two tests for TB screening is likely to give higher sensitivity and negative predictive value, thereby making it better to detect patients who are not likely to have TB. These markers however have been used as adjunct tests for diagnosis and we will explore whether it is possible to use a cut-off with higher sensitivity and lower specificity to re-position the test as a screening (not a diagnostic) test for TB.

Xpert MTB/RIF ULTRA: The ULTRA-sensitive Xpert MTB/RIF is a new version of the current Xpert- MTB/RIF test. It is expected to have high sensitivity (close to culture)

and a limit of detection of 10CFU/ml of MTB (ten times lower than the current Xpert MTB/RIF). It was developed to improve the detection of TB in smear-negative cases, which is commonly found among HIV patients. With this diagnostic device, the difficulty associated with TB diagnosis among HIV patients may be overcome, and there may also be no need for empirical TB treatment, because of the ability of the test to detect very low amount of MTB in sputum and other body fluids/tissues.

Participants' Selection

The participants for this objective will be newly diagnosed HIV-positive patients attending the same participating centres and who have symptoms of TB at the time of diagnosis of HIV and patients without symptoms who are unlikely to have TB.

As described for objective 1, newly identified HIV patients will be interviewed and screened for symptoms suggestive of TB using the WHO screening questionnaire. One third of the patients are expected to have TB symptoms. Patients with symptoms will be eligible to participate. A register of all the newly diagnosed HIV patients with their TB symptoms' screening outcome will be kept, whether they are recruited into the study or not. Patients without symptoms participating in the evaluation of the WHO algorithm will be included as controls. The number of participants without symptoms will be determined by the sample size calculations (see below).

Specimen collection

Each participant will be asked to produce three sputum samples, urine and blood, as described in the previous section. Instruction on how to produce good quality sputum samples will be given to each patient. Specimens will be examined to ascertain their quality and quantity, and specimens with deficient quality or quantity will be retaken. Urine samples and exhaled air will be collected at the same time of sputum collection, as described for objective 1.

Specimen Transportation

After specimen collection, specimens will be transported to ZMC as described for objective 1.

Specimen Processing

The specimens will be processed as follows:

Every participant will be tested with LED-FM, Xpert MTB/RIF ULTRA, the Xpert MTB/RIF, QuantuMDx Q-POC, xRapid-TB, and culture. The first sputum sample will be used for ULTRA-Xpert MTB/RIF, LED-FM, Genedrive and BlaC. The second sputum sample will be tested with culture, current Xpert MTB/RIF and LED-FM. The third sputum sample will be kept under a cold chain in case anything goes wrong with any of the first two samples.

Sputum:

In addition to the experimental tests described above, all sputum samples will undergo the following tests:

Smear Microscopy: smears will be prepared as described for objective 1. LED-FM will be used to describe the performance of the new TB diagnostics among smear-positive and smear-negative patients.

Culture: culture will be performed using two solid **Lowenstein-Jensen** (LJ) culture media slopes per patient and monitored for growth for 2 months. The time to growth and number of colonies will be documented. Drug sensitivity testing will be conducted on Xpert RIF-positive samples to determine the prevalence of Rifampicin mono-resistance and MDR-TB among the study population, and to refer patients for appropriate treatment.

Urine

Urine samples will be tested in two ways. Firstly, 2ml of fresh sample will be taken, concentrated by centrifugation, suspended in 0.75ml phosphate buffer, and then tested with the current Xpert MTB/RIF. Secondly, the remaining volume will be stored at -80^0C and samples will be defrosted, concentrated by centrifugation, and tested using ULTRA-Xpert MTB/RIF once the cartridge becomes available. The delay sampling of ULTRA is due to the cartridge only being released in the Spring of 2016. Once cartridges become available in the country, fresh urine samples will be tested in duplicate using Xpert and Ultra.

Blood

Participants will be directed to the phlebotomy unit (where blood samples for routine testing are being collected) where 5 – 10 ml of blood will be collected for CRP and IP-10 tests. After collection, specimen samples will be transported under appropriate condition to our research laboratory, where the samples will be analysed.

The test performance characteristics (sensitivity, specificity positive and negative predictive values of each test will be established using culture as the reference standard.

(3) To optimize the WHO algorithm for TB among HIV patients, using alternative diagnostics.

This third objective of the study will also be achieved by the combined analysis on participants in objectives 1 and 2 above. To achieve this objective, we will use the results of the TB diagnostics carried out in objectives 1 and 2 to determine which test or tests combination yields the highest proportion of the patients that are correctly diagnosed or ruled out of TB.

The diagnostic yield of the combination of tests listed below, will be determined, using culture as the reference standard. Tests' combinations were selected depending on the intended use, as defined by the manufacturers.

The analysis will determine the yield of (a) Xpert MTB/RIF alone (b) Xpert MTB/RIF Ultra alone (c) xRapid-TB alone (d) QuantuMDx Q-POC alone (e) (CRP + IP-10) alone (f) xRapid-TB followed by Xpert MTB/RIF (g) QuantuMDx Q-POC followed by Xpert MTB/RIF (h) (CRP + IP-10) followed by Xpert MTB/RIF (i) Xpert MTB/RIF Ultra and QuantuMDx Q-POC (l) xRapid-TB followed by Xpert MTB/RIF Ultra (k) (CRP + IP-10) followed by Xpert MTB/RIF Ultra

Two by n tables will be drawn to determine the sensitivity and specificity of each of the combination of tests, using culture as the reference standard. We will plot Receiver Operating Characteristic (ROC) curves for the tests, with the sensitivity on the vertical (Y) axis and False positive (1-specificity) on the horizontal (X) axis, and determine the Area Under the Curve (AUC). The higher the AUC for each test/tests combination, the better its diagnostic yield/performance.

(4) To assess the proportion of patients with HIV and or TB who have Type 2 Diabetes Mellitus (T2DM)

The objective will use a cross sectional study design to screen for type T2DM, all patients enrolled for objectives 1 and 2. Blood samples will be collected for Fasting Blood Sugar (FBS), for diagnosis to determine the proportion of the patients with T2DM. Patients with high FBS will be asked to repeat the test, as recommended by the WHO to confirm an abnormal result.

In addition, a cohort of TB patients who were diagnosed to have diabetes in Zankli Medical Centre, and have completed their TB treatment, will be contacted and followed up, to determine if they are still diabetic by collecting their blood and testing it with Glycosylate Haemoglobin (HbA1C) machine. Because, studies have shown that if diabetes is caused by insulin resistance associated with TB, once TB is treated, the diabetes may resolve or disappear.

(5) To compare the performance of FBS and Glycosylated Haemoglobin (A1C) for the diagnosis of type 2 M (T2DM) among patients with T2DM and HIV/TB

FBS has the disadvantage of being measured after several hours of fasting and is a one-point estimate of glucose homeostasis. A potentially better and easier marker of glucose homeostasis is the use of A1C, which measures the amount of haemoglobin that has been glycated and reflects the levels of glucose in blood in the 2-3 months before the measurement. The fifth objective will be achieved by a cross sectional survey using FBS and A1C to screen the patients in objectives 1 and 2. We will compare the agreement of the tests with the result of the reference standard DM diagnostic (2 consecutive FBS), to determine the performance of each of the A1C among the study population.

(6) To retrospectively determine the treatment outcome of patients with TB with and without HIV.

This will be a retrospective descriptive study of patients who have completed their TB treatment within the last two years at the Directly Observed Treatment Short-course (DOTS) clinic of University of Abuja Teaching Hospital (UATH).

With the permission of the UATH TB programme coordinator, all TB treatment registers of patients diagnosed to have TB in the centre who were recommended to initiate treatment in the centre from January 2013 to January 2015 will be retrieved from the clinic record room. The records will be reviewed to extract information regarding the patients' HIV status (positive, negative or undetermined), whether the diagnosis had microbiological confirmation or not (i.e. positive smear microscopy, Xpert MTB/RIF or culture) , clinical presentation (whether pulmonary or extra-pulmonary), anti-TB drug regimen started and date treatment initiated, treatment outcome according to the TB NTP outcomes (abandoned/Loss-to-follow up, cured, completed, treatment failure, relapse or transferred out or unknown) basic demographic characteristics (age, gender, educational status, occupation) and factors that may be associated with treatment outcomes (smoking and known diabetes). A data extraction form will be used and the proportion of missing records will be noted. No identifiable variables will be recorded. Patients will only be identified by the hospital record number for the purposes of database cleaning.

(7) To retrospectively determine the prevalence of hypertension and DM among newly diagnosed patients with HIV.

This will be a retrospective review of records of all patients newly diagnosed to have HIV who were enrolled at the UATH's ART site from July 2014 to July 2015.

Measurements of vital signs, including blood pressure, weight and height are routinely collected at first visit to the HIV site and at every follow up clinic appointment (except height). These measurements are filed in the patient register and kept in the hospital's central records by the M&E unit of the ART clinic and medical records of UATH and digitised into a unit database.

The data will be collected from M&E unit database, or if missing, from the patients' physical files records, the PhD candidate will extract the blood pressure, weight, height, body mass index, blood glucose, age, gender, level of education and occupation at the time of enrolment. After extraction, the data will be entered into a database.

(8) To retrospectively determine the incidence of hypertension and DM among the same patients one year after initiation of ART.

The records of all patients identified in objective one HIV patients who had normal blood pressure (diastolic BP <90 and systolic BP <120) will be reviewed for this objective to extract information on the blood pressure and blood sugar one year after enrolment. In addition, information will be extracted on weight, Body mass index, ART regimen/s used and its date of initiation, and co-morbidities/illnesses present at the time of follow up.

A.6	**PARTICIPANTS**: Please state the number of research participants to be recruited. If you are unable to give precise figures, please give estimates.

A.6.1 AGE/SEX	Neonates (<28 days)	Infants (1-11 months)	Young children (1-9 years)	Adolescents (10-18 years)	Adults (>18years)
Males					About 350 (35%) HIV positive patients.
Females					About 650 (65%) HIV positive patients.
Males					About 30 (60%) HIV negative patients
Females					About 20 (40%)HIV negative patients

A.6.2	**ELIGIBILITY CRITERIA**

Inclusion Criteria	**Exclusion Criteria**
(1) Confirmed HIV positive status (2) Age of 18 years and above (3) Newly diagnosed (4) Consent to participate in the study (5) Enrolled at the selected ART sites In addition, for the e-nose: 1. HIV- negative status 2. Age of 18 years and above 3. Newly diagnosed patient with smear positive TB 4. Consent to participate in the study	Patients on temporary enrolment Patients already diagnosed to have TB (except for the e-nose) Patients unable to provide informed consent Patients with a high risk of imminent death (these are patients with severe respiratory distress, loss of consciousness, un-recordable blood pressure or any other sign of life threatening conditions)

5. Enrolled at DOTS clinics of the participating health facilities	

Justification for eligibility criteria – if you are excluding a particular group you should justify their exclusion.

Newly diagnosed HIV patients on temporary enrolment in the selected health facilities are excluded from the study because they are usually on transit through or on a visit to Abuja and were screened for HIV because they became ill. These patients usually prefer to go to ART sites close to where they live and may not be available for all the required investigations and follow up. We decided to exclude all newly diagnosed HIV patients who have been diagnosed of TB (except those for the e-nose Proof of Concept) from this study, because this is the focus of the study.

A.6.3	**VULNERABLE GROUPS:** Please identify vulnerable groups that will be included in this study. Also state how you will minimise any harm to each group identified.

The likely vulnerable groups that may be included in this study are;

(1) Adults who may be too sick, that they cannot give informed consent for their participation in this study (that is, sick patients with no sign of life threatening condition)
(2) Pregnant women (mostly detected during antenatal care and referred to the ART clinics for Prevention of Mother-to-Child Transmission of HIV (PMTCT))
(3) Patients worried their family would stigmatize them if they know the diagnosis
(4) Uneducated patients who do not understand English and may not be able to read or write
(5) Patients who are very poor and may not be able to return for routine follow-up appointment

Methods to minimize harms to vulnerable groups

(1) For adults who are too sick or unable to give informed consent due to a disability, we will wait until the patients have improved in the hospital, to obtain informed consent. Patients will be approached for written informed consent after they are well informed about the study. In case of patients unable to give informed consent, the patient will be excluded from the study and a tally of these patients will be kept to inform the data analysis.
(2) Pregnant women will be included, as this is not an interventional study and all specimens for this study will be collected for routine investigations (FBC, LFT, RFT and CD4 count, except FBS) for HIV/AIDS care. The study is not considered harmful to pregnant women. The main difference to routine care is the use of TB tests which are not routinely done. These tests will be carried out at a well-equipped research laboratory located outside the selected ART sites.
(3) Patients who are worried of being stigmatized by family members/neighbours if they are aware of their HIV or TB status, will be sent to professional counsellors. Staff members are well trained, for the needed counselling and patients will be assured that no one will be informed of their HIV or TB status, without their

permission. They will be encouraged to join patients' support groups, where they can get practical experience of how other patients cope with stigma and discrimination.

(4) Uneducated patients will be approached by an interpreter, who will communicate with them in their language. The interpreter will read the patients' information sheet and consent form to them in the presence of two witnesses, and if they agree to participate in the study, they will be allowed to thumbprint the consent form.

(5) We will use the routine clinic appointment system for patients' follow-up. Patients who are very poor and unable to return to clinic appointments are routinely referred to the Home-based and patients' support unit. We will refer these patients to the unit for assistance, as needed.

A.6.4	**RECRUITMENT PROCESS:** Please detail the procedures for how you will be approaching each group of participants to take part in the project. Where will recruitment take place? Who will be responsible for recruitment of participants?

We will approach the management of each of the selected ART sites for permission to carry out the study. We will inform the health care providers in the ART sites about the purpose of the study and seek their cooperation/support. Each newly diagnosed HIV patients in the health facility will be contacted through the monitoring and evaluation (M&E) unit (which is responsible for the enrolment of all newly diagnosed HIV patients) and the Home-based Care unit (that takes a comprehensive history of each newly diagnosed HIV patient at his/her first visit, before allocating the patient to a doctor) and invited to participate, after informing the patient the purpose of the study.

We will determine the number of sample to be collected per clinic day by dividing the study's total sample size by the number of clinic days within the period of data collection. If the number of newly diagnosed HIV patients who meet the selection criteria is not more than the sample size per clinic day, all consecutive patients who agree to participate will be recruited into the study, otherwise an appropriate random sampling method will be used to recruit patients into the study. Every one of the study participants we will be screened for TB symptom, using the WHO TB symptoms screening questionnaire, after undergoing the routine vital signs' measurement. After the screening, patients will be classified into those with TB symptoms (symptomatic) and those without the symptoms (asymptomatic). Please notice the gender ratio stated (35% male and 65% female) reflect the male/female ratio observed in the clinics where the study will be conducted.

Recruitment of HIV negative patients in the DOTS clinics will be as follows; We will approach the head of the FCT-TBLCP to ensure collaboration of Gwagwalada and Wuse DOTS clinics staff and for the permission to carry out the study. Every consecutive newly diagnosed TB patient who is smear-positive will be eligible for this arm of the study, will be approached and invited to participate after being fully informed about the study and its procedures. Every patient that gives his/her written informed consent to participate, will be recruited into the study and will undergo similar tests as described for HIV-positive patients (culture, Xpert, BlaC, Genedrive).

A.7	**OUTCOMES:** What is the primary outcome measure for the study? What are the secondary outcome measures? (if any)

Primary Outcomes

For objective 1

(1) Proportion of patients correctly identified not to have TB by the WHO algorithm
(2) Proportion of patients with TB missed by the WHO algorithm

For objective 2

(1) Proportion of patients with TB correctly identified or ruled out by each diagnostic test used

Secondary Outcomes

(1) Diagnostic or combination of tests yields
(2) Proportion of HIV and HIV/TB patients with DM
(3) Agreement between FBS and Hb(A1C) for DM diagnosis among the study population
(4) E-nose proof of principle and initial performance characteristics.

A.8	**MAJOR METHODS OF ANALYSIS:** What are the major methods you intend to use to analyse the data?

Summary statistics will be presented with their 95% confidence intervals. The case detection rate will be calculated using culture as the gold standard. Rates will be adjusted by the expected sensitivity of the reference standard. The incremental detection yield of the tests will be calculated for different combinations of tests.

We will then use a two-by-two table to compare the results of the symptom screening with the result of the reference standard TB diagnostic (which is sputum culture) and determine the probability that the study's participants who do not have any TB symptom are truly free of TB .This will give us the predictive value of symptom screening to rule out TB .ROC curves will be plotted for the diagnostics and the Area Under the Curve(AUC) will be used to determine the performance of each of the diagnostics. The larger the AUC for a test, the better the performance of the test. The same curve and AUC will also be used to determine the diagnostic yield of each of the TB test or combination of tests.

McNemar's tests for matched data will be used to compare the sensitivity, specificity and predictive values of Genedrive, BlaC, current Xpert MTB/RIF and Xpert MTB/RIF Ultra, and will be adjusted for the expected sensitivity of culture.

The accuracy of BlaC, (at high and low sensitivity settings), Genedrive, current Xpert MTB/RIF and Xpert MTB/RIF Ultra will be compared against culture. The performance

of each test and combination of tests will be compared against culture. The latter is the approach we would expect to use if the test performs satisfactorily.

Each of the new TB diagnostics will be considered to be of sufficient value for deployment in the health system if its performance alone or combined with other test (at a high sensitivity setting) has

1. A higher accuracy than the expected 75% accuracy of TB diagnosis which the current Xpert MTB/RIF has when used among HIV positive patients or on smear-negative TB samples and
2. The specificity of the approach is not lower than 93% (in other words, the difference is not more than a -5% different than the expected Xpert MTB/RIF specificity of 98% on smear-negative samples).

Proportion of HIV and HIV/TB patients with DM will be determined by descriptive statistics.

The performance of glycosylated Hb (A1C) and FBS among the study population, will be evaluated by determining the agreement between the two tests.

A.9	**SAMPLE SIZE:** Please justify your choice of sample size (as described in A.6.1). Please ensure that the sample size calculation is based on the primary outcome measure as detailed in A7.

Sample size / statistical power

Comparisons of diagnostic tests

Sample size has been determined to ensure adequate statistical power to detect clinically significant differences (as measured by area under ROC curves) in the performance of pairs of diagnostic tests.

A total of 1000 HIV+ patients will be recruited across two centres. All of the study diagnostic tests will be performed on all recruited patients; patients either unable or unwilling to provide sufficient tissue for this will be replaced.

Assuming a 20% TB prevalence in this population, the study sample will be expected to contain 200 TB+ and 800 TB- patients. This will provide 80% (90%) power to detect a 4.5% (5.2%) difference (or 87.2% power to detect an exact 5% difference) in a comparison of the ROC curve AUCs for any two of the study diagnostic tests. This assumes an AUC of 0.70 for the poorer performing of the two diagnostic tests and up to 75% correlation between the results from the two diagnostic tests; should either the lower AUC or the between test correlation be higher than these assumed values, the statistical power of the study will increase.

Estimates of sensitivity and specificity

Asymptomatic and symptomatic patients combined.

The proposed sample will provide estimates of:

1. test sensitivity with a precision of between ±6.4% (for a true sensitivity level of 70%) and ±3.0% (for a true sensitivity level of 95%);
2. test specificity with a precision of between ±3.2% (for a true specificity level of 70%) and ±1.5% (for a true specificity level of 95%).

Symptomatic patients only

Based on data from a previous study, it is expected that one-third of patients (1000*0.333 = 333) will be TB+ symptomatic, of whom 33% (333*0.33 = 111) patients will be truly TB+ and 67% (222) will be truly TB-. This will provide estimates of:

1. test sensitivity with a precision of between ±8.5% (for a true sensitivity level of 70%) and ±4.1% (for a true sensitivity level of 95%);
2. test specificity with a precision of between ±6.0% (for a true specificity level of 70%) and ±2.9% (for a true specificity level of 95%).

Asymptomatic patients only

Based on the same study, it is expected that the remaining two-third of patients (1000*0.667 = 667) will be TB+ asymptomatic, of whom 11.1% (667*0.111 = 74) patients will be truly TB+ 88.9% (593) will be truly TB-. This will provide estimates of:

1. test sensitivity with a precision of between ±10.5% (for a true sensitivity level of 70%) and ±5.0% (for a true sensitivity level of 95%);
2. test specificity with a precision of between ±3.7% (for a true specificity level of 70%) and ±1.75% (for a true specificity level of 95%).

E-nose Proof of Concept

For the e-nose proof of concept, will be restricted to a small sample size, to assess whether larger studies are suitable. Exhaled breath will be collected from 300 symptomatic HIV positive cases and additional 50 adults who are confirmed to have smear-positive TB but who are HIV-negative. The additional set of patients will be recruited for the e-nose proof of concept sub-study because there is no data on HIV-negative patients and to explore whether HIV infection interacts with the analysis (because HIV infection may release the same VOCs identified and measured by the device).

A.10	**QUALITY ASSURANCE:** Quality assurance is more than just for data analysis – it should include procedures in design and data collection. What procedures are in place to ensure the quality of the data? What consideration has been given to methods of analysis to ensure efficiency of data use?

To ensure that every segment of this study is of good quality, we will do the following at each stage of the study;

Pre-data collection

(1) Extensive literature search and expert opinion seeking on the appropriate study design for the study.
(2) Design of appropriate data collection tools that have been piloted in situations similar to what is expected during data collection and found suitable.
(3) Statisticians will be contacted for their expert opinion/advice on the methods of data analysis needed for the study
(4) Necessary training for research assistants and others who will be involved in the study.
(5) We will ensure that all the needed Laboratory equipment/instruments and consumables are available and in good condition.
(6) The laboratory that will be used is well equipped and known for expertise in all the planned laboratory work
(7) Standard Operating Procedures (SOPs) and universal safety precaution measures will be made available where needed.

During Data collection

(1) We will ensure that every aspect of data collection is handled by individuals that are well trained.
(2) Inclusion and exclusion criteria will be properly applied to ensure that right participants are recruited,
(3) Patients will be taught how to produce adequate amount of good quality samples.
(4) Adequate and uncontaminated amount of appropriate specimen samples will be collected for each objective
(5) There will be regular monitoring/supervision of data collection processes, and lapses/deficiencies noted will be promptly attended to.
(6) Specimens' samples will be transported under cold chain/appropriate condition to the research Laboratory
(7) Double data entry will be used to enter the participants' socio-demographic and clinical information, and each of the participants will have research codes that will be used to link this information with his/her laboratory investigations.
(8) Each of the transported specimens' samples will be stored under appropriate conditions.

Laboratory Analysis of the samples

(9) Everyone participating in the laboratory analysis of the specimens' samples will be reminded of the SOPs and universal safety precaution measures, and handbills/posters containing the information, will be posted on the wall or at places where it can easily be seen in the Laboratory.
(10) There will be proper inspection/examination of each of the specimens' samples, diagnostics and consumables, to ensure they are in good condition before use.
(11) Manufacturers' instructions will be followed in the use of the diagnostics and consumables, so that good quality results can be obtained from them.

(12) Positive and negative controls will run on the diagnostics to ensure they are functioning well.
(13) There will be regular laboratory inspections by the PI to ensure that the SOPs and universal safety precaution measures are strictly adhered to, and to also know if there is any challenge/ need that requires urgent attention.
(14) Results of routine investigations will promptly be communicated to the physicians in charge of the patients' management for prompt treatment decisions
(15) Results of the laboratory investigations will be recorded in a register (which will be properly kept under lock and key) and a highly secured electronic database.
(16) There will also be regular meetings with all the research team members to discuss the ongoing research and address any identified challenge/constraint.
(17) Data will be checked for completeness, and missing data will be sought for.
(18) Assistance/expert input of the statisticians at LSTM will be sought to ensure high quality data analysis.

SECTION B

PROCEDURES AND PATIENT CARE

B.1	**PROCEDURES**	Please detail any clinical or other research procedures to which participants will be subjected.	
Procedure		**To be carried out by:**	**Who is the person employed by?**
1.Enrolment of newly diagnosed HIV patients into the HIV/AIDS Control Program of the selected ART sites		Routinely done by the record officers of the selected ART sites	Managements of the selected ART sites (UATH and Health Management Board of FCDA)
2. Participants invitation for participation in the study and obtaining informed consent		PI and/or research assistants	PhD Student at LSTM/Research Team
3. Routine patients' vital signs check		Routinely done by the Community Extension Health Workers(CHEWS) of the selected ART sites	Managements of the selected ART sites (UATH and Health Management Board of FCDA)

4. Routine Patients' first visit's detailed history taking and documentation	Routinely done by the staff of Home-based Care and Support unit of the selected ART sites	Managements of the selected ART sites (UATH and Health Management Board of FCDA)
5. Participants recruitment and TB symptoms' screening, physical examination, recording of the checked patients' vital signs, ordering of routine and other investigations, interpretation of the investigations' results, prescription of the needed medication, and appropriate referral when needed	PI and other research doctor(s)	(a). PhD Student at LSTM (b). Management of UATH (though the PI is currently on study leave) (3) Research Team
6. Sputum. Exhaled breath and urine samples collection	Research assistant	Research team
7. Blood samples collection for routine for HIV care, and blood sample for FBS	Routinely done by the Phlebotomists of the selected ART sites	Managements of the selected ART sites (UATH and Health Management Board of FCDA)
8. Samples/specimens Transportation to the research Laboratory	Assigned driver by the research Laboratory	Zankli Medical Centre
9. Specimens' analysis	Laboratory Scientists of the research Laboratory	Zankli Medical Centre
8. Data entry	PI / and a data entry Clark	PhD Student at LSTM
9. Data Analysis,	PI with expert advice from Statisticians in LSTM	LSTM
Continue if necessary		

B.2	**STANDARD PATIENT CARE:** Please explain if the procedures outlined in B.1 are part of the normal clinical work of the staff who will perform the procedure. If not applicable to your study, please write a sentence stating that this is not a clinical study.

The procedures outlined above are part of the normal clinical work of the staff who will be performing the procedures and most of these procedures are routinely done for every newly diagnosed HIV patient.

All the specimens' samples (except exhaled breath, which is non-invasive) that will be collected for this study, are routinely collected for routine investigations for HIV Care. All the specimen samples will be taken to a well-equipped research laboratory, where the routine investigations and some specialised ones will be carried out, and the investigations' results will be sent back to the clinics for the needed treatment decision.

B.3	**END OF TRIAL TREATMENT:** For intervention trials, what steps will be taken to make successful interventions or treatment available to all trial participants at the end of the trial? If not applicable to your study, please write a sentence stating that this is not a clinical study.

Though, this study is not an interventional study, the health facilities where the study will be carried out have both ART (for HIV care, treatment and support) and DOTS (for TB treatment) services, each of the participants will be given the standard treatment for his/her case.

Any case of DR-TB identified in the course of this research will be referred to an appropriate treatment centre for DR-TB. Every case of DM found during this study, will also be given appropriate referral.

B.4	**TRAINING**	Please indicate the basis on which the persons identified in B.1 are thought to be competent to carry out these procedures. List any training of staff which will be required prior to commencement of the study.	
Staff Member		**Experience/competencies**	**Training Required**
Record officers of the selected ART sites		Graduates of reputable Nigerian Universities/schools of Health Technology, with many years of working experience at the ART sites	None, already well trained
PI		A Public Heath Physician with more than ten years of working experience in	None, (PI has undergone a training on Good Clinical Practice(GCP), which was

	HIV/AIDS Control programs at the local, national and international levels. He has worked for more than six years at the UATH's ART site providing HIV/AIDS and HIV/TB co-infection prevention, care, treatment and support services. A current PhD student at LSTM	conducted in LSTM in November 2014, and has the certificate for the training)
Research Laboratory Scientists	University graduates with many years of experience in TB/HIV laboratory diagnosis	Good Clinical Laboratory Practice (GCLP)
Research Assistants	They will be University graduates with good knowledge/experience in sociology or medical/basic medical sciences	Necessary training may include; (1). Good Clinical Practice (2). How to interpret and administer questionnaire (3). Good interaction with the study participants (4). Other necessary trainings

SECTION C

RISKS AND CONSEQUENCES

<table>
<tr><td>C.1</td><td>ADVERSE EFFECTS, DISCOMFORT OR RISKS: Outline the potential adverse effects, discomfort or risks that may result from the study for participants, investigators and members of the public and how you will minimise them.</td></tr>
<tr><td rowspan="2">C.1.1

Participants</td><td>Potential adverse effects, discomfort or risks

This study is an observational study and most of the specimens' samples that will be used are routinely collected among the study population. As a result of this, adverse effects, discomfort or risks caused only by this research, if any, will be minimal, and may include;

(1) Discomfort during sputum induction (in case there is a need), in patients unable to produce sputum.
(2) Pain during blood collection for FBS (though, this will be taken at the same time with blood samples for other routine investigations for HIV care, and whether a patient participates in this study or not, he/she will still experience the pain).
(3) Fear of stigma and discrimination that may occur if unauthorised persons are aware of the participants' HIV or TB status
(4) Fear of not knowing/sure of what will really happen to their body fluids' samples that will be taken outside their health care facilities</td></tr>
<tr><td>Steps to be taken to minimise adverse effects, discomfort and risks

(1) For sputum induction, we will use the persons who are well trained and can use the method with minimal discomfort within the hospital.
(2) Blood sample collection for FBS will be done during blood collection for other routine investigations and by well-trained phlebotomists who have several years' experience in doing it at the ART sites.
(3) Participants will be given full assurance and thorough counselling that their TB or HIV status will not be disclosed to any unauthorized person.
(4) We will let them be aware that the laboratory where their specimens will be analyzed, is well-equipped and is collaborating with the National TB and Leprosy Control Program (NTLCP), and that nothing evil (a local common belief) will happen or be done to the samples collected. And we will also let them know we need to take the samples to the laboratory because the new tests are not available in the health facilities.</td></tr>
</table>

C.1.2 **Investigators**	Potential adverse effects, discomfort or risks (1) Accidental exposure to TB causing organisms (2) Needle prick injury and/or occupational exposure to HIV
	Steps to be taken to minimise adverse effects, discomfort and risks (1) To prevent occupationally acquired TB or HIV and other blood born infections, we will put in place SOPs and universal safety precaution measures, and encourage every investigator to adhere to the SOPs. (2) Adequate and appropriate protective devices will also be made available. (3) There will be regular monitoring of investigators' adherence to the SOPs and universal safety precaution measures. (4) Post exposure prophylaxis (PEP) against HIV infection, will be made available, in case there is any accidental exposure to any potential HIV-containing body fluid. (5) We will encourage occupational vaccination against hepatitis B infection for anyone working in the hospital and the laboratory who has not been vaccinated against the infection.
C.1.3 **Members of the public**	Potential adverse effects, discomfort or risks (a) Possibility of transmitting TB from one patient to another or from TB patients to health care workers or patients' relatives. (b) Transmission of TB during sputum specimen transport to the research laboratory (c) Stigma and discrimination against participants' family members if the HIV or TB status is known to the community
	Steps to be taken to minimise adverse effects, discomfort and risks a. Clinical triage will be done to separate patients who are seriously coughing from other patients b. Patients will be kept in a well-ventilated area of the clinics c. Sputum collection will be done in a well-ventilated area, using the necessary protective devices d. Sputum samples' specimens will not be transported with a public vehicle e. Participants' information will be kept confidential by the research team

C.2	**CONSEQUENCES FOR LOCAL HEALTH SERVICES**
C.2.1	**IMPACT ON LOCAL SERVICES:** What demands will this research place on local health services?

Most of the processes/procedures in this research follow the routine procedures in HIV/AIDS care (for instance, all the specimens' samples are routinely collected for HIV care) and the impact of this research on routine care and staff would be minimal.

This may include increased patients' waiting time, due to the time that will be spent on patients' information sheet and consent form.

However, all participants (who would have been seen by the local doctors) will be clinically assessed/seen by the research doctor(s) and their specimens' samples will be analysed at the research laboratory for both routine and specialised TB tests. As a result of this, both the hospitals clinical and laboratory workload of the selected facilities will be reduced.

C.2.2	**MINIMISING IMPACT ON LOCAL SERVICES:** Detail how the design of the research project takes into account the demands described above in C.2.1

To minimise the impact that increased patients' waiting time may have on local health services;

1. All the participants in this study will be seen the PI and/other doctors in the research team, who are well trained and have many years of experience in HIV/AIDS and TB prevention, care, treatment and support. As a result of this, the workload for the health facilities' doctors will be reduced, and patients will be able to leave the clinic on time.
2. As much as possible, all assessments that are additional to routine care will be conducted while the patients are waiting for other clinical assessment or testing.
3. Adequate and well-trained research assistants will be engaged in the research, so that the time spent on patients' recruitment will be reduced to the minimum.
4. The specimens' samples for this study will be transported to a well-equipped laboratory, where both routine and specialised investigations will be carried out on the specimens, and the investigations will be promptly communicated to the doctors in charge of the patients' management, for prompt treatment decision. By this, the workload of the local (health facilities') laboratories will be reduced, and the participants will be screened for TB with the best available diagnostics, thereby get the appropriate treatment.

SECTION D

PRIVACY AND INFORMED CONSENT

D.1	**INFORMED CONSENT (<u>please pay particular attention to the guidance notes for this section</u>)**
D.1.1	**OBTAINING INFORMED CONSENT**: Please give details of how you will obtain informed consent. You must include details of (i) information given to participants, (ii) who will deliver the information and (iii) consideration of local circumstances. Please note that reference of transport of samples to another country must be included in the consent forms. Please also give special consideration to whether proxy consent is required (for those lacking capacity to consent, minor etc) and give details as to how this would be obtained.

The Principal Investigator (PI) and trained research assistants will contact each of the newly diagnosed HIV patients in each of the selected health facilities through its monitoring and evaluation (M&E) unit (which is responsible for the enrolment of all newly diagnosed HIV patients) and the Home-based Care unit (that takes a comprehensive history of each newly diagnosed HIV patient at his/her first visit, before allocating the patient to a doctor) and invite them to participate in the proposed research, after being fully informed of the purpose of the study and its procedures. They will also be informed that their participation is voluntary and their refusal to participate in this study will not deny them of any of their rights as patients in the clinic. It will also be made known to them that they still reserve their right to withdraw from the study anytime they want to, without affecting their treatment.

Participants will be given 2 hours to consider and make up their mind about participating in the study. For patients who are illiterate (who cannot read or write), the research information sheet and consent certificate will be read to them in the language they understand, in the presence of two witnesses, to ensure that they are well informed about the research. Patients that agree to participate in the study and meet its selection criteria will be asked to give his/her written informed consent by signing or thumb-printing the consent certificate. In case there is any eligible and willing participant who cannot give his/her informed consent, as a result of severity of his/her illness or mental incapability, a legally assigned/approved relative of the patient will be allowed a proxy to obtain his/her consent.

D.1.2	**CONSTRAINTS:** Please outline any potential constraints to consent and indicate how you will reduce the impact of these constraints.

Some of the constraints that may be encountered in the course of obtaining consent from the study's participants are;

(1) Illiteracy: This challenge will be overcome by communicating to patients in the language he/she understands, using an interpreter (for Hausa and Igbo Languages), while the PI will interpret the information to Yoruba for any Yoruba speaking patients. Consent forms will be provided in Hausa, Igbo and Yoruba languages. If a patient does not understand these languages, we will use the services of a staff member who speaks the language. This is the current clinic procedure to explain all HIV service procedures in the study sites.
(2) Some women will be unable to provide informed consent (even after patient has made up her mind to participate) without first obtaining permission from her husband (this is based on local

cultural/religious practices). We will follow local customs and with her agreement, invite the patients' husband or father and they will be given information about the study, and request for their approval of the patients' consent for participation in the study. This additional procedure follows the same procedures used in the HIV services and considers that some women prefer not to participate.

(3) The thought that a patient may not be properly treated or attended to, by his/her doctor if he/she refuses to participate in the proposed study: It will be made clear to the prospective participants that they have the right to refuse participating in the study without being denied of any of their rights/privileges as patients in the clinic.

D.2	**ASSISTANCE:** Please outline any assistance (financial or otherwise) that will be offered to potential participants or individuals in return for their participation in this research.

All participants will be tested using Xpert MTB-RIF Ultra and culture (the reference standard TB diagnostic), which is not current routine standard at these sites and therefore patients will obtain optimal TB diagnosis.

The timing of sputum collection, the quality of smear microscopy and culture and other diagnostic results will be monitored, and therefore participants will be more thoroughly investigated and results are likely to be received earlier.

Prompt diagnosis and treatment of DM could improve treatment outcome and reduce the risk of developing TB among the participants.

Within the context of a research study, patients are also likely to benefit from good medical services. No re-imbursement of patients' costs will be offered as costs for patients will be equal or less than those incurred by attending the routine diagnostic services.

Incentives: There are no financial incentives for participating in the study.

D.3	**PRIVACY AND CONFIDENTIALITY:** Please describe how participant privacy and confidentiality will be maintained during data collection, analysis and storage. Please include what will be collected (data, samples etc.) and where and for how long it will be stored.

The information and other data that will be collected from this study's participants, will be kept strictly confidential under lock and key and in a secured database.

Only the authorised members of the research team, the research sponsors, the clinic officials and ethics committee members, may have access to the information/data.

We will give and attach a number instead of participants' names, to any information about each of them. The number given to each participant will only be known by the researchers and we will make sure that the information is kept where no one else can have access to it.

Sputum, blood, exhaled air and urine are the participants' biological specimens that will be collected during this study. Because the reference standard test for TB (sputum culture) and the new

diagnostics that we want to evaluate are not available at the study centres, the specimens will be stored under appropriate condition and transported to a research laboratory, where all the needed tests can be done. Some of the specimens (sputum, breathed air and urine) may be stored for some months for the evaluation performance of one of the new TB diagnostics, which will be available in the first quarter of next year (2016). As there is a need to transport breathed air outside Abuja, a separate consent will be taken from the participants for this purpose. All specimens will only be used for investigations/tests that are related to the study objectives and will be destroyed five years after this study

D.4	**DISSEMINATION:** Please outline what plans you have for dissemination of results

The results of this research will be disseminated through the following means;

(1) Discussion of the study's findings with the stakeholders (management, health care workers and patients) in the health facilities used for the study
(2) The findings of the study will be discussed in Abuja, Nigeria, immediately after the study, in a larger forum comprising the national TB and HIV stakeholders, such as National TB and Leprosy Control Program(NTLCP), National Agency for the Control of AIDS (NACA), National AIDS and STI Control Program (NASCAP), FCT's Agency for the Control of AIDS (FACA) and its TB control program (FTBLCP), United Nations' agencies, HIV/AIDS and TB control plans implementing partners, Network of People Living with HV in Nigeria (NEPLWHAN), traditional and religious leaders, academia and researchers.
(3) Academic data will be presented at selected scientific conferences (such as the conferences of the International Union Against Tuberculosis and Lund Diseases (The Union), International AIDS Society(IAS), and International Association of Providers of AIDS Care (IAPAC))
(4) We will publish this study's findings in reputable peer-reviewed Journals
(5) My PhD dissertation, which will be based on the study's findings, will be submitted to the University of Liverpool and LSTM.

SECTION E

MAJOR ETHICAL ISSUES

E.1	**MAJOR ETHICAL ISSUES** Outline what you consider to be the major ethical issues involved in this research. Please indicate how you plan to deal with these ethical issues.

The main ethical issues to be considered in this study are as follows:

- **Autonomy**

To ensure autonomy of every participant in this study, the investigators must make sure that every patient is fully/well informed about the research and its procedures, given enough time to consider his/her participation, make an informed decision whether to participate or not, without any coercion or undue inducement, and if he/she decides to participate, he/she should be able to

determine how the biological specimens and information collected from him/her should be stored and used.

In this proposed study, we consider autonomy in the following ways:

1. Informed Consent

To obtain informed consent from this study's participants, we will fully describe the processes and procedures that will be used in the study and also discuss the possible constraints that may be encountered in the process and modalities put in place to overcome these constrains. Please see sections D.1.1 and D.1.2 for more details.

2. Confidentiality

The information and other data that will be collected from this study's participants, will be kept strictly confidential under lock and key and in highly secured databases. Only the authorised members of the research teams will have access to the information/data. Full details on how this will be achieved is discussed in section D.3

3. Storage and further use of biological specimens

Sputum, blood, air and urine are the participants' biological specimens that will be collected during this study. Because the reference standard test for TB (sputum culture) and the new diagnostics that we want to evaluate are not available at the study centres, the specimens will be stored under appropriate condition and transported to a research laboratory where all the needed tests can be done. Some of the specimens (sputum, breathed air and urine) may be stored for some months for the evaluation of performance of one of the new TB diagnostics (Xpert MTB/RIF Ultra), which will be available in the first quarter of next year (2016). As there is a need to transport breathed air outside Abuja, a separate consent will be taken from the participants for this purpose. All specimens will only be used for investigations/tests that are related to the study objectives and will be destroyed five years after this study. The specimens' storage and further use plans are discussed in the patients' information sheet and section D.1.1

- **Non-maleficence**

Because this study is not an interventional study/a clinical trial, the possibility of any harm to the participants, investigators or public is minimal. The potential adverse effects, discomfort and risks that may result from this research and plans to prevent them, are outlined for each category of people, in sections C.1.1 to C.1.3.

- **Beneficence**

The proposed study will be highly beneficial to the participants and the public at large. Presently, it is difficult to diagnosed TB among HIV patients because of high proportion of smear-negative TB cases (which cannot be diagnosed by the commonly available diagnostic (smear microscopy)) and reduced sensitivity of Xpert MTB/RIF among them. Through this research, the reference standard TB diagnostic (sputum culture), which is not available at any of the selected ART sites, will be used in addition with routine diagnostics, for the participants. Thereby making their TB screening of very highly standard with resultant appropriate and prompt treatment. Validation of the current

WHO algorithms for TB among HIV patients, will also inform decisions by policy makers in TB/HIV control programs.

Evaluation of the TB diagnostics may also lead to discoveries that will solve the problem associated with TB diagnosis among HIV patients and thereby prevent/reduce the deaths and public health hazards that may be due to missed cases of TB among the study population.

Prompt diagnosis and treatment of DM will also improve treatment outcome and reduce the risk of developing TB among the participants. As a result of this, the policy makers in TB/HIV control program may include routine DM screening among TB and HIV patients in the national policy.

- **Justice considerations**

Improved TB diagnosis among HIV patients is highly needed in Nigeria and other high-burden TB and HIV setting, because a lot of TB cases among this population are diagnosed late or missed, and thereby making TB the commonest cause of death among them. In addition, public health hazards that may result from TB transmission from undetected cases, will also be prevented if they are diagnosed early and promptly treated.

This study will follow the routine HIV/TB care procedures, collect the same specimens' samples (except urine and breathed airs) for routine care, but take the samples to a well-equipped research Laboratory for both routine and specialised TB tests. As a result of this, patients participating in this research will have better/higher quality diagnosis than what they would have if they had not participated in the study.

This study will also reduce the clinical and laboratory workloads of the selected ART sites, because all the study participants will be seen by the research doctors who are well trained in TB/HIV care, and all the specimens will be tested in the research laboratory with the research fund.

Lastly, findings from this study will be of great benefit to the participants, other persons living with HIV, general public, and the nation (Nigeria) at large. Please, see sections C2.1 and C2,2, for more details on the impact of this research on local services

DECLARATION: TO BE SIGNED BY MAIN APPLICANT Applicants **must initial** each declaration or tick N/A in the right hand column if applicable	**Initial** (by hand)	**N/A**
(1) I confirm that the details of this proposal are a true representation of the research to be undertaken.		
(2) I will ensure that the research does not deviate from the protocol described.		
(3) If significant protocol amendments are required as the research progresses, I will submit these to the Liverpool School of Tropical Medicine Research Ethics Committee for approval.		
(4) Where an appropriate mechanism exists, I undertake to seek additional in-country Ethical Approval in the country(ies) where the research is to be carried out.		
(5) I agree to abide by the ethical principles underlying the Declaration of Helsinki and all relevant LSTM Standard Operating Procedures (SOP) relating to research conduct (available on LSTM intranet at http://pcwww.liv.ac.uk/lstmintranet/research_management/governance_polices_codes.htm or by contacting the Research Office).		
(6) I understand that all conditions apply to any co-applicants, researchers and other staff involved in the study, and that it is my responsibility to ensure that they abide by them.		
For studies over two years in duration (7) I will provide the Research Ethics Committee with an annual report.		
For studies using 'human tissue'* (8) I confirm I will abide by LSTM's Policies and Standard Operating Procedures relating to activities involving human tissue *Human tissue (relevant material) is defined as any material that has come from a human body and consists of, or includes, human cells, but excludes such things as blood and plasma. Further detail can be found at http://www.hta.gov.ac.uk/legislationpoliciesandcodesofpractice/definitionofrelevantmaterial.cfm.		
For studies that involve a clinical trial or participation of humans (the use of their data or tissue) (9) I confirm that I have completed a sponsorship and indemnity form http://pcwww.liv.ac.uk/lstmintranet/research_management/applyingforRG/intro.htm		

I expect the project to commence on (Date)		**Complete by (date)**	
Signed:		**Date:**	

From time to time the committee uses ethics applications for training purposes or to give examples to new applicants. In all cases the applications are anonymised. If you **DO NOT** consent to your application being used for these purposes please tick the box. ☐

8.4. Appendix 4. Patient Information Sheet

Title: Optimising diagnosis and management of Tuberculosis among HIV patients

Name of Principal Investigator: Dr Rotimi OWOLABI

Name of Organization: Liverpool School of Tropical Medicine

This Informed Consent Form is for men and women attending this clinic (Special Treatment Clinic), who we are inviting to participate in a research on how to improve detection of Tuberculosis (TB) and Diabetes among people living with HIV (PLHIV). The title of our research project is "**Optimising diagnosis and management of Tuberculosis among HIV patients in a high TB and HIV burden setting"**

This Informed Consent Form has two parts:

1. Information Sheet (to share information about the research with you)
2. Certificate of Consent (for signatures if you agree to take part)

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction

Good morning/afternoon. I am Dr Owolabi, doing a PhD program at the Liverpool School of Tropical Medicine (LSTM), United Kingdom. We are doing a research on Tuberculosis (TB), which are common in this country. I am going to give you information and invite you to be part of this research.

TB is a very common infection among people living with the Human Immunodeficiency Virus (HIV), and it is sometimes difficult to diagnosis the disease among them. Because people living with HIV (PLHIV) usually have low amount of TB germ in their sputum, which most of the current tests used for TB may not be able to detect. Also, because HIV reduces the ability of the body to fight other infections, PLHIV may not have the signs and symptoms which may make doctors or other health care workers to suspect this disease. Many PLHIV with TB were discovered very late or missed. To improve the way we identify and properly treat this disease, we decided to look for better ways of detecting it in anyone that has it. There are better methods to identify this disease by using new tests called Xpert MTB/RIF Ultra, Genedrive and BlaC. Apart from sputum which is commonly used for TB diagnosis by the currently used TB tests, some of these new tests can also identify TB causing germ in urine, saliva/particles in the mouth, and other body fluids.

People who have diabetes are more likely to have TB and people with HIV are more likely to develop diabetes. And if it occurs together with TB in a person, it makes tuberculosis treatment difficult, and poorly treated/control diabetes can lead to many long term health problems/disabilities like loss of vision, poor blood flow, kidney damage and damage to many organs/parts of the body.

You do not have to decide now whether or not you will participate in the research. We will give you about two hours to consider your participation, but if you want to take more time, we are happy to wait until you come back to your next visit". Before you decide, you can talk to anyone you feel comfortable with about the research.

If you decided to participate, we would ask you some questions about you and your current and previous illnesses. If there are some words in these pages or later in the interview that you do

not understand, please ask me to stop as we go through the information, and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose of the Research

We are investigating three diseases that are often found together and make the appropriate management of people with these diseases more complicated. These three diseases are diabetes, tuberculosis and HIV infection. Tuberculosis is a severe disease caused by a germ (bacterium) that infects the lung; it can cause cough, weight loss, hotness of the body (fever), general body weakness and night sweats. This disease is diagnosed by using a microscope to the look into sputum samples and check TB germ. Other tests that are sometimes performed for the diagnosis are culture and X-ray. With the current commonly used TB tests, a lot of cases of TB among PLHIV are not detected. Because of this, there is a need to look for better ways of identifying if someone among them has this disease or not.

In this research, which aim to examine current ways of identifying people who have or do not have TB and diabetes among PLHIV are good enough, and determine whether the new tests to find if a person has these diseases are better than the tests currently being used, in order to come up with better ways of detecting the diseases among them. To carry out this study, we will test the samples you provide with the new tests and compare its results with that of the current tests.

Participant Selection

All adults who have been diagnosed with HIV infection and are attending this clinic will be approached and asked to participate in this study, because TB is the most important opportunistic infection and commonest cause of death among PLHIV, but the death caused by TB can be prevented if this disease is properly identified and treated on time. Through this

research, we want to discover better methods of identifying persons with and without TB among PLHIV, so that persons with the disease among them can be properly treat on time and prevented from spreading the disease to others. While those found not have TB, can also be given drug to prevent them from developing the disease

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice to decide whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the test that is routinely offered in this clinic to find out if someone has TB or not, and we will tell you more about it late. You may change your mind later and stop participating, even, if you had agreed earlier

Procedures and Protocol

Because we do not know if the current ways of identifying whether someone with HIV infection has TB or not, is good enough, we need to use some specialised tests (Culture and Xpert MTB/RIF), which are not usually done for patients in this clinic, to know how good the current methods are. Because we also do not know if the new Tuberculosis tests are better than the tests currently available for diagnosing the disease, we need to compare them in the same samples. To determine which of the tests is better, all the people participating in this research will be tested with the current and the new tests. Participants will be tested with the standard diagnostic tests and with the new tests called Xpert MTB/RIF ultra, Genedrive, BlaC and e-nose. The usual test used at this centre is to check with a microscope whether your sputum has the Tuberculosis germ. This is called smear-microscopy. Apart from these, we will also conduct other tests that are more specialised, such as culture, current Xpert MTB/RIF and Xpert MTB/RIF ultra. These tests are currently reserved for special

circumstances, but we need to conduct them here to be able to determine how good the new tests are. This study's results will be used to make your treatment decisions by the staff of the clinic. Current treatment is decided on the basis of sputum specimens. The main difference is that we will also have Xpert and culture results and we will provide this additional information to your doctor. However, we will not provide the information on Xpert MTB/RIF ultra, Genedrive, BlaC and e-nose, because we need to be sure that the tests are working properly before using it for treatment decision. All patients that participate in the study will also receive the same treatment given to patients not participating in the study.

To check your blood sugar and determine whether you have diabetes or not, we will collect your blood. This will be collected at the same time your blood will be collected for other tests that are usually done for all patients in this clinic, before they can start treatment. The amount of blood to be collected for this test is small and can not affect you negatively.

Risks: This study is not associated with any additional risks.

Discomforts: The study will not cause you any additional discomforts.

Samples to be collected and stored: We will ask you to submit three sputum samples for examination (which is the same with what other patients who are not participating in this study will be asked to submit). Two on the spot, one, the first time we are seeing you, the second, one hour after the first, and the third one, in the morning of the second day. One of these samples will be cultured, to see if the germs grow in the following weeks. If the germs grow, we will store them in small tubes for further description (called genotyping) and to check if the germ is susceptible to the drugs for treatment. The isolates will be stored for 3 years to facilitate the completion of these tests and then will be destroyed. We will also collect your urine and some of air you breathe out, and check them with the new TB tests, to see if they contain the germ that causes the disease.

All the samples (sputum, blood and urine) that will be collected from you (except the air you breathe out/out-breathe air) will be taken to a well-equipped research laboratory here in Abuja, where it can be done, because the specialised (culture) and the new tests are not available in this clinic/hospital.

Because the test that we will use to check the air you breathe out and determine whether you have TB or not, is a new test and is not available in this hospital, our research laboratory or anywhere in Nigeria, we have to collect the air you breathe out, in a small bag and take it to a laboratory in the United Kingdom, where it can be done. Once we do it there, and we are sure that the test is working well, the machine/equipment needed for this test, will be brought to Nigeria and it can be available in this hospital for everyone that needs it.

Benefits: If you participate, we will test your sputum with culture and Xpert, which are the best tests for this disease available. This is not routinely offered in the diagnosis of TB in this centre. If culture is positive, we will call you back to start you on treatment.

You will also have your blood sugar tested to check if you have diabetes. If you do, you will be referred to the appropriate unit for its treatment.

Incentives: There are no financial incentives for participating in the study.

Confidentiality: We will not be sharing the identity of those participating in the research.

The information that we collect from this research project will be kept confidential. Information that will be collecting about you during the research will be properly kept from unauthorised persons and only the research sponsors, the institutional officials and ethics committee members may have access to the data. We will give and attach a number instead of your name, to any information about you. The number given to you will only be known by the researchers and we will make sure that the information is kept where no one else can have access to it.

Right to Refuse or Withdraw: You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

Who to Contact: If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following: [*name, address/telephone number/e-mail*].

8.5. Appendix 5. Informed Consent form

Consent Form

CONFIDENTIAL

Study Title: Optimising diagnosis and management of Tuberculosis among HIV patients in a high TB and HIV burden setting	
Principal Investigator: Dr Rotimi Owolabi	**Study Site:** Abuja, Nigeria

	Please initial box
(1) I confirm I have read and understood the information sheet dated..... (Version.....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
(2) I understand that participation in this study is voluntary and I am free to withdraw consent at any time, without giving a reason, without any penalties.	
(3) I understand that data collected during the study, may be looked at by individuals from LSTM and from regulatory authorities. I give permission for these individuals to have access to my records.	
(4) I hereby declare that I have not been subjected to any form of coercion in giving this consent.	
(5) I agree / do NOT agree to the data about me, collected in this study being stored for further use in the future.	
(6) I agree to take part in this study.	

Signing this declaration does not affect your right to decline to take part in any future study.

Name of participant	Date	Signature
Name of person taking Consent	Date	Signature

***For patients LARs who can not read/write**

Name of participant	Date	Thumbprint
Name of an impartial witness	Date	Signature

***For patients who are willing to participate but too sick to give consent**

Name of participant	Date	Signature
Name of a legally authorized representative	Date	Signature

When complete: 1 copy for participant; 1 copy (original) for research

8.6. Appendix 6. Questionnaires

8.6.1 Questionnaire on Optimising the Diagnosis and Management of Tuberculosis and Diabetes among HIV patients in a high TB and HIV burden setting

Facility Name _____ Date d d m m y y

Patient's Name _____ _____

Surname First name

Patient's Hospital Number _____ Enrolment Number _____

ART Clinic Enrolment Number _____ Research Code _____

ART Site' code (UATH, Gwagwalada = 1, Wuse General Hospital = 2) _____

Address _____

Mobile phone _____

Demographic characteristics

Age (Years) _____ Sex (Male=1, Female=2) _____

Marital status (Single=1, with partner/married=2, separated=3, divorced=4, widowed=5) _____

Education (None=0, primary=1, secondary=2, higher education=3, prefer not to answer=4) _____

Occupation (Student=1, Unemployed=2, Civil Servant=3, Artisan=4, Business/trading=5, Farming=6, House wife=7, other=8) _____

If occupation is others, please, specify _____

Ethicity (Hausa=1, Igbo=2, Yoruba=3, other=4) _____

If other ethinicity, please specify _____

Religion (Christianity=1, Islam=2, Traditional=3, other=4) _____

Family Type (Monogamy=1, Polygamy=2) ☐

Current Medical History

Presenting Complaints

How long ago did you notice the first symptom of this illness? Months ☐☐ Weeks ☐☐

Symptoms in the last months			**Duration**	
Fever	(Yes=1, No=2)	☐	If yes, duration (weeks)	☐
Night sweats	(Yes=1, No=2)	☐	If yes, duration (weeks)	☐
Muscle pain	(Yes=1, No=2)	☐	If yes, duration (weeks)	☐
Fatigue/weakness	(Yes=1, No=2)	☐	If yes, duration (weeks)	☐
Weight loss	(Yes=1, No=2)	☐	If yes, duration (weeks)	☐

Weight gain	(Yes=1, No=2)		If yes, duration (weeks)	
Cough	(Yes=1, No=2)		If yes, duration (weeks)	
Difficulty with breathing	(Yes=1, No=2)		If yes, duration (weeks)	
Chest pain	(Yes=1, No=2)		If yes, duration (weeks)	
Oral thrush	(Yes=1, No=2)		If yes, duration (weeks)	
Poor appetite	(Yes=1, No=2)		If yes, duration (weeks)	
Painful swallowing	(Yes=1, No=2)		If yes, duration (weeks)	
Nausea and/or vomiting	(Yes=1, No=2)		If yes, duration (weeks)	
Diarrhoea	(Yes=1, No=2)		If yes, duration (weeks)	
Abdominal pain	(Yes=1, No=2)		If yes, duration (weeks)	
Abnormal genital discharge	(Yes=1, No=2)		If yes, duration (weeks)	

Genital/rectal pain/sores (Yes=1, No=2) ☐ If yes, duration (weeks) ☐

Painful urination (Yes=1, No=2) ☐ If yes, duration (weeks) ☐

Headache (Yes=1, No=2) ☐ If yes, duration (weeks) ☐

Poor vision/visual loss (Yes=1, No=2) ☐ If yes, duration (weeks) ☐

Depression (Yes=1, No=2) ☐ If yes, duration (weeks) ☐

Memory loss (Yes=1, No=2) ☐ If yes, duration (weeks) ☐

Other (please, specify) _____

Past Medical History

Hypertension (Yes=1, No=2) ☐ If yes, duration (months) ☐

Diabetes (Yes=1, No=2) ☐ If yes, duration (months) ☐

Asthma (Yes=1, No=2) ☐ If yes, duration (months) ☐

Other (specify) _____

Are you currently on any medication? (Yes=1, No=2) ☐

If yes, what are you using the medication(s) for? _____

When did you first know you have HIV? d d m m y y

How long (in months) did you first know you have HIV? ☐

Where were you first tested for HIV? (In this health facility=1, other health facility within FCT=2, outside FCT=3, don't know=4, do not want to say=5) ☐

Have you taken HIV medications before? (Yes=1, No=2) ☐

If yes, purpose of the HIV medications: ○ PMTCT ○ Treatment ○ PEP ○ others ○ Not known (***please thick as many as appropriate from the options***)

Do you have allergy to any medicine? (Yes=1, No=2, don't know=3) ☐

If yes, what medicine(s) are you allergic to? ○ Sulfonamides ○ Chloroquine Penicillins

○ Don't know ○ Other (specify) _____

Family and Social History

Do you drink alcohol? (Yes=1, No=2, do not want to say=3) ☐

If yes, how frequently? (Occasionally=1, once a week=2, regularly=3) ☐

Do you use illicit drug(s)? (Yes=1, No=2, do not want to say=3) ☐

Do you take herbal supplements (Yes=1, No=2) ☐

Do you take traditional medicine(s) (Yes=1, No=2) ☐

Do you smoke? (Yes=1, No=2, ex-smoker=3, do not want to say =4) ☐

Do you have a sexual partner? (Yes=1, No=2) ☐

If yes, has he/she been tested for HIV? (Yes=1, No=2, don't know=3) ☐

If yes, does he/she have HIV? (Yes=1, No=2, don't know=3) ☐

Please, tick all the possible answers in the next question

Which of your relatives/partners have been tested for HIV? ○ Spouse ○ Sexual partners ○ Child/children ○ None ○ Don't know

Do you have any relatives who have HIV? (Yes=1, No=2, don't know=3) ☐

Please, tick all the possible answers in the next two questions

If yes, who? ○ Spouse ○ Son/Daughter ○ Father ○ Mother ○ Sibling(s)

Which of your relatives are receiving treatment for HIV? ○ Spouse ○ Sexual partners ○ Child/children ○ None ○ Don't know ○ Father ○ Mother ○ Siblings

Do you have any relatives who have diabetes? (Yes=1, No=2, don't know =3) ☐

Please, tick all the possible answers in the next question

If yes, who? ○ Father ○ Mother ○ Siblings ○ Uncle ○ Aunt ○ Child ○ Child/Children ○ Other _____

FOR FEMALE PATIENTS ONLY

Last menstrual period | d | d | m | m | y | y |

Are you currently pregnant? (Yes=1, No=2, uncertain=3) ☐

If yes, gestational age (weeks) ☐☐

TB Treatment

Have you ever been treated for TB? (Yes=1, No=2, don't know=3) ☐

If yes, currently or previously? (Currently on treatment=1, previously treated=2) ☐

If previously treated, how many months ago? ☐☐

TB symptom currently present? (Yes=1, No=2) ☐

TB status: (No signs or symptoms suggesting TB=1, signs or symptoms of TB present=2) ☐

Anthropometric Measurements

Height (m) ☐ Hip (cm) ☐

Weight (kg) ☐ Waist (cm) ☐

BMI (kg/m^2) ☐ Waist/Hip Ratio (WHR) ☐

Physical Examinations

General appearance (Normal=1, abnormal=2, not checked=3) ☐

Respiratory distress (Present=1, Absent=2) ☐

Pallor (Present=1, Absent=2) ☐

Jaundice (Present=1, Absent=2) ☐

Oral thrush (Present=1, Absent=2) ☐

Skin rashes (Present=1, Absent=2) ☐

Paedal oedema (Present=1, Absent=2) ☐

Temperature (^{0}C) ☐ Pulse Rate (beats/min) ☐

Systolic blood pressure (mmHg) ☐ Diastolic blood pressure (mmHg) ☐

Respiratory Rate (breaths/min) ☐

Functional status: (Working=1, Ambulatory=2, Bedridden=3) ☐

WHO clinical staging: (Stage 1=1, stage 2=2, stage 3=3, stage 4=4) ☐

Other clinical findings _____

Laboratory Investigations

Facility Name ____ Age (in years) ____

Date of specimens' collection d d m m y y Date received d d m m y y

Patient's Name ____ ____

Surname Other names

Patient's Hospital Number ____ Enrolment Number ____

ART Clinic Enrolment Number ____ Research Code ____

ART Site' code (UATH = 1, Wuse GH = 2) ____ Lab Number ____

Specimen code (Blood = 1, Sputum = 2, Urine=3) ____ Gender (Male=1, Female=2) ____

Tests	Results	
○ CD4		cells/uL
○ CD4%		%
○ WBC		X 10^9 /L
○ PCV		%
○ Hb		g/dl
○ Platelets		X 10^9 /L
○ HBsAg (Positive=1, Negative=2)		
○ Creatinine		umol/L

Tests	Results	
Viral Load		copies
FBS		g/dl
FBS		umol/L
HbA1C		%
HbA1C		umol/L
Total Cholesterol		umol/L
LDL		umol/L
HDL		umol/L

TB Screening Tests' Results Sheet

Facility Name _____ Age (in years) ____

Date of specimens' collection d d m m y y Date received d d m m y y

Patient's Name _____ _____

Surname First name

Patient's Hospital Number _____ Enrolment Number _____

ART Clinic Enrolment Number _____ Research Code _____

ART Site' code (UATH = 1, Wuse GH = 2) ____ Lab Number _____

Specimen code (Blood = 1, Sputum = 2, Urine=3) ____ Gender (Male=1, Female=2) ____

		Sputum 1	**Sputum 2**
Available	(Yes = 1, No = 2)		
Quality	(Saliva = 1, mucoid = 2, mucopurulent = 3, purulent = 4)		
Blood stained	(Yes=1, No=2)		
Amount > 2ml?	(Yes=1, No=2)		

If sputum is salivary or insufficient, request new samples

LED-FM (Neg = 1, Scanty = 2, + = 3, ++ = 4, +++ = 5) ☐ ☐

If scanty, number of FAB ☐ ☐

Xpert MTB (Not detected = 1, Detected = 2, Invalid = 3, Error = 4, No result = 5) ☐

If MTB detected (Very low = 1, Low = 2, Medium = 3, High = 4) ☐

Rif Resistance (Detected = 1, Not detected = 2, Indeterminate = 3) ☐

If Xpert is invalid, error or no result: repeat Xpert using sputum 1 or request a new sputum if specimen is insufficient

Xpert Ultra MTB (Not detected = 1, Detected = 2, Invalid = 3, Error = 4, No result = 5) ☐

If MTB detected (Very low = 1, Low = 2, Medium = 3, High = 4) ☐

Rif Resistance (Detected = 1, Not detected = 2, Indeterminate = 3) ☐

If Xpert Ultra is invalid, error or no result: repeat Xpert ultra using sputum 1 or request a new sputum if specimen is insufficient

Genedrive Done? (Yes = 1, No = 2) ☐ ☐

Genedrive machine used (1, 2, 3, 4 or 5) ☐ ☐

Genedrive consecutive number ☐ ☐

MTB (Undetected OK = 1, ☐ ☐

Detected sensitive = 2,

Detected resistant = 3, Retest = 4, Retest control failed = 5)

Observations_____

BlaC Done? (Yes = 1, No = 2) ☐

If, yes**,** MTB (Detected = 1, Not detected = 2, Indeterminate = 3) ☐

Culture Done? (Yes = 1, No = 2) ☐ (Pos = 1, Neg = 2, ☐

Cont = 3 Missing = 4)

Time to positive (days) ☐☐ Date d d m m y y

Number of colonies ☐☐☐☐ Sample saved ☐

(Yes = 1, No = 2)

DST (Sens = 1, Res = 2, Not done = 3) Rif ☐ INH ☐ ETH ☐ STR ☐

Urine Sample

Xpert Ultra

Specimen available? (Yes = 1, No = 2) ☐

Specimen in good conditions? (Yes = 1, No = 2) ☐

Ultra done? (Yes = 1, No = 2) ☐

MTB (Not detected = 1, Detected = 2, Invalid = 3, Error = 4, No result = 5) ☐

If MTB detected (Very low = 1, Low = 2, Medium = 3, High = 4) ☐

Rif Resistance (Detected = 1, Not detected = 2, Indeterminate = 3) ☐

E-nose

Date

Sample taken? (Yes = 1, No = 2) ☐ d d m m y y

Sample sent to UK? (Yes = 1, No = 2) ☐ d d m m y y

8.6.2 Questionnaire of evaluation of CRP and IP-10 as screening tool for active TB

Hospital Registration Number ☐ Date ☐☐☐☐☐☐ (d d m m y y)

Name of hospital ☐

Name _____ Address ☐

Mobile phone ☐ Other phone ☐

Age Years [not known = 99] ☐☐ Sex (Male=1, Female=2) ☐

Marital status [Single=1, with partner/married=2, divorced/separated=3, widowed=4] ☐

Patients is (New=1, Not new= 2) ☐

Treatment for TB (Never treated=1, previous treatment=2, currently on treatment=3) ☐

If treated before, classification: (Retreatment=1, Relapse=2, new treatment=3) ☐

If previously treated, months ago? ☐☐

How long ago did you notice the first symptom of this illness? Months ☐☐ Weeks ☐☐

For how many weeks have you had cough? Weeks ☐☐

		[Y =1, N=2]	**Weeks**
Do you have	Hemoptysis (nose bleeds)	☐	☐☐
	Fever	☐	☐☐
	Weight loss	☐	☐☐
	Chest pain	☐	☐☐
	Night sweats	☐	☐☐
	Weakness	☐	☐☐
	Loss appetite	☐	☐☐
	Other illness	☐	Specify _____

HIV [Positive = 1, negative = 2, Does not want to say = 3, unknown = 4] ☐

Laboratory

Hospital ☐ Patient Name ☐

Date sputum collected d d m m y y time h h m m

Date received d d m m y y time h h m m

Date blood collected d d m m y y time h h m m

Date received d d m m y y time h h m m

Laboratory Number ☐

		Sputum 1	Sputum 2	Sputum 3
Available	[Yes =1, No=2]	☐	☐	☐
Quality*	[saliva=1, mucoid=2, mucopurulent=3, purulent = 4]	☐	☐	☐
Blood stained	[Yes =1, No=2]	☐	☐	☐
Amount > 2 ml?*	[Yes =1, No=2]	☐	☐	☐

** if sputum is salivary or insufficient request further sputum samples*

Were further samples requested? [Yes =1, No=2] ☐

		Sputum 1	Sputum 2
ZN smear microscopy	[Neg =1, Scanty =2, + =3, ++ =4, +++ = 5]	☐	☐
	If scanty, N. of FAB	☐	☐

Culture Done? [Yes =1, No=2] ☐ Pos =1, Neg =2, Cont =3, Missing =4] ☐

Time to positive [days] ☐☐ Date d d m m y y

Number of colonies ☐☐☐☐ Sample saved [Y =1, N=2] ☐

DST [Sens =1, Res =2, Not done =3] ☐ Rif ☐ INH ☐ ETH ☐ STR ☐

Xpert Test used (Xpert= 1, Ultra =2) ☐

MTB [Not detected = 1, Detected =2, Invalid =3, Error =4, No result = 5]* ☐

If MTB detected [Very Low=1, Low =2, Medium=3, High =4] ☐

Rif Resistance [DETECTED=1, NOT DETECTED=2, INDETERMINATE=3] ☐

**If Xpert is invalid, error or no result: repeat Xpert using sputum 1, or request a new sputum if specimen is insufficient.*

CRP-ELISA Done? [Yes =1, No=2] ☐ Result: (**ng/mL**) ☐☐☐☐☐

CRP-rapid test Done? [Yes =1, No=2] ☐ result: (mg/l)

<10 ☐ (check one)

10-40 ☐

40-80 ☐

>80 ☐

IP-10-ELISA Done? [Yes =1, No=2] ☐ Result: (**pg/mL**) ☐☐☐☐☐

		Sputum 1	**Sputum 2**
xRapid	Done? [Yes =1, No=2]	☐	☐
	Result [Neg =1, Scanty =2, + =3, ++ =4, +++ = 5]	☐	☐
	If scanty, N of FAB	☐	
	Date/time examined	d d / / y y	h h : m m

Observations _____

		Sputum 1	**Sputum 2**
Fluorobot	Done? [Yes =1, No=2]	☐	Done? [Yes =1, No=2] ☐
	Result [Neg=1, Scanty=2, +=3, ++=4, +++= 5]	☐	[Neg=1, Scanty=2, +=3, ++=4, +++= 5] ☐
	If scanty, N of FAB	☐	☐

Date/time examined d d | | | y y h h m m

Observations _____

Sputum 1 **Sputum 2**

QuantumDx

Done? [Yes =1, No=2] ☐ Done? [Yes =1, No=2] ☐

Result [Neg=1, Scanty=2, +=3, ☐ [Neg=1, Scanty=2, +=3, ☐

++=4, +++= 5] ++=4, +++= 5]

If scanty, N of FAB ☐ ☐

Date/time examined d d | | | y y h h m m

Observations _____

HIV viral load

Done? [Yes =1, No=2] ☐ Result (cp/ml) ☐☐☐☐☐☐☐

Observations _____

8.6.3 Data abstraction/collection form for the study on TB treatment outcome

Enrolment Date | d | d | m | m | y | y | Data abstraction Date | d | d | m | m | y | y |

Patient's Name _____ _____

Surname First name

Patient's Hospital Number _____ Enrolment Number _____

Demographic characteristics

Age (Years) ☐☐ Sex (Male=1, Female=2, Not known = 9) ☐

Marital status (Single=1, with partner/married =2, separated=3, divorced=4, widowed=5) ☐

Education (No formal education=0, primary=1, secondary=2, higher education=3, prefer not to answer=4) ☐

Occupation (Student=1, Unemployed=2, Civil Servant=3, Artisan=4, Business/trading=5, Farming=6, Full House wife=7, other=8) ☐

If occupation is others, please, specify _____

Ethicity (Hausa=1, Igbo=2, Yoruba=3, other=4) ☐

If other ethinicity, please specify _____

Religion (Christianity=1, Islam=2, Traditional=3, other=4) ☐

Family Type (Monogamy=1, Polygamy=2) ☐

Baseline clinical history

Mode of entry to DOTs clinic (ART clinic =1, wards = 2, MOPD = 3, SOPD = 4, GOPD= 5, others =6) ☐

Smear microscopy (Positive = 1, Negative = 2, not done = 3) ☐

If smear-positive: smear grade (Scanty = 1, + = 2, ++ = 3, +++ = 4) ☐

Xpert MTB/RIF (MTB detected = 1, MTB not detected = 2, error = 3, not done = 4) ☐

If MTB detected, (Low = 1, medium = 2, high = 3) ☐ Rif resistance (Yes = 1, No = 2) ☐

X-rays: (Not suggestive = 1, Suggestive = 2, Not done = 3) ☐

Culture: (Positive = 1, Negative = 2, Contaminated = 3, Not done = 4) ☐

TB diagnosis: (PTB = 1, EPTB = 2) ☐

If EPTB: which types? (TB of lymph nodes = 1, Abdominal TB = 2, TB meningitis = 3, TB pleuritis = 4, Others = 5) ☐

If other forms of EPTB (please, specify) _____

TB classification based on treatment history (New = 1, re-treatment = 2, Not known = 9) ☐

TB drug susceptibility (Drug susceptible =1, MDR= 2, other forms of DR-TB = 3, Not tested = 4) ☐

Anthropometric Measurements

Height (m) ☐ Weight at 2 months (kg) ☐

Weight at start of treatment (kg) ☐ Weight at 5 months (kg) ☐

Systolic blood pressure ☐ Diastolic blood pressure ☐

HIV testing done (Yes=1, No =2, Not known = 9) ☐

HIV status (Positive = 1, Negative = 2, Undetermined =3, Not known = 9) ☐

If HIV positive, is the patient receiving HIV care in this facility? (Yes=1, No =2. Not known = 9) ☐

If HIV positive, is the patient on ART? (Yes=1, No =2, Not known = 9) ☐

If on ART, what regimen? (TDF/FTC/EFV = 1, TDF/FTC/NVP = 2, ZVD/3TC/NVP = 3, ZVD/3TC/EFV = 4, TDF/FTC/ATV = 5, TDF/FTC/LPV/r = 6, ZVD/3TC/ATV = 7, ZVD/3TC/LPV/r = 8, Others (please specify) _____ ☐

If HIV positive, is the patient on Co-trimoxazole preventive therapy (CPT) (Yes = 1, No =2) ☐

Past Medical History

Hypertension (Yes =1, No = 2) ☐

Diabetes (Yes =1, No = 2) ☐

Asthma (Yes =1, No = 2) ☐

Others (please, specify)----------

Date TB treatment started ☐☐☐☐☐☐ (d d m m y y)

Anti-TB regimen (Category 1=1, Category 2 = 2, second line =3) ☐

Duration of TB treatment (months) ☐☐

Follow up smear microscopy:

Month 2: done (yes = 1, no = 2, NK = 9) If done, (Sc = 1, + = 2, ++ = 3, +++ = 4, Neg = 5, result missing = 6) ☐

Month 6: done (yes = 1, no = 2, NK = 9) If done, (Sc = 1, + = 2, ++ = 3, +++ = 4, Neg = 5, result missing = 6) ☐

Treatment outcome (Cured = 1, treatment completed = 2, default/LTFU = 3, failure = 4, ☐

Transferred out = 5, died = 6, not evaluated = 7)

8.6.4 Data abstraction/collection form for the study on prevalence and incidence of hypertension among HIV patients

Enrolment Date | d | d | m | m | y | y | Data abstraction Date | d | d | m | m | y | y |

Patient's Name _____ _____

Surname First name

Patient's Hospital Number _____ Enrolment Number _____

Demographic characteristics on first attendance to UATH ART site

Age (Years) ☐☐ Sex (Male=1, Female=2) ☐

Marital status (Single=1, with partner/married=2, separated=3, divorced=4, widowed=5) ☐

Education (None=0, primary=1, secondary=2, higher education=3, prefer not to answer=4) ☐

Occupation (Student=1, Unemployed=2, Civil Servant=3, Artisan=4, Business/trading=5, Farming=6, House wife=7, other=8) ☐

If occupation is others, please, specify _____

Ethicity (Hausa=1, Igbo=2, Yoruba=3, other=4) ☐

If other ethinicity, please specify _____

Religion (Christianity=1, Islam=2, Traditional=3, other=4) ☐

Family Type (Monogamy=1, Polygamy=2) ☐

Baseline vital signs measurement

Anthropometric and Blood Pressure Measurements

Height (m) _____ Hip (cm) _____

Weight (kg) _____ Waist (cm) _____

Systolic blood pressure (mmHg) _____ Diastolic blood pressure (mmHg) _____

Please note that, if systolic blood pressure is >120mmHg or diastolic blood pressure >90mmHg, then, patient is hypertensive.

Is the patient hypertensive	(Yes = 1, No = 2)	☐
Functional status:	(Working=1, Ambulatory=2, Bedridden=3)	☐
WHO clinical staging:	(Stage 1=1, stage 2=2, stage 3=3, stage 4=4)	☐
Eligible for ART	(Yes=1, No=2)	☐

Routine Investigations

Baseline/at enrolment

Tests	**Results**	
CD4	☐☐☐	cells/uL
FBS	☐☐☐	mg/dl
WBC	☐☐☐	X 10^9 /L
PCV	☐☐☐	%
Hb	☐☐☐	g/dl
Platelets	☐☐☐	X 10^9 /L
HBsAg	(Positive=1, Negative=2) ☐	
Creatinine	☐☐☐	umol/L
ALT/SPGT	☐☐☐	U/L
Viral Load	☐☐☐☐☐☐☐	

1 year after enrolment

Tests	**Results**	
CD4	☐☐☐	cells/uL
FBS	☐☐☐	mg/dl
WBC	☐☐☐	X 10^9 /L
PCV	☐☐☐	%
Hb	☐☐☐	g/dl
Platelets	☐☐☐	X 10^9 /L
HBsAg	(Positive=1, Negative=2) ☐	
Creatinine	☐☐☐	umol/L
ALT/SPGT	☐☐☐	U/L
Viral Load	☐☐☐☐☐☐☐	

Date of attendance (1 year later): | d | d | m | m | y | y | Check here if patient did not return ☐

Functional status after 1 year: (Working=1, Ambulatory=2, Bedridden=3) ☐

WHO clinical staging after 1 year: (Stage 1=1, stage 2=2, stage 3=3, stage 4=4) ☐

ART status after 1 year? (On ART = 1, Not on ART = 2) ☐

If on ART, for how long (in month)? ☐☐

If on ART, what regimen? (TDF/FTC/EFV = 1, TDF/FTC/NVP = 2, ZVD/3TC/NVP = 3, ZVD/3TC/EFV = 4, TDF/FTC/ATV = 5, TDF/FTC/LPV/r = 6, ZVD/3TC/ATV = 7, ZVD/3TC/LPV/r = 8, ZVD/TDF/3TC/LPV/r = 9, ZVD/TDF/3TC/ATV = 10 ☐

Others (please specify) ----------

ART Class (Protease Inhibitor (PI) included = 1, PI not included = 2) ☐

ART regimen after 1 year, compared to the regimen at ART initiation (same = 1, substituted = 2, switched = 3) ☐

Patient's status 1 year after enrolment (On care/active = 1, Transferred out = 2, LTFU=3, Dead = 4) ☐

Anthropometric and Blood Pressure Measurements 1 year after enrolment

Height (m) ☐ Hip (cm) ☐

Weight (kg) ☐ Waist (cm) ☐

Systolic blood pressure (mmHg) ☐ Diastolic blood pressure (mmHg) ☐

Please note that, if systolic blood pressure is >120mmHg or diastolic blood pressure >90mmHg, then, patient is hypertensive.

Is the patient hypertensive (Yes = 1, No = 2) ☐

8.7. Appendix 7. Research Ethics approval letters

8.7.1 Ethics approval letter from the Research Ethics Committee of the University of Abuja Teaching Hospital, Abuja, Nigeria

UNIVERSITY OF ABUJA TEACHING HOSPITAL

P.M.B. 228, ABUJA - F.C.T. NIGERIA
07040045614, 09-2905535, 09-2904040
www. uath. ng.org.

Chief Medical Director
Dr. Peter Alabi
BM. BCH, FMCP

Chairman Medical Advisory Committee
Dr. A.S. Haruna
MBBS, FWACP

Chairman, Board of Management
Valentine I. Attah Ph D
Director of Administration
Musa Abdullahi
MPA, AHAN

Our Ref: FCT/UATH/HREC/PR/491

Your Ref: _____

Date: 10/2/16

Dr R S Owolabi
Liverpool School of Tropical Medicine (LSTM)
Pembroke Place,
Liverpool, L3 5QA
United Kingdom

Letter of Approval

Proposed Title: Optimising the diagnosis and management of tuberculosis (TB) and type diabetes (T2DM) among HIV patients in a high TB and HIV burden setting

Proposed Site: UATH

Sponsor: Liverpool School of Tropical Medicine

Your submission to the committee on UATH Health Research Ethics Committee on the above named protocol refers.

The Committee reviewed the following documents:

- A completed UATH HREC Application form
- Informed Consent Form
- Research Proposal
- Questionnaire/Proforma

The committee has considered the ethical merit of your submission and approved the protocol. The approval is for one year and will lapse on 9/2/17. It can be renewed on request. Modification of any part of the research methodology will require an approval.

Accept assurance of our highest regards.

Prof. B Ekele
Chairman UATH HREC

8.7.2 Initial ethics approval letter from the LSTM Research Ethics Committee (15.045)

Dr Rotimi Owolabi
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44(0)151 705 3100
Fax: +44(0)151 705 3370
www.lstmed.ac.uk

Monday, 15 February 2016

Dear Dr Owolabi,

Re. Research Protocol (15-045) Optimizing the Diagnosis and Management of Tuberculosis (TB) and Diabetes Mellitus (DM) among HIV Patients in a high TB and HIV burden setting.

Thank you for your letter of 11 February 2016 providing the necessary in-country approvals for this project. I can confirm that the protocol now has formal ethical approval from the LSTM Research Ethics Committee.

The approval is for a fixed period of three years and will therefore expire on 10 February 2019. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.
- Reporting of all severe unexpected Adverse Events to the Committee
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements will result in withdrawal of approval and may result in disciplinary action. The Committee would also like to receive copies of the final report once the study is completed.

Yours sincerely,

Angela Obasi

Dr Angela Obasi
Chair
Research Ethics Committee

8.7.3 LSTM Research Ethics Committee's approval letter for the consent form amendment

LSTM
LIVERPOOL SCHOOL
OF TROPICAL MEDICINE

Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44(0)151 705 3100
Fax: +44(0)151 705 3370
www.lstmed.ac.uk

Rotimi Owolabi
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

Wednesday, 09 December 2015

Dear Mr Owolabi

Re. Research Protocol (15.045) Optimizing the Diagnosis and Management of Tuberculosis (TB) and Diabetes Mellitus (DM) among HIV Patients in a high TB and HIV burden setting.

Thank you for your email of 01 December 2015 providing the committee with details of an amendment to the consent form (LSTM Consent_Form-2-revised).

This amendment has now been reviewed, noted and accepted on the behalf of the committee. Please continue to adhere to the conditions of approval and to update us of any further changes to the study that may arise.

Yours sincerely,

Angela Obasi

Dr Angela Obasi
Chair
LSTM Research Ethics Committee

8.7.4 Ethics approval letter from the Research Ethics Committee of the Nigeria's Federal Capital Territory (FCT), Abuja

FEDERAL CAPITAL TERRITORY
Health Research Ethics Committee
Research Unit, Room 10, Block A Annex, HHSS, FCTA Secretariat,
No. 1 Kapital Street Area 11, Garki, Abuja - Nigeria

Name of Principal Investigator:	Dr. Rotimi S Owolabi
Address of Principal Investigator:	Dept. of Medicine, University of Abuja Teaching Hospital, Gwagwalada, Abuja.
Date of receipt of valid application:	26/09/2016

Notice of Research Approval
Approval Number: FHREC/2016/01/76/17-10-16

Study Title: Optimising the Diagnosis and Management of Tuberculosis (TB) and Type 2 Diabetes (T2DM) among HIV Patients in a High TB and HIV Burden Setting.

This is to certify that the FCT Health Research Ethics Committee (FCT HREC) has approved the research described in the above stated protocol.

Effective Date: - 17/10/2016
Expiration Date: - 16/10/2017

Note that no activity related to this research may be conducted outside of these dates. Only the FCT HREC approved informed consent forms may be used when written informed consent is required. They must carry FCT HREC assigned protocol approval number and duration of approval of the study.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations, and with the tenets of the code. The FCT HREC reserves the right to conduct compliance visit to your research site without prior notification.

Modifications: Subsequent changes are not permitted in this research without prior approval by the FCT HREC.

Problems: All adverse events or unexpected side effects arising from this project must be reported promptly to FCT HREC.

Renewal: This approval is valid until the expiration date. If you are continuing your project beyond the expiration date, endeavor to submit your annual report to FCT HREC early, and request for renewal of your approval to avoid disruption of your project.

Closure of Study: At the end of the project, a copy of the final report of the research should be forwarded to FCT HREC for record purposes, and to enable us close the project.

Ikwubiela S. Adem
Secretary, FCT HREC
October 17, 2016

8.7.5 Ethics approval letter for research protocol amendment, from LSTM Research Ethics Committee

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44(0)151 705 3100
Fax: +44(0)151 705 3370
www.lstmed.ac.uk

Rotimi Owolabi
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

Monday, 19 December 2016

Dear Dr Owolabi

Re. Research Protocol (15.045) Optimizing the Diagnosis and Management of Tuberculosis (TB) and Diabetes Mellitus (DM) among HIV Patients in a high TB and HIV burden setting.

Thank you for your letter of 05 December 2016 providing the Committee with details of this protocol amendment 2, entailing:

1. Supplementation of study with audits based on TB registers and HIV care records, to mitigate low recruitment
2. Use of newly-available diagnostic platforms

This amendment has now been reviewed, noted and accepted on the behalf of the Committee. Please continue to adhere to the conditions of approval and to update us of any further changes to the study that may arise.

Yours sincerely,

Angela Obasi

Dr Angela Obasi
Chair
LSTM Research Ethics Committee