IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV POSITIVE PATIENTS ON HAART AT THE MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC

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DECLARATION

I declare that the work submitted in this dissertation has been done by me and has not been submitted for any other degree award in any university

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DEDICATION

I dedicate this piece of work to my dear wife Betty and my two lovely daughters Ivy and Erin

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
APC	Antigen Presenting Cell
ART	Antiretroviral Therapy
ATS	American Thoracic Society
BCG	Bacille Calmette Guerin
CBC	Complete Blood Count
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention
DTH	Delayed Type Hypersensitivity
HAART	Highly Active Antiretroviral Therapy
HBCs	TB High Burden Countries
HIV	Human Immunodeficiency Virus
IDC	Infectious Diseases Clinic
IGRA	Interferon Gamma release Assay
IL	Interleukin
LJ	Lowensen Jensen Media
LTBI	Latent Tuberculosis Infection
MTB	Mycobacterium Tuberculosis
MU-UMDNJ	Makerere University- University of Medicine and Dentistry of New Jersey
PLWA	People Living with HIV/AIDS
PPD	Purified Protein Derivative
TB	Tuberculosis

TBIRIS	Tuberculosis Immune Reconstitution Inflammatory Syndrome
TST	Tuberculin Sensitivity Test
TU	Tuberculin Unit
VL	Viral Load
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Positive TST: **S**kin induration of ≥ 5mm 48-72 hours after PPD administration

Negative TST: **S**kin induration of < 5mm 48-72 hours after PPD administration

ABSTRACT

Background: It is estimated that a third of the world's population is infected with tuberculosis (TB).

This epidemic is fueled by HIV, with reactivation rates of 8-10%/year and overall lifetime risk of 30% in HIV patients. Uganda has both a high TB and HIV prevalence of 646/100000 population and 6.4% respectively with over 1,000,000 people living with HIV and AIDS (PLWA).

HIV makes the diagnosis of TB infection difficult. Tuberculin Skin Test (TST) is negative in up to 70% of HIV patients. In Uganda about 80,000 people are on antiretroviral drugs (ARVS) .These drugs lead to recovery of the immune system. It is not known whether this immune system recovery leads to a corresponding improvement in the performance of the TST. This would allow use of TST in the diagnosis of latent TB infection and subsequently institution of prophylaxis therapy in HIV infected patients on HAART.

In this study we evaluated whether HAART restores PPD reactivity and the factors associated with this PPD reactivity.

Objective: The overall objective of this study was to determine the effect of HAART on the in vivo PPD reactivity and the associated factors among HIV patients at the Makerere University Adult Infectious Diseases clinic (IDC).

Methods: This was a prospective cohort study of 130 HIV positive patients who initiated antiretroviral therapy at the adult IDC at Mulago Hospital. We enrolled consenting HIV positive ART naïve adults who had history and physical examination done to exclude active

tuberculosis (TB). All patients had a chest X-ray examination. 0.1ml of purified protein derivative (PPD) (RT-23) was administered. Patients with negative baseline TST received a second TST at 2 months and those who were still negative at this time got a third TST at 6 months. At all these intervals patients were clinically evaluated to exclude active TB and blood samples for CD4 cell counts were taken at the six months interval. Patients with positive TST but no active TB were given a 9 month course of isoniazid prophylaxis.

Data was abstracted from the patients' cohort files using pre-tested tools and entered into Epidata version 3.1 and later exported to STATA version 10 for analysis.

Patients with skin indurations of 5mm and over were considered positive. We calculated the proportions of patients with positive PPD at enrollment and after two and six months of HAART. We compared the clinical, radiological and CD4 cell counts of TST positive and negative patients at enrollment and follow up.

Results: of the 155 patients screened 130 fulfilled eligibility criteria. Forty one (31.5%) were TST positive at baseline and this was significantly associated with history of weight loss and cough, as well as higher CD4 cell counts. Twelve patients (13.5%) had TST conversion during six months of follow up of whom 8 were in the first 2 months and this was associated with a greater increase in CD4 cell counts after six months of HAART (mean CD4 rise in converters 174 vs. 91, p=0.03). Five out of 89 (5.6%) patients followed up to 6 months developed active TB

Conclusions: Among ART naïve HIV positive patients TST positivity is low and after six months of HAART, less than 50% were TST positive. Patients with higher CD4 cell count increase are more likely to have TST conversion during HAART.

Recommendations: Diagnosis of latent TB in HIV patients is difficult and repeat TST testing should be considered for those HIV positive patients with negative TST before ART who have experienced favorable immunological recovery if clinicians have a need to diagnose latent TB infection.

Active TB screening is needed before ART and during the early months of ART because the burden of TB is high during this period as shown in this study.

TB control measures are needed in HIV care settings to prevent TB transmission as we showed in this study that 1 in 10 of the pre-HAART patients have undiagnosed active TB

CHAPTER ONE

1.0 BACKGROUND AND INTRODUCTION

Tuberculosis (TB) remains an important infectious disease and it is estimated that one-third of the world's population is infected with Mycobacterium tuberculosis. Previous successes in control of this disease have been reversed by the HIV epidemic and infection rates are increasing in most parts of the world especially Sub-Saharan Africa which accounts for 80% of the new TB infections (WHO, 2006). Uganda being among the 22 TB High Burden Countries (HBCs) has annual TB incidence, prevalence and mortality rates of 402/100,000, 646/100,000, and 92/100,000 respectively (WHO, 2006)

The HIV prevalence has declined in Uganda over the last two decades. Prevalence is currently estimated at 6.4% down from about 18% in the early nineties (Uganda HIV/AIDS Serobehavioral survey, 2004/2005).

Despite reducing prevalence rates, about 1,000,000 people are estimated to be living with AIDS in Uganda (Uganda HIV/AIDS Sero-behavioral Survey 2004/2005)

The WHO estimates that 39% of Ugandan TB patients are co-infected with HIV (WHO, 2009). However studies in TB patients attending the National TB Treatment Centre at Mulago Hospital reported prevalence of up to 60% (Isabirye 2002).

Access to antiretroviral therapy (ART) has improved from 10,000 before the launch of the 3 by 5 initiative to 80,000 of the 120,000 people in need of ART (Uganda HIV/AIDS Serobehavioral survey, 2004/2005).

The sensitivity of the TST/Mantoux Test (which involves an intradermal injection of a TB product, the Purified Protein Derivative, PPD) is reported to be as low as 30% in a number studies involving HIV patients. The anergy rate in those with CD4 <200 ranges from 40-70% (Jones 2006, Belete 2006, Elliott 1995, Graham1992, Markowitz 1993, Rangaka 2007)

Highly active antiretroviral therapy (HAART) use leads to immune system recovery and reduction in the incidence of opportunistic infections including TB as well as the morbidity and mortality associated with it (Dheda 2004, Mocroft 2003, MU-UMDNJ 2007, Wagner 2001, Whalen 2000, Narita1997). Steven Lawn and others found reductions in TB incidence of more than 80% (Lawn 2005)

It is not known whether this immune system recovery will permit the use of TST in HIV infected patients taking HAART especially in our setting where TB infections rates are high and patients start HAART at advanced levels of immune-suppression.

1.1 Problem Statement

HIV is associated with an increased risk of TB infection. However diagnosis of TB infection among HIV infected patients is difficult because all available tests (including TST) are based on measurement of cell mediated immune response to the TB antigens.

HIV depletes the cells of the cell mediated immunity reducing the accuracy of these tests especially TST.

More HIV positive patients are accessing antiretroviral therapy with recovery of their immune system.

There is scanty data in resource limited, high tuberculosis burden settings like Uganda regarding improvement in performance of TST in patients on HAART.

1.2 Justification

The treatment of latent tuberculosis infection in HIV-infected individuals greatly reduces the risk of active tuberculosis. Tuberculin skin test (TST) is at present is the only standardized method of identifying individuals with latent tuberculosis infection especially in resource limited settings. However, the sensitivity of this test is greatly reduced in HIV-infected individuals. Highly active antiretroviral therapy (HAART) leads to restoration of immune system function and of delayed-type hypersensitivity response to mycobacterial antigens.

It is recommended that PPD-positive HIV patients receive isoniazid (INH) prophylaxis, in order to prevent the likely progression of tuberculous infection to active TB. Active TB in an HIV-infected patient may lead to increased HIV replication, accelerated immunosuppression, and more rapid progression to death.

Current guidelines (US) recommend that clinicians should consider repeating TST for individuals whose initial skin test was negative and whose immune function has improved in response to HAART(ATS, CDC 2000). However, scant data is available on the rate of conversion from negative to positive TST reaction and on factors predictive of this conversion after the initiation of HAART in patients without active tuberculosis in high TB burden settings like Uganda.

1.3 Research Questions

1. What is the proportion of TST conversion among HIV patients attending the adult IDC within 6 months of initiating HAART?
2. What is the association between TST conversion and CD4 cell count, clinical and radiological factors within 6 months of HAART among HIV patients attending the adult IDC?

1.4 Hypothesis

Null hypothesis

1. HAART is not associated with TST conversion among HIV patients within 6 months of initiating HAART.
2. There is no association between TST conversion and CD4 cell count, clinical and radiological factors within 6 months of HAART among HIV patients attending the adult IDC?

1.5 General Objective

Determine the in vivo TST reactivity and associated factors among HIV patients on HAART at the adult IDC

Primary Study Aim

The primary aim of this study is to determine the in vivo TST reactivity among HIV patients attending the adult IDC following six months of HAART.

Secondary Aim

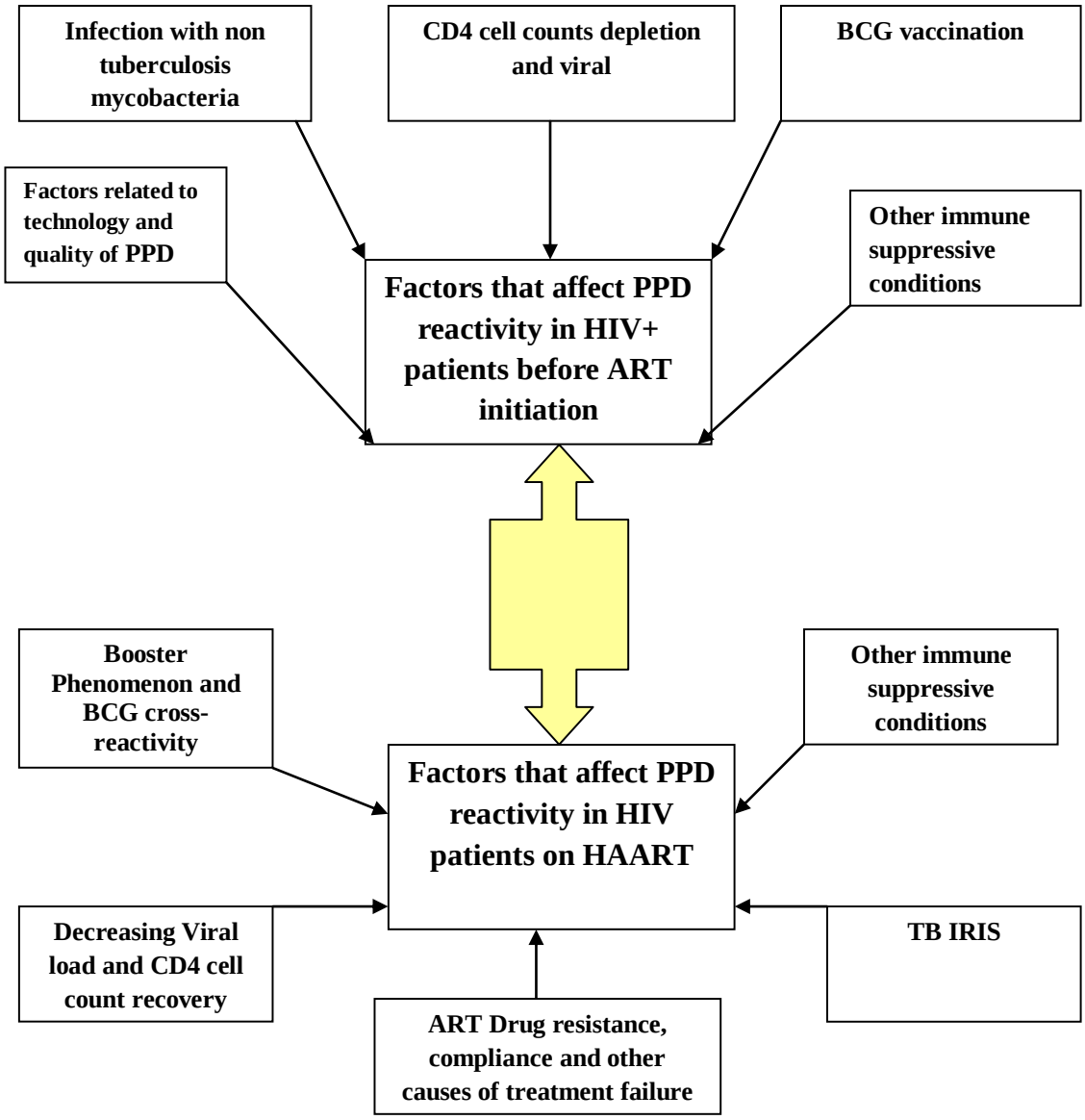
Determine the association between TST conversion with CD4 cell count, clinical, and radiological factors within 6 months of HAART among HIV patients attending the adult IDC.

1.5.1 Specific Objectives

1. Determine the proportion HIV patients with positive baseline TST before initiation of HAART
2. Determine the proportion of HIV patients with negative baseline TST who test positive on the same test 2 and 6 months after HAART
3. Determine the association between TST conversion and CD4 cell count raise, clinical and radiological factors within 6 months of HAART among HIV patients attending the adult IDC.

1.6 Conceptual Frame work

Factors associated with in vivo PPD reactivity among HIV positive patients before and after HAART



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Tuberculosis/ HIV infection interaction.

TB and HIV are both intracellular pathogens and known to have profound influence on the progression of each other.

HIV infection brings about the depletion of CD4+ T cells, which play a major role in immunity to TB by the production of interferon gamma. Apart from the reduction in number, HIV also causes functional abnormality of CD4+ and CD8+ cells. HIV in addition to T-cells also binds APCs (macrophages) via its gp120 and thus impairing their ability to present antigens to the T-cells.

Likewise, TB infection also accelerates progression of HIV disease from infection, AIDS and death. TB stimulates macrophages to secrete TNF-alpha, which is a potent activator of HIV replication within T cells (Collins 2002, Goletti 1996, Whalen 2000).

Patients infected with the human immunodeficiency virus (HIV) are at high risk to develop active TB disease after infection with Mycobacterium tuberculosis. For this reason, all persons not already known to be reactive should receive a purified protein derivative (PPD) test as soon as possible after HIV is diagnosed (CDC., 1998)

Prospective studies performed before the widespread use of highly active antiretroviral therapy found that the rate of progression to active tuberculosis in HIV-infected patients who are TST positive was 3.4 to 9.7 percent per year (Selwyn 1989, Guelar 1993) which is worse than a cumulative lifetime risk of 10 percent in immune-competent patients who are TST positive. The lifetime risk for reactivation for an HIV-infected patient was calculated to be 30 percent by one

investigator based upon prospective cohort studies and assumptions about the rate of decline in risk over time. Isoniazid (INH) treatment of TST-reactive HIV-infected patients reduces the incidence of active TB by 71% over a 3 year period and the lifetime risk of active tuberculosis to 4 percent or less (Whalen 1995, Fitzgerald 2000, Pape 1993). Whalen, Okwera and others in a study of 2736 Ugandans found a reduction of incidence of active TB of 67% among PPD positive HIV patients who received INH TB preventive therapy (Whalen 1997). However this protection lasts 2-3 years and the impact of therapy on community TB incidence is unclear.

Despite use of ART, TB in HIV infected patients still leads to increased mortality. Moore and others in a study of 1044 HIV patients on HAART in Tororo, Uganda found that the Cumulative mortality was 17.9/100 person-years for patients who had TB compared to 3.8/100 person-years for those without TB (P<0.001)(Moore 2007)

In areas of high TB incidence, routine isoniazid preventive therapy may have a role regardless of PPD testing results. In a study of 1655 HIV-infected male employees of a South African gold-mining company, INH was prescribed to all patients who had no evidence of active TB by chest x-ray or by sputum culture. Patients were not screened for PPD status. TB incidence declined by 46 percent with the introduction of INH (Grant, 2005).

2.2 Diagnosis of TB infection among HIV patients

Diagnosis of TB infection among HIV patients poses a challenge because all available tests are reported to have reduced sensitivity. Up to 70% of HIV patients are anergic to TST (Jones 2006, Belete 2006, Elliott 1995, Graham 1992, Markowitz 1993, Rangaka 2007). Studies of TST reactivity among HIV patients on HAART are few and most are in HIV patients with active TB. Narita and others reported restoration of PPD reactivity of 89% among HIV/TB patients on

HAART (Narita, 1997). Among HIV patients without active TB in low TB prevalence settings restoration of PPD reactivity is between 5-12% after HAART and is associated with a favorable immune system recovery (Girardi, 2002, Fisk, 2003). One study by Fisk TL and others of HIV patients without active TB and initiating ART at CD4 cell counts < 100 cells and who had been on ART for at least 2 months at a hospital in Atlanta Georgia USA reported PPD reactivity of 11% and anergy in these patients reduced by 94% in this group of patients. Reactivity restoration was associated with a CD4 cell count increase of 100 or greater (Fisk, 2003).

2.3 Tuberculin Skin Testing (TST)

The tuberculin Skin Test (or Tuberculin Sensitivity Test, PPD test or Mantoux test, Pirquet test) has been used to diagnose TB infection since it was first introduced by Robert Koch in 1890 as an extract of boiled tubercle bacilli (old tuberculin).The test is named after Charles Mantoux, a French physician who developed on the work of Koch and Clemens Von Pirquet to create his test in 1907

This test uses the Purified Protein Derivative (PPD) prepared by precipitation of protein components from culture filtrate of Mycobacterium tuberculosis or the recombinant PPD which preliminary results show to be more specific than PPD (Coler, 2000). The precipitation removes some of the large carbohydrate antigens thus making it more specific than old tuberculin. The test becomes positive about 6 weeks following infection, wit a of range 3-12 weeks.

2.4 Indications for TST Testing

Indications for annual tuberculin skin testing in asymptomatic individuals include in low TB settings include: HIV infection, ongoing potential close contact with cases of active TB (includes health care workers, prison guards, Mycobacteriology laboratory personnel), presence of a medical condition that increases the risk of active TB (eg, silicosis, diabetes mellitus, anticipated or actual long-term therapy with glucocorticoids or other immunosuppressive medications, hematologic or reticuloendothelial malignancies, end-stage renal disease, hemodialysis patients, alcoholism, gastrectomy, jejunoileal bypass, solid organ transplant recipients, or conditions associated with rapid weight loss or chronic malnutrition, residence in a long term care facility (eg correctional institutions, mental institutions, and nursing homes) (American Thoracic Society, 2000)

2.5 Administering a Tuberculin Skin Test

There are two methods of tuberculin skin testing: the intradermal Mantoux method and the multiple-puncture test.

Intradermal Mantoux method — The Mantoux skin test involves the intradermal injection of 0.1 ml of PPD generally into the volar surface of the forearm in an area free of lesions and away from veins. Creation of a visible wheal is crucial; subcutaneous administration results in a false-negative test.

The standard dose of PPD (Tubersol or Aplisol) used for this test is 5 tuberculin units (TU), which was formerly known as "intermediate strength." The standard dose of RT-23 is 2 TU. "First strength" (1 TU) and "second strength" (250 TU) doses of tuberculin were frequently used

in the past. However, these doses are not standardized, are usually unavailable, and are of no utility in diagnosing tuberculous infection.

Multiple puncture devices — Multiple-puncture tests (eg, the Heaf or tine test) are done by puncturing the skin of the forearm with a set of short prongs or tines coated with tuberculin. Multiple-puncture tests are easy to give, and they are convenient because they do not require a needle and syringe. However, in the multiple-puncture test the amount of tuberculin that actually enters the skin cannot be measured and thus this test technique results in inadequate sensitivity and specificity.

2.6 Reading a Tuberculin Skin Test

Skin tests should be interpreted 48 to 72 hours after intradermal administration of the antigen. The transverse diameter of induration should be measured and recorded in millimeters. Although erythema is usually also present, only induration should be measured.

Two methods for reading the test are in common usage. The ballpoint pen method involves drawing a line toward the area of induration and noting an increase in resistance to movement of the pen when the border of the indurated region is reached. The procedure is repeated on the other side of the skin test reaction, and the overall width of the indurated region can then be measured. The palpation method involves using the finger to feel the borders of the area of induration.

In a systematic study of skin test measurements, the interobserver reliability was slightly higher for the ballpoint pen method than for the palpation method (Pouchot, 1997). Intraobserver reproducibility for the ballpoint pen method was high but, in 5 percent of tests, a second

measurement by the same observer could be at least 2.7 mm less to 3.0 mm more than the first measurement.

2.7 Skin test performance

Ten to 25 percent of patients with newly diagnosed pulmonary TB will have a false negative skin test. This is due to a combination of factors, including: Inadequate nutrition anergy, the inability to react to skin tests because of a weakened immune system (eg, HIV-infected patients, malignancy) Specific cytokines which inhibit delayed hypersensitivity and are liberated in active disease and severe TB (eg, miliary disease). The simultaneous presence of an immunosuppressive disorder, concurrent viral infection and corticosteroid therapy

2.8 Sensitivity and specificity

The sensitivity and specificity of the TST is dependent on the cut off points of induration that defines a positive result in a specific population. The specificity is increased (but sensitivity decreased) by progressively increasing the reaction size for a positive test.

The ATS/CDC guidelines place individuals in one of three pretest risk levels, on the basis of the prevalence of infection in the risk group and an assessment of risk for the progression of latent to active infection, in interpreting a positive test. These criteria maximize the sensitivity and specificity of the test

Approximately 95 percent of patients with a history of active TB, who have received adequate anti-tuberculosis therapy, will have a positive skin test if they do not have a concurrent underlying immunosuppressive disorder. The sensitivity of the TST in asymptomatic infected persons is unknown, but it is assumed to be close to the 95 percent reactor rate seen among patients in whom the disease has been successfully treated.

The specificity of the skin test is somewhat variable and is dependent primarily upon the likelihood of cross-reactions in geographic areas where non-tuberculosis mycobacteria are common and use of BCG is high.

2.9 Test interpretation

The results of this test must be interpreted carefully. The person's medical risk factors determine at which increment (5 mm, 10 mm, or 15 mm) of induration the result is considered positive. A positive result indicates TB exposure.

Indurations >5 mm: HIV-positive persons and children, Recent contacts of TB case, Fibrotic changes on chest radiograph consistent with old TB, Patients with organ transplants and other immune-suppressed patients (receiving the equivalent of >15 mg/d Prednisone for >1 mo)

Indurations >10 mm**:** HIV negative persons and adults**.** Recent arrivals (<5 yr) from high-prevalence countries**,** Injection drug users**,** Residents and employees of high-risk congregate settings: prisons and jails nursing homes and other health care facilities and homeless shelters**,** Mycobacteriology laboratory personnel**,** Persons with clinical conditions that make them high-risk: silicosis, smoking, diabetes mellitus, chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignancies (eg, carcinoma of the head or neck and lung), weight loss of >10 percent of ideal body weight, gastrectomy, jejunoileal bypass**,** Children <4 yr of age or infants, children, and adolescents exposed to adults in high-risk categories (American Thoracic Society 2000)

Indurations >15 mm: Persons with no risk factors for TB (American Thoracic Society 2000)

2.10 False Positive Tests

False positive results may be caused by non-tuberculous mycobacteria infection or prior BCG vaccination. Prior BCG may result in a false-positive result for many years afterwards. A review of the literature on this subject since 1966 found BCG after the first year of life to be associated with a false positive rate of 41.8% compared to 12% if BCG was given in the first year of life. Non tuberculous mycobacteria did not significantly influence results (Farhat 2006).

2.11 False Negative Tests

False negatives may be a result of:

Infections such as viral (HIV, measles, mumps, chickenpox) ,Bacterial (typhoid fever, brucellosis, typhus, leprosy ,pertussis, overwhelming tuberculosis, tuberculous pleurisy), Fungal (South American blastomycosis), Metabolic derangements (chronic renal failure), Low protein states (severe protein depletion, afibrinogenemia), Diseases affecting lymphoid organs (Hodgkin's disease, lymphoma, chronic lymphocytic leukemia, sarcoidosis), Drugs (corticosteroids and many other immunosuppressive agents), Age (newborns, elderly patients with "waned" sensitivity), Recent (within the past 10 weeks) or overwhelming infection with M. tuberculosis, Live attenuated vaccines such as measles, mumps, rubella, varicella, oral polio, BCG, and oral typhoid (TY21a) may temporarily suppress the DTH response to a TST. If the TST is indicated after a live attenuated vaccine, it will likely be most accurate if 6 weeks has passed since vaccine administration, Stress (surgery, burns, mental illness, graft versus host reactions)

BCG immunization has a variable effect on TSTs. A minority of vaccinated children has a TST > 10 mm and older children are more likely to have a positive TST suggesting the cumulative

effect of exposure to TB disease and the risk of acquiring TB infection. Children who receive BCG after infancy or those who receive more than one BCG immunization also have an increased rate of positive TST (Farhat 2006)

Factors related to the tuberculin used that may result in false negative include:

Improper storage (exposure to light and heat), improper dilutions, Chemical denaturation, Contamination, Adsorption (partially controlled by adding Tween 80)

The method of administration and method of reading and recording may also cause a falsely negative result: Injection of too little antigen, Subcutaneous injection, Delayed administration after drawing into syringe, Injection too close to other skin tests, Inexperienced reader, Conscious or unconscious bias, Error in recording

CHAPTER THREE

3.0 STUDY METHODS

3.1 Study design

This study was a prospective cohort study. HIV positive patients about to start HAART had baseline TST done and those with negative TST at enrollment received repeat TST at two and six months if still negative, "a before and after design". We chose this design because it would be unethical to have patients with comparable immune-suppression but withhold ART for study purposes.

This study was nested in a cohort study that was designed to investigate the incidence and pathogenesis of TBIRIS at the IDI through clinical monitoring for symptoms and signs of tuberculosis after initiation of HAART and laboratory monitoring using sputum and immunological tests. This cohort included HIV positive patients with CD4 cell count less than 200 cells/uL residing with 20 km radius from IDI and who had negative tests for active TB. Patients in this cohort were evaluated at baseline, 2 weeks and monthly thereafter for six months and 3 months afterwards for 2 years. Because patients at this clinic are enrolled into the IDI cohort we maintained an IDI cohort form in the patients' files and collected the required information at each visit. The required number for the TBIRIS cohort was 200 patients and we included in this study the first 130 patients enrolled who had been enrolled by the end of the study period in April 2009.

3.2 Setting

The study was conducted at the Makerere University Infectious Diseases Institute clinic.

The clinic has over 25,000 registered patients of whom over 8,000 have been initiated on ART. The clinic is operated four days a week, excluding Wednesday and weekends and offers

diagnosis, care and treatment at no cost to the patients. About 40-60 patients initiate HAART per week.

3.3 Study period

The study started in February 2008 and ended April 2009

3.4 Population

3.4.1 Target population

Adult HIV positive patients initiating HAART

3.4.2 Accessible population

Adult HIV positive Patients initiating HAART at the adult IDC during the study period.

3.4.3 Study population

All HIV positive patients initiating HAART at the Adult IDC who meet the inclusion criteria within the study period.

3.5 Participants selection

3.5.1 Inclusion criteria

We included HIV positive patients of 18 years of age and over, ART naïve patients with CD4 cell count< 200 cells/uL and residing within 20 km radius from the IDI.

3.5.2 Exclusion criteria

Patients with active TB and other forms of immune-suppression such as cancer chemotherapy, radiotherapy as well as pregnant women and those in whom samples could not be obtained for various reasons were excluded.

3.6 Sampling methods

3.6.1 Sampling unit

HIV positive patient due to initiate HAART.

3.6.2 Sampling procedure

Consecutive sampling was used and participants who met the inclusion criteria were enrolled until 130 patients had TST done.

3.7 Sample size estimation

The sample size was estimated based on the hypothesis that the number of patients converting to a positive test will be greater than zero taking patients with negative TST at enrollment as controls and the same patients after six months of HAART as the experimental group.

Using the formula for determining sample size for dichotomous variables, rates or proportions, (Fleiss 1981)

$$n = C + p_c q_c + p_e q_e / d^2 + 2/d + 2$$

Where n is the required sample size, $qc = 1-p_c$; $q_e = 1-p_e$; and $d = |pc-pe|$. d is the difference between p_c and p_e, expressed as positive value. C is a constant that depends on the values chosen for alpha and beta.

We set alpha in this study at 0.05 and beta 0.80 and C was therefore 7.85

Fisk et al found a TST conversion of 11.9% among patients on ART. We planned to detect a conversion proportion equal to or higher and substituting in the above formula

$n = 7.85 + 0 \times (1-0) + 0.119 \times 0.881/0.119^2 + 2/0.119 + 2 = 93$

We planned therefore to enroll 93 patients with negative TST and evaluate the same number after six months of HAART.

However due to logistical and time constraints we were able to follow up a total of 89 patients.

3.8 Data collection and Study Measurements/variables

Clinical data measurements

We collected demographic data, TB and HIV related history and physical examination findings at enrollment. The same history and physical examination was repeated at month two follow up interval and at month six follow up for those patients with negative TST at enrollment and month two respectively.

TB history collected included: history of prior TB treatment, prior TST, cough, night sweats, loss of weight and appetite and swelling of glands anywhere on the body. We asked patients if they had ever taken ARV drugs for any length of time.

Clinical parameters collected included vital signs: axillary body temperature, blood pressure, body weight and height, Karnofsky score, respiratory rate and pulse rate.

We also examined for the presence of a Bacille Calmette Guerin (BCG) scar.

Body Systems examination was conducted for the presence of signs of TB: signs of pulmonary consolidation, pleural effusion, wheezing. We looked for presence of ascites, abdominal tenderness, enlarged liver and spleen. Other physical examination findings were recorded as and when found.

Diagnosis of active tuberculosis

Patients were considered to have active tuberculosis when they had a two positive sputum smears, positive mycobacterium culture on Lowenstein Jansen media and if they had TB symptoms (any one of cough of more than 2 weeks, fever, loss of appetite, night sweats or loss of weight) and a chest x-ray suggestive of active tuberculosis as interpreted by a radiologist.

Sputum tests were performed at enrolment and during follow up visits as indicated by the clinical presentation of the patients.

All patients received a chest radiograph before enrollment into the study and this could be repeated during follow up if indicated.

CD4 cell count measurement

We collected blood for CD4 cell count measurement at enrollment and at month six follow up visit. Lymphocyte subsets (CD4 cell counts) were analyzed by standard three-color flow cytometry (FACScan; Becton Dickinson, San Jose, CA)

TST

All eligible patients were injected 0.1ml of PPD (RT-23 SSI 2TE; Statens Serum Institute, Copenhagen, Denmark), on the volar aspect of the left arm. The area of injection was swabbed with alcohol and allowed to dry before injection. Patients were given instructions to return after 2-3 days for reading of the test. Results of the TST were recorded in the TST results form in the patients study file

Patients with negative TST (i.e. skin indurations <5mm) received a repeat test after two months of ART and those who were still negative received a third TST after six months of ART. TST was administered and read according to a standard Operating Procedure (SOP) developed before the study

At all points of TST testing, patients with positive TST were evaluated for prophylaxis eligibility. Eligible patients were treated with oral isoniazid 300mg once a day for nine months.

3.8.1 Table of Study Measurements

Variable	Interval		
	Baseline/Enrollment, M0	**Follow up, M2**	**Follow up, M6**
Demographics	**X**		
Clinical evaluation	**X**	**X**	**X**
CXR	**X**	**+/-X**	**+/-X**
Sputum smears	**+/-X**	**+/-X**	**+/-X**
CD4 cell count	**X**		**X**
PPD	**X**	**+/-X**	**+/-X**
Key: X= measurement was done at that study point, +/-= measurement done as clinically indicated			

3.8.2 Schedule of study measurements

Once patients were recruited into the study, they continued their IDC scheduled visits. The study measurements were done at enrolment, 2 and 6 months intervals.

3.9 Data Management, quality control and analysis

Data was collected with the help of trained medical officers and research nurses and the Principal investigator checked all the clinical review forms (CRF) at the end of each day for completeness and missing data was obtained and filled into the CRF as soon as possible. We entered the data in Epidata version 3.1 and exported it to STATA version 10 for analysis. Before export the principal Investigator cleaned the data and the cleaning was also done when data was in STATA.

With the help of a statistician, data analysis was done using STATA 10 statistical software. Descriptive statistics were used to summarize data of each variable in the data set. Categorical variables such as sex, marital status, and the level of education as well as presence or absence of clinical and radiological parameters were summarized into frequencies and proportions and tested

for significant association with Fisher Exact test. Continuous variables such as age, weight, CD4 cell counts, pulse rate, respiratory rate were summarized into means and medians as appropriate and their association with TST tested with t test, paired and unpaired as appropriate.

We calculated proportions of patients with positive TST at enrollment, two and six months after ART.

Bivariate analysis: We compared clinical, radiological and CD4 cell counts between patients with positive TST tests at enrollment and between converters and non converters at the end of follow up. Using multivariable logistic regression we assessed factors that were independently associated with positive TST at baseline and during follow up.

The 95% confidence intervals were constructed around the estimates and the P values used as a measure of statistical significance. A P-value of 0.05 or less was considered significant. The Chi-square tests were computed and the Fisher's exact test was used for cell frequencies less than 5. Results were summarized in tables and graphs.

3.10 Quality control

Data collection tools were pre tested in a pilot study for clarity and standardization. We trained the research assistants in the use of the data collection tools especially the CRF, administration and reading of the TST. The principal investigator cross-checked the CRF every day for completeness.

3.11 Ethical considerations

This study was approved by the Institutional review boards of the Department of Medicine, and the research and ethics committee of the Faculty of Medicine.

All participants provided written informed consent and patients who did not consent to participate in this study continued to receive standard care from the IDC. Patients were free to withdraw their consent and indeed a few did withdraw their consent from further study participation.

Confidentiality was observed throughout all the study procedures. Patients who developed active TB were treated according to the Uganda National TB and Leprosy Program recommendations and the attending clinicians were involved in the management of these patients.

3.12 Dissemination of results

The results of the study will be disseminated to the Department of Medicine, Makerere University Faculty of Medicine and School of Graduate Studies, Sir Albert Cook Medical Library and IDI. The results will be presented at meetings both local and international and published in an appropriate peer reviewed journal.

CHAPTER FOUR

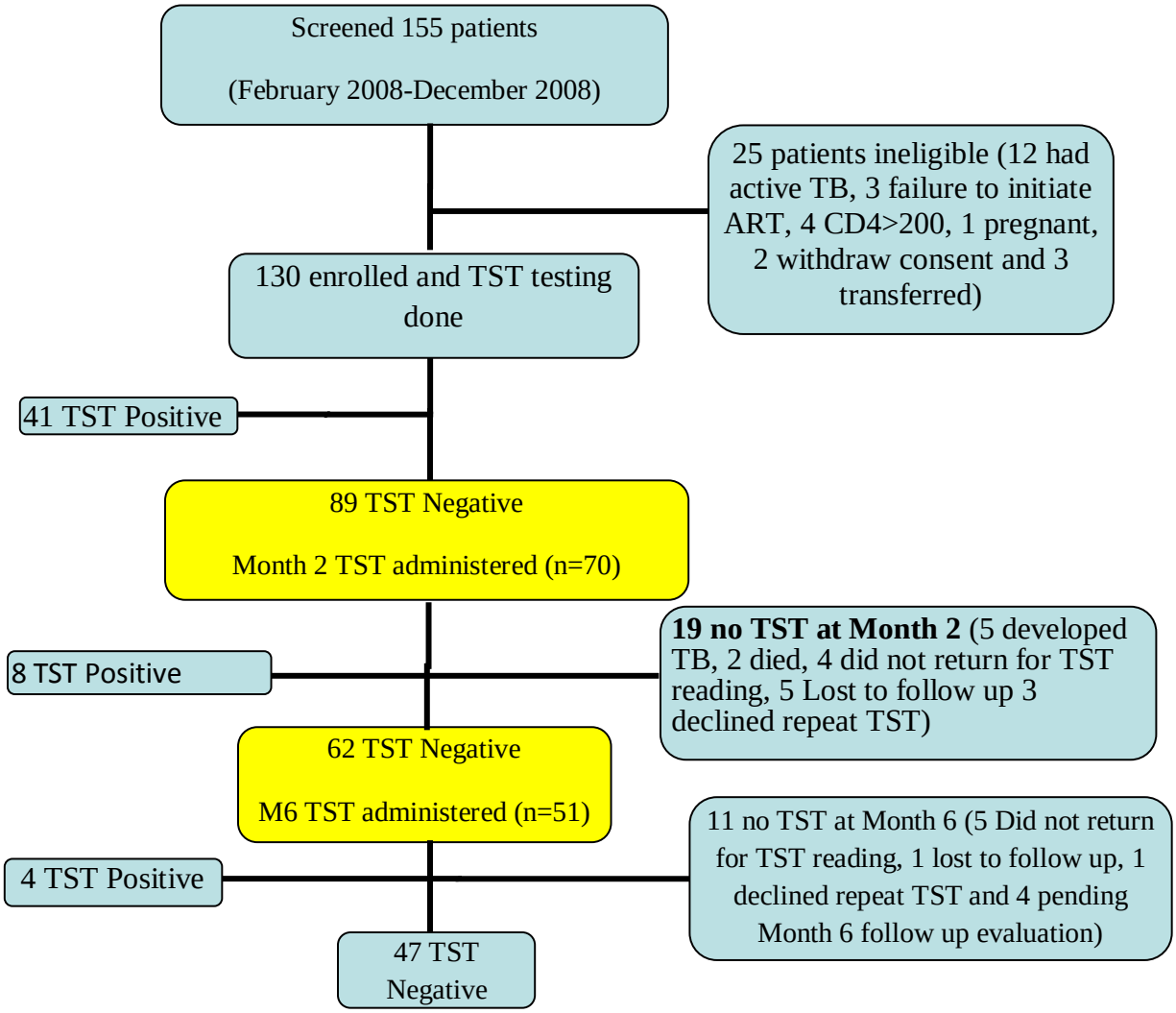
4.0 Results

From February 2008 through December 2008, 155 patients who were initiating ART at the IDI were consecutively screened, after providing a signed informed consent. Out of these, 130 were enrolled and had TST done before ART initiation. Twelve of those not eligible for TST had diagnosis of active tuberculosis (7.7% of all patients screened) after clinical, mycobacteriological and radiological assessment of whom 9 were culture positive. Other reasons for non eligibility included failure to start ART within a week after TST administration (n=3), CD4 cell counts > 200 cells/µL (n=4), withdraw of consent (n=2), pregnancy (n=1) and transferred to other HIV care centers (n=3), figure 1

At enrollment all patients returned for TST reading within the required 72 hours.

Six patients were lost to follow up (6.7%), five of them were lost between enrollment and the month 2 follow up interval.

Figure 1: Study Profile



4.1 Cohort demographic, clinical, radiological and immunological characteristics

Cohort baseline characteristics showed that sixty nine percent (90/130) of the patients were female and the mean age ± standard deviation (SD) of 36 ± 1.8 years. 53.8% were in the age group 30-39 years.

One hundred and twelve (86.2%) of enrolled patients had mostly primary education (44.6%). Forty of the patients were unemployed (30.8%) and of those employed 48.5% were self employed (table1a).

Table 1a: Cohort baseline demographic Characteristics (N=130)

Characteristics	Number, n	Percentage, %
Sex		
Female	90	69.2
Age		
20-29	21	16.2
30-39	70	53.8
40-50	23	17.7
50+	16	12.3
Education		
No education	18	13.8
Primary	58	44.6
Secondary	49	37.7
Tertiary	5	3.9
Marital Status		
Married	56	43.1
Single	43	33.1
Widowed	11	8.5
Cohabiting	2	1.5
Other	18	13.9
Occupation		
Unemployed	40	30.8
Self employed	63	48.5
Professional	2	1.5
Other	25	19.2

Almost half of the patients had BCG scar and most had CD4 cell counts below 200 cells/µL (table 1b)

Of the 130 patients, 122 had Chest x-rays of whom 21 had abnormal chest x-rays. Majority of the patients were treated with Combivir and nevirapine HAART (60.3%) (table 1b).

Table 1b: Cohort baseline clinical Characteristics (N=130)

Characteristic	Number, n	Percentage, %
BCG scar		
Present	61	46.9
Absent	53	40.8
Unclear	16	12.3
Baseline CD4 cell count		
<200cell/uL	118	90.8
>200cell/uL	12	9.2
Baseline Chest X-ray*		
Abnormal	21	17.2
Normal	101	82.8
ART Regimen initiated**		
CBV/NVP	73	60.3
CBV/EFV	**32**	26.5
TRUVADA/EFV	**7**	5.8
T30	**7**	5.8
D4T/3TC/EFV	**1**	0.8
OTHER	**1**	0.8

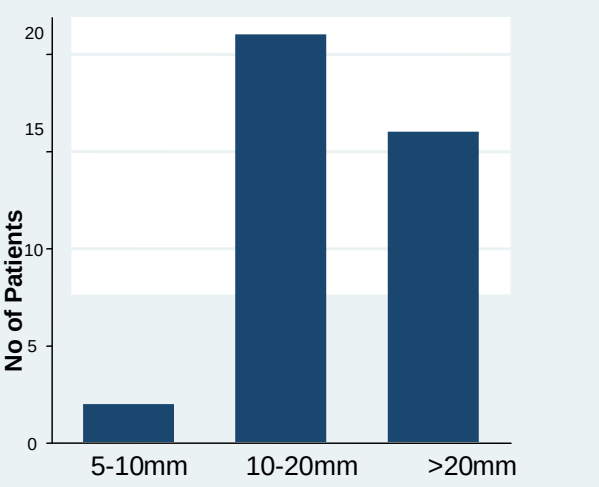
*** n=122, ** n=121**

Of the 130 patients enrolled, 41 were TST positive at baseline (31.5%±4.0) (table 2). Of these most had TST induration size of above 10mm with a median reading of 20mm (range 8-33mm) (graph 1)

Table 2: TST status at different study intervals

Study Interval	Total with TST done	No Positive	Percentage
Baseline	130	41	31.5
Month 2	70	8	11.4
Month 6	51	4	7.8

Graph 1: Distribution of the 41 TST positive patients in the different induration cutoffs at baseline



(TST positive patients with no TB symptoms and normal chest radiographs received latent TB therapy with 300 mg of isoniazid once a day for 9 months.

Table 3: Comparison of Baseline Clinical, radiological and CD4 cell counts of patients at enrollment by TST results

CHARACTERISTIC	BASELINE TST STATUS		P-VALUE
	TST NEGATIVE, n=89	TST POSITIVE, n=41	
Gender, n (%)			
Female	63(71.6)	26(63.4)	0.414
Male	25(28.4)	15(36.6)	
Age, mean	36.3	37.0	0. 71
History of TB treatment, n (%)	6(6.7)	4(9.8)	0. 50
Lymph gland swelling: n (%)	3(3.4)	3(7.3)	0.38
Chest pain, n (%)	5(5.7)	1(2.5)	0.66
Loss of appetite, n (%)	13(14.8)	6(15.4)	1. 0
Loss of weight, n (%)	18(20.5)	16(40.0)	**0.03**
Night sweats n (%)	5(5.7)	4(10.0)	0.46
Cough>2 weeks, n (%)	22(25)	14(35)	**0.17**
Heart rate (bpm), mean	92	86	**0.004**
Respiratory rate (bpm), mean	17	17	0.30
Systolic(mmHg), mean	105.6	106.9	0.57
Diastolic(mmHg), mean	68.5	69.4	0.65
Temperature, ^{o}C, mean	36.3	36.2	0.40
Karnofsky score(%), mean	92	94	**0.09**
Body weight (kg), mean	59.9	59	0.71
Lymphadenopathy n (%)	9(10.3)	6(14.6)	0.56
BCG scar, n (%)	58(66.7)	17(41.4)	**0.012**
Abnormal baseline CXR, n (%)	11(58.0)	8(42.1)	0.43
Baseline CD4 cell count, cells/ul, mean	109	152	**0.0006**
Baseline CD4 percentage, mean	7.8	14.1	**0.002**

4.2 Factors associated with positive TST at enrollment

History of weight loss was significantly associated with a positive baseline TST (p=0.03). Cough tended to be higher among TST negative patients but this did not reach statistical significance (table 3)

Patients with positive TST results had lower average pulse rate, 86 bpm compared to 92 bpm among those with negative results (p=0.004).

Seventy five of the patients (57.7%) had a BCG scar. BCG scar presence was significantly lower among TST positive patients compared to the TST negative ones (41.4% vs 66.7 %), OR 0.31 95% CI (0.13-0.72, p=0.012)

In multivariate logistic regression analysis higher baseline CD4 cell count, absence of a BCG scar and a lower pulse rate remained significantly associated with a positive baseline TST, p=0.004, 0.019 and 0.004 respectively.

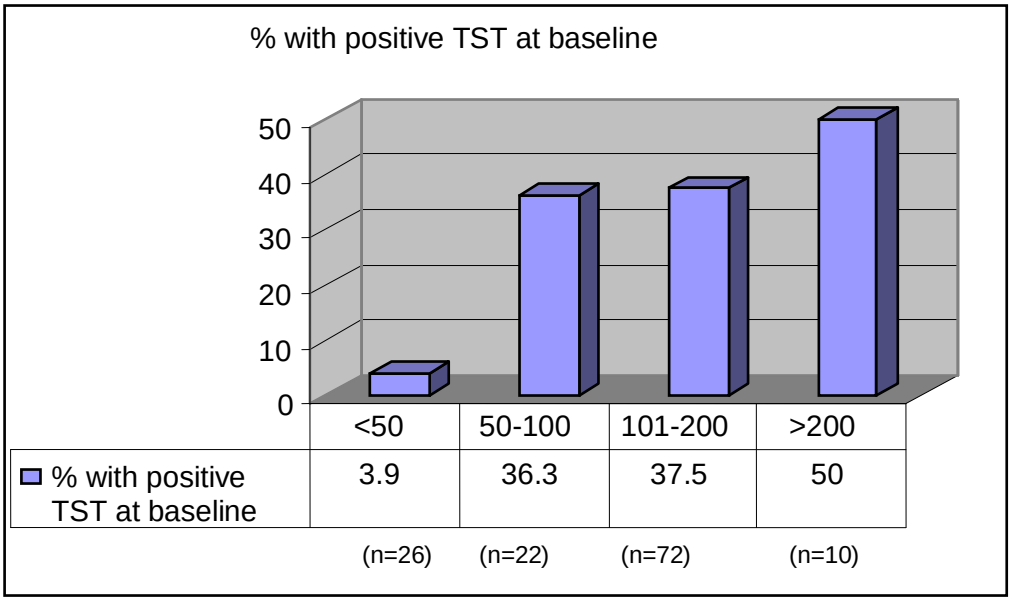
One patient had features of lung consolidation, one had a pleural effusion and 2 had abdominal tenderness and one patient had moderate ascites. One patient had hepatomegally and 3 had splenomegally. Two of the three with splenomegally were TST positive with skin induration 15mm (data not shown).

The average (±SD) absolute CD4 cell count was higher in patients with a positive TST, 152±8 95% CI (136-168) cells/µL compared to 109±8 95% CI (94-123) cells/µL, p=0.0006 table 3. The average (±SD) CD4 percentage followed similar trends.

Over all most patients with negative TST had TST reading of zero with none having a measurement between 1-5 mm, only 2 below 5-14mm indicating a strong response among those who were positive.

Also as shown in graph 2 63.1% of the TST positive patients had CD4 cell count above 100, and half of those with CD4 cell count about 200 had positive TST.

Graph 2: Prevalence of TST positivity by CD4 groups (<50, 50-100, 101-200 and >200)



Chest radiograph abnormalities were not associated with a positive TST p=0.43 (table 3)

Twenty patients were able to provide sputum samples and three were positive for TB. Twelve of these had TB cultures done on LJ medium ordered and 6 grew MTB. Both sputum and culture results were not associated with positive TST results. Patients with TB were given TB treatment according to the national TB treatment guidelines.

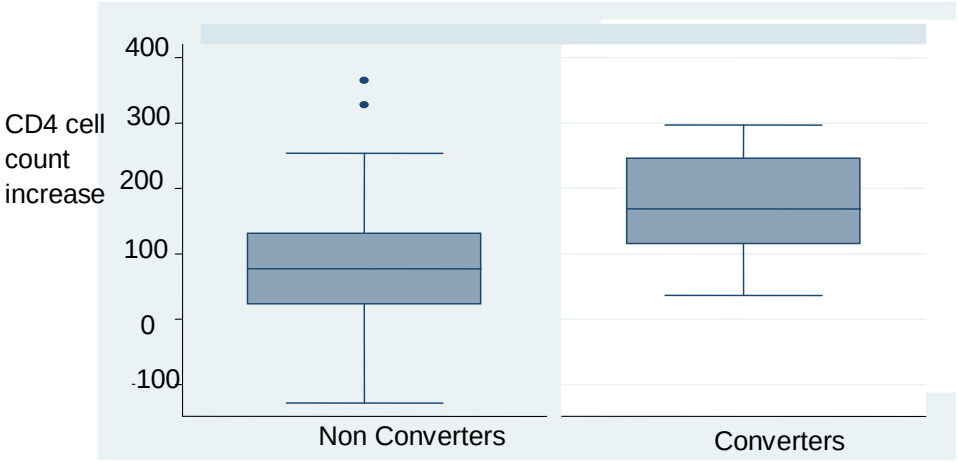
4.3 TST Conversion and associated factors

TST conversion: Twelve of 89 patients with negative TST at baseline (13.5%) converted to positive TST during HAART. Eight of these patients (66.7%) converted after two months of HAART and four after six months (table 2).

4.4 Factors associated with PPD conversions

Patients with TST conversion had significantly higher CD4 cell counts increase after six months of HAART with mean ±SD of 174±89 95 % CI (100-249) cells/µL compared to 91±96 95% CI (60-118) cell/uL in non converters, p=0.02.

Figure 2: CD4 T cell increase among TST non converters and converters.



There were more patients with abnormal baseline chest x-rays among the non-converters. Also BCG scar was more prevalent in the non converters but these difference did reach statistical significance, p=0.19 respectively.

Clinical factors like night sweats, weight loss, loss of appetite, lymphadenopathy, features of consolidation and pleural effusion were not associated with TST conversion (data not shown).

Multivariate analysis showed a trend of higher CD4 cell count increase being associated with TST conversion, p=0.057. Other clinical and radiological factors were not associated with TST conversion.

Table 4: Risk of TST conversion during follow up by selected Clinical, radiological and immunological Characteristics

CHARACTERISTIC	TST conversion Status		RR, 95% CI	P-VALUE
	Positive(n=12)	Negative(n=60)		
Gender, female n (%)	9 (75)	41 (68.6)	0.77 (0.23-2.59)	0.68
Productive cough during follow up, n (%)	1(8.3)	7(11.7)	0.68(0.10-4.60)	0.68
BCG scar, n (%)	6 (50)	41 (69.5)	0.51(0.18-1.41)	0.19
Abnormal baseline CXR, n (%)	1 (8.3)	6 (11.1)	0.78 (0.12-5.17)	0.09
Month CD4 cell count_ mean cells/μL	262	203		0.16
CD4 cell count increase_ mean cells/μL	**174**	**91**		**0.02**

After month two of HAART, 5 out of the 89 patients with negative TST (5.6%) had been diagnosed and treated for active tuberculosis. These were patients in whom the initial TB wok up was negative and were therefore unmasking TBIRIS cases. We observed no case of active TB between month 2 and month 6 follow up interval.

Patients who had a diagnosis of active TB were treated and excluded from repeat TST.

CHAPTER FIVE

5.1 Discussion

In this prospective cohort of ART naïve HIV positive patients 31.5% of the enrolled patients had positive TST at baseline. Another 13.5% with negative baseline TST converted to positive TST within six months of initiating ART. TST conversion was significantly associated with higher CD4 cell count increase. Patients with BCG scar were significantly likely to be TST positive while those without a BCG scar tended to have more TST conversions but this did not reach significance levels. We did not find any clinical or radiological predictors of TST conversion in this study.

The low number of positive TST at baseline is in keeping with the advanced immune-suppression as majority of the patients had low CD4 cell counts. The skin induration that is measured in the TST is a delayed type hypersensitivity reaction that results from the patients CD4 cells recognizing and reacting within the dermis of the skin. In patients with HIV there is CD4 depletion and therefore not enough to react with the PPD antigen. The conversions could be due to the improved immune status of the patients as converters had significantly higher CD4 cell count increase than non converters. We also note however that most conversions (8 of the 12 converters) occurred during the first two months of HAART at which point we do not expect significant quantitative CD4 recovery although qualitative function may have improved greatly accounting for the response. On the other hand this could be a marker of TBIRIS that was sub-clinical to be diagnosed. We performed repeat TST testing in the same patients and there is a possibility of the early conversions (Month 2) being due to booster phenomenon. However our repeat TST was done after 8 weeks at which point we do not expect many patients to have a booster phenomenon as it wanes with time.

After HAART initiation most of the patients experienced favorable immune reconstitution and this could explain why we failed to detect any significant clinical predictors of TST conversion. Secondly at each interval patients were vigorously investigated for TB and those found with active TB received TB chemotherapy and were excluded from repeat TST testing.

Our findings are comparable to those found in other studies. PPD reactivity among previously TST negative HIV patients is reported to be between 5-12% after HAART and is associated with a favorable immune system recovery (Girardi 2002, Fisk 2003).

Fisk et al in a cohort of HIV patients without TB and initiating ART at CD4 cell counts < 100 reported TST reactivity of 11.9% and anergy in these patients reduced by 94%. Reactivity restoration was associated with a CD4 cell count increase of 100 or greater (Fisk, 2003).

TST conversions with favorable immune system recovery has implication for LTBI therapy as it means TST testing can be repeated for those with negative pre-HAART TST. The CDC in its MMWR of April 2009 has recommended repeat TST testing provided an HIV patients CD4 cell count has increased above 200 cells/uL (CDC MMWR 2009).

LTBI therapy has been shown in a number of studies to reduce incidence of active TB (Whalen 1995) but this is in only TST positive patients. In settings like Uganda where the Interferon gamma release assays LTBI tests are not available this finding means that TST is still an important tool of diagnosing LTBI in HIV patients with advanced immune-suppression provided they experience favorable immunological recovery after HAART. This would guide therapy and reduce active TB. Reduction of active has public health implication as it would eventually lead to reduced transmission.

The low TST positivity among HIV patients with advanced immune-suppression has been reported in many other studies.(Jones 2006, Belete 2006, Elliott 1995, Graham 1992, Markowitz, 1993, Rangaka 2007). Jones et al in a study among Uganda HIV positive patients reported comparable TST positivity (Jones 2006). In the same study higher CD4 cell counts were associated with a positive TST. Belete found higher TST reactivity prevalence among HIV patients with absolute CD4 cell counts of ≥200 cells/µl (24.1% versus 62.8%, $P < 0.001$), whereas anergy (no response) was higher in those with <200 cells/µl (72.4% versus 30.8%, $P < 0.001$).

The lower rates of BCG scar among TST positive patients was unexpected as BCG is reported in a number of studies to cross react with PPD giving false positive results. This could be due the fact that most of the studied patients had advanced immune-suppression and positive TST could be a marker of sub-clinical active TB diseases other than remote latent TB infection with the association of BCG scar being completely unrelated. We have not found data to corroborate this finding in the literature. However Jones et al in study among Ugandan HIV positive patients found that BCG presence did not differ significantly between anergic and non anergic patients (Jones 2006).

A review of the literature on this subject since 1966 found BCG after the first year of life to be associated with a false positive rate of 41.8% compared to 12% if BCG was given in the first year of life (Farhat 2006). Most BCG vaccinated people in Uganda received BCG vaccination shortly after birth and probably we do not expect BCG to significantly affect TST.

5.2 Conclusion

Among ART naïve HIV positive patients TST positivity is low and after six months of HAART, less than 50% were TST positive. Patients with higher CD4 cell count increase are more likely to have TST conversion during HAART.

5.3 Recommendations

1. Repeat TST testing should be considered for those HIV positive patients with negative TST before HAART who have experienced favorable immunological recovery.

2. Active TB screening is needed before ART and during the early months of ART because all 5 cases of active TB we report in this study occurred within 2 months of HAART.

3. TB control measures are needed in HIV care settings to prevent TB transmission as we showed in this study that 1 in 10 of the pre-HAART patients have undiagnosed active TB

5.4 Limitations

- The major limitation in this study was lack of a diagnostic tool to separate those infected but non reactive to PPD at baseline from those who could have been truly negative. So we cannot tell whether the converters were incident infections or just restored reactivity.

- TST reactivity could also have been affected by non tuberculosis mycobacteria which we know are so prevalent in our setting.

- Lastly we recruited 89 patients instead 93 required to achieve the study power because of time constraints

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APPENDICES

Appendix I: Baseline Evaluation form

IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV POSITIVE PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC.

Baseline Evaluation

Identification and socio-demographics

1. **Study IDNO** _____ 2. **Date of enrollment** _ _/__/__ __ __ ___

3. **IDC NO**_____ 4. **Age**_____

5. **Sex** 1= Female 2= Male

6. **Address**:
Village/LC1_____Subcounty/Division_____District_____ _____

7. **Marital status**1=married 2= single 3=widowed4=cohabiting 5=other_____

8. **Level of education:** 1= No education 2= Primary 3= Secondary 4= Tertiary

9. **Occupation:** 1=Unemployed 2= Self employed 3= Professional

4= other (specify) _____

TB Disease History

10. **Have you ever been treated for TB** 1=Yes 2=No If yes site of TB 1=Pulmonary 2=Extra pulmonary

11. a)**Past history of TST** 1=Yes 2=No b) If Yes date of TST___ ___/___ ____/___ ___ ___ ___

12**. Does the patient have any of the following symptoms?**

a) Cough of more than 2 weeks duration 1= Yes 2= No

If yes productive 1= Yes 2= No

b)Night sweats 1= Yes 2= No c) Weight loss 1= Yes 2 = No

d)Loss of appetite 1= Yes 2 No e) Chest pain 1= Yes 2= No

f)Shortness of breath 1=Yes 2=No g) Swollen glands (lymph nodes) 1= Yes 2 =No

HIV Disease History

13. a)**Has the patient been on ARVS prior to placement of baseline TST** 1=Yes 2=No

b)If Yes regimen_____, Duration_____weeks . Start date __ ___/__ __/__ __ __ __

Other past Medical History

14. **Has the patient ever had any other immunosuppressant condition other than HIV** 1= Yes 2= No

If yes circle whichever applies:

1= Diabetes 2=Cancer 3=Chronic liver disease 4= Chronic Kidney disease

5= cancer chemotherapy 6= long term use of corticosteroids 7 other _____

Physical Examination findings

15. **General examination findings**

a)Temperature_____0C b) Pulse_____beats/min c) Blood Pressure_____mmHg

d)Karnofsky Score_____% e) Respiratory rate_____breaths/min 20 f) Height_____(cms)

g) Weight_____(Kgs)

h) **Lymphadenopathy** 1= present 2= absent

If present, site of lymphadenopathy(circle all that apply)

1=Cevical 2=Axillary 3=Supraclavicular 4=Epitroclea r 5=Femoral 6=other_____

i) BCG scar 1= present 2= absent 3= not sure

15. **Respiratory system**

a) Consolidation (crepitations/or Bronchial Breathing) 1= present 2= absent

b) Pleural. Effusion 1=present 2=absent c) Wheezing/rhonci 1=present 2=absent

16. **Abdomen**: a**)** Tenderness 1=present 2=absent b) Ascites 1=present 2= absent

c) Hepatomegally 1=present 2= absent d) Splenomegally 1= present 2=absent

17. **Other abnormal physical findings (specify finding and system)**

_____

18. **ART regimen:** 1= CBV/NVP 2=CBV/EFV 3=Truvada/EFV 4=T_{30} 5= d4T/3TC/EFV 6=TDF/CBV

7=TDF/3TC/NVP

8=other_____

19. **Baseline CXR reading** 1= Abnormal CXR 2= Normal CXR

If abnormal, CXR findings: 1=infiltrates 2= fibrosis 3= cavities 4= military TB

5= adenopathy 6= pleural effusion 7= pleural thickening 8= 0ther_____

For abnormal CXRs, likelihood TB 1=Unlikely 2= possible 3= probable 4=Definite

20. **Sputum smears for TB results** 1= positive 2= Negative 3= not applicable (no/dry cough) 4= not done

If positive 0=Scanty 1 = 1+ 2 =2+ 3 = 3+ 4 = 4+

21. **Sputum culture results for TB 1**= positive 2= Negative 3= not applicable (no/dry cough) 4= not done

22. **M0 PPD administered** 1=Yes 2=No 25. **Date of PPD__ __/__ __/__ __ ___ ____**

23. **PPD results** 1=positive 2 negative (00 or <5 mm)

If Yes (positive) size of induration: across _____mm

Along_____mm

24. **CD4 cell count at enrollment**_____cells/uL 25 CD4 percentage_____%

Appendix II: Follow up review forms (M2 and M6)

M2 follow up form

VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC.

1. **Study IDNO**_____ 2. **Date of enrollment** _ _/__/__ __ __ _

3**. Follow up interval** _____weeks

3. **Date of follow up** __ __/__ __/__ __ ___ ____

4. **Enrollment TST** 1= positive 2= Negative.

If Positive, stop here. If negative fill in M2 Evaluation

5. **Since the last visit has the patients experienced any of the following symptoms**

a) Cough of more than 2 weeks duration 1= Yes 2= No

If yes productive 1= Yes 2= No

b)Night sweats 1= Yes 2= No c) Weight loss 1= Yes 2 = No

d)Loss of appetite 1= Yes 2 No e) Chest pain 1= Yes 2= No

f)Shortness of breath 1=Yes 2=No g) Swollen glands (lymph nodes) 1= Yes 2 =No

6. **ART medication compliant % of prescribed medications taken by the patients in the past 30days**

1= 10% 2= 20% 3= 30% 4= 40% 5= 50 6= 60% 7= 70% 8= 80% 9= 90% 10= 100%

7. **Has the patients ART regimen changed since last visit 1= Yes 2= No**

If yes date of change ___ ___/__ ___/__ ___ ____ ___

Reason of change 1= Toxicity/complication 2= Failure 3= new drug available 4=Drug out of stock

5= Patients decision 6= other_____

New regimen **1**= CBV/NVP 2=CBV/EFV 3=Truvada/EFV 4=T_{30} 5= d4T/3TC/EFV 6=TDF/CBV

7=TDF/3TC/NVP

8=other_____

8. **General examination**

a)Temperature_____0C b) Pulse_____beats/min c) Blood Pressure_____mmHg

d)Karnofsky Score_____% e) Respiratory rate_____breaths/min 20 f) Height_____(cms) g) Weight_____(Kgs)

h) Lymphadenopathy 1= present 2= absent

If present site of lymphadenopathy(circle all that apply)

1=Cevical 2=Axillary 3=Supraclavicular 4=Epitroclea 5=Femoral6=other_____

9**. Respiratory examination findings**

a) Consolidation (crepitations/or Bronchial Breathing) 1= present 2= absent

b) Pleural. Effusion1=present 2=abs c) Wheezing/rhonci1=present 2=absent

10. **Since last visit has the patient been treated for TB** 1= Yes 2= No

If yes type of TB 1= PTB 2= EPTB 3=Empirical therapy

11. Follow up CXR done 1= Yes 2= No, if yes, CXR reading 1= Normal 2= Abnormal

If abnormal, CXR findings: 1=infiltrates 2= fibrosis 3= cavities 4= military TB 5= adenopathy 6= pleural effusion 7= pleural thickening 8= 0ther_____

For abnormal CXRs, likelihood TB 1=Unlikely 2= possible 3= probable 4=Definite

12 **Sputum smears for TB results** 1= positive 2= Negative 3= not applicable (no chronic cough) 4= not done

13. **Sputum culture results for TB** 1= positive 2= Negative 3= not applicable (no positive smear) 4= not done

14. **M2 PPD administered** 1=Yes 2=No **Date of PPD**__ __/__ __/__ __ ___ ____

15**. M2 PPD results** 1=positive 2 negative (00 or <5 mm)

If Yes (positive) size of induration: a) across _____mm b) Along_____mm

M6 follow up form

VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC.

1. **Study IDNO**_____ 2. **Date of enrollment** _ _/__/__ __ __ _

3**. Follow up interval** _____weeks

3. **Date of follow up** __ __/__ __/__ __ ___ ____

4. **Enrollment TST** 1= positive 2= Negative.

If Positive, stop here. If negative fill in M2 Evaluation

5. **Since the last visit has the patients experienced any of the following symptoms**

a) Cough of more than 2 weeks duration 1= Yes 2= No

If yes productive 1= Yes 2= No

b)Night sweats 1= Yes 2= No c) Weight loss 1= Yes 2 = No

d)Loss of appetite 1= Yes 2 No e) Chest pain 1= Yes 2= No

f)Shortness of breath 1=Yes 2=No g) Swollen glands (lymph nodes) 1= Yes 2 =No

6. **ART medication compliant % of prescribed medications taken by the patients in the past 30days**

1= 10% 2= 20% 3= 30% 4= 40% 5= 50 6= 60% 7= 70% 8= 80% 9= 90% 10= 100%

7. **Has the patients ART regimen changed since last visit 1= Yes 2= No**

If yes date of change ___ ___/__ ___/__ ___ ____ ___

Reason of change 1= Toxicity/complication 2= Failure 3= new drug available 4=Drug out

of stock 5= Patients decision 6= other_____

New regimen **1**= CBV/NVP 2=CBV/EFV 3=Truvada/EFV 4=T_{30} 5= d4T/3TC/EFV 6=TDF/CBV

7=TDF/3TC/NVP

8=other_____

8. **General examination**

a)Temperature_____0C b) Pulse_____beats/min c) Blood Pressure_____mmHg

d)Karnofsky Score_____% e) Respiratory rate_____breaths/min 20 f) Height_____(cms) g) Weight_____(Kgs)

h) Lymphadenopathy 1= present 2= absent

If present site of lymphadenopathy(circle all that apply)

1=Cevical 2=Axillary 3=Supraclavicular 4=Epitroclea 5=Femoral6=other_____

9**. Respiratory examination findings**

a) Consolidation (crepitations/or Bronchial Breathing) 1= present 2= absent

b) Pleural. Effusion1=present 2=absent c) Wheezing/rhonci1=present 2=absent

10. **Since last visit has the patient been treated for TB** 1= Yes 2= No

If yes type of TB 1= PTB 2= EPTB 3=Empirical therapy

11. Follow up CXR done 1= Yes 2= No, if yes, CXR reading 1= Normal 2= Abnormal

If abnormal, CXR findings: 1=infiltrates 2= fibrosis 3= cavities 4= military TB 5= adenopathy 6= pleural effusion 7= pleural thickening 8= 0ther_____

For abnormal CXRs, likelihood TB 1=Unlikely 2= possible 3= probable 4=Definite

12 **Sputum smears for TB results** 1= positive 2= Negative 3= not applicable (no chronic cough) 4= not done

13. **Sputum culture results for TB** 1= positive 2= Negative 3= not applicable (no positive smear) 4= not done

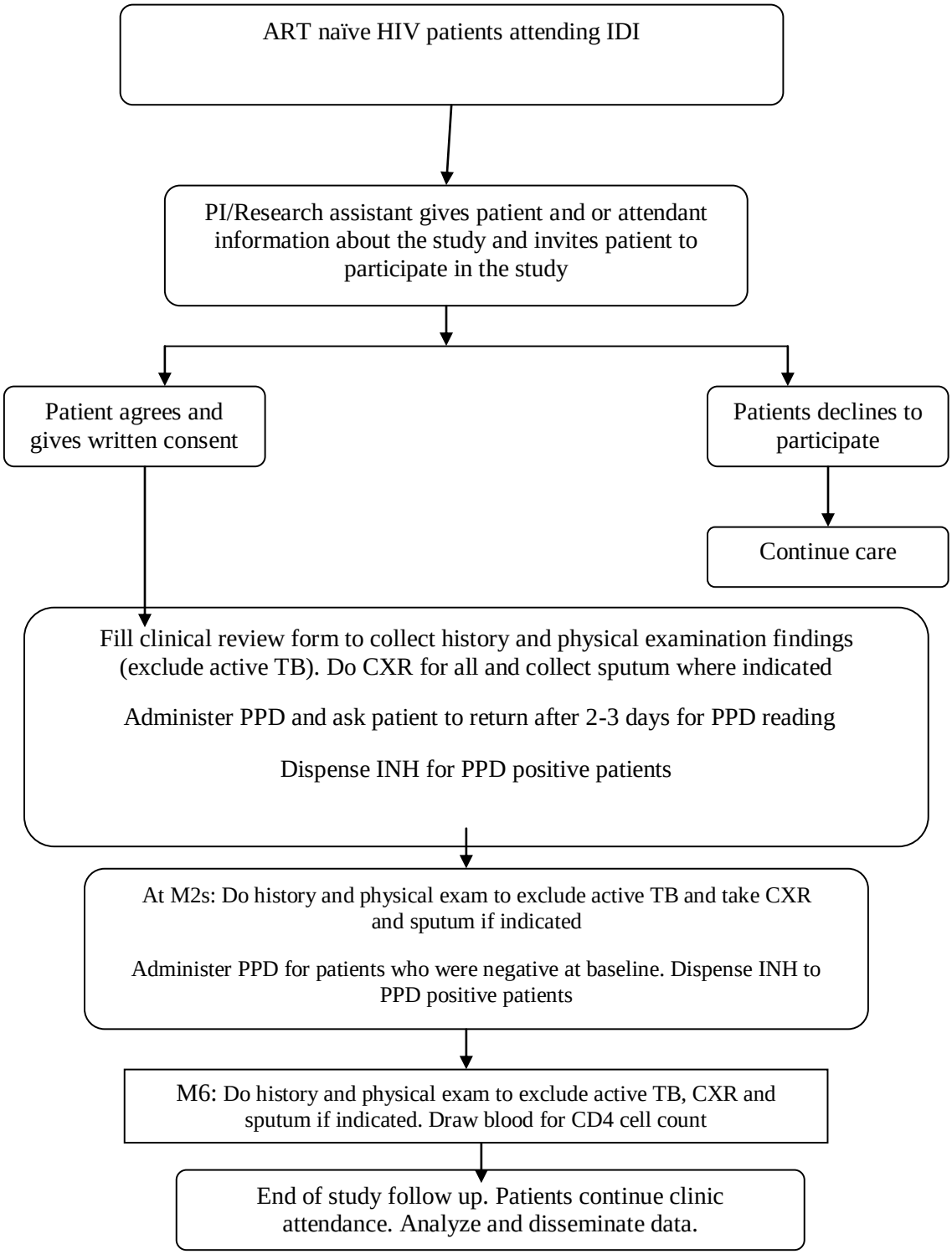
14. **M6 PPD administered** 1=Yes 2=No **Date of PPD**__ __/__ __/__ __ ___ ____

15**. M6 PPD results** 1=positive 2 negative (00 or <5 mm)

If Yes (positive) size of induration: a) across _____mm b) Along_____mm

16. Follow up (M6) CD4 cell count_____cells/u 17. CD4 percentage_____%

Appendix III: Study flow chart



Appendix IVIa: English Consent

Makerere UNIVERSITY

FACULTY OF MEDICINE

DEPARTMENT OF MEDICINE

STUDY TITLE: IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC

INVESTIGATOR: Dr. James Bruce Kirenga

We are asking you to participate in research study title: IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC This study seeks to find ways of diagnosing TB infection among HIV positive patients. Once one is exposed to TB germs infection is established but majority of people do not develop symptoms of TB disease immediately. Symptoms may come later. Treatment is available for this symptom less form of TB to prevent it from turning into TB with symptoms. However we must have ways of diagnosing this kind of TB. The Tuberculin skin test is one of these but because of HIV the test performs poorly because of the damage HIV causes to the body's defense system and we have no test in poor countries like Uganda of diagnosing this kind of symptom less TB (latent TB).

Because the drugs called antiretroviral drugs, the ones you are about to start cause repair of the body defense systems we are seeking to find out if this will also improve the performance of the tuberculin skin test.

This research study will recruit 200 patients and we shall request you to see the study health workers for a maximum of six months.

When you agree to part in this study by signing at bottom of this form, you will be asked some questions about your health and thereafter we shall perform a clinical examination and a chest x-ray and or sputum examination may be requested to exclude TB disease (TB with symptoms). We shall then take off about 6 ml of blood to check your CD4 and perform another blood test for latent TB. We shall then inject the TST drug using a small needle like for diabetics at the front of your left arm. You will be required to return after 2 days for us to read and interpret this test.

If you are found to have this latent TB, you be provided with treatment that reduces the chance of this TB from progressing to TB with symptoms.

If this test is negative you will be requested to see the study team at 2 months and 6 months from the time you started taking your ARVs. At each of these points we shall examine you to see if you have TB disease and if your previous tuberculin was negative we shall repeat it. At the point when you test positive you will stop seeing the study team but continue your follow up at the IDC and receive treatment for latent TB.

Risks and Discomforts

The tuberculin skin test is generally a very safe procedure. However you may experience some pain during the injection of the drug. You may also experience itching a few days after administration of the drug. Blood draws are also associated with some pain.

Benefits
You benefit by having results of samples taken especially the CD4 cell counts. The time taken to have a diagnosis for your problem is also expected to reduce and if you are found to have latent TB, treatment will be provided. In addition, this study may help us better understand how to diagnose TB infection in HIV patients

Alternatives
If you choose not participate in this study you will receive standard care available in the clinic.

Confidentiality
Information obtained about you for this study will be kept private to the extent allowed by law. However, the following groups will be able to view your medical records and have access to private information that identifies you by name: your doctor; members of the study team, members of the Mulago hospital research committee .The results of the study may be published for scientific purposes. These results could include your lab tests and X-rays. However, your identity will not be given out.

Refusal or Withdrawal without Penalty
Your taking part in this study is totally voluntary. There will be no penalty if you decide not to be in the study. If you decide not to be in the study, you will not lose any benefits you are otherwise owed. You are free to withdraw from this research study at any time. Your choice to leave the study will not affect your relationship with this institution.

Cost of Participation and payment for participation and injuries
There will be no cost to you from taking part in this study and there will be no payment to participate in this study. However all tests will be done at no cost and results will be availed to the doctors attending to you in this clinic. If any injuries accrue from this study you will be given the standard of care for such injuries as available in Mulago Hospital. We shall also reimburse your transport costs at a rate of 5000 USH when you come for study visits

Questions
If you have any questions, concerns, or complaints about the research or a research- related injury, please contact Dr. Bruce Kirenga on 0782 404 431 or the chairman of the Makerere Medical school research and ethics committee on

Signatures
Your signature below indicates that you agree to participate in this study. You will receive a copy of this signed document.
Signature of Participant_____Date_____
Signature of Investigator _____Date_____

*Signature of Witness*_____ *Date*_____

Appendix VIb Luganda Consent: Consent

Makerere UNIVERSITY

FACULTY OF MEDICINE

DEPARTMENT OF MEDICINE

STUDY TITLE: IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC

Akulira okunonyereza: Dr. James Bruce Kirenga

Tu kusaba okwetaba mukunonyereza kuno okumanyidwa mulungereza nga: IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC. Ekigendererwa mu kunonyereza kuno kwe kuzuula engeri gye bakebera n'okuzzuula obulwadde bwa kafuba(TB)nga tebunanyikiira nyo mu abo abalwadde ba'mukenenya (HIV). Bwebazuula omulwadde nga alina obubonero oba obuwuka obwefananyiriza obwa kafuba (TB) naye abantu abasinga tebatera kufuna mangu bubonero bwa bulwadde bwa kafuba(TB). Obubonero buno buyinza okulabika oluvanyuma lwekisera. Obujjanjabi bwo'obulwadde bwakafuba nga tebunatandika kuraga bubonero webuli okuziyiza obubonero obwo okuvayo. Naye tulina okuba ne ngeri gye tukeberamu ekika kyo bulwadde buno obwakafuba. Okukebera kuno okwa Tuberculin skin testing kulungi naye olwo bulwadde bwa mukenenya (HIV) ebiva mukukeberwa kuno tebirambikibwa bulungi olwokuba obulwadde bwamukenenya (HIV) buba bwata dda abasilikale abakuuma omubiri ate nga mu Nsi enjjavu nga Uganda tetulina byuuma bisobola kukebera nakuzuula kafuba nga tekanaba kuleta bubonero.

Edagala eriyiitibwa ARVs lyojja okutandikira kwo okumira lizamu amanyi gabasilikale abakuuma omubirigwo, twagala okuzuula oba okukerwa tuberculin skin test kuna'avayo bulungi.

Mu kunonyereza kuno tujja kuwandiika abalwadde 130 era ojja kusabibwa okulaba abakolakubyobujjanjabi mu banga lya myezi mukaaga.

Bwonooba okiriza okwetaba mukunonyereza kuno nga otadde ekinkumu (signinga) wansi kulupapula luno. Ojja kubuzibwa ebibuuzo ebikwata kubulamu bwo noluvanyu tujja kukebera mu x-ray ekifuba kyo wamu nekikondolwa kyo kikeberebwe oba temuli bubonero bwa kafuba (TB).

Tujja kujjako akajiko komusaayi tukebere obunji bwamanyi gabasilikale abakuuma omubirigwo(CD4) era nomutindo gwobulwadde bwa TB gwoliko.

Noluvanyuma tujja kukuba akayiso ke dagala lya TST akefananyirizaako akabalwadde ba sukaali. Ojja kukomawo oluvanyuma lwe naku biri (2) onyonyolebwe ebinaaba bivudde mu kukeberebwa okwo.

Bwo'nozulibwa ne TB ngatanaba kulaga oba okulinya wagulu, tujja kuwa edagala erinamukendeza aleme okulinya wagulu okulaga obubonero.

Bwo'nozuulibwa nga tolina TB ojja kusabibwa okudda olabe abasawo abali mu kunonyereza ku mubanga lya myezi ebiri (2) ku mukaaga (6) okuva ekisera wonotandikira okumira ARVs.

Tujja kuba tukukebera kubuli mitendera gino tulabe oba olina obulwadde bwa kafuba (TB) nebwoba nga wazulibwa nga tolina bulwadde bwa kafuba(TB) tujja kudamu okukebera.

Ekisera webana'nazuulira nti olinamu akafuba (TB) ojja kulekera awo okulaba abakola mukunonyereza kuno naye ogende mumaso ngofuna obujjanjabi bwo mu IDC.

Obuzibu n'obutewulira bulungi
Ekikyuma e kyeyambisibwa mu ku keberera obulwadde bwa kafuba (tuberculin skin test) kiri mu nkola nungi era tekilina mutawana gwona eri obulamu bwo.

Wayinza okubaawo okusiyiibwa n'okulumizibwa mu kukujjako omusaayi oba obutewulira bulungi nga wakatandika eddagala naye ebyo byona tebilina kyabulabe kyona kyebigenda kukola ku bulama bwo.

Byoonoganyulwa mu
Ojja keberebwa bazuule amanyi gabasilikale abali mu mubiri gwo, Obudde bwebanamala nga bakunonyereza'ko embera yo bulamubwo ejja kugenda elongokoka era bwe banakusanga nga olina akafuba ojjakuwebwa obujjanjabi. Okunonyereza kuno kuyinza okutuyamba okukebera netuzuula obulwadde bwa kafuba nga tebunakula mu abo abalwadde ba mukenenya.

Okwesalirawo
Bwono'ba tokiriza kwetaba mu kunonyereza kuno tewali kigenda kutabula bujjanjabi bwolina kufuna mu dwaliro lino

Okukuuma ebyaama ebikukwaatako
Okwetaba mu kunoonyereza kuno kuyinza okukwekula ebyafaayo oba ebyaama byo. Nkukakasa nti ebiwandiiko ebiriko ebikukwatako bigenda kukumibwa bulungi mukifo ekyekusifu nga kyaakyaama ekitatuukibwako muntu mulala yenna okuggyako akuliira okunoonyerereza kuno nabo abali kukakiiko akanonyereza mu dwaliro lye Mulago. Elinya oba ebiwandiiko ebikukwatako bigenda kuwandikiibwaako mu alipoota yona okugya ko olwe nsonga yo bulamu bwo.

Olina eddenbe okugaana oba kusazaamu okwetaba mu kunoonyereza kuno
Oyinza okugaana okwetaba mu kunoonyereza kuno oba okukuvaamu ekisera kyona.

Bino byona tebigenda kutabula bujjanjabi bwolina kufuna mu dwaliro lino

Ebyo okusasulibwa
Tojja kusasulwa olw kwetaba mukunonyereza kuno era tolina kusasula kukwetabamu. Wabula ojja kukeberebwa ku bwerere era ebinazuulibwa oluvanyuma bijja kuwebwa omusawo wo.Bwewana'bawo obuzibu bwona obukutuka'ko mukunonyereza kuno ojja kujanjabibwa bulungi. Era tujja kusasulira shs 5,000/= zono'ba okozeseza nga oze okwetaba mukunonyereza kuno.

Ebibuuzo

Bwoba olina ebibuuzo byona ebikwatagana no kunonyereza kuno, labagana ne Dr. Bruce Kirenga ku simu 0782 404 431 oba Chairman wa Makerere Medical school research and ethics committee ku simu 0712-120-552

Signatures

Omukono oba ekinkumu kyo wamanga kiraga nti osazewo okwetaba mukunoonyereza ku kyeyagalire era osomye byona ebikwata kukunoonyereza kuno nobitege'ra bulungi.

.....	.....
Ennaku z'omwezi	Erinya mu kyapa n'omukono oba ekinkumu ky'oyo agenda okwetabamu.
.....	.....
Ennaku z'omwezi	Erinya mu kyapa n'omukono oba ekinkumu ky'oyo akulira okunonyereza kuno.
.....	.....
Ennaku z'omwezi	Erinya mu kyapa n'omukono oba ekinkumu ky'oyo abadewo.

Appendix V: TST Standard Operating Procedure

Standard Operating Procedure for tuberculin skin testing

Administering the Mantoux Tuberculin Skin Test

Make sure that the area for administering the test has a firm, well-lit surface, and that equipment and supplies are ready. The Mantoux tuberculin skin test consists of an intradermal injection of exactly one tenth of a milliliter (mL), which contains 5 tuberculin units.

Look at the vial label to make sure the vial contains the tuberculin that you want to use, including the tuberculin unit strength.The label should indicate the expiration date. If it has been open more than 30 days or the expiration date has passed, the vial should be thrown away and a new vial used.

When you open a new vial, write the date and your initials on the label to indicate when the vial was opened and who opened it.

To avoid reducing the potency of the tuberculin, store it inside a refrigerator so that it remains between 2 and 8 degrees Centigrade. Also store and transport the tuberculin in the dark as much as possible and avoid exposure to light.

On a firm, well-lit surface, expose the patient's arm and slightly flex it at the elbow. The injection should be placed on the palm-side-up surface of the forearm, about 2 to 4 inches below the elbow. Your local institutional policy may specify the right or the left forearm for the skin test. The area selected should be free of any barriers to placing and reading the skin test such as muscle margins, heavy hair, veins, sores, or scars.

After choosing the injection site, clean the area with an alcohol swab by circling from the center of the site outward. Allow the site to dry completely before the injection. Because some of the tuberculin solution can adhere to the inside of the plastic syringe, the skin test should be given as soon as possible after the syringe is filled.

Pick up the syringe and be sure to fasten the needle tightly on the syringe by holding the cap and twisting it onto the tip of the syringe. Next, remove the needle cap.

The needle bevel should be perpendicular to the flange of the syringe. If necessary, turn and tighten the needle to line up the bevel correctly with the flange.

Place the vial on a flat surface, hold the vial between the thumb and fingers, and insert the needle through the neoprene stopper.

Invert the vial while keeping a firm hold on the syringe and plunger. The tip of the needle should be below the fluid level in the vial.

Pull back on the plunger and draw out slightly more than the one tenth of a milliliter needed for the test.

Remove the needle from the vial. Hold the syringe in an upright position, then draw back slightly on the plunger. Tap the syringe lightly to break up air bubbles, then push forward.

Expel all air and excess fluid from the syringe and needle, leaving exactly one tenth of a milliliter of tuberculin solution in the syringe.

The second step in administering the Mantoux tuberculin skin test is injection. You'll inject the tuberculin, discard the needle and syringe, check that the skin test was administered properly, and repeat the test if needed.

Stretch taut the selected area of skin between the thumb and forefinger.

For an intradermal injection, the needle bevel is advanced through the epidermis, the superficial layer of skin, approximately 3 mm so that the entire bevel is covered and lies just under the skin.

The injection will produce inadequate results if the needle angle is too deep or too shallow.

When the needle is inserted at the correct angle you can see the bevel of the needle just below the skin surface. Next, release the stretched skin and hold the syringe in place on the forearm.

Grip the flange of the syringe between your first and middle fingers. Use your thumb to press on the plunger.

Now, slowly inject the tuberculin solution. You should feel fairly firm resistance as the tuberculin enters the skin. A tense, pale wheal that's 6 to 10 mm in diameter appears over the needle bevel. Remove the needle without pressing or massaging the area.

Next, discard the used syringe immediately in the designated puncture-resistant container

Reading the Mantoux Tuberculin Skin Test To begin, collect the following supplies: a small, plastic, flexible ruler marked in millimeters to measure the test, a pen to mark the edges of the induration, and an alcohol pad to clean off the pen marks. You'll need the tuberculin testing forms for documenting the measurement results.

The skin test should be read between 48 and 72 hours after the skin test has been administered.

The basis of reading the skin test is the presence or absence of induration, which is a hard, dense, raised formation. This is the area that is measured.

EQUIPMENT AND SUPPLIES

1. A single-dose disposable tuberculin syringe
2. Cotton balls and antiseptic
3. Purified protein derivative
4. A ruler with millimeter (mm) measurements
5. Forms to record placing of PPD and reading of skin test
6. Fridge for storing reagents
7. A puncture-resistant sharps disposal container
8. A pen

REFERENCES

- Diagnostic Standards / Classification of TB in Adults and Children (PDF) - Am J Respir Crit Care Med 2000; 161: 1376-1395

- Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection - MMWR 2000; 49 (No. RR-6)

Appendix VI: TST results form

Baseline TST					**M2 TST**				**M6 TST**			
Patient IDNO	**Date of PPD admin**	**PPD reading date**	**Ind size (mm)**	**TST Result(+ve, -ve, Anergic**	**Date of PPD admin**	**PPD reading date**	**Ind size (mm)**	**TST Result(+ve, -ve, Anergic**	**Date of PPD admin**	**PPD reading date**	**Ind size (mm)**	**TST Result(+ve, -ve, Anergic)**

Appendix VII: Schedule of activities

IN VIVO PPD REACTIVITY AND ASSOCIATED FACTORS AMONG HIV PATIENTS ON HAART AT THE MAKERERE UNIVERSITY INFECTIOUS DISEASES CLINIC

Schedule of Study Activities

Time	Jan08-jul08	Aug08	Sept08	Oct08	Nov08	Dec08	Ja09	Feb09	Mar09	Ap09	May09
Activity											
Proposal writing	X										
Dept proposal presentation	X										
FOM proposal presentation	X										
Data collection-enrolment of patients		X	X	X							
Data collection-follow up					X	X	X	X	X	X	
Data analysis											X
Dissertation writing											X
Dissertation defense											X