University of Bergen

Predictors of sputum conversion among pulmonary tuberculosis patients in Mwanza, Tanzania

Thesis submitted in partial fulfilment of the requirements for the degree of Master of Philosophy in International Health

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ACKNOWLEDGEMENTS

I would like to acknowledge the ongoing nutrition, diabetes and pulmonary TB/HIV study in Mwanza, Tanzania, conducted as a joint collaborative project between the National Institute for Medical Research (NIMR) Mwanza and University of Copenhagen and sponsored by The Danish Medical Research Council and Danish Council for Development Research for allowing me participate in this study and to use information from the study - Thank you.

I am grateful to the Norwegian State Education Loan Fund (Staten Lånekassen), for awarding me the scholarship to pursue my studies at the University of Bergen and to NUFU: (NUFUPRO-2007/10183): Research and capacity building to address the diagnostic and therapeutic challenges in tuberculosis for facilitating this study. I thank the Centre for International Health for the training I acquired in the form of courses. Likewise I would like to acknowledge the United Republic of Tanzania through National Institute for Medical research (Mwanza centre) for allowing me to utilize this opportunity to pursue my studies. I would like to thank Mr. John Changalucha, The center director of NIMR, Mwanza for allowing me to study abroad and for his additional support. Thank you very much.

I convey my heartfelt gratitude to my supervisors, Professor Harleen Grewal, Professor Henrik Friis, Professor Aase B. Andersen and Dr. Nyagosya Range for having faith in me from the start and believing that I had the capacity to carry out this study. I am greatly indebted to them for their valuable advice, constructive criticism, encouragement and support throughout my study period. I am truly inspired, thank you.

My utmost appreciation goes to Dr. George Praygod, and Dr. Daniel Faurholt for their exceptional role played on accomplishing this work. I would like to extend my sincere appreciation to the TB-Nutrition study team for their contribution to make this work possible. Also, I would like to thank the patients and their treatment supervisors who participated in this study.

My honest appreciation goes to my colleagues Dr. Mbazi Senkoro, Dr. Pilly Chillo, Dr. Bernard Ngowi and Dr. Kijakazi Mashoto, for the special support and co-operation of the Tanzania community in Bergen for many interactions. I would like to express a special note of thanks to my classmates Linn Fossdal, Astrid Spjældnes, Catherine Skogen and Jonas Finger for their special friendship, and their efforts to introduce me to the Norwegian way of life and tradition.

I give thanks to my best friend Jackline Patrick Babu who always kept my working spirit up. I express my heartfelt to my guardians Mr. and Mrs. D.M. Mbosoli and my brother Nkanda Kidola and his wife for the all encouragement, special support and love.

I thank my late parents for giving me a foundation and for being my inspiration

Lastly, I acknowledge the contribution of many people who were directly or indirectly involved with realization of this work and to the Almighty God for keeping me strong and healthy throughout my study period.

ABBREVIATIONS

AIDS	Acquired immuno deficiency syndrome
AFB	Acid fast bacilli
BMI	Body mass index
BMC	Bugando medical centre
CD4+	Cluster of differentiation antigen 4
DOTS	Directly observed treatment short course
E-P	Energy protein
EDTA	Ethylene diamine-tetra-acetic acid
ELISA	Enzyme immunosorbent assay
HIV	Human immunodeficiency virus
Hb	Haemoglobin
IGRAs	Interferon gamma release assays
M.tb	*Mycobacterium tuberculosis*
MOHSW	Ministry of Health and Social Welfare
MUAC	Mid upper arm circumference
MMN	Multi-micronutrients
NTLP	National Tuberculosis and Leprosy Programme
NIMR	National Institute for Medical Research
PTB	Pulmonary tuberculosis
PCR	Polymerase chain reaction
TB	Tuberculosis
TLC	Total lymphocyte count

WHO World Health Organization

WBC White blood cell count

ZTRL Zonal tuberculosis reference laboratory

SUMMARY

Background: Tuberculosis (TB) is a major cause of morbidity and mortality worldwide. TB kills 2 million people each year and 98% of these deaths occur in developing countries. TB remains a burden especially in Africa, Eastern Mediterranean and South-East Asia regions.

Among 22 countries with the highest TB burden in the world, Tanzania ranks as number 14 globally and is the 6th in Africa. The number of TB cases in Tanzania has increased six folds in the past two decades. Case detection rate in Tanzania is still below the global estimated target of 70%. Seven percent of the population in Tanzania is infected with HIV and 50% of TB patients are co-infected with HIV. In addition, the majority of TB patients are malnourished.

Because resources are limited, it is imperative to focus the efforts of the health system on those patients needing the most care. Lack of sputum conversion in pulmonary tuberculosis patients on anti-TB treatment is an indicator of potential treatment failure. These patients represent a source of continued TB transmission. Such patients may in the worst case turn out to be harbouring drug resistant *Mycobacterium tuberculosis*.

Predicting treatment outcome in TB patients receiving anti-TB treatment at an early stage, by identifying factors among patients who are more likely to end up in treatment failure, would benefit the TB program overall and allow the TB staff to take special care of those patients who could be at an increased risk of not sputum converting at the end of the 2nd month of the intensive phase of anti-TB treatment. The aim of this study was to identify risk factors that may be associated with persistent sputum smear positivity at the end of the 2nd month after initiation of anti-TB treatment among pulmonary TB patients in Mwanza, Tanzania.

Methods: A prospective cohort study conducted within ongoing randomised multi-micronutrient supplementation trials among smear positive pulmonary TB patients in Mwanza city was undertaken from April 2006 to November 2008. All patients recruited were interviewed to obtain social demographic and TB medical history. In addition, anthropometric measurements (height, weight, and arm circumference and body mass index [BMI]) were recorded at baseline and at 2 months post initiation of anti-TB treatment. HIV counselling followed by HIV testing was undertaken for all patients. Laboratory investigations for CD4+ count, white blood cell count

(WBC), haemoglobin (Hb), sputum smear microscopy and sputum culture were undertaken.

Results: A total of 601 PTB patients were enrolled of which 249 (41%) were HIV+. Five hundred and sixteen (86%) patients were followed to the end of the 2nd month anti anti-TB treatment. The overall sputum smear conversion rate at the end of the 2nd month of the intensive phase was 76. 4%, while the sputum culture conversion rate was 94. 2%. Factors associated with persistence of sputum smear positivity at the end of the 2nd month of intensive phase were: male sex (OR 1.92, 95%CI 1.16; 3.18, P=0.01), 3+ initial pre-treatment AFB smear grading (OR 1.9, 95%CI 1.17; 3.19, P=0.01) and a BMI of < 18.5kg/m^2 (OR 2.17, 95%CI 1.24; 3.7, P=0.006). The presence of a BCG scar was found to be associated with a 43% lower risk of persistence of sputum smear positivity at the end of the 2nd month after initiation of anti- TB treatment (OR 0.57, 95%CI 0.35;0.95, P=0.032). The presence of a BCG scar was also strongly associated with a lower risk of persistent sputum culture positivity (OR 0.28, 95%CI 0.35; 0.95, P=0.002). HIVstatus, smoking, CD4 count, total WBC count and Hb levels were not significantly associated with sputum smear microscopy and sputum culture conversion post anti-TB treatment.

Conclusion: Pulmonary TB patients with an absence of BCG scar, male sex, high initial AFB sputum grading, and a low body mass index, are at a risk of remaining culture and/or sputum smear positive at the end of 2nd month of the intensive phase of anti-TB treatment. Closer attention should be provided to such patients while on anti- TB treatment. Additionally, BCG vaccination should be continued to be promoted and supported. Larger studies also in other areas are needed to assess the role of BCG vaccination on sputum conversion.

1.0 INTRODUCTION

1.1. TB natural history

Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis* (M.tb), an organism that was discovered by a German physician Robert Koch in 1882 (1). TB is a disease that affects a number of different organs of the human body, but primarily the lung. The mode of spread is from an infected person to an uninfected person by inhalation of infected droplet nuclei with M.tb.

The period from which a person is infected by M.tb up to the development of active disease varies from individual to individual (2, 3). The development of infection to active disease can take many years, or the infection may remain dormant for life. Only 10% to 20% of all individuals who are infected with M.tb end up with active disease in their life time (2, 3).

Common clinical presentations of pulmonary TB (PTB) include, coughing more than two weeks, fever, night sweat, haemoptysis and wasting (4). The health status of an individual is an important determinant for the risk of progression to TB disease. The progress of the disease depends mainly on tissue destructive delayed-type hypersensitivity reaction to M.tb and cell mediated immunity (macrophage-activating responses). In general, type 1 T helper cells provide protective immunity while type 2 T helper cell response leads to tissue destruction. Following infection, TB may either progress to disease immediately (like infants or severely immunosuppressed individuals) or the bacteria may be contained by the immune system. This progress phenomena theoretically is divided into primary and post-primary forms and both have quite different pathological features (5). Following an initial infection, the inhaled mycobacteria via the respiratory tree reach the alveoli where they are engulfed by alveolar macrophages, which comprise the initial lesion (Ghon focus). If the initial defence fails to contain the bacteria, M.tb continues to multiply intracellularly leading to cavity formation. Once a cavity is formed a large number of bacilli are excreted in the sputum and a patient becomes an infectious case. Twenty per cent of cases of open cavitating TB resolve without treatment (5).

1.2. TB burden

Despite the existence of TB control programmes globally, TB remains one of the major publichealth problems worldwide. It is one of the greatest challenges that face health systems in the 21st century. The total numbers of new TB cases is rising worldwide. From 1997 to 2000 the

number of new TB cases increased at a rate of 1.8 % per year (3). They were an estimated

8.3 million new cases in 2000 and the number rose up to 9.2 million in 2006 (3, 6, 7). The most affected region is, Africa which accounts for 31% of the global TB cases while the western pacific and South-East Asia regions together account for 55% (6-8). In sub-Saharan Africa, the TB incidence rate is twice that of the South East Asia region at nearly 350 cases per 100,000 population (8).

Tanzania ranks 14th among the 22 countries with the highest TB burden worldwide and 6th in Africa (6, 7). The case detection rate in Tanzania is still below the global estimated target of 70 % (6, 7). The majority of the cases appear to be in young adults aged 15-45 years, the same age group that is affected by HIV/AIDS (3, 9). Approximately, 50% of all TB patients in Tanzania are co-infected with HIV (9). TB cases in Tanzania have increased from 11,753 in 1983 to 65,665 in 2004 (9). This increase is largely attributed to HIV/AIDS (9). It is estimated that about 7% of the general population in Tanzania is infected with HIV (10). The life time risk to develop TB in HIV negative individuals is 5-10% compared to 50% in HIV positive individuals (9).

1.3. TB and HIV interaction

TB and HIV are difficult to separate from each other as both have an impact on one another. While HIV increases the risk of developing active TB, TB on other hand adds to the risk of progression from HIV to AIDS (8). Dual infection with HIV/AIDS and TB has contributed largely to an increase in number of new TB cases worldwide and remains a major cause of death in an HIV infected person (7, 9). The African region has 85% of all TB cases with HIV (7). South Africa has 28% of all TB/HIV case globally and contributes 33% of all cases in Africa (7). Data from East Africa shows Kenya to contribute 10% of all HIV/ TB cases while Tanzania has 3% and Uganda 2% (7). TB patients co-infected with HIV are at risk for a poor treatment outcome (11). Sputum conversion at two months post anti-TB treatment has been reported to be important interim indicator of treatment success (12). A cohort study conducted in North Carolina, U.S.A. reported HIV infection as one of the important predictors of lack of sputum conversion in TB patients (11). The study showed that 46% of TB patients co- infected with HIV had a lower rate of sputum conversion compared to non-HIV infected TB patients (11). A study from Uganda, undertaken by Bwire *et al* showed no difference in sputum conversion rate at 8 weeks post anti-TB treatment in TB patients with HIV and those without HIV (78% vs.76%), however TB

with HIV were more likely to die (P=0.017) (13).

1.4. TB and nutrition

Malnutrition has also been associated with increasing susceptibility to TB. Studies have shown TB patients suffer from wasting and micronutrient deficiency (14). Concurrent macro and micronutrient deficiency compromises the immune system function which in turn increases the risk of TB reactivation (15). A study conducted to assess nutritional status between TB patients and healthy controls, showed that 66% of the TB patients were underweight as compared to 10% of the healthy controls (P<0.001) (16). Furthermore, the study also showed that the plasma retinol concentration in TB patients was lower than that for healthy individuals (16). Vitamin A deficiency has been shown to be a risk factor for developing TB (17). Various mechanisms like, poor dietary intake due to loss of appetite, poor absorption of nutrients from the intestine and increase uptake of nutrients by specific target tissue due to increase body metabolism, were associated with nutritional deficiency in TB patients (17). Since anti-TB treatment is given to malnourished TB patients, there is a possibility that nutritional deficiency may impair treatment outcome. A study from Indonesia by Karyadi *et al* reported that micronutrient supplementation resulted in an earlier elimination of tubercle bacilli from the sputum (18). The number of patients with sputum smears negative for tubercle bacilli were higher in the micronutrient supplemented group than in the placebo group (23% vs. 13%) (18). However, a recent study conducted in Mwanza, Tanzania on the effect of micronutrient supplementation on treatment outcome in PTB patients showed that neither multi-micronutrient nor zinc supplementation had a significant effect on sputum culture conversion, although multi-micronutrient supplementation was significantly associated with the weight gain (0.78 kg; P=0.02) (19).

1.5. Laboratory diagnosis and treatment

TB case identification depends on availability of sensitive and specific diagnostic tools. A part from presumptive diagnosis of TB based on clinical symptoms and chest x-ray findings, a definitive diagnosis of TB relies upon the identification of the causative agent in clinical samples. Detection of M.tb in clinical specimens can be undertaken by various methods such as microscopy, culture, and the newer polymerase chain reaction (PCR) based assays. The commonest method in use for diagnosing TB cases is still sputum examination by microscopy in high TB burden countries. Microscopy is cheap, easy to perform and does not require highly skilled technicians. Despite being widely used, microscopy has limitations as it is not specific for

the detection of acid-fast bacilli (AFB) in sputum and can only detect AFB in 60-70% of culture positive specimens (20, 21). Ziehl-Neelsen staining (ZN stain) and fluorescent staining are the current microscopic methods in use. ZN microscopy is still widely used in low and middle income countries as it is relatively cheap. Fluorescent microscopy is more sensitive and is faster thereby decreasing the time required to examine slides although the related expenses do limit its wide use (21).

Culture remains the cornerstone of TB diagnosis. Compared to microscopy, culture is more sensitive as it can detect as few as 10 bacteria per ml of sputum and it has a sensitivity of 80-85% and specificity of 98% (22). Despite the benefit that culture methods have, the cost and time taken to obtain a result (4-8 weeks) limit their wide spread use. Apart from the methods mentioned, there are other rapid diagnostic methods which are relatively expensive and thus are not affordable in high TB burden countries. These include DNA and RNA based PCR methods. Newer commercially available bloods test (Quantiferon, TB-Gold in tube - Cellestis, T-spot.TB - Oxford immunotec) that measure interferon gamma responses to M.tb specific antigens are also being promoted. These tests do not accurately distinguish active TB disease from latent infection (4, 5). Large prospective longitudinal studies are required to assess the diagnostic and prognostic value of these interferon gamma release assays (IGRAs).

An identified case of active TB needs to be treated appropriately in order to stop the chain of transmission. Anti-TB drugs are either bactericidal or bacteriostatic. The bacteriostatic drugs are not included in standard drug regimens of TB treatment. The anti-TB drugs which are bactericidal can effectively sterilize the TB lesion. World Health Organization (WHO) promotes a combination of different anti-TB drugs in order to prevent the emergence of drug-resistant strains. The current regime in the management of TB is divided into 2 months of intensive phase and 4 months of continuation phase of anti-TB therapy. During the intensive phase, the drugs in use are rifampicin, isoniazid, pyrazinamide and ethambutol. Usually in the first and second week following the initial infection there are large numbers of actively replicating bacilli present in the pulmonary cavities. Most bacilli are initially killed by isoniazid with the help of rifampicin and ethambutol. These drugs quickly render the patient non- infectious. In the following weeks of the intensive phase the remaining bacilli are killed by pyrazinamide and rifampicin. In the continuation phase the main drugs used are rifampicin and isoniazid. All remaining dormant bacilli are killed by rifampicin and those that are rifampicin- resistant are killed by isoniazid (5).

1.6. TB control strategy in Tanzania

TB control in Tanzania is performed by the National Tuberculosis and Leprosy Programme (NTLP). Formulation, policy development and implementation is undertaken at the central level in line with guidelines developed by WHO and other related partners. At the regional level TB/leprosy coordinators interpret policy guidelines and monitor implementation at the district level. The NTLP under the Ministry of Health and Social Welfare (MOHSW) has successfully extended activities to include TB/HIV control in the whole country. The TB case detection rate was 46% and treatment success rate 82 % in 2005 (7).

1.7. Indicators of TB treatment success

Early case identification and treatment of all TB cases is a priority. It has been shown that sputum smear conversion is an important indicator of both the effectiveness of treatment and infectivity of the patients (23). Persistent smear positivity at the end of the 2^{nd} month of treatment has been cited as one of the main predictors for treatment failure (23, 24).

Various studies have been undertaken in different settings to identify factors associated with sputum conversion. It has been shown that factors like an initial pre-treatment high sputum AFB smear grading is associated with persistent sputum positivity at 2 months post anti-TB treatment (25). Cure rates and conversion rates decrease as initial smear grading increases (26). Thus, a study conducted in Thailand showed that sputum conversion after 2 months of the intensive phase was 61.7% in patients with pre-treatment strongly positive smears (grading 2+ or greater) compared to 91% in patients with weakly positive smears (grading less than 1+) (27). A study undertaken in Gambia showed that the proportion of smear converters at 2 months was inversely related to the bacterial load in the initial sputum smear (96.2%, 85.8% and 81.8% for initial smear grading of 1+, 2+ and 3+ respectively $P<0.001$) (28).

Other findings from studies assessing predictors for sputum conversion show factors such as, age of the patient, duration of smoking, and gender, may have significant impact on sputum conversion (23, 25, 29). A study undertaken to identify, simple clinical, microbiological and radiological factors that were associated with a delay in sputum sterilization in Saudi Arabia, showed that age above 40 years was significantly associated with persistent sputum smear positivity (24). In another study from Guinea Bissau using a simple clinical score based system to measure a TB patient's clinical status at repeated visits, showed that 21% of mortality occurred in

patients with a higher score compared to 13% mortality in patients with a low score (30).

The cluster differentiation antigen 4 (CD4+) counts, are important in monitoring the immune system in TB patients co-infected with HIV, however, CD4+ counts together with Hb in TB patients have not been shown to be among significant factors predicting sputum conversion (29). Other blood parameters like platelet levels and erythrocyte sedimentation rate have been shown to be significantly associated with time to sputum conversion (25, 29).

2.0 RATIONALE

Predicting treatment outcome among TB patients receiving anti-TB treatment under directly observed treatment short course (DOTS) strategy helps in recognizing cases that are more likely to fall in the treatment failure group. In Tanzania, a routine procedure used for diagnosing new PTB cases is sputum smear microscopy. The approach in which every suspected TB case should submit 3 sputum specimens for smear microscopy examination, detects up to 80% of smear positive patients on first specimen examination. Fifteen percent of smear positive patients can be detected on a second sputum specimen examination and 5% on the third sputum specimen (9). In 2006, Tanzania introduced a change in the short course treatment regimen for TB under the DOTS strategy. The change in treatment regimen goes hand in hand with changes in the diagnostic sputum submission procedure from 3 samples of sputum specimens to only 2 samples, one produced on the spot and one morning sputum specimen on the following day. The short course regimen for 8 months was discontinued and switched to a 6 months short course regimen. The new regimen is divided into 2 months of intensive phase and 4 months of continuation phase. In this regimen drugs are given to patients in a fix dose combination (FDC) of 4 drugs (rifampicin, isoniazid, pyrazinamide and ethambutol) during the 2 months of intensive phase and 2 drugs in a FDC (rifampicin, isoniazid) during the 4 month of continuation phase (9). In general, anti-TB drugs are effective and most TB patients are cured. However, it is a challenge that some patients fail to convert after anti-TB treatment, the so-called treatment failure patients. Various studies in different settings worldwide have tried to identify factors that may be associated with sputum conversion. Many factors such as clinical, microbiological, radiological and social demographic factors may be associated with sputum smear and culture conversion. Little is known about factors that may be associated with persistent sputum AFB positivity in Tanzania. With this objective in mind, we undertook a prospective study among sputum smear positive PTB patients in Mwanza, Tanzania. Factors that could be associated with persistent sputum positivity at the end of 2 months

after initiation of anti-TB treatment were assessed. Delineating factors associated with delayed sputum conversion could help in strengthening TB control programmes, especially in a low income country like Tanzania.

3.0 OBJECTIVES OF THE STUDY

3.1. Study goal

To improve TB treatment outcome in Tanzania

3.2. General objective

To assess the role of HIV and other predictors of sputum smear and culture conversion at 2 months following initiation of anti-TB treatment among sputum smear positive PTB patients.

3.3. Specific objectives

1. To determine the sputum smear and culture conversion rates at the end of 2 months after initiation of anti-TB treatment.

2. To determine the relationship between AFB smear and culture grading at baseline and sputum conversion at the end of 2 months after initiation of anti-TB treatment.

3. To determine the relationship between HIV status and CD4+ counts at baseline and sputum conversion at the end of 2 months after initiation of anti-TB treatment.

4. To assess the effect of anthropometric (BMI, arm circumference), and bio-chemical parameters (Hb, WBC count) on sputum smear conversion (at the end) of 2 months after initiation of anti-TB treatment.

4.0 METHODOLOGY

4.1. Study setting and design

4.1.1. Study setting

The study was conducted in the city of Mwanza which is constituted by two districts namely Ilemera and Nyamagana. Mwanza city is the second largest in Tanzania after Dar es Salaam. Mwanza city is located on the southern shore of lake Victoria in the northern part of Tanzania (**Figure**.**1**). It covers an area of 1,325 sq km of which 425 is dry land and 900 sq km is covered by water from Lake Victoria. Of the 425 sq km dry land area, only 86.8 sq km is urbanized. According to the 2002 National census, Mwanza city has a total population of 476,646 (Nyamagana District 210,735 and Ilemera 265, 911). The current population is estimated to be just above half a million people, with an annual growth rate of 3.2%. Rural to urban migration is approximately 8% (31).

Mwanza city is divided by the NTLP into 3 operating TB districts. These are Mwanza urban North, Mwanza urban East and Mwanza urban South. These 3 NTLP districts cover the entire Mwanza city. Mwanza city has a TB notification rate (smear positive) of 50-100 per 100,000 (32). Daily TB clinic services in the city are provided by both the government and private hospitals. Each district has at least one main health facility where TB patients are registered after being diagnosed with TB at a nearby clinic. The health facilities at each district are Bugando Medical Centre and Butimba health centre for Mwanza urban South district. Buzuruga health centre for Mwanza urban East district, and Sekou- Toure regional hospital for Mwanza urban North districts. Together these health facilities provide health services to inhabitants of Mwanza city. and those from outside Mwanza city.

4.1.2. Study design

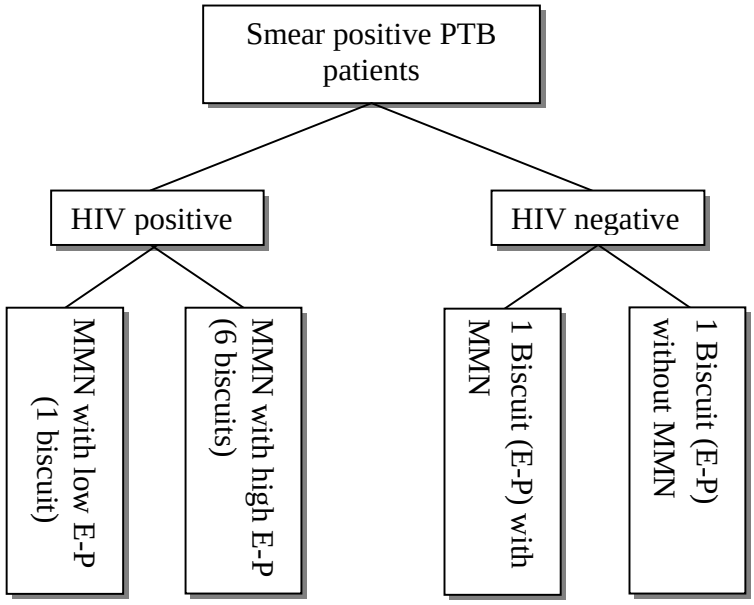
A prospective cohort study conducted within ongoing randomised clinical trials.

Figure 1. Map of Tanzania showing the location of Mwanza city

4.2. Study population

All patients diagnosed with sputum smear positive PTB recruited from the 3 NTLP districts and attending the four major health facilities from April 2006 to November 2008 in Mwanza city were considered for recruitment. Sputum smear positive PTB patients who consented and were participating in the ongoing randomised nutritional supplementation trials in Mwanza and met the inclusion criteria, were recruited. The ongoing randomised nutritional supplementation trials are aimed at evaluating the effect of energy protein and micronutrients supplementation on various TB treatment outcomes. The trials commenced in 2006 in Mwanza city, in which patients who were diagnosed to be smear positive PTB with HIV were randomised to either biscuits containing multi-micronutrients (MMN) with high or low energy-protein (E-P). Patients who were diagnosed as smear positive PTB without HIV, were randomised to receive one E-P biscuit with or without MMN (**Figure 2**).

Figure 2. A profile of trial participants receiving either the multi-micronutrient (MMN) or the energy-protein (E-P) intervention



4.3. Inclusion and Exclusion criteria

4.3.1. Inclusion

1. Sputum smear positive PTB patients recruited at the local health facilities and confirmed as sputum smear positive at the Zonal reference laboratory.

2. Patients aged 15 years or above.

4.3.2. Exclusion

1. Pregnant women.
2. Terminal illness (from TB or any other serious infection unlikely to survive more than 48 hours).
3. Non – residence (patients who will not stay in the study area for the entire period of six months for anti -TB treatment).
4. Not willing to participate.

4.4. Sample size

All consecutive smear positive PTB patients confirmed as smear positive at Zonal tuberculosis reference laboratory (ZTRL) from April 2006 to November 2008 were recruited. For calculating the sample size the proportion of patients that failed to convert by sputum culture at the end of 2nd months of the intensive phase was assumed to be 14% in Mwanza city (19)**.** The absolute estimated precision of the proportion of sputum culture non-converters was set to be 0.03 i.e. its 95% confidence interval was targeted to be 0.05.

The following formula was used for sample size calculation.

$n= (Z_{1-\alpha/2})^2 \times p \times (1-p)/d^2$

Where:

n = required sample size

Z = reliability coefficient (1.96)

p = estimated proportion of sputum culture non-conversion among sputum culture positive PTB patients in Mwanza city (14%)

d = absolute estimated precision error required on either side of proportion (0.03)

$n= (1.96)^2 \times 0.14 \times (1-0.14)/0.03^2 =514$

The sample size was increased by a factor of at least 15% to compensate for those who could be lost to follow up, for those with negative culture results at baseline, for contaminated culture results and for those who could die before the 2 months visit. From April 2006 to November 2008 a total of 601 smear PTB patients were recruited.

4.5. Patient enrolment, data collection and follow up procedure

4.5.1. Patient enrolment procedure

A total of 601 PTB patients who were smear positive regardless of their HIV status were enrolled into this study. Patients diagnosed as sputum smear positive by microscopy using Ziehl Neelsen staining at the first visit at a diagnostic health facilities, were referred to any of the four main recruitment health facilities. At the recruitment health facility, patients were provided information on the study and those who were eligible and willing to participate

were asked for an oral and written consent. Those who consented and for whom one or both spot and next day early morning sputum samples were ZN smear microscopy positive, were requested to provide an additional early morning sputum specimen. The early morning sputum specimens were collected in sterile universal bottles and sent to ZTRL for smear microscopy examination using Auramine O staining and for culture using Lowenstein- Jensen solid media. Sputum results for AFB were graded at the ZTRL according to the WHO/IUATLD recommendations (9). Smear result was reported as "0" if there were no AFB seen per 100 fields, "1-9" if exact number of AFB seen per 100 fields, "1+" if AFB seen were 10-99 per 100 fields, "2+" if AFB seen per field were 1-10 and "3+" if AFB seen per field were more than 10 per field (9). Regarding culture results, grading was reported as "0" if no growth, "1+" growth between 1-100 colonies; "2+" more than 100 colonies but non confluent growth and "3+" confluent growth (19). Only PTB patients who were sputum smear positive by microscopy at both local health facilities and at the ZTRL were recruited into the study.

4.5.2 Data collection procedure

Participant's information was collected through structured questionnaires at the first visit, and at the end of 2nd month after initiation of anti-TB treatment (**see appendices**). Information on social demographic characteristics was ascertained only at the first visit. Information such as medical history, smoking history and anthropometric measurements (height, weight, arm circumference) were collected at baseline and at the 2nd months visit. Patients' weight, height, and arm circumference were measured using a digital weighing scale, height board and tape measure, respectively. Body mass index was calculated by dividing weight over height square. Additionally, HIV counselling and testing was done on all recruited participants. Participants were then requested to provide blood for a HIV test, total lymphocyte count (TLC) and CD4 cell counts. Ethylene diamine-tetra-acetic acid (EDTA) tubes were used to collect blood for Hb, WBC counts, and CD4 counts while plain vacutainer tubes were used to collect blood for the HIV test. Blood for HIV testing and other blood parameters were examined on the same day as collection at the National Institute for Medical Research (NIMR) laboratory. HIV testing was done using 2 different rapid antibody tests; Determine HIV-1/2 (Inverness Medical Japan co ltd, Abbot, Japan) and Capillus HIV-1/2 (Trinity Biotech, Ireland). Discordant samples were tested using Uniform II vironostika-HIV Ag/Ab Micro-Elisa system (Biomerieux bv, The Netherlands). CD4+ counts

were measured using a Partec cyflow counter machine (Germany). TLC counts were determined manually by a senior laboratory technician.

4.5.3 Follow up

All patients that were seen at baseline were reviewed again at the end of the 2nd month post anti-TB treatment. Information that was collected at baseline was repeated at 2 months except for social demographic information and HIV testing.

4.6. Data management and analysis

Patient information was double-entered using EpiData version 3.1 and the data were analysed using STATA program version 10.1. The differences in proportions between groups were compared using chi–square test. Student *t* -test was used to compare the differences in means of continuous variables. Evaluation of risk factors of non-conversion by sputum smear and culture examination at the end of the 2nd month of intensive phase was undertaken by logistic regression. For purpose of logistic regression analysis, dummy variables were used for independent variables with more than two categories. Additionally, initial sputum smear and culture grading were dichotomized into ≤ 2+ or 3+. All binary categorical variables were coded as 0 and 1. Height, weight and arm circumference was grouped in equal categories based on sex-specific tertiles (at 33.3 and 66.7 percentiles). The logistic regression analyses were adjusted for age and sex. Finally multivariate logistic regression analysis was undertaken by including factors found significant or marginally ($P<0.10$) significant in univariate logistic analysis, and adjusting for age, sex and HIV status irrespective of their association with the outcome. A P- value of 0.05 was set for statistical significance.

4.7. Definitions

Sputum smear conversion; defined as the proportion of initial smear-positive patients with a negative smear at the end of 2 months of the intensive phase of anti-TB treatment (33).

Sputum culture conversion; defined as the proportion of initial culture-positive patients with negative culture at the end of 2 months of intensive phase of anti-TB treatment (33).

Background characteristic in this study include; age, sex, marital status, occupation, religion and smoking.

Infectious background characteristics include; initial AFB smear grading, initial tubercle bacilli culture grading, BCG scar, CD4 count, haemoglobin and white blood cell count.

Anthropometric background characteristics include; height, weight, body mass index, and arm circumference.

Anaemia; defined as haemoglobin less than 120g/l for females and less than 130g/l for males.

4.8. Ethical consideration

The study was conducted within the framework of the two ongoing randomised nutritional supplementation trials, which were granted permission from National ethics committee in Tanzania on 10th February 2006 (please see appendix for a copy). These studies, with registration number NCT00311298 follow guidelines for clinical trials. Oral and written information in Swahili was provided to all participants prior to obtaining informed oral and written consent. Guardian or parents were requested for consent for those who were under 18 years of age.

5.0 RESULTS

A total of 601 PTB positive patients were enrolled into this cohort from April 2006 to November 2008, of which 249 (41%) were HIV+. Of these 601 patients, 516 (86%) patients showed up at the end of 2nd month of intensive phase treatment, and were included in the final analysis for calculation of conversion rates based on sputum smear examination. Of the 85 patients excluded from the study, 24 patients failed to come at the 2nd month visit, 11 patients died before completing the 2nd month visit, and 50 patients failed to provide sputum at the 2nd month visit. Among those recruited at baseline, only 447 (74.4%) patients were included in the final analysis for calculating the sputum culture conversion rate, as 26 (4.3%) patients were excluded after their culture results were found to be contaminated at baseline. An additional 21 (4.1%) patients were excluded after having contaminated culture results at the 2nd month of follow up and for 22 (3.7%) patients the baseline sputum cultures were negative. (**Figure 3 and 4).**

5.1. Characteristics of sputum smear positive pulmonary TB patients recruited

For the patients recruited, the mean age was 35 years (range, 15 to 84 years) for HIV- patients and 34.5 years (range, 18 to 80) for the HIV+ patients (P=0.51). The proportion of females was higher among the HIV+ compared to HIV- patients (49% and 29% respectively, P<0.001). The proportion of patients who were separated, widowed or divorced was higher among HIV+ compared to HIV- (24.4% vs 15.1%, P=0.01). The proportion of patients who never smoked (72.3%) was higher among the HIV+ group compared to the HIV- group (61.7%) [P=0.001]. They were no difference observed between the HIV+ and HIV- groups with respect to occupation and religion (**Table 1**).

5.2. Infectious and anthropometric background characteristics for patients recruited

A higher proportion of HIV- patients had a baseline AFB smear grading of 3+ as compared to HIV+ patients (72.5 % HIV- and 55.8% HIV+, P<0.001). The mean CD4 count was 315 (95% CI 287; 343) for HIV+ and 553 (95% CI 518; 588) for HIV- patients (P<0.0001). The proportion of patients with anaemia was higher among HIV+ patients as compared to the HIV- patients (96.8% vs. 85. 2% P, <0.001). As expected, patients who were HIV+ had a lower mean WBC (10^9cells/l) [6.6 (95%CI 6.3; 7.0)], weight (kg/m^2) [50.3 (95%CI 49.2; 5I.4)], and arm circumference (cm) [22.3(95%CI 21.9; 22.7)] as compared with HIV- patients.

[WBC; 7.4(95%CI 7.2; 7.7)], [weight; 51.7 (95%CI 50.9; 52.6)], and [arm circumference; 22.9 (95%CI 22.6; 23.2)] (**Table 2).**

Figure 3. Flow chart for PTB+ patients followed for sputum smear conversion at the end of the 2nd month of the intensive phase treatment.

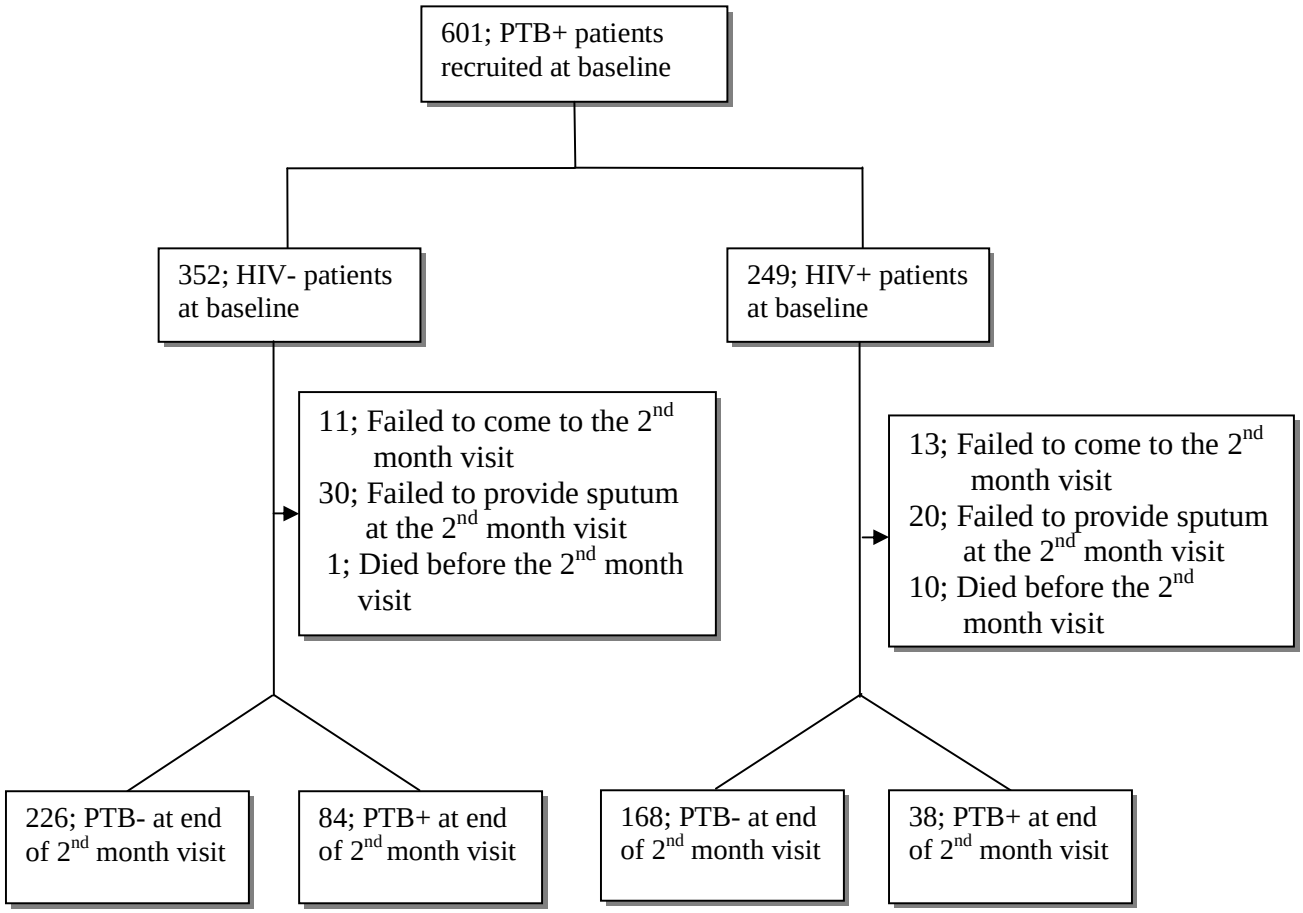
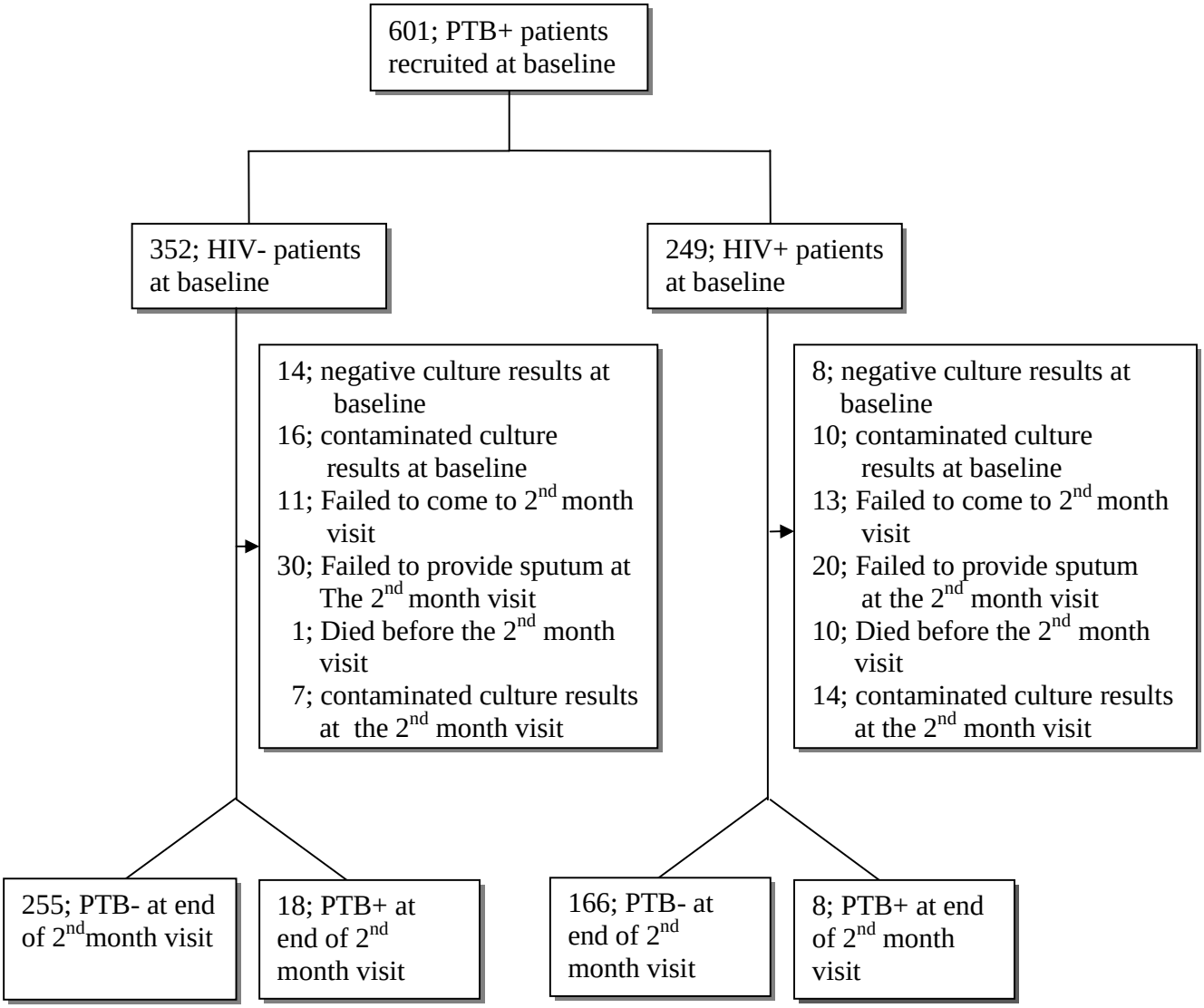


Figure 4. Flow chart for PTB+ patients followed for sputum culture conversion at the end of the 2nd month of the intensive phase treatment.



5.3 Sputum smear conversion at the end of two month of intensive phase

Sputum smear conversion among the 516 PTB positive patients was observed in 394 (76.4%) patients. Fifteen percent females and 28.9% males (P<0.001) remained sputum smear positive at the end of the 2nd months of anti-TB treatment. There was no difference observed in sputum smear conversion among PTB positive patients as categorised by age, occupation and smoking (**Table 3**.). Patients with an initial pre-treatment AFB smear grading of 3+ had a lower conversion rate (72.1%) compared to patients with other sputum smear grading categories (P=0.004). Among patients with a BCG scar, 78.9% achieved sputum smear conversion as compared to 66% in patients who did not have a BCG scar (P=0.006) **(Table 4).** Of those patients with a body weight of ≥18.5kg/m^2, 15.3% did not sputum smear convert at the end of the 2nd month of anti-TB treatment as compared to 30.3% who had a weight of ≥17- <18.5 kg/m^2 and 29.2% with weight <17 kg/m^2 [P=0.001]. Patients with an arm circumference in the lower tertile were observed to have a lower sputum smear conversion rate as compared to those in middle and upper tertiles (69.5%, 78.9%, 80.3%, respectively P=0.04). There was no difference observed between sputum smear conversion rates among the PTB patients as categorised by HIV status, anaemia, CD4 count, WBC counts and height (**Table 4).**

5.4. Sputum culture conversion at the end of two month of intensive phase

Of the 447 patients analysed based on sputum culture, 26 patients (5.8%) remained sputum culture positive at the end of the 2nd month of the intensive phase. There was no observed difference between sputum culture conversion among patients as categorised by age, sex, marital status, occupation, smoking and religion (**Table 5**). However, a higher proportion of patients who had a pre-treatment AFB grading of 3+ failed to convert as compared to patients with a baseline sputum grading of ≤2+ (7.3% and 1.0%, respectively P=0.016). Thirteen percent of those who did not have a BCG scar did not sputum culture convert as compared to 4.2% of those who had a BCG scar (P=0.002). No significant difference was observed in sputum culture conversion among PTB patients as categorised by HIV status, CD4 count, WBC count, Hb levels and BMI (**Table 6**).

5.5. Factors associated with sputum smear and culture conversion

Logistic regression was performed to identify factors that were independently associated with persistence of sputum smear and culture positivity at the end of the 2nd month post anti-TB treatment as categorised by background, infectious and anthropometric factors. Thus, being of male sex (OR 2.11, [95%CI 1.31 ; 3.40] P=0.002), an initial AFB smear grading of 3+ (OR 2.24, [95%CI 1.38 ; 3.65], P=0.001), presence of a BCG scar (OR 0.54, [95%CI 0.33 ; 0.88], P=0.015), having a BMI of ≥17- <18.5 kg/m^2 (OR 2.30, [95%CI 1.33 ; 3.99], P=0.003), and BMI of <17 kg/m^2 (OR 2.42, [95%CI 1.46 ; 4.02], P=0.001) were significantly related to persistence of sputum smear positivity at the 2nd month following anti-TB treatment (**Table 7**). Regarding factors associated with persistence of sputum culture positivity, the presence of a BCG scar (OR 0.27, [95%CI 0.11; 0.62], P=0.002) remained significant (**Table 8**).

In the multivariate regression model, factors that remained independently associated with persistent sputum smear positivity included:- male gender (OR 1.92, [95% CI 1.16; 3.18], P=0.01), an initial AFB smear grading of 3+ (OR 1.9, [95% CI 1.17; 3.19] P=0.01), having a BMI of <17 kg/m^2 (OR 2.18, [95%CI 1.31; 3.64], P=0.01), and BMI of ≥17- <18.5 kg/m^2 (OR 2.17, [95%CI 1.24; 3.79], P=0.006) (**Table 9**). There was a 43% (OR 0.57, [95%CI 0.35; 0.95], p=0.032) and 72% (OR 0.28, [95%CI 0.11; 0.62], P =0.002) lower probability of not sputum smear converting and culture converting respectively, at the end of the 2nd month of intensive phase therapy if the patients had a BCG scar (**Table 9**).

6.0 DISCUSSION

6.1. Methodological issues.

6.1.1. Validity

The extent to which study cofounders are successfully eliminated and independent variables produce an observed effect are measures of internal validity, while if the study findings can be generalised to a wider population the study is said to have a high external validity (34).

6.1.2. Internal validity

In our study the following factors might have affected the validity of our findings**:**

6.1.2.1. Instrumentation bias

Digital weighing scales, partec cyflow CD4 count machine, measuring boards, tape measures and microscopy were used in this study. Reliability of the readings provided by these instruments is dependent upon continuous calibration as well as the proficiency of an observer. Error was minimised by providing regular training to the study team on use of instruments. Furthermore instruments were calibrated at regular intervals to ensure consistency in results.

6.1.2.2. Recalling bias

There is a possibility that patients do not recall past history and/or may not provide an accurate history of smoking. However, this bias should be taken care of by the randomization undertaken for the ongoing nutritional intervention trials from where the patients for the present study were recruited.

6.1.2.3. Confounding factors

Age and gender influences the chance of being exposed to infections as well as the risk of developing primary disease (35, 36). Age has been shown to have an impact on sputum conversion (25, 37). Factors such as age and gender may act as confounders and bias our outcomes. In our study, biases that may have risen due to these factors were controlled in a logistic analysis.

6.2.3. External validity

Our study was undertaken in the northern part of Tanzania. Northern Tanzania has a mixture of different tribes from almost all parts of country. Despite limiting ourselves in recruiting only patients residing in the study area, our results on the influence of age, gender, and initial sputum smear grading and BMI on sputum smear conversion do not differ significantly from those reported in similar studies conducted elsewhere (26-28).

6.2 Discussion based on results

The overall sputum smear conversion rate was 76.4% and the sputum culture conversion rate was 94.2%. Twenty seven percent of patients who were HIV- and 18.5 % of patients who were HIV+ did not convert by sputum smear examination while 6.6% of HIV- and 4.6% of HIV+ patients did not convert at the end of the 2^{nd} month of intensive phase as examined by sputum culture. The high conversion rates observed is a reflection of the success of the NTLP in the overall control of TB in the area where this study was undertaken. Drug susceptibility testing of the 26 isolates from the patients who did not convert based on sputum culture examination has not been performed as yet. A recent study from Tanzania on drug susceptibility testing of 111 M.tb isolates from PTB patients indicate that 10.8% of the isolates were resistant to at least one anti-TB drugs and 2.7% were resistant to isoniazid and rifampicin (multi-drug resistant TB) (38). A higher conversion rate observed by sputum culture examination as compared with sputum smear examination may not be surprising. Sputum culture examination has a higher sensitivity and specificity as compared to sputum smear examination (9). Moreover, sputum samples contain dying or dead AFB that may contribute to positive sputum microscopic results but such samples are negative on sputum culture examination.

Our study shows that the sputum conversion rate observed varied among the different patient categories. PTB patients with pre-treatment initial AFB smear grading of 3+ had a significantly lower sputum smear and culture conversion rate (for smear 72.1% and for culture 93.1%) as compared with patients with an initial AFB smear grading of 1+ or $\leq$ 2+ (for smear 82.4% and for culture 96.5%). These results concur with findings from other studies undertaken in for e.g. India, Thailand and Gambia (26-28). As AFB multiply in the lung, there is an increase in the cavitating lesions that appear in the lung (39). Bacillus load correlates with an increase in consolidation which usually manifests as an exudative or caseous necrosis (39). The initial action of anti-TB

drugs is to decrease the numbers of bacilli in lung lesions resulting in improvement of lung consolidation. Thus, patients with an initial low mycobacteriological load (low AFB grading) tend to convert earlier (39).

Our study shows that there was no significant difference in conversion rates among TB patients co-infected and those not infected with HIV. Neither sputum smear microscopy (72.9%, HIV- vs. 81.5% HIV+) nor sputum culture (93.4%, HIV- vs. 95.4%, HIV+) conversion rates were significantly different for PTB patients with or without HIV co-infection. These findings are in contrast with a similar study undertaken in North Carolina, USA where it was reported that TB patients co-infected with HIV have a lower rate of sputum conversion than did PTB patients without HIV (for HIV- 73.6% and for HIV+ 57.8%) (11). A study undertaken in Uganda on tuberculosis chemotherapy and sputum smear conversion among HIV- and HIV+ PTB patients, found no significant difference in conversion among HIV+ and HIV- patients (13). Previous studies have documented that HIV+ PTB patients have fewer cavitating lung lesions than do HIV- PTB patients (40). Thus anti-TB drugs would presumably clear bacilli equally effectively in HIV+ patients as well as in HIV- PTB patients. To maintain these high conversion rates DOT providers and clinicians should continue to emphasise a strict adherence to anti-TB drugs in both HIV+ and HIV- PTB patients.

CD4+ counts despite playing a role in immunity did not significantly influence the sputum conversion rate. This study shows no difference in sputum conversion rates with different CD4+ counts. Patients with CD4+ counts of ≤ 200, 201-350 and >350 had sputum smear conversion rates of 81.3%, 75.8% and 74.6%, respectively and sputum culture conversion rate of 97.8%, 91.6% and 93.9 respectively. Similar results were observed in a study by Singla *et al* in Saudi Arabia (24), and by Domínguez- Castelleno *et al* in Spain (29), who showed no relation between CD4+ counts and sputum conversion rates. Furthermore, in concordance with other studies (25), we show that the Hb level and total WBC counts did not seem to influence the sputum conversion rate.

Interaction between malnutrition and TB is associated with complex mechanisms (41). TB disease has an effect on the nutritional status by affecting dietary intake due to loss of appetite, poor absorption of nutrients from the intestine and increase uptake of nutrients by specific target tissue due to increase of body metabolism (17). Both micro and macro nutrient deficiency can influence susceptibility to TB. Severe malnutrition has a profound effect on cell-mediated immunity (41).

BMI is one of indicators of nutritional status and was below 18.5 kg/m^2 in majority of the TB patients in our study at the time of TB diagnosis. A study conducted in Tanzania on nutritional status and weight gain in patients with pulmonary TB showed 77% of males and 58% of females had a BMI below 18.5kg/m^2 at the time of admission (41). Furthermore, a study from Malawi, showed that there was a reduction of 20% in BMI (from 21.7 to 17.3) among PTB patients as compared with matched controls (15). In this study, the mean BMI among PTB patients with HIV and those without HIV was 18.4 and 18.3, respectively, at the time of recruitment. The sputum smear conversion rate was 69.7% for patients with BMI <18.5kg/m^2 and 84.7% for BMI ≥18.5kg/m^2 while the culture conversion rate was 90.3% for BMI <18.5 kg/m^2 and 95% for ≥ 18.5 kg/m^2 at the end of the 2nd month of intensive phase. Thus, PTB patients who are malnourished are at a risk of sputum smear persistence at the end of 2nd month post anti-TB treatment.

Multivariate analysis was performed to document those variables independently associated with sputum smear and culture conversion at the end of the 2nd month following anti-TB treatment. The factors that showed a statistically significant and independent relationship with sputum smear conversion were; initial (pre-treatment) smear grading, male gender, BMI, and the presence of a BCG scar. The presence of a BCG scar was also positively associated with sputum culture conversion at the end of the 2nd month of the intensive phase of anti-TB treatment.

Association with initial smear grading, male gender and BMI on sputum conversion has also reported on previous studies. (23-25, 27, 40). Patients with numerous AFB in the sputum reflect the high numbers of bacilli in the lung, with a corresponding increase in cavity formation. Thus, a delayed sputum conversion in patients with high initial bacillary loads in the sputum is not surprising (39, 40). Male gender was reported by Banu Rekha *et* and Güler *et al* as an independent factor associated with sputum conversion (23, 25). Males are generally more likely to be smokers and to consume alcohol. Smoking and alcohol have been observed to be associated with a delay in seeking health care as well as contributing to a poor compliance to anti-TB medication. Thus, these factors may contribute to a delayed sputum conversion (42, 43).

The BCG vaccine is still the only vaccine in use against TB, although recently efforts to develop new vaccines are in progress (44). Globally, the BCG vaccine is delivered to approximately 90% of all infants (44). Meta analysis of BCG trials has reported the protective efficacy of the vaccine to vary across populations and range from 0% to 86% (45, 46). The protective effect against

severe forms of meningeal and miliary TB was 79% (46). The risk of acquiring TB was reduced by 50% in vaccinated individuals (45). Meta analysis of BCG trials data also suggested that the protective efficacy of BCG may persist 10 years or more after infant vaccination (47). A recent study by Weir *et al* showed that BCG vaccination during infancy and adolescence can induce immunological memory to mycobacterial antigens that is measurable up to 14 years (48). Additionally, a study looking at long term BCG efficacy among American Indians and Alaska natives, showed that protection could last up to 60 years (49). The BCG vaccine is given to all infants in Tanzania at birth and is included in the expanded programme of immunization (EPI). Factors like differences in exposure to different strains of M.tb as well as to atypical mycobacteria from one geographical area to another, different BCG vaccines strains used, genetic characteristics of the population, and nutritional differences among different populations could contribute to the variable efficacy of BCG(50).

In this study interestingly, the presence of a BCG scar was significantly associated with sputum smear and culture conversion. Thus, patients with a BCG scar were 43% less likely by sputum smear microscopy and 72% less likely by sputum culture to remain positive at the end of 2nd month of intensive phase of anti-TB treatment. This exciting finding needs to be confirmed in larger studies in Tanzania as well as in other geographical locations and populations.

7.0 CONCLUSIONS

We show that initial (pre-treatment) high smear grading, male gender, low BMI, and the absence of a BCG scar are associated with persistent sputum smear positivity (at the end of the intensive phase of anti-TB treatment). The absence of a BCG scar was also positively associated with persistent sputum culture positivity. HIV status, CD4 counts and Hb levels were not associated with delayed sputum conversion.

1. The presence of a BCG scar should be documented and included in models predicting sputum conversion.
2. Clinicians should carefully assess PTB patients who are at risk of not converting using identified predicting factors.
3. Emphasis on nutritional health education, promotion and support should be considered for all TB patients regardless of HIV status.

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9.0 TABLES

Table 1. Background characteristics for 601 sputum positive pulmonary TB patients categorised by HIV status [1]

		HIV status		
	Total n	Negative n=352	Positive n=249	*P* value[2]
Age (years)	601	35.0 (15-84)	34.5 (18-80)	0.51
Sex	601			
Female		102 (29.0)	122 (49.0)	<0.001
Male		250 (71.0)	127 (51.0)	
Marital status	598			
Married or cohabiting		199 (56.5)	122 (49.6)	0.01
Single		100 (28.4)	64 (26.0)	
Separated, widowed or divorced		53 (15.1)	60 (24.4)	
Occupation	600			
Farmer		135 (38.4)	81 (32.7)	0.35
Businessman		79 (22.4)	63 (25.4)	
Other		138 (39.2)	104 (41.9)	
Religion	601			
Christian		257 (73)	191 (76.7)	0.09
Muslim		78 (22.2)	54 (21.7)	
Other		17 (4.8)	4 (1.6)	
Smoking	601			
Current smoker		86 (24.4)	27 (10.8)	0.001
Past smoker		49 (13.9)	42 (16.9)	
Never smoker		217 (61.7)	180 (72.3)	

[1] Data are provided as mean (range) for age or n (%).
[2] X^2 – test used for categorical variables and the t- test for continuous variables.

Table 2. Infectious and anthropometric background characteristics for 601 pulmonary TB categorised by HIV status[1]

		HIV status		
	Total n	Negative n=352	Positive n=249	*P* value[2]
Initial AFB smear grading[3]	601			
≤1+		43 (12.2)	57 (22.9)	<0.001
2+		54 (15.3)	53 (21.3)	
3+		255 (72.5)	139 (55.8)	
Initial AFB culture grading[4]	546			
1+		24 (7.6)	26 (11.4)	0.14
2+		44 (13.8)	39 (17.1)	
3+		250 (78.6)	163 (71.5)	
BCG scar	600			
No		71 (20.2)	47 (19.0)	0.71
Yes		281 (79.8)	201 (81.0)	
CD4 count (10^6 cells/ml)	600	553 (518 ; 588)	315 (287 ; 343)	<0.0001
Haemoglobin	600			
Non anaemic		52 (14.8)	8 (3.2)	<0.001
Anaemic		300 (85.2)	240 (96.8)	
WBC (x10^9cells/l)	600	7.4 (7.2 ; 7.7)	6.6 (6.3 ; 7.0)	0.0003
Height (cm)	599	167.8 (166.9 ; 168.7)	165.6 (164 ; 166.6)	0.0009
Weight (kg)	600	51.7 (50.9 ; 52.6)	50.3 (49.2 ; 51.4)	0.03
BMI (kg/m^2)	599	18.4 (18.1 ; 18.6)	18.3 (18.0 ; 18.7)	0.92
Arm circumference (cm)	601	22.9 (22.6 ; 23.2)	22.3 (21.9 ; 22.7)	0.03

[1] Data are mean (95% confidence interval) or n (%).
[2] X^2 – test used for categorical variables and the t- test for continuous variables.
[3]Smear grading by auramine microscopy defined as ≤1+ if is 1-99 AFB per 100 fields; 2+ = 1-10 AFB per field; 3+ = more than 10 AFB per field.
[4] Culture grading defined as 1+ = 1-100 colonies; 2+ = more than 100 colonies and 3+ = confluent growth colonies.
BMI= body mass index; AFB= acid fast bacilli; WBC= White blood cell count; BCG =Bacilli Calmette-Guérin.
Anaemia defined as haemoglobin less than 120g/l for females and less than130g/l for males.

Table 3. Sputum smear conversion among 516 pulmonary TB patients at the end of the 2nd month of intensive phase categorised by background characteristics [1]

		Smear results		
	Total n	Converted n=394	Not converted n=122	*P* value[2]
Age (years)	516			
15-24		71 (78.0)	20 (22.0)	0.08
25-34		157 (80.9)	37 (19.1)	
35-44		99 (75.0)	33 (25.0)	
≥45		67 (67.7)	32 (32.3)	
Sex	516			
Female		165 (85.0)	29 (15.0)	<0.001
Male		229 (71.1)	93 (28.9)	
Marital status	516			
Married or cohabiting		217 (78.1)	61 (21.9)	0.27
Single		109 (77.3)	32 (22.7)	
Separated, widowed or divorced		68 (70.1)	29 (29.9)	
Occupation	515			
Farmer		134 (74.0)	47 (26.0)	0.07
Businessman		105 (84.0)	20 (16.0)	
Other		154 (73.7)	55 (26.3)	
Religion	516			
Christian		296 (77.3)	87 (22.7)	0.39
Muslim		90 (75.0)	30 (25.0)	
Other		8 (61.5)	5 (38.5)	
Smoking	516			
Current smoker		69 (70.4)	29 (29.6)	0.08
Past smoker		55 (70.5)	23 (29.5)	
Never smoker		270 (79.4)	70 (20.6)	

[1] Data are n (%).
[2] X^2 – test for categorical variables.

Table 4. Sputum smear conversion among 516 pulmonary TB patients at the end of the 2nd month of intensive phase categorised by infectious and anthropometric characteristics[1]

	Total n	Converted n=394	Not converted n=122	*P* value[2]
Initial AFB smear grading[3]	516			
≤1+		70 (82.4)	15 (17.6)	0.004
2+		76 (87.4)	11 (12.6)	
3+		248 (72.1)	96 (27.9)	
Initial AFB culture grading[4]	468			
1+		34 (79.0)	6 (21.0)	0.25
2+		49 (71.0)	20 (29.0)	
3+		276 (76.9)	83 (23.1)	
BCG scar	515			
No		68 (66.0)	35 (34.0)	0.006
Yes		325 (78.9)	87 (21.1)	
HIV	516			
Negative		226 (72.9)	84 (27.1)	0.16
Positive		168 (81.5)	38 (18.5)	
Haemoglobin level	516			
Non anaemic		45 (83.3)	9 (16.7)	0.20
Anaemic		349 (75.5)	113 (24.5)	
CD4 count (10^6cells/ml)	516			
≤200		91 (81.3)	21 (18.7)	0.38
201-350		97 (75.8)	31 (24.2)	
>350		206 (74.6)	70 (25.4)	
WBC (10^9 cells/l)	516			
<4.0		46 (82.1)	10 (17.9)	0.36
4.0-11.0		331 (76.1)	104 (23.9)	
>11.0		17 (68.0)	8 (32.0)	
Height[5]	516			
Lower tertile		130(76.5)	40 (23.5)	0.32
Middle tertile		138 (79.8)	35 (20.2)	
Upper tertile		126 (72.8)	47 (27.2)	
Weight[5]	516			
Lower tertile		129 (73.7)	46 (26.3)	0.03
Middle tertile		120 (71.9)	47 (28.1)	
Upper tertile		145 (83.3)	29 (16.7)	
BMI (kg/m^2)	516			
<17		126 (70.8)	52 (29.2)	0.001
≥17- <18.5		85 (69.7)	37 (30.3)	
≥18.5		183 (84.7)	33 (15.3)	
Arm circumference[5]	516			
Lower tertile		116 (69.5)	51 (30.5)	0.04
Middle tertile		127 (78.9)	34 (21.1)	
Upper tertile		151 (80.3)	37 (19.7)	

[1] Data are n (%): [2] X^2 – test for categorical variable.

[3]By auramine microscopy defined as ≤1+ = 1-99 AFB per 100 fields; 2+ = 1-10 AFB per fields; 3+ = more than 10 AFB per field.

[4] 1+ = 1-100 colonies; 2+ = more than 100 colonies and 3+ = confluent colonies.

[5]Height, weight and arm circumference divided to 33.3 and 66.7 percentile and based on sex specific tertiles.

Lower tertile cut off; height <158.2 for females and <167.1 for males, weight <43.1 for females and <49.7 for males, arm circumference <20.6 for females and <21.1 for males.

Middle tertile cut off; height 158.2-164.2 for females and 167.1-173 for males, weight 43.1-50 for females and 49.7-55.3 for males, arm circumference 20.6-23 for female and 21.1- 23.4 for males.

Upper tertile cut off; height ≥164.3 for females and ≥ 173.1 for males, weight ≥ 51 for females and ≥ 55.4 for males, arm circumference ≥ 24 for females and ≥23.5 for males. BMI= body mass index; BMI graded according to WHO recommendations.

Anaemia defined as haemoglobin less than 120g/l for females and less than130g/l for males.

Table 5. Sputum culture conversion among 447 pulmonary TB patients at the end of the 2nd month of the intensive phase categorised by background characteristics[1]

		Culture results		
	Total n	Converted n=421	Not converted n=26	*P* value[2]
Age (years)	447			
15-24		72 (90.0)	8 (10.0)	0.18
25-34		170 (96.1)	7 (3.9)	
35-44		100 (92.6)	8 (7.41)	
≥45		79 (96.3)	3 (3.67)	
Sex	447			
Female		158 (96.3)	6 (3.7)	0.14
Male		263 (92.9)	20 (7.1)	
Marital status	447			
Married or cohabiting		235 (95.9)	10 (4.1)	0.18
Single		115 (91.3)	11 (8.7)	
Separated, widowed or divorced		71 (93.4)	5 (6.6)	
Occupation	446			
Farmer		139 (93.3)	10 (6.7)	0.84
Businessman		112 (94.9)	6 (5.1)	
Other		169 (94.4)	10 (5.6)	
Religion	447			
Christian		308 (94.5)	18 (5.5)	0.21
Muslim		104 (94.6)	6 (5.4)	
Other		9 (81.8)	2 (18.2)	
Smoking	447			
Current smoker		80 (90.9)	8 (9.1)	0.32
Past smoker		64 (94.1)	4 (5.9)	
Never smoker		277 (95.2)	14 (4.8)	

[1] Data are n (%).
[2] X^2 – test used for categorical variables.

Table 6. Sputum culture conversion among 447 pulmonary TB patients at the end of the 2nd month of the intensive phase categorised by infectious and anthropometric characteristics[1]

	Total n	Converted n=421	Not converted n=26	*P* value[2]
Initial AFB smear grading[3]	447			
≤2+		139 (96.5)	5 (3.5)	0.14
3+		282 (93.1)	21 (6.9)	
Initial AFB culture grading[4]	446			
≤2+		102 (99.0)	1 (1.0)	0.02
3+		318 (92.7)	25 (7.3)	
BCG scar	446			
No		74 (87.0)	11 (13.0)	0.002
Yes		346 (95.8)	15 (4.2)	
HIV	447			
Negative		255 (93.4)	18 (6.6)	0.38
Positive		166 (95.4)	8 (4.6)	
Haemoglobin level	447			
Non anaemic		43 (93.5)	3 (6.5)	0.83
Anaemic		378 (94.3)	23 (5.7)	
CD4 count (10^6cells/ml)	447			
≤200		90 (97.8)	2 (2.2)	0.17
201-350		98 (91.6)	9 (8.4)	
>350		233 (93.9)	15 (6.1)	
WBC (10^9 cells/l)	447			
<4.0		45 (97.8)	1 (2.2)	0.51
4.0-11.0		356 (93.7)	24 (6.3)	
>11.0		20 (95.2)	1 (4.8)	
Height[5]	447			
Lower tertile		139 (95.2)	7 (4.8)	0.20
Middle tertile		143 (96.0)	6 (4.0)	
Upper tertile		139 (91.4)	13 (8.6)	
Weight[5]	447			
Lower tertile		144 (93.5)	10 (6.5)	0.70
Middle tertile		130 (95.6)	6 (4.4)	
Upper tertile		147 (93.6)	10 (6.4)	
BMI (kg/m^2)	447			
< 17		149 (94.9)	8 (5.1)	0.15
≥17- <18.5		93 (90.3)	10 (9.7)	
≥18.5		179 (95.7)	8 (5.3)	
Arm circumference[5]	447			
Lower tertile		132 (93.6)	9 (6.4)	0.73
Middle tertile		127 (93.4)	9 (6.6)	
Upper tertile		162 (95.3)	8 (4.7)	

[1] Data are n (%).

[2] X^2 – test used for categorical variable.

[3] By auramine microscopy defined as ≤2+ = 1-99 AFB per 100 fields or 1-10 AFB per field; 3+ = more than 10 AFB per field.

[4] Culture grading defined as ≤2+ = more than 1 colony; 3+ = confluent colonies.

[5]Height, weight and arm circumference divided to 33.3 and 66.7 percentile and based on sex specific tertiles.

Lower tertile cut off; height <158.2 for females and <167.1 for males, weight <43.1 for females and <49.7 for males, arm circumference <20.6 for females and <21.1 for males.

Middle tertile cut off; height 158.2-164.2 for females and 167.1-173 for males, weight 43.1-50 for females and 49.7- 55.3 for males, arm circumference 20.6-23 for females and 21.1- 23.4 for males.

Upper tertile cut off; height ≥164.3 for females and ≥173.1 for males, weight ≥51 for females and ≥55.4 for males, arm circumference ≥24 for females and ≥23.5 for males. BMI graded according to WHO recommendations.

Anaemia defined as Hb <120g/l for females and <130g/l for males.

Table 7. Analysis of factors associated with persistent sputum smear positivity at the end of the 2nd month of intensive phase

	Converted	Not converted	Crude	Adjusted[1]	
	n	n	OR (95% CI)	OR (95%CI)	P value
Age (years)[2]					
15-24	71	20	1.19 (0.64 ; 2.20)	1.16 (0.62 ; 2.16)	0.63
25-34	157	37	1.0	1.0	
35-44	99	33	1.12 (0.93 ; 1.34)	1.09 (0.90 ; 1.30)	0.36
≥45	67	32	1.19 (1.03 ; 1.37)	1.13 (0.98 ; 1.30)	0.09
Sex[2]					
Female	165	29	1.0	1.0	
Male	229	93	2.31 (1.45 ; 3.66)	2.11 (1.31 ; 3.40)	0.002
Religion					
Christian	296	87	1.0	1.0	
Muslim	90	30	1.13 (0.70 ; 1.82)	1.14 (0.70 ; 1.86)	0.58
Other	8	5	1.46 (0.82 ; 2.58)	1.30 (0.72 ; 2.34)	0.38
Marital status					
Married or cohabiting	217	61	1.0	1.0	
Single	109	32	1.04 (0.64 ; 1.69)	1.2 (0.68 ; 2.08)	0.54
Separated, divorced & widowed	68	29	1.52 (0.90 ; 2.54)	1.7 (1.00 ; 3.04)	0.049
Occupation					
Farmer	134	47	1.0	1.0	
Businessman	105	20	0.54 (0.30 ; 0.97)	0.57 (0.31 ; 1.05)	0.07
Other	154	55	1.01 (0.65 ; 1.60)	1.10 (0.69 ; 1.76)	0.68
Smoking					
Current smoker	69	29	1.62 (0.97 ; 2.69)	1.10 (0.62 ; 1.92)	0.74
Past smoker	55	23	1.61 (0.92 ; 2.80)	1.13 (0.62 ; 2.06)	0.68
Never smoker	270	70	1.0	1.0	
Initial AFB smear grading[3]					
≤2+	146	26	1.0	1.0	
3+	248	96	2.2 (1.35 ; 3.51)	2.24 (1.38 ; 3.65)	0.001
Initial AFB culture grading[4]					
≤2+	83	26	1.0	1.0	
3+	276	83	0.96 (0.58 ; 1.59)	0.93 (0.56 ; 1.57)	0.79
BCG scar					
No	68	35	1.0	1.0	
Yes	325	87	0.52 (0.32 ; 0.83)	0.54 (0.33 ; 0.88)	0.02
HIV status					
Negative	226	84	1.0	1.0	
Positive	168	38	0.60 (0.39 ; 0.93)	0.70 (0.44 ; 1.10)	0.12
Haemoglobin					
Non anaemic	45	9	1.0	1.0	
Anaemic	349	113	0.61 (0.78 ; 3.42)	1.78 (0.83 ; 3.79)	0.14

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Table 7-continued

	converted	Not converted	Crude	Adjusted[1]	
	n	n	OR (95% CI)	OR (95%CI)	P value
White blood cell count (10^9cells/l)					
<4.0	46	10	0.69 (0.33 ; 1.410	0.75 (0.36 ; 1.55)	0.44
4.0 -11.0	331	104	1.0	1.0	
>11.0	17	8	1.14 (0.85 ; 1.52)	1.12 (0.83 ; 1.51)	0.43
CD4 count (10^6cells/ml)					
≤200	91	21	0.68 (0.39 ; 1.17)	0.79 (0.44 ; 1.40)	0.43
201-350	97	31	0.94 (0.58 ; 1.53)	0.98 (0.59 ; 1.61)	0.93
>350	206	70	1.0	1.0	
Height[5]					
Lower tertile	130	40	1.21 (0.7 ; 2.02)	1.22 (0.71; 2.06)	0.47
Middle tertile	138	35	1.0	1.0	
Upper tertile	126	47	1.47 (0.89 ; 2.42)	1.56 (0.93 ; 2.60)	0.09
Weight[5]					
Lower tertile	129	46	1.78 (1.06 ; 3.00)	1.72 (1.01 ; 2.93)	0.045
Middle tertile	120	47	1.96 (1.16 ; 3.30)	1.98 (1.16 ; 3.36)	0.01
Upper tertile	145	29	1.0	1.0	
BMI (kg/m^2)					
<17	126	52	2.29 (1.39 ; 3.74)	2.42 (1.46 ; 4.02)	0.001
≥17- <18.5	85	37	2.41 (1.41 ; 4.12)	2.30 (1.33 ; 3.99)	0.003
≥18.5	183	33	1.0	1.0	
Arm circumference [5]					
Lower tertile	116	51	1.66 (0.93 ; 2.94)	1.61 (0.91 ; 2.88)	0.10
Middle tertile	127	34	1.04 (0.57 ; 1.92)	1.01 (0.55 ; 1.88)	0.95
Upper tertile	151	37	1.0	1.0	

[1]OR adjusted for age and sex.
[2]OR for effect of age adjusted for sex and OR for effect of sex adjusted for age.
[3]Smear grading by auramine microscopy defined as ≤2+ = 1-99 AFB per 100 fields or 1-10 AFB per field; 3+ = more than 10 AFB per field.
[4]Culture grading defined as ≤2+ = more than 1colonies; 3+ = confluent colonies.
[5]Height, weight and arm circumference divided to 33.3 and 66.7 percentile and based on sex specific tertiles.
Lower tertile cut off ; height <158.2 for females and <167.1 for males, weight <43.1 for females and <49.7 for males, arm circumference <20.6 for female and <21.1 for males.
Middle tertile cut off; height 158.2-164.2 for females and 167.1-173 for males, weight 43.1-50 for females and 49.7- 55.3 for males , arm circumference 20.6-23 for females and 21.1- 23.4 for males.
Upper tertile cut off; height ≥164.3 for females and ≥173.1 for males, weight ≥ 51 for females and ≥ 55.4 for males, arm circumference ≥24 for females and ≥ 23.5 for males.
OR= odd ratio; BMI= Body mass index; 95% CI= 95% confidence interval.

Table 8. Analysis of factors associated with persistent sputum culture positivity at the end of the 2nd month of intensive phase

	Converted	Not converted	Crude	Adjusted[1]	
	n	n	OR (95% CI)	OR (95%CI)	P value
Age (years)[2]					
15-24	72	8	2.7 (0.94 ; 7.72)	2.61 (0.91 ; 7.51)	0.08
25-34	170	7	1.0	1.0	
35-44	100	8	1.94 (0.68 ; 5.52)	1.8 (0.62 ; 5.06)	0.29
≥45	79	3	0.92 (0.23 ; 3.66)	0.75 (0.19 ; 3.05)	0.69
Sex[2]					
Female	158	6	1.0	1.0	
Male	263	20	2.00 (0.79 ; 5.09)	2.1 (0.82 ; 5.45)	0.12
Religion					
Christian	308	18	1.0	1.0	
Muslim	104	6	0.98 (0.38 ; 2.55)	0.94 (0.36 ; 2.46)	0.90
Other	9	2	3.80 (0.76 ; 18.91)	5.20 (0.91; 29 ; 96)	0.06
Marital status					
Married or cohabiting	235	10	1.0	1.0	
Single	115	11	2.24 (0.93 ; 5.45)	1.86 (0.67 ; 5.18)	0.23
Separated, divorced & widowed	71	5	1.65 (0.54 ; 5.00)	1.83 (0.57 ; 5.83)	0.30
Occupation					
Farmer	139	10	1.0	1.0	
Businessman	112	6	0.74 (0.26 ; 2.11)	0.66 (0.23 ; 1.92)	0.44
Other	169	10	0.82 (0.33 ; 2.03)	0.71 (0.28 ; 1.82)	0.48
Smoking					
Current smoker	80	8	1.98 (0.80 ; 4.88)	1.96 (0.70 ; 5.48)	0.19
Past smoker	64	4	1.24 (0.39 ; 3.88)	1.27 (0.37 ; 4.33)	0.70
Never smoker	277	14	1.0	1.0	
Initial AFB smear grading[3]					
≤2+	139	5	1.0	1.0	
3+	282	21	2.07 (0.76 ; 5.60)	1.93 (0.70 ; 5.27)	0.20
Initial AFB culture grading[4]					
≤2+	102	1	1.0	1.0	
3+	318	25	8.01 (1.07 ; 59.92)	6.98 (0.92 ; 52.46)	0.06
BCG scar					
No	74	11	1.0	1.0	
Yes	346	15	0.29 (0.12 ; 0.66)	0.27 (0.11 ; 0.62)	0.002
HIV status					
Negative	255	18	1.0	1.0	
Positive	166	8	0.68 (0.29 ; 1.60)	0.84 (0.34 ; 2.06)	0.70
Haemoglobin					
Non anaemic	43	3	1.0	1.0	
Anaemic	378	23	1.15 (0.33 ; 8.98)	1.23 (0.32 ; 3.96)	0.85

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Table 8-continued

	Converted	Not converted	Unadjusted	Adjusted[1]	
	n	n	OR (95% CI)	OR (95%CI)	P value
White blood cell count (10^9cells/l)					
<4.0	45	1	0.32 (0.04 ; 2.49)	0.37 (0.48 ; 2.82)	0.34
4.0-11.0	356	24	1.0	1.0	
>11.0	20	1	0.74 (0.09 ; 5.76)	0.71 (0.09 ; 5.66)	0.75
CD4 count (10^6cell/ml)					
≤200	90	2	0.34 (0.08 ; 1.54)	0.41 (0.09 ; 1.89)	0.25
201-350	98	9	1.43 (0.60 ; 3.37)	1.69 (0.69 ; 4.09)	0.24
>350	233	15	1.0	1.0	
Height[5]					
Lower tertile	139	7	1.20 (0.39 ; 3.66)	1.17 (0.37 ; 3.65)	0.79
Middle tertile	143	6	1.0	1.0	
Upper tertile	139	13	2.22 (0.82 ; 6.03)	2.26 (0.82 ; 6.23)	0.11
Weight[5]					
Lower tertile	144	10	1.02 (0.41 ; 2.52)	0.88 (0.35 ; 2.23)	0.79
Middle tertile	130	6	0.68 (0.23 ;1.92)	0.62 (0.21 ; 1.76)	0.37
Upper tertile	147	10	1.0	1.0	
BMI (kg/m^2)					
<17	149	8	1.20 (0.44 ; 3.29)	1.09 (0.39 ; 3.03)	0.87
≥17- <18.5	93	10	2.40 (0.91 ;6.30)	1.95 (0.73 ; 5.29)	0.18
≥18.5	179	8	1.0	1.0	
Arm circumference[5]					
Lower tertile	132	9	1.38 (0.52 ; 3.67)	1.23 (0.46 ; 3.35)	0.67
Middle tertile	127	9	1.43 (0.53 ; 3.82)	1.35 (0.49 ; 3.65)	0.56
Upper tertile	162	8	1.0	1.0	

[1]OR adjusted for age and sex.
[2] OR for effect of age adjusted for sex and OR for effect of sex adjusted for age.
[3]Smear grading by auramine microscopy defined as ≤2+ = 1-99 AFB per 100 fields or 1-10 AFB per field; 3+ = more than 10 AFB per field.
[4] Culture grading defined as ≤2+ = more than 1colonies; 3+ = confluent colonies.
[5]Height, weight and arm circumference divided to 33.3 and 66.7 percentile and based on sex specific tertiles.
Lower tertile cut off ; height <158.2 for females and <167.1 for males, weight <43.1 for females and <49.7 for males, arm circumference <20.6 for females and <21.1 for males.
Middle tertile cut off; height 158.2-164.2 for females and 167.1-173 for males, weight 43.1-50 for females and 49.7- 55.3 for males , arm circumference 20.6-23 for females and 21.1- 23.4 for males.
Upper tertile cut off; height ≥164.3for females and 173.1 ≥ for males, weight≥51 for females and ≥55.4 for males, arm circumference ≥24 for females and ≥ 23.5 for males.
OR= odd ratio; BMI= body mass index; 95% CI= 95% confidence interval.

Table 9. Analysis of the predicting factors independently associated with persistent sputum smear and culture positivity at the end of the 2nd month of intensive phase[1]

	Sputum smear conversion				Smear culture conversion			
	Converted	Not converted	Adjusted OR (95% CI)	P value	Converted	Not converted	Adjusted OR (95% CI)	P value
Sex								
Female	165	29	1.0		-		-	-
Male	229	93	1.92 (1.16 ; 3.18)	0.01				
Initial AFB smear grading[2]								
≤+2	146	26	1.0		-		-	-
+3	248	96	1.9 (1.17 ; 3.19)	0.01				
BMI (kg/m^2)								
<17	126	52	2.18 (1.31 ; 3.64)	0.003	-		-	-
≥17- <18.5	85	37	2.17 (1.24 ; 3.79)	0.006				
≥18.5	183	33	1.0					
BCG scar								
No	68	35	1.0		74	11	1.0	
Yes	325	87	0.57 (0.35 ; 0.95)	0.032	346	15	0.28 (0.11 ; 0.62)	0.002

[1]OR adjusted for age, sex, and HIV status irrespective of their association with outcome.
[2]Smear grading by auramine microscopy defined as ≤2+ = 1-99 AFB per 100 fields or 1-10 AFB per field; 3+ = more than 10 AFB per field.
BCG =bacillus Calmette-Guérin; CI= 95% confidence interval; OR = Odds ratio.

10.0 APPENDICES.

10.1. Registration form

PART A - (NAMES, ADDRESS, etc)

District: _____ DTLC: _____District TB No. _____

Patient's full name: _____

Age: _ _ (Years) Sex: ☐M ☐F Enrolment date: _ _/_ _/200__ TB case: ☐New

☐Relapse

Patient's full address: Street/Area: _____Village: _____

Telephone/mobile of patient or relative: _____ (specify relationship) _____

Full name and address of patient's relative: _____

Full name & address of Sub-Village/Street Leader: _____

Name of Town/City/Area: _____

PART B – (DIAGNOSIS OF TB)

Local Microscopy: ☐Positive ☐Negative Date sputum collected for BMC Lab: _ _/_ _/200__

Date sputum collected	Specimen	3+	2+	1+	1-9	Negative	Not done
	1						
	2						
	3						

Chest x-ray: ☐ ☐ If Done: ☐ ☐ ☐

Done Not done Normal Abnormal Not Read

If not done: _____ if abnormal: ☐ ☐

(Specify reason) TB suggestive TB not suggestive

PART C – (BLOOD, PHYSICAL EXAMINATION)

Blood drawn: ☐Yes ☐No, if no specify reason:_____

Date blood drawn: _ _/_ _/200__ BCG-scar (TB scar): ☐No ☐Yes ☐Cannot be seen

Arm circumference: _ _, _ cm Triceps skinfold thickness: _ _, _ mm.

Waist circumference: _ _ _, _ cm Hip circumference: _ _ _, _ cm

Height: _ _ _, _ cm Weight: _ _ _, _ kg Grip strength: _ _ _, _ kg

PART D – (QUESTIONNAIRE)

General information:

1. Religion? ☐Christian ☐Muslim ☐Orthodox

☐Hindu ☐other, specify: _____

2. Marital status? ☐Married ☐Single ☐Separated ☐Divorced

☐Widow(er) ☐cohabiting

3. Tribe? _____

4. Occupation? ☐Farmer ☐Fisherman ☐Business ☐Housewife

☐Unemployed ☐Employed, skilled ☐Employed, unskille

☐Other, specify: _____

Morbidity questions:

1. During the last one month did you have any of the following?

a) Cough (more than 2 weeks) ☐ No ☐ Yes
b) Fever ☐ No ☐ Yes
c) Chest pain ☐ No ☐ Yes
d) Shortness of breath ☐ No ☐ Yes
e) Excessive sweating at night ☐ No ☐ Yes
f) Loss of body weight ☐ No ☐ Yes ☐ Do not know
g) Cough blood ☐ No ☐ Yes

2. Have you ever been treated for TB? ☐ No ☐ Yes, if yes when _ _/__

(mm/yy).

3. Have you ever lived together with a TB patient? ☐ No ☐ Yes

Smoking and alcohol drinking questions:

4. Do you smoke (prior to disease/TB)? ☐ No ☐ Yes (skip to 4 a) If no to 4.
Have you ever smoked? ☐ No (skip to 5) ☐ Yes If yes to a,

how long time ago did you smoke (months/years)? _____

b) If yes to 4/4a., what do you smoke? ☐Cigarettes ☐Pipe ☐ other, specify_____

c) If yes to 4/4a., how many per day? ☐<1 ☐1-5 ☐6-10
☐11-15 ☐16-20 ☐20+

d) If yes to 4/4a., how many years? ☐<5 ☐6-10 ☐10+

5. Do you take alcohol (prior to disease/TB) ☐ No (end) ☐ Yes

a) Which type of alcohol did you take? (You can choose >1) ☐Local brew ☐Beer
☐Hard liquor

b) If you took local brew, how often? ☐<1/week ☐1-3/w ☐4-6/w
☐ daily

c) If you took beer, how often? ☐<1/week ☐1-3/w ☐4-6/w
☐ daily

d) If you took hard liquor, how often? ☐<1/week ☐1-3/w ☐4-6/w
☐ daily

10.2: Baseline laboratory form

PART E – (PULMONARY TB STUDY FORM: BACTERIOLOGICAL, ZTRL)

Date sputum collected: _ _/_ _/200__

Date sputum received at BMC: _ _/_ _/200__

TB case: ☐New ☐Relapse ☐Follow up at month _____

Microscope result: At month: _____ ☐Positive ☐Negative ☐Not done

Specimen 1: Smear Result **Culture Result:** At month _____

3+	2+	1+	Neg.	Not done

3+	2+	1+	1-19 Colonies	Neg.	Not Done	Contaminated

PART F: LABORATORY RESULTS FORM

District / Unit Reg. No.:_____ PTB sputum status: ☐ Positive ☐ Negative

Test Date: |__|__| / |__|__| / |__|__|
DD MM YY

HIV Rapid Tests				ELISA
Test	Capillus	Determine	Conclusive	
Result	☐ Neg ☐ Pos ☐ Equiv	☐ Neg ☐ Pos ☐ Equiv	☐ Neg ☐ Pos ☐ Equiv	☐ Neg ☐ Pos
Tube info	Lot/Batch No.: |__|__|__|__|__|__|__|__|__| Expiry date: |__|__|/|__|__|/|__|__| DD MM YY	Lot/Batch No.: |__|__|__|__|__|__|__|__|__| Expiry date: |__|__|/|__|__|/|__|__| DD MM YY		
Lab Tech Preparing				
Lab Tech Reading				

Other Tests									
Test	W.B.C						HemoCue	HIV	Serum Samples Taken (√)
	Total (mm^3)	Differential							
		Lym	Neu	Mon	Eos	Bas	Hg (g/dL)	CD4 Count	
Result									
Lab Tech Preparing Test									
Lab Tech Reading Test									

10.3. Follow up questionnaire and laboratory form

☐ 2nd MONTHS

PART C – (BLOOD, PHYSICAL EXAMINATION)

Date blood drawn: ___/___/200__

Height: _____, __ cm Weight: _____, __ kg Grip strength: _____, __ kg

Arm circumference: ____. ___ cm Triceps skinfold thickness: _____. ___ mm.

Waist circumference: ____. ___ cm Hip circumference: _____. ___ cm.

PART D – QUESTIONNAIRE

Morbidity questions:

1. During the last one month did you have any of the following?

a) Cough (more than 2 weeks)	☐ No	☐ Yes
b) Fever	☐ No	☐ Yes
c) Chest pain	☐ No	☐ Yes
d) Shortness of breath	☐ No	☐ Yes
e) Excessive sweating at night	☐ No	☐ Yes
f) Cough blood	☐ No	☐ Yes

Smoking questions:

2. Do you smoke? ☐ No ☐ Yes

a) If yes to 2, what do you smoke? ☐Cigarettes ☐Pipe ☐Other, specify _____

b) If yes to 2, how many per day? ☐<1 ☐1-5 ☐6-10 ☐11-15 ☐16-20 ☐20+

PART F: (Laboratory form results 2 and 5 months**)**

Study ID. No.:_____ Test Date: |__|__| / |__|__| / |__|__|

DD MM YY

Test	W.B.C.						HemoCue	HIV	Serum Samples Taken (√)
	Total (mm^3)	Differential					Hb (g/L)	CD4 Count	
		Lym	Neu	Mon	Eos	Bas			
Result									
Lab Tech Preparing Test									
Lab Tech Reading Test									

10.4. Study Consent Form

You are asked to consent for your participation in a study aimed at finding predictors of sputum smear conversion that are more likely to be associated with slow response/treatment failure in tuberculosis patients. This study is undertaken as a sub study, as part of a major study done by The National Institute for Medical Research (NIMR) in Collaboration with the University of Copenhagen, Denmark in Mwanza, Tanzania in order to improve TB treatment outcome. We are requesting you to participate because you have been found to have TB. Your participation might benefit other TB patients like you in the future. If you agree to participate you may be expected to answer some questions asked by clinic staff and to provide specimens for TB, and HIV diagnosis.

What you are expected to do

You will be given information about the study, and if you agree to participate you will undergo a brief interview and pre-test counselling for HIV. Then you will be requested to provide blood specimens for TB and HIV.

Confidentiality

The information regarding your test results will be confidential and will only be made known to yourself or any other person you choose to tell the results to and the clinicians treating you. Your name will not be mentioned or used in any papers or study reports; and only study identification numbers will be used.

Benefits

In case you are diagnosed with TB, or HIV, you will get treatment or be referred accordingly. Treatment for TB is provided free of charge. The same applies if you are diagnosed with HIV, you will be referred to the treatment and care clinic for management including provision of ARV as per National HIV/AIDS Treatment and Care guidelines.

Participation

In case you do not want to be enrolled in the study or do not want to have your blood drawn for testing, that decision will not in any way interfere with your right to get TB treatment. You are also free to withdraw from the study anytime you feel to do so for whatever reason(s). Your withdrawal from the study will not affect your right to treatment

Summary statement

I have been told about the objectives of the study and the benefits of being included in the study. I have clearly understood that decision to be recruited in the study is upon me to decide and that I can refuse or withdraw from the study at any time and that my refusal will not interfere with my right to TB treatment

Consent:

I ---------- (name of the client/patient) agree to participate in the study and my blood to be drawn for testing TB, and HIV.

Signature of the client/patient _____

Thumb of the client (*in case not able to write*) _____

Witnessed by (name) ---------- (if patient is not able to write)

Signature _____ Date _____/_____/200 _____

Name of Clinician advising/ counselling the patient _____

Signature of Clinician/Counsellor _____

Date: _____/_____/200_

NB: In the field application, this form is translated into Swahili language

10.5 Ethical clearance

17/02/2006 16:59 +225-22-2121360 NAT INST FOR MED RES PAGE 02

ATTN: PROF. FRIIS H.

THE UNITED REPUBLIC OF TANZANIA

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10th February 2006

Dr N Range
NIMR Muhimbili Research Centre
P O Box 3436
Dar es Salaam

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: The role of diabetes and nutritional support during treatment of Pulmonary TB: Two randomized nutritional supplementation trials in Tanzania *(Range N et al)* whose Principal Investigator is Nyagosya Range, has been granted ethics clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is made available to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine.

Name: Dr Andrew Y Kitua

Signature

CHAIRMAN
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Gabriel L Upunda

Signature

CHIEF MEDICA L OFFICER
MINISTRY OF HEALTH