

Clinical diagnosis of smear negative pulmonary tuberculosis in HIV-positive patients at Athlone Hospital in Botswana

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DECLARATION

I, DR TAURAYI ADRIANO TAFUMA, hereby declare that the work for this dissertation, unless where acknowledged, is my own. It is being submitted in partial fulfilment for the degree Master of Public Health, in the School of Health Care Sciences, University of Limpopo, Medunsa Campus.



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ABSTRACT

Background and aim: Smear-negative pulmonary tuberculosis (SNPTB) has become an increasingly important clinical and public health problem, especially in areas that are affected by the dual infection of TB and human immunodeficiency virus (HIV) (Mello et al, 2006; WHO, 2006; Harries et al, 1998). There are recommended guidelines for diagnosing SNPTB to reduce misdiagnosis in sub-Saharan Africa, but there is little information on whether these guidelines are followed correctly (Harries et al, 1998). The aim of this study was to investigate the clinical diagnosis of SNPTB in HIV-positive patients at Athlone Hospital in Botswana.

Methods: This was a quantitative, descriptive study which used two sources of data and data collection methods: a 4 year retrospective records review and questionnaires for clinicians. All clinicians responsible for treating HIV-positive patients (n=8) were asked to complete a questionnaire on self-reported (1) compliance with the guidelines (2) use of other methods to diagnose SNPTB and (3) reasons for not complying with the guidelines. All records on SNPTB in HIV-positive patients from 2006 to 2009 (n=281) were reviewed to establish the compliance and use of other methods to exclude other respiratory infections.

Results: The response rate for clinicians was 87.5% (7/8). All clinicians (100% [7/7]) reported (a) always complying with using chest x-rays (CXRs), but (b) only sometimes complying with using 3 sputum results. Most clinicians (a) considered the duration of cough before making a diagnosis of SNPTB (57.1% [4/7]), and (b) placed patients on a trial of broad spectrum antibiotics before starting PTB treatment (85.7% [6/7]). The main reasons for non-compliance were: the inability of patients to submit sputum (100% [7/7]), delays in the laboratory (71.43% [5/7]), and lack of feedback from Botswana National Tuberculosis Program (BNTP) (57.14% [4/7]). Only 2.1% (6/281) of the records showed that other methods were used to rule out other respiratory infections, and overall compliance with the recommended guidelines was only 13.5% (40/281).

Conclusion: The compliance with the recommended guidelines in making a diagnosis of SNPTB was very poor in this study. The unavailability of user-friendly and fast diagnostic methods resulted in many cases being treated for SNPTB with inadequate investigations.

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LIST OF ABBREVIATIONS

AFB	Acid-fast bacilli
AFS	Acid-fast stain
AIDS	Acquired immuno-deficiency syndrome
BCG	Bacille Calmette-Guérin
BNTF	Botswana National TB Program
CXR	Chest-X-ray
DOTS	Directly observed therapy short course
DNA	Deoxyribonucleic acid
E-AMTDT	Enhanced Amplified MTB Direct Test
FDC	Fixed-dose drug combination
FDA	Food and Drug Administration
HIV	Human immunodeficiency virus
IUATLD	International Union Against TB and Lung Disease
INH	Isoniazid
IPT	Isoniazid Preventive Therapy
UNAIDS	United Nations Programme on HIV/AIDS
LTBI	Latent TB infection
LDHT	Lobatse District Health Team
LJ	Lowenstein-Jensen
MREC	Medunsa Research and Ethics Committee
MDR-TB	Multi-Drug-Resistant-TB
MTB	Mycobacterium TB
NTP	National TB Control Programme
NAA	Nucleic Acid Amplification
PLWHA	People living with HIV/AIDS
PCP	Pneumocystis Carinii (Jiroveci) Pneumonia
PCR	Polymerase Chain Reaction
PT	Preventive Therapy
PPM	Public-Private Mix

PTB	Pulmonary TB
RNA	Ribonucleic acid
SNPTB	Smear-negative pulmonary TB
SPPTB	Smear-positive pulmonary TB
MCS	Microscopy, Sensitivity; Culture
TST	Tuberculin Skin Test
TB	Tuberculosis
UVGI	Ultraviolet Germicidal Irradiation
WHO	World Health Organization
ZN	Ziehl–Neelsen

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CHAPTER 1– INTRODUCTION

1.1. BACKGROUND AND RATIONALE FOR THE STUDY

Tuberculosis (TB) is an ancient disease but it remains one of the deadliest diseases in the world. It is a social disease with medical implications. It has always occurred disproportionately among disadvantaged populations such as the homeless, malnourished, and overcrowded. Within the past decade it also has become clear that the spread of human immunodeficiency virus (HIV) infection and globalisation have resulted in increased numbers of TB cases (American Thoracic Society, 2000). In particular, smear-negative pulmonary TB (SNPTB) has become an increasingly important clinical and public health problem, especially in areas that are affected by the dual infection of TB and HIV, such as sub-Saharan Africa (Mello et al, 2006; WHO, 2006; Harries et al, 1998).

Botswana has one of the highest TB notification rates in the world and has consistently reported in excess of 590 cases per 100 000 population annually since 2000 (Botswana National TB Program [BNTP], 2007). Based on studies of TB and HIV co-infection it is apparent that the increase in TB incidence can be attributed to the increasing prevalence of HIV in Botswana (BNTP, 2007). SNPTB is defined as symptomatic illness in a patient with at least two sputum smear examinations negative for acid-fast bacilli (AFB) on different occasions in whom pulmonary tuberculosis is later confirmed by culture or other investigations (Siddiqi et al, 2003). SNPTB cases are on the rise in Botswana; as a result clinicians are inducted on the management of TB (including SNPTB) through in-service lectures, refresher courses or the use of National TB Program Manuals which highlights the guidelines used in the management of TB.

The BNTP (2007) recommends the use of these guidelines, which they adopted and adapted from the recommendations made by World Health Organization (WHO), the International Union Against TB and Lung Disease and United States Centers for Disease Control. If the guidelines are not properly used by clinicians, other opportunistic respiratory infections are misdiagnosed as smear negative PTB in HIV co-infected patients.

1.2. PROBLEM STATEMENT

The low sensitivity and specificity of the microscopic examination of sputum for TB in HIV-positive patients has resulted in increasing cases of SNPTB. The adopted and adapted BNTP diagnostic guidelines were developed to assist the clinicians with the diagnosis of SNPTB in Botswana, but as in other sub-Saharan countries, there is little information about whether these guidelines are used in suspected cases of TB (Harries et al, 1998).

1.3. PURPOSE OF THE STUDY

1.3.1. Research questions:

The purpose of this study was to answer the following research questions:

- To what extent do clinicians comply with the recommended national guidelines in the diagnosis of SNPTB in HIV-positive patients?
- To what extent do clinicians request other methods to rule out other respiratory conditions and confirm a diagnosis of SNPTB?
- What reasons do clinicians give for not complying with the national guidelines?

1.3.2. Aim:

This study aimed to investigate clinical diagnosis of SNPTB in HIV-positive patients at Athlone Hospital in Botswana.

1.3.3. Objectives:

- To establish the extent to which clinicians comply with the recommended guidelines when making a diagnosis of SNPTB in HIV-positive patients (number of sputum results used, use of antibiotics before starting anti-TB drugs, if chest X-ray was done and reported and period of coughing before a diagnosis of SNPTB is made).
- To establish the extent to which other methods were used in ruling out other respiratory conditions and confirming a diagnosis of SNPTB.
- To establish the reasons clinicians give for not complying with the recommended national guidelines.

CHAPTER 2 – LITERATURE REVIEW

2.1. EPIDEMIOLOGY OF PTB

2.1.1. The causative organism

There are five closely related mycobacterium grouped in the *Mycobacteria tuberculosis* complex: *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium microti*, and *Mycobacterium canetti*. Pulmonary TB (PTB) is caused mainly by *M. tuberculosis* (MTB), the natural reservoir of which is human beings. This is a fastidious, slow growing, strictly aerobic, lipid-rich, hydrophobic and acid-fast bacterial rod (Aderaye, 2007; American Thoracic Society, 2000). MTB has no outer membrane, but has a cell wall made up of three macromolecules, the peptidoglycans, arabinogalactan, mycolic acid and lipopolysaccharide which is anchored to the plasma membrane. It is the mycolic acid which resists discolorization by acid-alcohol (Aderaye, 2007).

2.1.2. Transmission

MTB is transmitted by patients with PTB. Virtually all new infections are acquired via airborne transmissions (Tostmann et al, 2008; Aderaye, 2007; American Thoracic Society, 2000). Seminal experiments demonstrating airborne TB transmission by droplet nuclei were performed by Riley and co-workers in the 1950s–1960s. They noted that guinea pigs acquired TB by breathing exhaust air from a TB ward (Escombe et al, 2008). Droplet nuclei are commonly produced when persons with pulmonary or laryngeal TB cough, sneeze, speak, or sing. These droplet nuclei are so small that once inhaled they reach the alveoli within the lungs, where the organisms replicate (American Thoracic Society, 2000).

There are several factors which facilitate the likelihood of transmission of MTB, namely (1) the number of MTB organisms being expelled into the air (increased with lung cavitations, coughing frequency and cough-inducing procedures), (2) the concentration of

organisms in the air determined by the volume of the space and its ventilation, (3) the length of time an exposed person breathes the contaminated air, and (4) presumably the immune status of the exposed individual (Escombe et al, 2008; American Thoracic Society, 2000). It is vital to note that patients with PTB and producing a smear negative sputum are capable of transmitting MTB as was revealed in a study from the Netherlands, where approximately 12.6% transmissions were caused by SNPTB patients (Tostmann et al, 2008). However, organisms deposited on intact mucosa or skin does not invade tissue (American Thoracic Society, 2000).

2.1.3. Pathogenesis

After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. MTB gets its first contact with resident macrophages and alveolar epithelial type II pneumocytes. This type II cell is found in greater numbers than macrophages in alveoli. In addition, dendritic cells play a very important role in the early stages of infection since they are much better antigen presenters than are macrophages and presumably play a key role in activating T cells with specific MTB antigens. The dendritic cells are migratory unlike differentiated macrophages; hence they play a vital role in dissemination of MTB (Aderaye, 2007; Smith, 2003; American Thoracic Society, 2000).

The infected macrophages in the lung are responsible for the production of chemokines which will attract inactivated monocytes, lymphocytes, and neutrophils of which none will kill the bacteria very efficiently (Smith, 2003). Out of this, granulomatous focal lesions composed of macrophage-derived giant cells and lymphocytes will begin to form. In persons with intact cell-mediated immunity this will limit the multiplication and spread of the organism (Smith, 2003; American Thoracic Society 2000). As cellular immunity continues to develop, macrophages loaded with bacilli are killed, and this results in the formation of the caseous centre of the granuloma, surrounded by a cellular zone of fibroblasts, lymphocytes, and blood-derived monocytes (Smith, 2003). Despite the acidity, low availability of oxygen and presence of toxic fatty acids in the caseous tissue

of the granuloma, small numbers of MTB will remain alive and dormant for decades (Smith, 2003; American Thoracic Society, 2000). This enclosed infection is referred to as latent or persistent TB and can persist throughout a person's life in an asymptomatic and nontransmissible state (Smith, 2003; American Thoracic Society, 2000).

However, if an infected person cannot control the initial infection in the lung or if a latently infected person's immune system becomes weakened by immunosuppressive drugs, HIV infection, malnutrition, aging, or other factors, the granuloma centre can become liquefied by an unknown process and then serves as a rich medium in which the now revived bacteria can replicate in an uncontrolled manner (Smith, 2003). This will then lead to active PTB and the bacilli can spread to other organs via the lymphatic system and the blood. When this happens, the person becomes infectious and requires therapy for him/her to survive (Adeyare, 2007; Smith, 2003).

Death will result if the MTB growth is uncontrolled, through the extensive lung damage leading to suffocation due to insufficient oxygen. This anoxia occurs due to the obliteration of lung parenchymal cells involved in oxygen uptake as well as obstruction of bronchiolar passages by granulomatous growths and by blood released during the rupture of liquefied granuloma in adjacent lung tissue (Smith, 2003).

It is estimated that approximately 10% of individuals who acquire TB infection and are not given preventive therapy will develop active TB. The risk is highest in the first 2 years after infection, when half the cases will occur (American Thoracic Society, 2000). Of note is that, HIV-positive persons infected with MTB have a 50% chance of developing reactivation (post primary) TB at some time in their lives (Smith, 2003). In sub-Saharan Africa, it has been shown that various forms of TB occur across the spectrum of HIV associated immunodeficiency (Adeyare, 2007) and these are PTB, TB meningitis, lymphatic TB, and disseminated TB. Most of the cases are CD4 dependant, thus PTB occurs at CD4+ counts of 300-500 cells/mm³ or less, though in some cases PTB can be found at higher CD4+ counts, while disseminated TB occurs at lower values of CD4 count.

2.1.4. Incidence of TB

2.1.4.1. Global incidence

In 2008, there were an estimated 9.4 (range, 8.9–9.9 million) million incident cases (equivalent to 139 cases per 100 000 population) of TB globally, 9.6–13.3 million prevalent cases of TB, 1.1–1.7 million deaths from TB among HIV-negative people and an additional 0.45–0.62 million TB deaths among HIV-positive people (WHO, 2009). Out of the 5.7million cases of TB notified in 2008, approximately half of these were SNPTB cases (WHO, 2009) which makes it pertinent to consider the impact of SNPTB in our TB programmes. This observation reflects strong association between the two conditions but there is a possibility of misdiagnosis of other HIV-related respiratory infections as TB (Aderaye, 2007), probably due to limited use of the recommended guidelines by the clinicians or lack of other diagnostic modalities to exclude other opportunistic infections in resource-poor settings.

HIV and TB co-infection presents special challenges to the expansion and effectiveness of directly observed therapy short course (DOTS) programs and the Stop TB Strategy. TB accounts for one-third of acquired immuno-deficiency syndrome (AIDS) deaths worldwide and is one of the most common causes of morbidity in people living with HIV/AIDS (PLWHA) (WHO, 2009; Tsiouris et al, 2007). Currently, about 33 million people are HIV infected, and at least one-third are also infected with TB. The dual epidemics of TB and HIV are particularly pervasive in sub-Saharan Africa, where HIV has been the single most important factor contributing to the increasing incidence of TB over the last 10 years. In some countries in sub-Saharan Africa, up to 70 percent of patients with active TB disease are also HIV-positive (WHO, 2009). The dual epidemics are also of growing concern in Asia, where two-thirds of TB-infected people live and where TB now accounts for 40 percent of AIDS deaths. Eastern Europe and the former Soviet Union have the fastest growing HIV epidemic in the world, and HIV co-infection is a factor that could exacerbate problems with the multidrug-resistant TB (MDR-TB) epidemic in these regions (WHO, 2009). The overlap of TB/HIV co-infection with MDR-

TB and extensively drug-resistant TB presents a tremendous challenge and threatens progress in controlling both TB and HIV/AIDS (WHO, 2009).

The frequency of HIV-associated PTB depends on the prevalence of TB and HIV in the society and the overlap between the two populations. Because of the high risk of developing TB in HIV-infected people by either endogenous reactivation or de-novo infection, a tremendous increase in the case load in high HIV, and TB endemic areas has occurred, particularly in sub-Saharan Africa where reported cases rates has increased by over 50% (WHO, 2009).

2.1.4.2. Incidence in sub-Saharan Africa

Sub-Saharan Africa has the highest prevalence of HIV infection and had the highest incidence of TB before the HIV/AIDS era (Tsiouris et al, 2007; Corbert et al, 2006). In the six southern African countries with adult HIV prevalence of more than 20%, TB case-notification rates are 461–719 per 100 000 per year; by comparison, the notification rate in the USA was 5 per 100 000 per year. True yearly rates in sub-Saharan Africa are likely to be even higher because of under diagnosis and under-reporting (Corbert et al, 2006).

In this region, TB is often the first manifestation of HIV infection, and it is the leading cause of death among HIV-infected patients. In hospital-based studies, 40–65% of HIV-infected sub-Saharan African patients with respiratory disease had TB (Corbert et al, 2006). In primary health and chest clinic settings, TB was confirmed in 43–70% of adults with cough for 3 weeks or longer (chronic cough) in Zimbabwe, Kenya, and Malawi (Corbert et al, 2006).

The high case rate in sub Saharan Africa contributed to a global rise in TB incidence of 1% in 2003, despite stable or declining rates in the rest of the world (WHO, 2009).

2.1.4.3. Incidence in Botswana

Botswana has one of the highest TB notification rates in the world and has consistently reported in excess of 590 cases per 100 000 population annually since 2000. A decline in TB notifications rates of 506/100 000 to 199/100 000 was noted from 1975 to 1989. However TB rates began to rise in the early 1990s and reached a peak in 2002 with a notification rate of 623/100 000 (BNTP, 2007).

Currently, TB is a major public health problem in Botswana responsible for more than 10% of all institutional admissions and over 5% of Out Patient Department attendances. It is the second most important health problem after HIV/AIDS, being one of the leading causes of death in adults (Rakgailwane & Sekgwake, 2007). Based on studies of TB and HIV co-infection it was apparent that the increase in TB incidence was attributed to the increasing prevalence of HIV in Botswana (BNTP, 2007).

2.2. HIV / PTB CO-INFECTION

2.2.1. The impact of HIV on PTB

Before the beginning of the epidemic of infection with HIV, approximately 85% of reported TB cases were limited to the lungs, with the remaining 15% involving only non-pulmonary or both pulmonary and non-pulmonary sites (Verma & Maharjan, 2008; American Thoracic Society, 2000). With advanced HIV infection approximately 53-62 % of reported cases are extra pulmonary TB. Studies from India reported that extra pulmonary TB by itself was not associated with decreased CD4 but patients with a combination of pulmonary and extra pulmonary TB had significantly lower CD4 counts (Verma & Maharjan, 2008).

HIV has created an enormous impact on the epidemiologic and clinical features of TB worldwide, particularly in resource-poor countries (DeReimer et al, 2007). The epidemic has led to large increases in the frequency of SNPTB, which has made laboratory-confirmed diagnosis of PTB difficult thus resulting in poor treatment outcomes and excessive early mortality compared with smear-positive disease (Morse et al, 2008;

Getahun et al, 2007; Colebunders & Bastian, 2000). A study which was done in Malawi confirmed the clinical impression that HIV-positive patients with SNPTB had higher mortality rate than HIV-positive patients with smear-positive PTB when they are all on treatment. The mortality rate was 3.9 times higher among SNPTB patients (Colebunders & Bastian, 2000).

In particular, SNPTB has become an increasing clinical and epidemiological problem, especially in areas that are affected by the dual infection TB/HIV e.g. sub-Saharan Africa (Mello et al, 2006; WHO, 2006; Harries et al, 1998). For example, a study which was done in Zambia found out that 43% of 72 HIV-positive patients with culture-proven pulmonary TB in Lusaka, were smear-negative compared with 24% of 37 HIV-negative cases (p -value = 0.003) (Colebunders & Bastian, 2000). This predominance of smear-negative disease may be partly due to 1) heavy workloads increasing the likelihood of false-negative laboratory errors, and 2) misdiagnosis of other HIV-related pulmonary conditions as smear-negative TB (Colebunders & Bastian, 2000). Overall, population trends and most clinical based studies do suggest that HIV-positive patients have a higher rate of smear-negative disease.

The features of PTB and the clinical presentation are influenced by the degree of immuno-suppression. Patients with a well preserved CD4 count are likely to present with symptoms and signs similar to HIV-negative patients (i.e. cough for at least three weeks, night sweats and weight loss). Patients with more advanced immuno-suppression often present with more atypical symptoms and signs. Symptoms may be nonspecific or absent and it may be difficult to distinguish from HIV disease or other opportunistic infections (Elston & Thaker, 2008). Haemoptysis which is usually considered to be the hall mark of PTB in developing countries is not as frequently reported by patients with advanced HIV infection (Aderaye, 2007). Because of this clinicians need to have a high index of suspicion for TB in symptomatic HIV-positive patients (Elston & Thaker, 2008).

PTB nearly always causes abnormalities on the radiological films of the chest. Endogenous reactivation of latent infection usually causes abnormalities in the upper

lobes of one or both lungs and cavitation is common. As TB progresses, infectious material may be spread via the airways into other parts of the lungs, causing patchy opacities (bronchopneumonia). Dissemination of the infection in the lungs may lead to a miliary pattern on the chest film (evenly distributed small nodules) (American Thoracic Society, 2000). However in HIV-positive patients with PTB, the nature of the radiographic findings depends to a certain extent on the degree of HIV-related immunosuppression (Aderaye, 2007).

In the early course of HIV infection typical radiological findings as described above are common. With advanced HIV disease the radiographic findings become more “atypical”: cavitation is uncommon and lower lung zone or diffuse infiltrates and intrathoracic adenopathy are frequent and at times a normal chest film is found (American Thoracic Society, 2000; Harries et al, 1998). Advanced HIV infection increases the risk of reactivation of latent TB infection and exogenous re-infection, the infection progresses more rapidly to active disease, pulmonary cavitations is less likely to occur, adverse drug reactions are more common and death rates are higher (Tostmann et al, 2008; Getahun et al, 2007; DeReimer et al, 2007; American Thoracic Society, 2000; Colebunders & Bastian, 2000).

A study which was done in San Francisco where HIV prevalence is high suggested that high HIV prevalence amplified the local TB epidemic: 13.7% of San Francisco’s TB cases were attributable to HIV in the population and most of them were due to reactivation of latent TB infection (DeReimer et al, 2007). This was similar to the findings (14%) in Harare, Zimbabwe (Corbet et al, 2006). DeReimer et al (2007) noted that transmission of MTB to HIV-positive persons lead to rapid progression to disease in the newly infected individuals as compared to an HIV negative patient. In most European countries where HIV prevalence is low TB clinics are encouraged to increase HIV testing during consultation and HIV clinics should keep TB high on the list of differential diagnosis in any difficult clinical cases (Zumla et al, 2000).

Corbet et al (2006) cited other studies stating that, in Africa, TB is often the first manifestation of HIV infection, and it is the leading cause of death among HIV-infected patients. HIV has caused patients with TB to commonly present with atypical symptoms: MTB was isolated from 9% of adults with acute pneumonia in Kenya, 35% of people with cough for less than 3 weeks in Malawi, 23% of febrile HIV-infected inpatients in Tanzania, and 13% of HIV-infected patients with chronic diarrhoea in Kenya (Corbet et al, 2006).

2.3 LABORATORY DIAGNOSIS OF PTB

2.3.1. Microscopy

The microscopic examination of the stained sputum smear is the corner stone of PTB diagnosis in resource-poor settings because it is cheap, quick and easy to activate and provides preliminary confirmation of the diagnosis (Matee et al, 2008; Tostmann et al, 2008; Long, 2001; Colebunders & Bastian, 2000). Sputum smear microscopy is done using the acid-fast staining procedure. Two procedures are commonly used for acid-fast staining: the carbolfuchsin methods, which include the Ziehl-Neelsen (ZN) and Kinyoun methods, and a fluorochrome procedure using auramine- O or auramine-rhodamine dyes. Studies have found that there must be 5,000 to 10,000 bacilli per millilitre of specimen to allow the detection of bacteria in stained smears (Aderaye, 2007; American Thoracic Society, 2000).

ZN staining with light microscopy is the most commonly used method; however it is time consuming and has low sensitivity (Salami, 2006). It provides the physician with a preliminary confirmation of the diagnosis and a quantitative estimation of the number of bacilli being excreted which is of clinical and epidemiologic importance in assessing the patient's infectiousness (American Thoracic Society, 2000).

The standard WHO recommendation for TB diagnosis in the DOTS program is the use of direct sputum microscopy on 3 stained sputum specimens. First and third are on spot, while the second is the early morning sample (Matee et al, 2008; BNTP, 2007; Mello et

al, 2006; WHO, 2006). Identification of sputum smear-positive patients is crucial for the control of TB as it is estimated that one undiagnosed case of smear-positive TB patient infects 10-20 contacts over a one year period if untreated (Aderaye, 2007).

The TB burden and the HIV epidemic have over stretched the meagre diagnostic facilities and manpower in developing countries leading to under-reading of most slides. Such under-reading has resulted in 21-29% of patients being registered as smear-negative PTB who were confirmed to be smear-positive when re-examined at reference laboratories (Aderaye, 2007). The worst scenario was in Botswana in 1992 where studies reveal that approximately 48% of the patients reported to have PTB had no sputum smears examination performed (Colebunders & Bastian, 2000). Currently there is a debate about whether two or three sputum specimens should be examined for diagnosis of PTB as some suggest that reducing the recommended number of specimens examined to two could benefit TB control programs by using fewer resources and by reducing the time spent on case detection (Matee et al, 2008).

In a study which was done in Tanzania, the incremental diagnostic value was 92.1%, 1.8% and 7.1% for the first, second and third smears, respectively. The higher diagnostic yield value of the third smear found in this study differs from most other studies (Matee et al, 2008). When they did a systematic review of 37 studies the third specimen lead to increase in sensitivity by 3.1% (95% CI, 2.1 to 4.2%) (Matee et al, 2008). The difference between the second and the third specimen yield reflect poor instructions for specimens collected at home or very good instructions for the spot third specimens and might also be due to improved cough technique by the time of the third specimen (Matee et al, 2008).

With HIV infection the sensitivity of sputum microscopy is greatly reduced because of the lower rate of caseation necrosis, and consequent lower numbers of AFB in the airway (Matee et al, 2008). In patients who can produce sputum, several studies have demonstrated that sputum acid-fast stain (AFS) after concentration with sodium hypochlorite partially restores the already lost sensitivity of sputum direct microscopy

(Aderaye et al, 2007). However, many patients with advanced HIV and suspected PTB do not produce sputum spontaneously making the diagnosis difficult. Induction of sputum is an option in this case (Morse et al, 2008). Currently, health care providers in Botswana are being trained on how to use sputum inducers, though on a small scale. Inhalation of an aerosol of sterile hypertonic saline (3– 15%), usually produced by an ultrasonic nebulizer, is used to stimulate the production of sputum. The aerosol-induced specimens are thin and watery, and if not labelled as induced sputum they can be discarded by the laboratory as an inadequate specimen (American Thoracic Society, 2000).

A study which was done in Botswana where HIV prevalence is high revealed that sputum induction on its own had increased diagnostic benefit for the detection of PTB and these results were consistent with a study which was done in Malawi (Morse et al, 2008). In addition, a study which was done in Ethiopia, showed that bronchoalveolar lavage and induced sputum if concentrated with sodium hypochlorite (5%) and then stained by the Ziehl Nielsen technique improves detection of AFB in both HIV-infected and non-infected patients (Aderaye et al, 2007). With this, Aderaye et al (2007) concluded that the technique of sputum concentration can be applied to resource-poor settings as it is quick, simple and affordable. Furthermore, it will avoid delays in making the diagnosis and allow immediate initiation of treatment for TB in HIV-positive patients where the sputum is likely to be negative and the chest X-ray is usually misinterpreted as pneumocystis pneumonia or bacterial infection.

Induced sputum is easy to perform, inexpensive and well-tolerated (Morse et al, 2008). Despite these advantages, sputum induction and sputum concentration are not widely used in sub-Saharan Africa (Aderaye et al, 2007). This then contributes to poor TB diagnosis and control.

Fluorescence microscopy increases the probability of detecting acid-fast bacilli, especially if the sputum contains few bacteria, and hence improves the sensitivity of microscopy in HIV-positive patients. The use of fluorescence microscopy in resource-constrained settings is limited by high costs because fluorescence microscopy is four to

five times more expensive than light microscopy and the light bulbs must be replaced after 200 h of use (Getahun et al, 2007). Other difficulties are the need for a reliable electricity supply and the presence of naturally fluorescent particles in sputum that can be confused with acid-fast bacilli (Getahun et al, 2007).

Advantages are that time needed to examine the smear is much lower and nearly 15 times as many fields of view can be scanned in the same period. In a systematic review, Getahun et al (2007) noted that 43 of 44 studies that used fluorescence microscopy showed that on average, in comparison with Ziehl-Neelsen microscopy, fluorescence microscopy showed a 10% increase in sensitivity and 9% incremental yield, and this improvement was not affected by HIV status. Its use could also improve the diagnosis of other opportunistic infections that are common in people with HIV infection or AIDS such as *Pneumocystis carinii* pneumonia (PCP) (Getahun et al, 2007).

In reading smears, the laboratory usually provides the clinician with a rough estimate of the number of AFB detected as shown in Table 2-1. In our setting, clinicians are provided with the quantity report only for quick and easy interpretation.

Table 2-1 Quantitation scale for AFB smear according to stain used

Carbolfuchsin (× 1,000)	Fluorochrome (× 250)	Quantity Reported
No AFB/300 fields	No AFB/30 fields	No AFB seen
1–2 AFB/300 fields	1–2 AFB/30 fields	Doubtful, repeat test
1–9 AFB/100 fields	1–9 AFB/10 fields	Rare (+1)
1–9 AFB/10 fields	1–9 AFB/field	Few (+2)
1–9 AFB/field	10–90 AFB/field	Moderate (+3)
>9 AFB/field	>90 AFB/field	Numerous (+4)

Source: American Thoracic Society (2000)

2.3.2 Culture

Ideally all clinical specimens suspected of containing MTB should be inoculated onto culture media for four reasons: 1) sputum cultures increase the sensitivity of diagnosis substantially; thus sensitivity and specificity are about 80 and 98 percent, respectively; 2) growth of the organisms is necessary for precise species identification; 3) drug susceptibility testing; and 4) genotyping of cultured organisms would be useful for identification of epidemiological links between patients as well as to detect laboratory cross-contamination (American Thoracic Society, 2000). However, all this can only be achieved at an extra cost which is usually not feasible in developing countries.

There are three types of traditional culture media: egg based (Lowenstein-Jensen [LJ]), agar based (Middlebrook 7H10 or 7H11) and liquid (Middlebrook 7H12 and other commercially available broths). In developing countries, LJ medium is the most commonly used medium for culture of MTB, which is also recommended by WHO (Mathur et al, 2009; American Thoracic Society, 2000). Growth in liquid media is faster (1 to 3 weeks) than growth on solid media (3 to 8 weeks). When sputum is inoculated on LJ, macroscopic colonies of MTB appear in 2-6 weeks, and a negative culture report cannot be given before eight weeks. Growth tends to be slightly better on egg-based media, but growth is more rapid on agar media (Mathur et al, 2009; American Thoracic Society, 2000).

The liquid media allows scientists to detect the carbon dioxide production or oxygen consumption using the BACTEC and MGIT systems respectively. The agar medium permits examination of colony morphology and detection of mixed cultures. It is important to note liquid media can be used for primary isolation of mycobacteria from non-sterile sites only if supplemented with an antibiotic cocktail. The technology and cost needed to operate the broth-based culture systems makes their availability in resource-poor settings limited (Wong, 2008).

Specimens should be inoculated onto at least one container of solid medium. This culture system should be used in conjunction with a liquid broth culture system. Lowenstein-Jensen slants are a useful backup for detection of rare mycobacterial strains that may not grow on other media. Automated liquid systems should be examined for growth at least every 2 to 3 days; solid media should be examined for growth once or twice weekly (American Thoracic Society, 2000).

2.3.3. Molecular techniques

Molecular techniques have been developed with the intent to overcome the limitations associated with the traditional laboratory diagnostic methods thus leading to improved quality of diagnosis and faster identification of MTB infections (Scherer et al, 2009). The discovery of the polymerase chain reaction (PCR) led to the development of nucleic acid amplification (NAA) tests for rapid detection and identification of MTB in clinical samples, sometimes with limited determination of drug resistance. This works through identification of the MTB specific DNA (Deoxyribonucleic acid) or ribosomal RNA (Ribonucleic acid) sequences by PCR. These tests amplify target nucleic acid regions in viable or nonviable bacilli, which uniquely identify MT complex (Tsara et al, 2009; Salami, 2006; Shamputa et al, 2004).

There are in-house assays and commercial kits. The in-house tests are based on PCR assays developed by each laboratory and are dependent on laboratory methods (Tsara et al 2009). Commercial test available at the moment for the direct detection of MTB in clinical specimens are four: i) the Enhanced Amplified Mycobacterium TB Direct Test (E-AMTDT), ii) the AmplicorA *Mycobacterium TB* Test (Amplicor MTB test) and its automated version the Cobas Amplicor MTB test, iii) the BDProbe Tec ET test, and iv) the INNO-LiPA-Rif. TB test (Shamputa et al, 2004).

In a study which was done in Brazil, it was found that the AFB smear plus PCR dot-blot (PCR in-house) was more cost-effective than AFB smear plus culture, confirming the potential use of in-house PCR techniques in the diagnostic routine for ruling out of TB

diagnosis (Sherer et al, 2009). With PCR there is less time consumed in making a diagnosis, the method was patient friendly, it reduces costs to patients and, most importantly, PCR is not influenced by the HIV status of the patient. In addition, it showed a great improvement in sensitivity, negative predictive value, and offered an improvement for the ruling out of pulmonary TB diagnosis though generalisations of these findings could not be made as in-house PCR is setting dependent (Sherer et al, 2009). Botswana is a middle class country so it can try to apply these methods as was done in Brazil and would reduce the economic burden of managing false-negative results.

The E-AMTDT (Gen-ProbeA, Inc., San Diego, CA) is approved by the Food and Drug Administration (FDA) for the direct detection of MTB in both smear-positive and smear-negative respiratory specimens from patients suspected of having TB making it relevant in regions with TB/HIV co-infection. Results are available within 3.5 h after specimen decontamination. The sensitivity of this test in respiratory specimens compared with culture ranged from 85.7% to 97.8% and was higher in smear-positive specimens (91.7% to 100%) compared to between 40.0% and 92.9% for smear negative samples (Shamputa et al, 2004).

The clinical utility of the commercial NAA tests are that sputum specimens be collected on 3 different days for AFB smear and culture. The NAA test should be performed on the first specimen collected, the first smear-positive specimen, and additional specimens if needed. If the first result is both AFB smear-positive and NAA-positive, the patient can be presumed to have TB. However, if the specimen is smear-positive but NAA-negative, a test for inhibitors should be performed. This option is available in the Amplicor test, but if the E-MTD test is used, a specimen to which MTBDNA has been added must be analyzed. If inhibitors are not detected and additional specimens remain NAA-negative, the patient can be presumed to have non-tuberculous mycobacteria. However, if inhibitors are detected, the NAA test does not offer any diagnostic help (Soini & Musser, 2001).

If a specimen is smear-negative and NAA-positive and the same result is obtained with an additional specimen, the patient can be presumed to have TB. In the case that all sputum

specimens remain smear-negative and NAA-negative, the patient can be presumed to be not infectious; however, this does not exclude the possibility of active TB, and clinical judgment must be used in decisions regarding TB therapy (Soini & Musser, 2001).

Generally, all the NAA tests have higher sensitivity compared to sputum smear and approach the sensitivity of culture while providing results in a very short period of time (Tsara et al, 2009; Wong, 2008; Glassroth, 2004). Salami (2006), pointed out that NAA are also important for epidemiologic purposes, as they help in studying patterns of infection within a population through identification of the points of transmission by "DNA fingerprinting," which allows differentiation of unrelated strains of MTB by demonstration of nucleotide sequence differences at selected sites in their DNA genome. In addition, it helps differentiating MTB from other atypical mycobacteria if different DNA probes are used that are specific for the common species of mycobacterium (Wong, 2008).

Associated limitations of these molecular techniques are the naturally occurring inhibitors and, because of their high sensitivity, are more easily contaminated leading to falsely positive results. In addition, they are more expensive on a per test basis than conventional culture systems particularly in laboratories processing a low volume of specimens (Glassroth, 2004).

In conclusion, the currently available NAA tests can enhance diagnostic speed, but they do not replace AFB smear or culture (Tsara et al, 2009; Wong, 2008; Soini & Musser, 2001). Because these tests can only detect MTB, cultures are still needed for identification of non-tuberculous mycobacteria and for drug susceptibility testing. NAA tests cannot distinguish between live and dead organisms, so they cannot be used to monitor TB therapy. Clinicians should interpret the NAA test results based on the clinical situation, and the tests should usually be performed at the request of the clinician (Soini & Musser, 2001).

2.4. PREVENTION AND CONTROL OF PTB

2.4.1. TB Preventive Therapy

HIV infection increases the risk of reactivation of latent TB infection (LTBI) to active disease as was mentioned above. LTBI may be treated using one or more of the available anti-TB drugs as a means of preventing disease in HIV-infected persons (Guwatudde et al, 2004). Clinical trials have shown that primary TB preventive therapy (PT) (i.e., treatment to prevent a first episode of TB among those with no history of the disease) reduces TB incidence among HIV-infected individuals (Date et al, 2010; Grant et al, 2005). Results of a meta-analysis suggest that Isoniazid Preventive Therapy (IPT) reduces TB incidence by 33-42% overall, or by 60-64% among individuals who have positive tuberculin skin tests (Date et al, 2010; Grant et al, 2005). In view of these findings, the WHO, the Joint United Nations Programme on HIV/AIDS (UNAIDS), and the International Union Against TB and Lung Disease (IUATLD) recommend PT for tuberculin skin test (TST)-positive, HIV-infected persons who do not have active TB (Date et al, 2010; Grant et al, 2005; Guwatudde et al, 2004).

However, this recommendation has not been widely implemented in many countries, partly because of operational obstacles such as attrition during assessment for IPT, particularly non-return for tuberculin skin test results in low prevalent TB countries or logistic difficulties in performing tuberculin skin tests to diagnose latent TB infection in high incident countries and inadequate intensified case finding (Date et al, 2010; Grant et al, 2005). In addition, this strategy may be compromised by low adherence over the many months necessary to complete a single course of IPT (Cohen et al, 2006) for example in Botswana one need to be on IPT for six months. Furthermore, some clinicians and policy-makers still have the fear that using Isoniazid (INH) mono-drug therapy will increase the risk of promoting INH resistance and the current uncertainty about the long-term benefits of IPT (Date et al, 2010; Grant et al, 2005). However studies from the pre-HIV era did not support this concern, as long as close monitoring of resistance patterns to TB drugs is done (Grant et al, 2005).

In Botswana a successful pilot study on usefulness and feasibility of implementing IPT was discovered in 2001 and from then all districts offer IPT to HIV infected individuals. These individuals are thoroughly investigated for active TB before commencing IPT. Being an endemic area of TB, TST is not requested prior to IPT (BNTP, 2007). A study which was done in South Africa revealed that if IPT is routinely provided to HIV infected individuals; it reduces the incidence of TB by 38% overall and by 46% among individuals with no history of TB (Grant et al, 2005). Despite the above evidence, it is not clear that communitywide IPT will prove effective at controlling TB in areas with a high burden of HIV as recent theoretical work suggest that exogenous re-infection is common in high incidence areas hence individuals are likely to be re-infected after completion of a single course of IPT (Cohen et al, 2006).

2.4.2. Directly Observed Therapy Short Course (DOTS)

The DOTS strategy for TB control was launched by the World Health Organization (WHO) in 1995. It has four key technical pillars: detection of smear-positive pulmonary TB (SPPTB) using sputum microscopy, in patients presenting themselves to public clinics; directly observed treatment with short-course chemotherapy of which the main aim is to reduce the average number of people infected by each infectious case sufficiently to interrupt transmission; guaranteed continuous drug supply; and a case recording system tracking treatment outcomes (Obermeyer et al, 2008; WHO, 2002). Through the strategy, TB is controlled by rapid identification of SPPTB and treating these cases thus detection of 70% of new smear positive cases, and cure of 85% of such cases, (Wood et al, 2007; WHO, 2006).

DOTS strategy has been effective in many settings, particularly where levels of HIV are relatively low. Its implementation in 182 countries has helped in improving national TB control programmes (NTPs) in controlling TB. By 2004, more than 20 million patients had been treated in DOTS programmes worldwide and more than 16 million of them had been cured. Mortality due to TB has been declining and incidence diminishing or stabilizing in all world regions except sub-Saharan Africa and, to some extent, Eastern

Europe (WHO, 2006). The reasons for this are not very clear as approaches to increase treatment adherence e.g. involvement of community health workers and community members are implemented in this region. Results from five pilot projects (in Botswana, Kenya, Malawi, South Africa and Uganda) emphasising the roles of community and primary care facility in TB treatment show that these approaches are generally lower cost and more cost-effective, while maintaining satisfactory effectiveness (treatment success), compared to the traditional approach using hospital in-patient care (WHO, 2002).

After a decade of its implementation, DOTS failed to contain TB in sub-Saharan Africa, largely because of the parallel epidemic of HIV in the region; lack of infrastructure and adequate resources (Wood et al, 2007). Even in well run DOTS programmes, TB notifications rates remained high because the initial symptoms of TB are non-specific; even when a sputum smear is ordered, the sensitivity of this test is too low to detect active TB. In addition, patient delays in seeking care and healthcare worker delays in recognising and starting treatment for TB which would contribute to transmission (Brewer & Heymann, 2004). Furthermore, DOTS pays little or no attention to smear negative cases yet these patients can and do transmit TB infection to others (Tostmann et al, 2008; Brewer & Heymann, 2004) even though smear positive patients are more infectious, hence reduce its effectiveness. As a result, controlling TB requires much more than paying attention to people with sputum positive disease.

Because of the limitation of DOTS and dynamics in the TB disease, WHO launched Stop TB strategy in 2006 so to address major challenges in TB control thus expanding access to diagnosis and treatment through community TB care, and public-private mix (PPM) approaches aimed at engaging all care providers – state and non-state – in DOTS implementation. Innovative mechanisms have been developed to improve access to quality-assured and affordable drugs in resource-poor settings such as the Global Drug Facility and the Green Light Committee. The collaborative activities that need to be implemented by TB and HIV/AIDS control programmes have been developed to deal with emerging types of TB like HIV-TB and multidrug-resistant TB (MDR-TB) (WHO, 2006). Thus TB programmes and HIV programmes should share mutual concerns:

prevention of HIV should be a priority for TB control; TB care and prevention should be priority concerns of HIV/AIDS programmes especially in high TB/HIV prevalent areas.

The aim of TB treatment is to achieve cure, to prevent death and relapse, and to render patients non-infectious as rapidly as possible, as well as to prevent the emergence of drug resistance. Anti-TB agents are, therefore, selected (1) to kill the actively metabolizing bacilli in the cavities, (2) to destroy less actively replicating bacilli in the acidic and anoxic closed lesions, and (3) to kill near-dormant bacilli that might otherwise cause a relapse of the disease. The most effective agents for the destruction of tubercle bacilli are, respectively, isoniazid, pyrazinamide, and rifampicin (Onyebujoh et al, 2007). These 3 agents form the basis of the recommended WHO DOTS regimens as is shown below. Botswana follows the WHO guidelines on patient categorization and management. Currently they are using fixed-dose drug combinations (FDCs) to facilitate adherence to treatment and to reduce the risk of the development of drug resistance.

Table 2-2 Recommended treatment regimens in Botswana

TB Diagnostic Category	TB Patient	Initial Phase Regiment	Continuation Phase Regiment
1	All new cases of TB and severe cases of TB in children	2HRZE (2HRZS in TB meningitis)	4HR
2	Previously treated cases of TB -retreatment after relapse -retreatment after default -retreatment after treatment failure	2HRZES/1HRZE	5HRE
3	Less severe cases of TB in children	2HRZ	4HR
4	Chronic and MDR-TB cases (still sputum positive after supervised retreatment)	Specially-designed standardized or individualized regimens are recommended.	

Adapted from; BNTP Manual (2007) (*H=isoniazid, R=rifampicin, Z=pyrazinamide, E=ethambutol, S=streptomycin*). Number refers to number of month on a regiment.

The ultimate goal of eliminating TB depends on new diagnostics, drugs and vaccines. Clinical studies were conducted by the South African Medical Research Council in Durban, South Africa and concluded that treatment with either the gatifloxacin- or moxifloxacin-containing regimen (which are new drugs) was significantly more efficacious than either the standard 6-month regimen (2ERHZ/4HR) or an ofloxacin-containing regimen (the same regimen, with ethambutol replaced by ofloxacin (2ORHZ/4HR) after 2 months of treatment. Currently, a phase 3 clinical trial is ongoing

to evaluate the efficacy and safety of gatifloxacin-containing TB treatment in a mixed population of HIV-infected and -uninfected patients with TB (Onyebujoh et al, 2007). Hopefully this will enhance the NTPs to control TB.

2.4.3. Infection Control

2.4.3.1. Education of patients and community awareness

According to WHO (1999), more than one-third of HIV-infected persons living in areas with widespread TB will develop TB disease during their lifetime so educating communities and patients to recognize symptoms of TB and to seek health care and further investigations should be routine in these settings. Patients should understand how to protect themselves, and others, from exposure to TB by simple cough hygiene measures such as covering their noses and mouths when coughing or sneezing (WHO, 1999).

2.4.3.2. Environmental control measures

These are measures that are used in high-risk areas e.g. hospitals, prisons, etc to reduce the concentration of droplet nuclei in the air. They include ventilation (natural and mechanical), filtration, and ultraviolet germicidal irradiation (UVGI). The last two are technologically complex and expensive therefore are only practical in the private sector and in countries where resources are not limited (WHO, 1999). However, controlled natural ventilation can be effective in reducing the risk of spreading TB.

In areas where ventilation is assumed to not provide adequate protection and the country can afford UVGI and filter units, these should be used. Studies have shown that MTB is killed if the organisms are exposed to sufficient UVGI. Continuous upper air irradiation is the most applicable method in most resource-limited countries. The advantage of this technology is that the upper air is continuously being irradiated; thus, it provides some protection while the infectious patient is in the room. This requires good air mixing to be effective. Structural features such as ceiling height may limit the feasibility and

usefulness of UVGI. In addition, adverse reactions, such as acute and chronic skin and eye changes from overexposure are common if the UVGI is not installed and maintained properly (WHO, 1999).

2.4.3.3. Personal respiratory protection

Respirators are expensive to purchase and require specialized equipment to determine proper fit. Frequently, they are unavailable in resource-limited settings. If a respirator is needed, a certified N95 (or greater) or certified FFP2 (or greater) respirator should be used.

Respirators are different from face masks, such as surgical masks made of cloth or paper. Use of a face mask does not protect health care workers, other staff, patients, or visitors against TB. Therefore, it is NOT recommended that health care workers and other staff or visitors wear them. The N95 masks give adequate protection against inhalation of infectious droplet nuclei that may contain M.TB (BNTP, 2007; WHO, 1999).

It is important to note that Bacille Calmette-Guérin (BCG) vaccination does not reduce the risk for infection but it decreases the risk for progression from latent TB infection to active TB, especially disseminated or central nervous system disease in children (WHO, 2006; WHO, 1999).

2.4.4. Botswana National TB Control Programme (BNTP)

The Ministry of Health is the central government organ with the portfolio of responsibility for overall improvement and maintenance of national health. In line with this responsibility, the Ministry of Health sets broad policy directives, goals and strategies for health development and delivery. It established the Botswana National TB Control Programme in 1975, with technical assistance from WHO (Rakgailwane & Sekgwake, 2007; BNTP, 2007). Rakgailwane & Sekgwake (2007) noted that, the major objective of the TB Programme was to reduce the number of TB cases in the country, bearing in mind the financial implications. For that purpose, the Ministry of Health

developed a TB manual to be followed to ensure that the objectives of the TB Programme were met.

In these TB manuals the guidelines on the management of TB (including SNPTB) are clearly described. SNPTB is common among HIV-positive patients (Getahun et al, 2007; Aderaye, 2007; Colebunders & Bastian, 2000; Harries et al, 1998;) as a result clinicians in Botswana are inducted on the management of TB (including SNPTB) through in-service lectures, refresher courses or the use of National TB Program Manuals. The Botswana National TB Program (2007) recommends the use of these guidelines which they adopted and adapted from the recommendations made by World Health Organization, the International Union Against TB and Lung Disease and United States Centers for Disease Control, so if the guidelines are not properly used by clinicians other opportunistic respiratory infections are misdiagnosed as smear negative PTB in HIV co-infected patients.

However, it was observed that the BNTP did not have a clear mechanism in place to ensure that the said guidelines were understood and if they were properly used in the field (Rakgailwane & Sekgwake, 2007). In addition, there is little information in sub-Saharan Africa on whether these guidelines are used for diagnosing suspected TB (Harries et al, 1998). Furthermore, with the low sensitivity and specificity of the microscopic examination of the sputum in HIV-positive patients and increasing cases of SNPTB, the adopted and adapted diagnostic guidelines are hoped to assist the clinicians with the diagnosis of SNPTB in Botswana. The BNTP guidelines for the diagnosis of SNPTB are: (1) cough for at least 2 weeks, (2) at least two smear negative results including at least 1 early morning specimen, (3) chest X-ray (CXR) findings consistent with PTB, and (4) lack of response to a course of broad spectrum antimicrobial agents (BNTP, 2007).

For the guidelines number 2 and 3 the BNTP (2007) specifically states that a CXR may be done earlier but **always** submit sputum for acid fast bacilli examination.

2.4.5. Clinical diagnosis of SNPTB

Diagnosis of SNPTB is a difficult task, and in developing countries, the majority of these cases has been treated only on the basis of clinical and chest radiographic findings. The sensitivity of the AFB smear result is known to be poor, varying between 30% and 70% depending on a number of factors relating to how the test is implemented (Long, 2001). Without a standardized clinical work up, the misdiagnosis rates have been estimated as high as 35% to 52% (Mello et al, 2006). Getahun et al (2007) states that patients should be correctly diagnosed and treated for SNPTB but treating those without the disease should be avoided.

New diagnostic tools such as nucleic acid amplification (NAA) tests are not affordable in developing countries. Hence more effort has been employed to develop guidelines which can assist clinicians in diagnosing SNPTB. The recommended guidelines have resulted in a longer health-service delay in making the diagnosis of SNPTB than SPPTB because the diagnostic guidelines needs not less than seven days to establish the diagnosis of SNPTB under the most optimistic scenarios, if applied in a linear fashion. Such delays in diagnosis could be life-threatening (Getahun et al, 2007).

In some countries, clinical peer review of the suspected case of SNPTB by a clinical team was used to establish the diagnosis of SNPTB (Getahun et al, 2007). This shows how difficult it is to make a diagnosis of SNPTB in HIV-positive patients. However, before the HIV epidemic some national TB programs (NTP) were not monitoring the outcome of SNPTB as the majority had a good outcome, with some patients recovering without TB treatment (Hargreaves et al, 2001). In Malawi the reported case fatality of SNPTB in 2000 was 25% which was the same as smear positive PTB. This then prompted the Malawi NTP to make routine follow-up of SNPTB (Hargreaves et al, 2001) but the spread of TB had occurred as these cases do transmit MTB.

Twenty per cent of patients with suspected PTB in Botswana were smear-negative but culture-positive (Morse et al, 2008); nevertheless, cultures are not routinely performed on smear-negative sputum in Botswana. Autopsy studies of HIV-positive patients in

Ethiopia have shown that TB was the cause of death in 14% -54% of deaths, and TB was not diagnosed before death (Aderaye, 2007). In other studies it was noted that the high prevalence of SNPTB was attributed to the clinicians' non-adherence to diagnostic guidelines and absence of diagnostic tools to rule out other HIV-related pulmonary infections (Mesfin et al, 2005).

In addition, in a study in Uganda, patients with respiratory symptoms for more than three weeks and infiltrates on chest-x-ray (CXR) were sent for bronchoscope and bronchoalveolar lavage. The fluid was stained for alcohol and acid fast bacilli and PCP and cultured for bacteria and fungi. The major cause of disease was found to be PCP (38.6%), *Mycobacterium TB* (24%), to a lesser extent pulmonary Kaposi sarcoma (11%), pyogenic bacteria (8%) and no obvious cause in the remainder (Worodria et al, 2003).

In developing countries with high HIV prevalence, human error due to fatigue and demotivation induced by the lengthy and monotonous process of examining the smears as well as low remuneration affect the performance of laboratory personnel. It could also be presumed that many patients are misclassified due to insufficient time for laboratory technicians to examine properly the large number of sputum smears for presence of AFB (Mfinanga et al, 2007; Harries et al, 1998). Without a standardized clinical work up, the misdiagnosis rates have been estimated to be as high as 35% to 52%.

Table 2-3 Other causes of false-negative sputum smear findings

Stages	Cause
Sputum collection	Inadequate sputum sample Inappropriate sputum container Sputum stored too long before microscopic examination
Sputum processing	Faulty sampling for smear Faulty smear preparation and staining
Smear examination	Inadequate time spent examining smear Inadequate attention to smear
Administration	Misidentification of patient Incorrect labelling of sample Mistakes in documentation

Source: Long (2001)

2.4.5.1. Sputum Microscopy

The standard WHO recommendation for TB diagnosis in the DOTS program is the use of direct sputum microscopy on 3 stained sputum specimens (Matee et al, 2008). This is the same recommendation in Botswana. First and third are on spot, while the second is the early morning sample for outpatients (Matee et al, 2008; BNTP, 2007). For inpatients, three early morning specimen collected on three consecutive mornings is recommended (BNTP, 2007). Like other sub-Saharan countries, Botswana has the greatest TB disease burden and limited resources (only two laboratories which can perform sputum culture) hence culture is not routinely recommended as diagnostic tool for SNPTB (BNTP, 2007).

As was mentioned above, the sensitivity of sputum microscopy is low and is further reduced by HIV to confirm PTB in suspected cases. HIV also reduces the specificity of sputum microscopy by increasing the proportion of patients with non-tuberculous mycobacteria (Matee et al, 2008). A patient would be considered to have SNPTB if he presents with 1) three negative smear results and radiological findings and doctor's decision to treat for PTB 2) if the smear results are negative and a positive culture result

for MTB, and 3) patient is unable to produce sputum and has highly suspicious radiological and clinical findings and doctors decision to treat for PTB (BNTP, 2007).

2.4.5.2. *Chest X-ray (CXR)*

Chest radiograph (X-ray) is an important tool in supporting the diagnosis of PTB in symptomatic patients whose sputum is negative for AFB, but it is not possible to diagnose PTB using CXR only (van Cleeff et al, 2005). The following radiological appearances suggest active PTB, and in HIV-positive patients, the picture depends on the immunological status: 1) shadows in one or both upper zones, 2) cavities in one or both upper zones, 3) miliary pattern, 4) persistent shadows after pneumonia treatment, 5) pleural effusion 6) a combination of any of the above (BNTP, 2007; van Cleeff et al, 2005).

However, the use of chest radiography for diagnosis of PTB can be compromised by poor film quality, low specificity, and difficulties with interpretation. HIV infection further diminishes the reliability of chest radiographs since the disease commonly presents with an atypical pattern (Getahun et al, 2007). According to van Cleeff et al, (2005), restricting CXR for the diagnosis of smear-negative TB among smear-negative suspects, the proportion of over-diagnosis is as high as 23%. The chest radiograph can be normal in up to 14% of HIV-infected patients with sputum-culture-positive TB (Getahun et al, 2007). The timing for the CXR varies in different countries. In Botswana, a CXR can be done after collection of 2-3 sputum specimen or before collection of the sputum (BNTP, 2007) while in other countries it is done after examination of up to nine sputum smears (Getahun et al, 2007). Botswana's practice is consistent with the WHO recommendations which suggest that CXRs should be performed early in the course of investigation of a TB suspect so as to shortening delays in diagnosing SNPTB (WHO, 2006).

Reading of CXR depends on the experience and the interpretation skill of the reader making it subject to intra- and inter-reader variation. Studies conducted in the 1950s showed that readers have a tendency to under-read (21 – 39%) rather than to over-read

(2–19%), with less discrepancy when readers were more experienced (van Cleeff et al, 2005). A study in Japan using Miniature Mass Radiography found that around 20% of the cases with active TB were missed. In another study on X-ray classification in which 1,100 films were read by 90 experienced physicians and radiologists from 9 countries, up to 34% disagreement on the question: "is the film normal?" and a 28% disagreement on the question: "is there a cavity present?" were found (van Cleeff et al, 2005).

Several diseases including PCP, bacterial infections and cancerous conditions produce a similar picture to PTB especially in HIV infected patients such that the over-diagnosis of PTB is high while under-diagnosis of other conditions is low (Aderaye, 2007).

2.4.5.3. Use of antibiotics

Treatment with broad-spectrum antibiotics is used to exclude infections other than TB, and to improve the specificity of the diagnosis (Aderaye, 2007; Getahun et al, 2007). However, the WHO (2006) states that the primary role of antibiotics should not be as a diagnostic aid; they should be used to treat concomitant bacterial infection in people living with HIV/AIDS with cough or serious illness. Of note is that evidence for the use of empirical antibiotic treatment to rule out PTB as a cause of cough in HIV-infected persons is very limited, hence response to antibiotics does not exclude TB in TB suspects living in HIV-prevalent settings, though non-response make PTB more likely (WHO, 2006). Patients with TB can lose their respiratory symptoms after a course of antibiotics possibly due to the mycobacteriostatic properties of some of the drugs, and more likely due to the presence of superimposed infections (Aderaye, 2007; Getahun et al, 2007).

The WHO (2006) recommends that seriously ill patients with symptoms suggestive of TB should be treated empirically with broad-spectrum antibiotics because the benefits outweigh the risks. In Botswana this applies to patients who are going to be admitted and a short course of broad-spectrum antibiotics should be used to reduce the time delay for TB diagnosis. In this setting the choice of antibiotics should focus more on the treatment of PCP (BNTP, 2007). Fluoroquinolones are not recommended as antibiotics to be tried

in PTB suspects as they may cause undue delay in the diagnosis of TB and lead to drug resistance (WHO, 2006). In some countries (eg: Botswana) they are used as second line anti-TB drugs (BNTP, 2007). Common conditions which might and might not respond to antibiotics in HIV-positive patients are bacterial pneumonia, cryptococcosis, pulmonary nocardiosis, silicosis, Kaposi sarcoma and disseminated carcinoma. This has caused a dilemma in making a diagnosis in a resource-limited setting.

A trial of amoxicillin treatment screening in a setting where HIV prevalence is low, found that amoxicillin treatment had a significant impact on making a diagnosis of PTB, as 94% of patients who failed to respond to the antibiotic had a final diagnosis of PTB (Kudjawu et al, 2006). Also, some studies in South Africa, a country with a high prevalence of HIV infection, where screening with amoxicillin was followed, in the absence of a clinical response, by a course of erythromycin (broad spectrum antibiotic), found that this does improve diagnostic accuracy among SNPTB suspects with abnormal CXRs (Kudjawu et al, 2006). Duration of antibiotic prescriptions varies in different countries, ranging from 5 days to 28 days (Getahun et al, 2007). In Botswana, 3-5 days improvement should be ascertained before TB treatment is initiated (BNTP, 2007) though you will need to continue until a one week course is finished.

2.4.5.4. Duration of the cough

The diagnosis of PTB is influenced by the severity of the patient's illness, HIV status and on the level of care services available at facilities that patients present to. According to BNTP (2007), the key symptom to suspect PTB is a chronic cough (2-3 weeks).

However, in a study of persons with HIV infection living in Southeast Asia it was noted that, asking patient only about chronic cough was an insensitive approach to screening for TB (Cain et al, 2010). A similar study in Zimbabwe confirmed the same as they noted that the duration of cough had limited diagnostic utility in HIV-positive patients as both acute and chronic cough were having similar odds ratios for TB. In addition, acute cough, with or without radiological changes, has been reported to have a relatively high positive

predictive value for TB in areas where HIV is prevalent, and so should ideally be considered as a TB symptom in these contexts (Corbett et al, 2010).

The combination of symptoms such as cough of any duration, fever of any duration, or night sweats lasting 3 weeks or more during the previous 4 weeks accurately rules out TB in the vast majority of patients (Cain et al, 2010). A study in Zimbabwe however, evaluated the three symptoms: cough, drenching night sweat and weight loss and they noted that the sensitivity and specificity were similar for HIV-positive and HIV negative participants, while the presence of symptoms in HIV-positive participants had a higher positive predictive value and a lower negative predictive value (Corbett et al, 2010).

2.5. COMPLIANCE TO GUIDELINES FOR MAKING A DIAGNOSIS OF SNPTB

2.5.1. Studies outside Africa

It is vital to note that, any practitioner treating a patient for TB is assuming an important public health responsibility to prevent ongoing transmission of the infection and the development of drug resistance (WHO, 2009). A systematic approach needs to be applied to avoid both over- and under-diagnosis of TB, with the need for prompt treatment in a patient with an illness that is progressing rapidly. Over-diagnosis of TB when the illness has another cause will delay proper diagnosis and treatment, whereas, under-diagnosis will lead to more severe consequences of TB, including disability and possibly death, as well as ongoing transmission of MTB (WHO, 2009; Harries et al 1998).

Little is known about the adequacy of TB care but evidence from studies conducted in many different parts of the world show great variability in the quality of care, and poor quality care continues to plague global TB control efforts (Chiang et al, 2008; WHO, 2006). The WHO survey and other studies have shown that clinicians, especially those working in the private healthcare sector, often deviate from standard, internationally recommended, TB management practices. These deviations include under-utilization of sputum smear microscopy for diagnosis, generally associated with over-reliance on radiography; use of non-recommended drug regimens and mistakes in both drug dosage

and duration of treatment; and failure to supervise and assure adherence to treatment (WHO, 2009).

There have been increasing efforts to influence change in health professionals' behaviour and practice mainly driven by a realisation of unacceptable variations in service provision in healthcare (Siddiqi et al, 2008; Chiang et al 2008). Siddiqi et al, (2008) did a study in Cuba, Peru and Bolivia over two years with refresher courses in between so as to improve the diagnostic care for patients suspected of TB. Their findings were that clinical audit was most effective in improving standards of care in Cuba, but this had mixed results in Bolivia and limited success in Peru. Limited improvements were in some cases due to lack of coordination between the laboratories and health centres, lack of resources, political interference and perceived patients' beliefs.

In Pakistan a country with low HIV prevalence, the private sector makes a major contribution to providing health care for all kinds of health problems including TB. However, it was noted that none of the practitioners perform sputum microscopy for suspected case of PTB, these findings being similar to a study done in India (Shah et al, 2003). Similarly, in a Taiwanese study on general health facilities, it was found that clinician's practice in diagnosing PTB was not standardised as a high proportion of TB suspects were promptly put on anti-TB treatment based on CXR findings before sputum examination (Chiang et al, 2008). Self confidence of clinicians in interpreting CXR was the most facilitating factor in this sector. Recommendations were that clinicians should discontinue this practice and prescribe sputum examinations before making the decision to diagnose a patient with TB (Chiang et al, 2008).

2.5.2. Studies from sub-Saharan Africa

Techniques that are widely available in industrialized countries for obtaining pulmonary specimens such as fibre-optic bronchoscopy with bronchoalveolar lavage and for analysing them (such as culture, antigen detection and polymerase chain reaction) are beyond the resources of most hospitals in sub-Saharan Africa (Harries et al 1998). In

Ethiopia the National TB and Leprosy Control Program recommends good adherence to their guidelines since these other resources are not easily available. However the observed high prevalence of SNPTB in Ethiopia was attributed to the clinicians' non-adherence to diagnostic guidelines, and absence of diagnostic tools to rule out other HIV-related pulmonary infections (Mesfin et al, 2005).

Several studies were cited by Mesfin et al (2005) stating that improving adherence to the national diagnostic algorithm would increase the detection of smear positive PTB and in areas where HIV/TB co-infection is high, the use of rigorous diagnostic algorithms is recommended to improve detection of SNPTB. In their study, Mesfin et al (2005) found that non-adherence to the national diagnostic algorithm is a common problem in hospitals, contributing to the over-diagnosis of SNPTB in the districts. Of the 101 SNPTB patients, only 3 were diagnosed as per the national diagnostic criteria. The diagnostic algorithm used for PTB screening in Ethiopia was reported to be less sensitive and specific among HIV-infected suspects (Mesfin et al, 2005).

However, in Malawi nearly 60% of patients had all four diagnostic guidelines used by hospital staff in reaching the SNPTB diagnosis while 90% had used three or more (Harries et al, 2001). This is not unreasonable because the researchers did not strictly rely on history of cough and chest radiographic abnormalities provided there was a chronic respiratory illness, though these are requirements of their guidelines. Instead symptoms like weight loss were used. Reasons for this were that some patients with SNPTB cannot produce sputum, smear microscopy services may be interrupted because of staff shortages, microscope dysfunction or lack of reagents, and chest X-ray services may be interrupted for similar reasons (Harries et al, 2001). Nevertheless they accepted that non-adherence to diagnostic criteria, and mistakes in clinical diagnosis contributed, amongst other factors, to an over-recording of smear-negative PTB cases in Malawi (Harries et al, 2001).

In a South African study it was revealed that doctors and nurses do not have adequate knowledge or do not comply with the National TB Program and WHO guidelines when

diagnosing and treating TB patients (Loveday et al, 2008). In the hospital, the doctors lack clarity about sputum investigation and depend exclusively on CXRs to diagnose TB. This has led to many patients being placed inappropriately on TB treatment and others who may be smear or culture-positive to not be detected because of a failure to take sputum samples for microscopy and subsequent culture, as per the protocols in place (Loveday et al, 2008). The same was noted in Botswana as almost two thirds of the clinicians did not use smear microscopy as a diagnostic test for PTB (Huebener et al, 1997).

From the above it is clear that many African countries do not have sensitive diagnostic tools for TB hence the use of rigorous diagnostic algorithms is recommended to improve detection of PTB. According to Siddiqi et al (2008) different studies have shown that clinical audits do drive improvements in the quality of clinical care by encouraging use of guidelines in resource-poor settings. This study describes the clinicians' use of the national guidelines to make a diagnosis of SNPTB in HIV-positive patients considering that they are given all possible resources to use the guidelines. Furthermore it explores if other methods to exclude respiratory diseases that resemble SNPTB are being used. This has been noted to be the most cost effective response to the challenge caused by SNPTB on TB programs and the quality of care given to the HIV/TB co-infected patients.

CHAPTER 3 – METHODS

3.1. STUDY DESIGN

This was a quantitative descriptive study design which used two sources of data: Retrospective records and questionnaires for clinicians. Firstly, audits of the files of those patients who had completed SNPTB treatment and are HIV-positive were used and parameters used were compared with those in the guidelines. It was also determined from these files what other methods to exclude PTB were ever used before the diagnosis was made. Secondly, clinicians were asked to complete a structured questionnaire in order to establish the extent to which they report (1) compliance with the guidelines (2) use of other methods to diagnose SNPTB and (3) reasons for not complying with the guidelines.

3.2. STUDY SITE

Lobatse is one of the oldest towns in Botswana and is approximately 70km south of Gaborone. The town occupies 40 square kilometers and is surrounded by farms and villages. The population is projected to be 31000 by 2011 and currently is approximately 29700. The 1993 literacy survey showed that 81.3% of the population was literate. Women were more literate at 86.9% against 73.9% for men. Athlone is a general district hospital which provides services to the people from the five clinics within the town and many others in the nearby villages and some surrounding districts like Goodhope as well as Gaborone (Lobatse Urban Development Committee, 2003).

HIV/AIDS is one of the greatest concerns in the town. In 2000, 30% of deaths among the hospital patients were a result of AIDS. The most HIV/AIDS affected people are the young, productive age group of both males and females. By 2006 the prevalence of HIV in Lobatse was approximately 30.5% (WHO, 2008).

3.3. STUDY POPULATION

These were two study populations: (1) Retrospective records of SNPTB in HIV-positive patients, and (2) Clinicians who diagnose and treat these patients. The average number of

HIV-positive patients registered as smear negative TB cases within Athlone hospital per year was approximated to be 96. Thus the number of files which were meant to be reviewed was expected to be 384 after multiplying the number of patients per year by the number of years to be considered in the study which is from 2006-2009. There are eight doctors who diagnose and treat these patients, and all 8 were given a questionnaire to complete.

3.4. SAMPLE

There was no sampling and all the files and doctors were used by the researcher because the 2 study populations were relatively small. Only the records of those who had completed treatment with a diagnosis of SNPTB and were HIV-positive were considered. Sputum positive patients, HIV negatives and those with unknown HIV status were excluded.

3.5. DATA COLLECTION METHODS

Data Extraction Sheet and Structured Questionnaire:

A data extraction sheet (Appendix A) and a self administered questionnaire (Appendix B) were used to collect data.

Data collected from files:

Files of those patients who had completed SNPTB treatment and were HIV-positive were retrieved from the hospital records and were then audited by the researcher. The data extraction sheet covered all the parameters in the guidelines, as well as an item on whether other methods to exclude PTB were ever used before the diagnosis was made (Appendix A).

Data collected from clinicians:

An appointment was made to see each clinician in his / her office. The study was explained to the clinician, who was then asked to sign informed consent (Appendix C) if he / she was interested in taking part in the study. Thereafter the researcher left the questionnaire (Appendix B) with the doctor, and arranged for a time to collect it. The

questionnaire covers doctors' (1) compliance with the guidelines, (2) use of other methods to diagnose SNPTB, and (3) reasons for not complying with the guidelines. Data capturing was done using Microsoft Excel 2003.

3.6. DATA ANALYSIS

Descriptive statistics were used to analyze the data from the data extraction sheet and the questionnaire. Frequency distributions of all variables in the data extraction sheet (numbers of times guidelines were adhered to for: sputum results, weeks of cough, cases receiving antibiotics, CXR consistent with TB, other methods used to rule out TB) and the variables from the questionnaire (reported compliance, other methods used in making a diagnosis of SNPTB and reasons for not complying to guidelines) were calculated using Epi-Info version 3.4.3. Results were presented in the form of tables, pie charts and graphs. The clinicians' practice was rated against the recommended guidelines which were used as a reference for coding and capturing data.

3.7. RELIABILITY AND VALIDITY

The files that were used are the same files which were used for reporting to the Botswana National TB Program. The data extraction sheet contained the recommended guidelines and these were compared with what the clinicians were doing. A quality controlled data collection procedure was followed. Pre-testing of the checklist and questionnaire tools was done with a small number of files from the study admitted and outpatient cards, and doctors from the local clinics in the same district were used. Editing of the data collection tools was done after assessing the responses. Data on compliance with the guidelines was obtained from two sources (reported compliance from the questionnaire, and compliance according to patient records). This strengthened the validity of the study.

3.8. BIAS

Information bias was encountered when collecting data from files. Firstly, there was missing data from some of the files e.g. period of the cough was not always recorded.

Secondly, when extracting information from the records the researcher had difficulties with reading some of the handwriting, which may have resulted in errors in data. Also, information bias may also have been a problem with the questionnaires, since doctors may have been embarrassed about not following the guidelines, and this might have resulted in them giving incorrect information in the questionnaire. To reduce the errors introduced by this bias, verification of the data collected from records was done by asking the TB coordinator or health care provider to read the file as well, and doctors' identities were not included on the questionnaire. Also, information bias was minimized because the results from the records were used to verify the results from the questionnaire.

3.9. ETHICAL CONSIDERATIONS

Approval from the Medunsa Research and Ethics Committee was obtained first (MREC number, MREC/H/24/2010: PG, see Appendix D) and then permission from the Health Research and Ethics in the Ministry of Health of Botswana was obtained (Appendix E). After that the management of Athlone Hospital and the Lobatse District Health Team (LDHT) were approached with letters asking for permission to carry out the study (see Appendix F). Permission was granted in a short period of time from the hospital but the one from the LDHT took almost 2 months to be received. Informed consent was obtained from clinicians (Appendix C). Patients' records and doctors' completed data sets were kept strictly confidential.

CHAPTER 4 – RESULTS

4.1. RESPONSE RATE OF CLINICIANS AND DEMOGRAPHIC PROFILE OF RECORDS REVIEWED

4.1.1 Response rate of clinicians:

Of the eight clinicians given questionnaires only 87.5% (7/8) managed to complete the questionnaire which was designed to identify their compliance to the BNTP guidelines; use of other methods and reasons for not complying with guidelines when making a diagnosis of SNPTB in HIV-positive patients. The eighth clinician went on leave (outside the country) without submitting the questionnaire.

4.1.2 Demographic profile of records reviewed:

A total of 281 files were available that fitted the inclusion criteria and had complete data, and these were all reviewed. The mean age of patients in the records reviewed was 38±13 years (range: 15 to 82 years).

Table 4-1 Distribution of patients with respect to gender and age groups

Age group (years)	Males n (%)	Females n (%)	TOTAL n (%)
15-25	17 (45.9)	20 (54.1)	37 (100)
26-35	51 (49)	53 (51)	104 (100)
36-45	42 (59.2)	29 (40.8)	71 (100)
46-55	20 (55.6)	16 (44.4)	36(100)
56-65	13 (65)	7 (35)	20 (100)
>65 years	13 (100)	0 (0.0)	13 (100)
TOTAL	156(55.5)	125(44.5)	281(100.0)

n = number of patients

4.2. CLINICIANS' COMPLIANCE WITH RECOMMENDED GUIDELINES

4.2.1. Results from questionnaire:

4.2.1.1. Number of sputum results used:

Clinicians do not always use three sputum results to make a diagnosis of SNPTB in HIV-positive patients as shown in Table 4-2. The reasons ticked by the clinicians were 1) patients do not always submit the third specimen for sputum analysis (100% [7/7]); 2) having two sputum results and a CXR is adequate to make a diagnosis (42.9% [3/7]); 3) delays in getting results from the laboratory (85.7% [6/7]); 4) clinical history can be adequate to make a diagnosis (85.7% [6/7]).

Table 4-2 Use of 3 sputum results before making a diagnosis of SNPTB

3sputum results	Frequency	Percent	Cum Percent
Never	0	00.0%	00.0%
Sometimes	7	100.0%	100.0%
Always	0	00.0%	100.0%
Total	7	100.0%	100.0%

4.2.1.2. Period of cough considered before a diagnosis of SNPTB is made:

Clinicians valued the period of coughing differently in this study before they make a diagnosis of SNPTB as shown in Table 4-3. Reasons ticked by the 42.9% (3/7) clinicians who do not always consider the period of coughing before diagnosing SNPTB were 1) patients have a poor recall of the history of coughing (100% [3/3]); 2) some patients do not have a coughing history (66.7% [2/3]) and 3) coughing history does not differentiate PTB from other causes of chest infections (66.7% [2/3]).

Table 4-3 Period of cough considered before a diagnosis of SNPTB

Period of cough vital	Frequency	Percent	Cum Percent
Never	0	00.0%	00.0%
Sometimes	3	42.9%	42.9%
Always	4	57.1%	100.0%
Total	7	100.0%	100.0%

4.2.1.3. Trial of broad spectrum antibiotics before PTB treatment:

Most of the clinicians (85.7% [6/7]) ticked that they always start their patients on antibiotics before they start them on PTB treatment for SNPTB (Table 4-4). The reason given by the clinician (14.3% [1/7]) who only sometimes starts patients on antibiotics, was the fear of losing the patient to follow-up once he / she is on antibiotic treatment.

Table 4-4 Trial of antibiotics before PTB treatment

Trial of antibiotics	Frequency	Percent	Cum Percent
Never	0	00.0%	00.0%
Sometimes	1	14.3%	14.3%
Always	6	85.7%	100.0%

4.2.1.4. Period patients are prescribed the broad spectrum antibiotics:

The period of the antibiotic prescriptions were variable in this study as shown in Figure 4-1. Of the 57.1% (4/7) clinicians who do not always prescribe one week of antibiotics their reasons were: the fear of losing the patients (75% [3/4]) and to a lesser extent that some patients need more than one week of treatment (50% [2/4]). No clinician was afraid of introducing drug resistance due to trial of antibiotics.

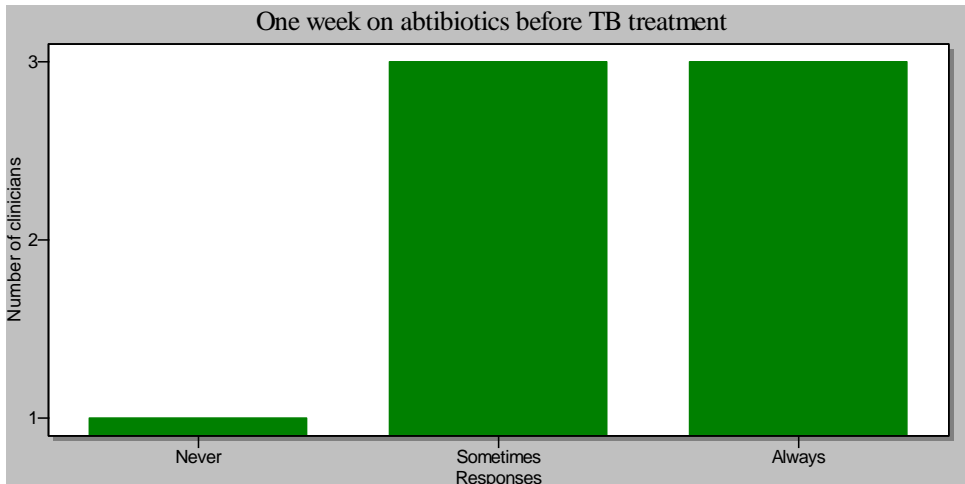


Figure 4-1 Trial of patients on antibiotics for one week

4.2.1.5. Use of CXR in diagnosing SNPTB:

All the clinicians (100% [7/7]) do a CXR before a diagnosis of SNPTB is made.

4.2.1.6 Diagnosis made on the CXR with features only of PTB:

The reliance on the CXR to make a diagnosis of PTB when it has features consistent of PTB is shown in Figure 4-2. For the 71.4% (5/7) who never or sometimes make a diagnosis on CXR findings which are consistent with PTB, 100% (5/5) reasoned that the CXR might be normal at the time of review. Others (60% [3/5]) suggested that the history is usually obvious hence the CXR findings will not contribute much. However, 40% (2/5) ticked that other respiratory causes should be excluded for it to be appropriate to start patient on TB treatment.

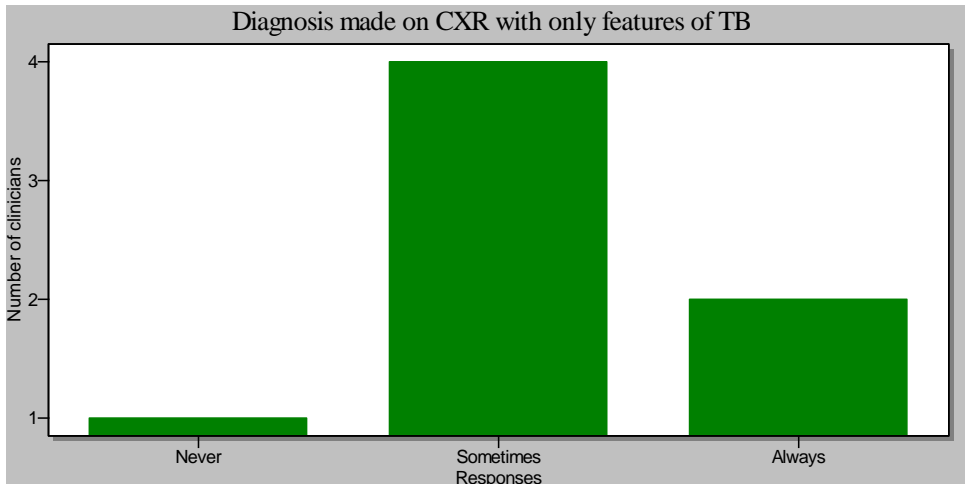


Figure 4-2 Making a SNPTB diagnosis only when CXR is typical of PTB

4.2.2. Results from records review:

4.2.2.1. Number of sputum results used:

The majority of the records (47.3% [133/281]) did not have the patients' sputum results when a diagnosis of SNPTB was made and only 22.8% (64/281) had 3 sputum results used in making the diagnosis (Figure 4-3).

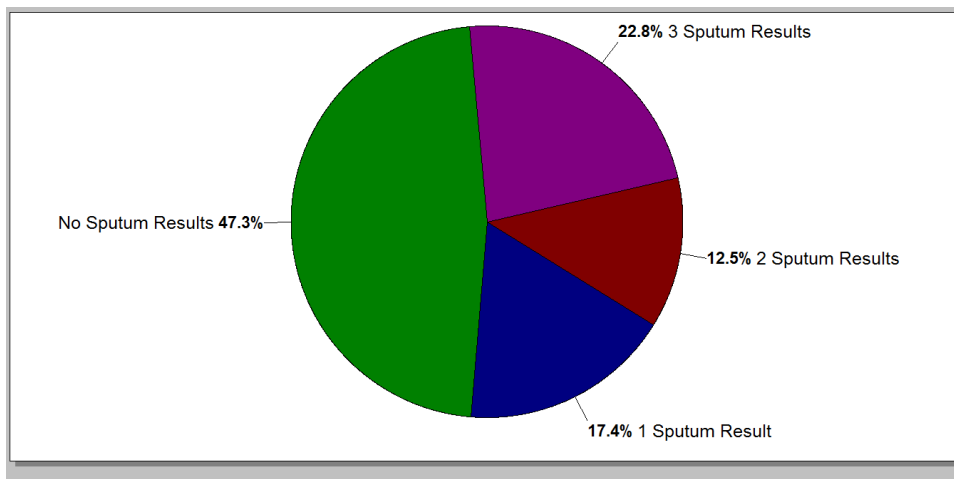


Figure 4-3 Distribution of sputum results

4.2.2.2. *Period of cough considered before a diagnosis of SNPTB is made:*

There was a variation in considering the period of coughing before patients were diagnosed with SNPTB as shown in Figure 4-4.

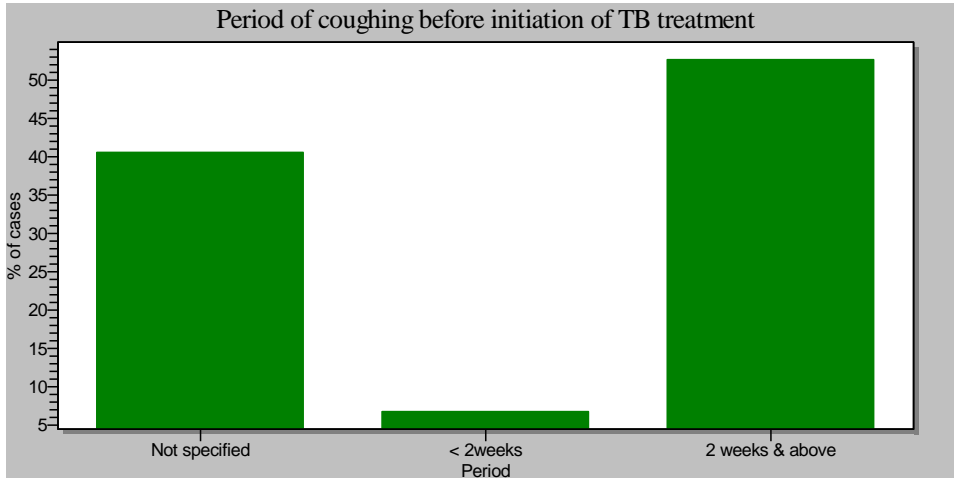


Figure 4-4 Period of coughing

4.2.2.3 *Trial of antibiotics and the period patients are kept on them:*

Use of antibiotics was only assessed among the inpatient as shown in Table 4-5 because the outpatient cards did not have an allocated space where a clinician is obliged to indicate if a trial of antibiotics was done. There were variations in the duration of the prescriptions of antibiotics as shown in Figure 4-5. A manual count of the number of days patients were kept on antibiotics was done on reviewing the drug charts of inpatients.

Table 4-5 Antibiotics given among admitted cases

antibiotics given	Frequency	Percent	Cum Percent
No	29	22.7%	22.7%
Yes	99	77.3%	100.0%
Total	128	100.0%	100.0%

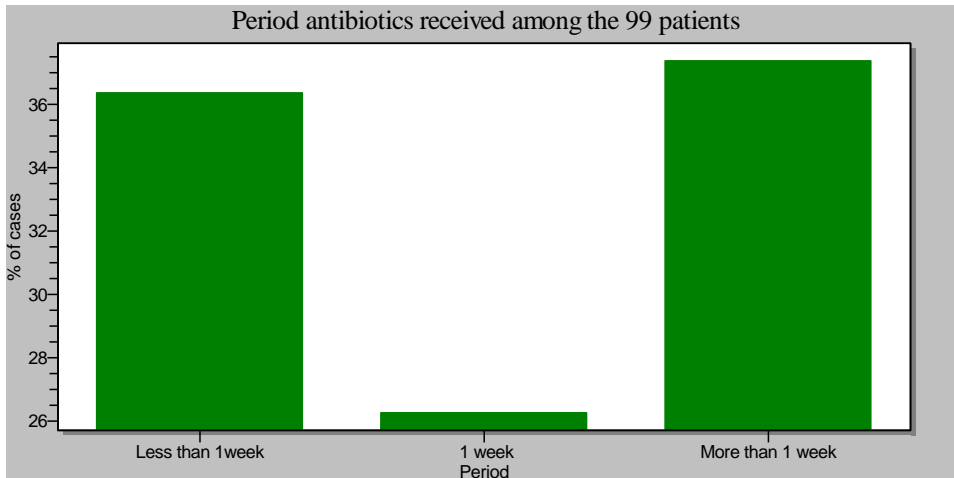


Figure 4-5 Period patients are kept on antibiotics

4.2.2.4. Use of CXR and the comments given regarding the CXR findings:

Out of the 281 records reviewed only one patient was started on TB treatment without a CXR. Of the 280 CXRs only 41.1% (115/280) were described as typical of PTB according to the common features typical of active PTB. More than half (55.4% [155/280]) had other descriptions such as “haze features”, “multiple patchy opacities”, “hilar patch infiltrates”, “fluffy infiltrates”, “severe lesions with consolidation”, “bilateral extensive infiltrates”, etc and some were just graphical presentations on the TB card. Very few records (3.6% [10/280]) had a normal CXR report.

4.2.2.5. Overall compliance to recommended guidelines:

Very few files (13.5% [40/281]) showed that patients were commenced on TB treatment according to the recommended guidelines (at least 2 sputum results; a cough of at least 2 weeks, CXR and with/out a trial of antibiotics).

4.3. USE OF OTHER METHODS TO RULE OUT OTHER RESPIRATORY INFECTIONS

4.3.1 Responses from the clinicians:

Responses to the possible methods which could be used in ruling out other respiratory infections as well as confirming the diagnosis of SNPTB are shown in Tables 4-6 to 4-12.

Table 4-6 Blood Cultures

Blood Culture	Frequency	Percent	Cum Percent
Never	1	14.3%	14.3%
Sometimes	6	85.7%	100.0%
Always	0	00.0%	100.0%
Total	7	100.0%	100.0%

Table 4-7 Bronchoscope for biopsy and aspiration

Bronchoscope	Frequency	Percent	Cum Percent
Never	6	85.7%	85.7%
Sometimes	1	14.3%	100.0%
Always	0	00.0%	100.0%
Total	7	100.0%	100.0%

Table 4-8 Sputum Culture for MTB

Culture	Frequency	Percent	Cum Percent
Never	2	28.6%	28.6%
Sometimes	5	71.4%	100.0%
Always	0	00.0%	100.0%
Total	7	100.0%	100.0%

Table 4-9 Chest-X-Ray

CXR	Frequency	Percent	Cum Percent
Never	0	00.0%	00.0%
Sometime	7	100.0%	100.0%
Always	0	100.0%	100.0%
Total	7	100.0%	100.0%

Table 4-10 Fluorescent Microscopy (FM)

FM	Frequency	Percent	Cum Percent
Never	7	100.0%	100.0%
Sometimes	0	100.0%	100.0%
Always	0	100.0%	100.0%
Total	7	100.0%	100.0%

Table 4-11 PCR for MTB

PCR	Frequency	Percent	Cum Percent
Never	7	100.0%	100.0%
Sometimes	0	100.0%	100.0%
Always	0	100.0%	100.0%
Total	7	100.0%	100.0%

Table 4-12 Sputum for microscopy, culture, and sensitivity

Sputum MCS	Frequency	Percent	Cum Percent
Never	0	00.0%	00.0%
Sometimes	5	71.4%	71.4%
Always	2	28.6%	100.0%
Total	7	100.0%	100.0%

4.3.2. Results from records review:

Besides the CXR and sputum for AFBs which are in the guidelines, only 2.1% (6/281) of the records reviewed had other methods used to rule out other respiratory conditions. These methods included (a) blood culture (50.0% [3/6]), (b) sputum for microscopy and culture for bacteria and fungus (33.3% [2/6]), and (c) sputum culture for MTB (16.7% [1/6]).

4.4 DIFFICULTY IN COMPLYING WITH THE GUIDELINES

All clinicians (100% [7/7]) in this study were aware of the BNTP recommended guidelines for the diagnosis of SNPTB. Only 14.3% (1/7) clinician did not have a copy of the guidelines at the time of the research. Guidelines were found to be difficult to use by 42.9% (3/7) while a minority 28.6% (2/7) ticked that they were not relevant to clinical practice. All clinicians (100% [7/7]) ticked that not all cases of suspected PTB were able to submit sputum for analysis while 57.1 % (4/7) noted that patients do not submit sputum on two consecutive days (one spot and early morning specimen).

The majority of the clinicians (71.4% [5/7]) ticked that the turnover of the results at the laboratory (waiting more than 24hrs) had an effect to the flow of their management while 14.3% (1/7) did not trust the accuracy of the results. The fear of losing patients during investigations was ticked by 42.9% (3/7) while absence of the Lung Disease physician was another reason ticked by all clinicians (100% [7/7]) as reasons not to comply. Of the 7 clinicians 4 (57.1%) noted that (a) the lack of feedback from the BNTP on the

clinicians' practice contributed to the non-compliance, and (b) feedback from BNTP would make a difference in the clinical practice.

CHAPTER 5 – DISCUSSION AND CONCLUSION

In Botswana, TB is a major public health problem and second most important health problem after HIV/AIDS, being one of the leading causes of death in adults (Rakgailwane & Sekgwake, 2007). The aim of accurate diagnosis and treatment of PTB is to achieve cure, to prevent death, TB relapse, render patients non-infectious as rapidly as possible, as well as to prevent the emergence of drug resistance (Onyebujoh et al, 2007). The aim of this study was to investigate the clinical diagnosis of SNPTB in HIV-positive patients at Athlone Hospital in Botswana by establishing the extent to which clinicians comply with the recommended national guidelines; extent to which other methods were used in ruling out other respiratory conditions and confirming a diagnosis of SNPTB and the reasons they give for not complying to guidelines.

5.1. CLINICIANS' COMPLIANCE TO GUIDELINES AND REASONS FOR NON-COMPLIANCE

This study has shown that clinicians have difficulties in complying with the BNTB guidelines when making a diagnosis of SNPTB which could lead to misdiagnosis. All clinicians who completed the questionnaire did not always use three microscopic sputum results when making a diagnosis of SNPTB. This result was supported by the records reviewed which found that only 22.8% of SNPTB cases with HIV were started on TB treatment with exactly three recommended sputum results. The reasons for not complying with this guideline were that, patients do not always submit the third specimen for sputum analysis; delays in releasing the results from the laboratory; adequacy of clinical history which would warrant a diagnosis with no further investigations; and to a lesser extent two sputum results and CXR findings.

Currently, there is a debate about whether two or three sputum specimens should be examined as some have suggested that reducing the recommended number of specimens examined to two could benefit TB control programs by using fewer resources and by reducing the time spent on case detection (Matee et al, 2008). However, the third sputum specimen has been reported to be important when investigating for PTB. For example in

a systematic review of 37 studies it was noted that the third specimen increased the sensitivity of microscopy by 3.1% mainly due to very good instructions for the spot third specimens and improved cough technique by the time of the third specimen (Matee et al, 2008). This could be the reason for the standard WHO and BNTP recommendation for PTB diagnosis in the DOTS program to use direct sputum microscopy on 3 stained sputum specimens (WHO, 2006; BNTP, 2007).

There was a better compliance to the consideration of the period of coughing before starting patient on TB treatment as was revealed by the completed questionnaire and the records reviewed. The few clinicians who do not follow this guideline reasoned that patients had a poor recall of the history of coughing, some would not have a coughing history and such a history does not differentiate PTB from other respiratory infections.

Although clinicians complied very well with the consideration of the period of coughing, there are studies which have reported that the duration of cough had limited diagnostic utility in HIV-positive patients as both acute and chronic cough had similar odds ratios for PTB (Corbett et al, 2010). Absence of a combination of symptoms such as cough of any duration, fever of any duration, or night sweats lasting 3 weeks or more during the previous 4 weeks accurately rules out tuberculosis in the vast majority of patients (Cain et al, 2010) other than relying on one symptom. However, the BNTP manual specifies that the key symptom to suspect PTB is a chronic cough (2-3 weeks) (BNTP, 2007).

In this study, trial of antibiotics was well adhered to although it was assessed only among admitted patients. All clinicians except one ticked that they always try their patients on antibiotics before a diagnosis of SNPTB is made. This was confirmed from the records reviewed. Records of outpatients by virtue of their design do not oblige a clinician to record antibiotics given before he/she initiates a patient on TB treatment. The reason for not complying was the fear to lose patients while on trial of antibiotics.

The role of antibiotics is controversial as different studies highlighted different evidence in using them. For example, WHO (2006) states that the primary role of antibiotics

should not be a diagnostic aid; they should be used to treat concomitant bacterial infection in people living with HIV/AIDS. They went on to say that response to antibiotics does not exclude TB in tuberculosis suspects living in HIV-prevalent settings (WHO, 2006). However, in South Africa, a country with high HIV prevalence, amoxicillin course followed by erythromycin course improved diagnostic accuracy among SNPTB suspects with abnormal CXRs (Getahun et al, 2007). With this, use of broad-spectrum antibiotics was noted to increase the specificity of PTB diagnosis (Aderaye, 2007; Getahun et al, 2007).

Not all clinicians were always giving a weeks' prescription of the antibiotics and this was confirmed from the records reviewed. The clinicians reasoned that they were afraid to lose patients while on antibiotics and some of the patients would want more than one week of treatment. However, no one was afraid of introducing drug resistance.

Anecdotally, there are common conditions which resemble PTB and do not respond to antibiotics in HIV-positive patients such as, cryptococcosis, pulmonary nocardiosis, silicosis, Kaposi sarcoma and disseminated carcinoma resulting in prolonged periods on antibiotics. In addition, Botswana has clinicians who have trained in different countries which might contribute to the variation of periods of prescriptions, which have been found to vary from 5-28 days in different countries for patients suspected of PTB with HIV (Getahun et al, 2007).

All clinicians do CXRs before making a diagnosis of SNPTB. However from the records reviewed there was only one case (0.4%) without a CXR done. Furthermore, very few clinicians (28.6% [2/7]) ticked that they always make a diagnosis of SNPTB when the CXR findings are consistent with PTB. Those who sometimes or never rely on CXR alone reasoned that the CXR can be normal in active PTB; other respiratory infections would need to be excluded before making a diagnosis and there is need of history to support the CXR.

A CXR is an important tool in supporting the diagnosis of PTB in symptomatic patients whose sputum is negative for AFB, but it is not possible to diagnose PTB using CXR only (van Cleeff et al, 2005). There was an observed over reliance on CXR for the diagnosis of PTB because of the ease and availability of radiological investigations in Botswana (Huebner et al, 1997). This could have fuelled the overwhelming requests of CXRs over sputum investigations in this study.

However, the over reliance on CXR for diagnosing PTB is usually compromised by poor film quality, low specificity, difficulties with interpretation and HIV infection which further diminishes its reliability since the disease commonly presents with an atypical pattern (Getahun et al, 2007). Different studies have noted that reading of CXR depends on the experience and the interpretation skill of the reader making it subject to intra- and inter-reader variation (van Cleeff et al, 2005). This was noted in our study as more than half of the records (55.4% [155/280]) had other descriptions of the CXR findings such as haze features, multiple patchy opacities, hilar patch infiltrates, fluffy infiltrates, severe lesions with consolidation, bilateral extensive infiltrates, etc., and some were just graphical presentation on the TB card. Some of these descriptions are not consistent with the expected findings in PTB cases but they were labelled SNPTB cases.

Other reasons which were not directly associated with the specific guidelines but contributed to the non-compliance were absence of the Lung Disease specialist at the hospital; some clinicians not having the copy of the guidelines; the lack of relevance of the guidelines and the difficulties in using them in clinical practice. Furthermore, the BNTP was not reviewing and providing feedback on the clinical practice for SNPTB diagnosis and the clinicians highlighted that a lot could be achieved if BNTP provides feedback and recommendations. According to Rakgailwane & Sekgwake, (2007) the BNTP has been lagging for a long time to apply mechanisms to ensure that PTB guidelines were understood and applied in clinical practice.

Overall compliance with the recommended guidelines was very poor in this study as we noted that only 13.5% of files audited had at least three criteria used among the patients

(thus at least 2 sputum results, cough for at least 2 weeks, a CXR and with/out trial of antibiotics). In countries with high burden of HIV, an increase in SNPTB diagnosis was attributed to HIV co-infection as other HIV-related pulmonary diseases that clinically resemble PTB were found to increase the diagnosis of SNPTB in resource poor African countries where other diagnostic techniques are unavailable (Mesfin et al, 2005). This study was done in a location where HIV prevalence is high at approximately 30.5% and in 2000, 30% of deaths among the hospital patients were a result of AIDS (WHO, 2008).

This poor compliance could explain the rise in the number of notified cases of TB in Botswana if our results could be generalised.

5.2. USE OF OTHER METHODS TO RULE OUT OTHER RESPIRATORY CONDITIONS AND CONFIRMING SNPTB

The options for performing other methods when confronted by a PTB suspect were very limited in our study. Though the majority of the clinicians highlighted that they can do sputum microscopy, sensitivity and culture (MCS) for bacteria and fungus in addition to blood cultures for ruling out other respiratory conditions, only 2.1% of the records reviewed established that other methods were used. This could mainly be due to the over reliance on the CXR by our clinicians when making a diagnosis of SNPTB as there was an observed easy availability of radiological investigations in Botswana (Huebner et al, 1997).

Other methods are very important to rule out other respiratory infections as was noted in a study done in Uganda where patients suspected of PTB (respiratory symptoms for more than three weeks and infiltrates on CXR) were sent for bronchoscope and bronchoalveolar lavage. The fluid was sent for MCS to detect PCP, bacteria and fungi and the findings were PCP (38.6%), MTB (24%), to a lesser extent pulmonary Kaposi sarcoma (11%), pyogenic bacteria (8%) and no obvious cause to the remainder (Worodria et al, 2003). Thus, miss-diagnosis of PTB is reduced if appropriate investigations are followed particularly in HIV-high burden countries where clinical signs and /or radiological findings have low diagnostic specificity (Mesfin et al, 2005).

New diagnostics tools such as the NAA tests were not available at our study site. These molecular techniques have been developed with the intent to overcome the limitations associated with the traditional laboratory diagnostic methods (Scherer et al, 2009). Though an AFB smear plus PCR dot-blot (in-house PCR) was more cost-effective than AFB smear plus culture in Brazil (Scherer et al, 2009), this test is not available in the government hospitals in Botswana. With PCR there is less time consumed in making a diagnosis, the method is patient friendly, reduces costs to patients and, most importantly, PCR is not influenced by the HIV status of the patient (Scherer et al, 2009).

Bronchoalveolar lavage improves detection of AFB in both HIV-infected and a non-infected patient (Aderaye, 2007) but this was not commonly practiced at our site. If this method could be used, in addition to sputum induction it can overcome the problem associated with non-sputum production among other HIV-positive patients. Fluorescence microscopy is four to five times more expensive than light microscopy and the light bulbs must be replaced after 200 h of use (Getahun et al, 2007) hence this was not used in our study site considering it is located in a poorly resourced setting.

CONCLUSION

From our study we can conclude that clinicians' compliance to the national SNPTB guidelines is very poor. Hence the increase in TB notifications in Botswana could be attributed to non-adherence to diagnostic algorithm among other causes. Proper interventions need to be employed as the wrongly labeled SNPTB cases will strain the meagre resources of TB programs. Furthermore inadequate investigations would miss the more infectious sputum positive cases as many studies have shown that compliance to the diagnostic guidelines do improve detection of smear positive PTB (Mesfin et al, 2005) considering that PTB can occur at a CD 4+ of up to 500 cells count/mm³ in HIV-positive patients (Aderaye, 2007).

We would like to recommend that the BNTP should make routine follow-ups to hospitals so that proper uses of guidelines are made before new methods are adopted into the Botswana clinical practice. A larger study would be appropriate such that we can make concrete conclusions pertaining to the use of BNTP guidelines at different hospitals in the country. Furthermore, BNTP should constantly provide feedback to clinicians such that modifications to the guidelines can be effected; and make guidelines easy and relevant to clinical practice. Stationed TB coordinators should be empowered to question clinicians where a diagnosis is not properly made. The BNTP should liaise with other stakeholders such that they can employ other diagnostic methods such as in-house PCR which can be used in making a SNPTB diagnosis.

Documentations and file storage needs to be improved for TB cases as in our study some of the files were not available or spoiled due to poor storage. This would make those who are involved in treating TB accountable to their diagnostic methods when clinical auditing is done. Anecdotally, this has been the case in HIV/AIDS programs where documentations are well secured and accounted for. SNPTB should be taken seriously as it is infectious. Patients should be put on treatment only after adequate investigations have been conducted; otherwise we are still doing trial of TB treatment among HIV-positive patients. This usually leads to unnecessary increased pill burden leading to poor adherence to treatment, side effects from TB drugs and probably reduced bioavailability of other HIV drugs for those on HIV treatment. In many African countries where other diagnostic tools are unavailable and HIV-TB co-infection is high, rigorous use of diagnostic algorithms has been recommended so as to improve detection of PTB (Mesfin et al, 2005). All these and other recommendations can assist us to eliminate this ancient and treatable condition (TB) from our societies especially where HIV and poverty are prevalent.

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Appendices

Appendix A: Data extraction sheet:

Part A

Station: Athlone Hospital. Lobatse. Botswana

Date of registration: _____

File Number: _____

Sex: _____

Age: _____

Part B: Please tick where appropriate.

PARAMETER

MEASURE

Period of cough before patient starts TB treatment	<2weeks		≥ 2weeks		Not specified
Number of sputum collected	0	1	2	3	
Antibiotics given	Yes			No	
Period patient received the antibiotics	<1 week		1 week		>1 week
Chest X-ray done	Yes			No	
Comment of Chest X-ray	Typical of Pulmonary TB		Normal		Other specify
Other methods used to rule out other respiratory diseases	Yes and specify			No	

Appendix B : Questionnaire for Clinicians

Name of Study: **Clinical diagnosis of smear negative Pulmonary Tuberculosis (SNPTB) in HIV-positive patients at Athlone Hospital in Botswana.**

Station: Athlone Hospital. Lobatse. Botswana

Date: _____

Please tick where appropriate.

Compliance with the guidelines

1a) Do you use three sputum results before you diagnose a HIV-positive patient with sputum negative PTB?

a) Always	b) Sometimes	c) Never
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1b) If your answer to this questions was “sometimes” or “never”, please tick one or more of the following reasons why you do not always do this:

i) Patients do not always submit the third specimen	
ii) Two results plus a Chest X-ray are enough to make a diagnosis	
iii) The laboratory takes long to release the results	
iv) Clinical history is adequate, there is no need for lab results	
v) Other (please specify)	

2a) Do you consider period of coughing important before a diagnosis of SNPTB in HIV-positive patients is suspected?

a) Always	b) Sometimes	c) Never
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2b) If your answer to this questions was “sometimes” or “never”, please tick one or more of the following reasons why you do not always do this:

i) Patients do not recall exact period	
--	--

ii) Some patients do not have history of coughing	
iii) There is no difference from other causes of chest infections most of the time	
iv) Other (please specify)	

3a) Do you try suspected SNPTB HIV-positive patients on a course of broad spectrum antibiotics before you make a diagnosis of SNPTB?

a) Always	b) Sometimes	c) Never
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3b) If your answer to this questions was “sometimes” or “never”, please tick one or more of the following reasons why you do not always do this:

i) Fear of loss to follow-up of patients while on trial of antibiotics	
ii) May mask the clinical progression of TB	
iii) Causes unnecessary drug resistance	
iv) It increases pill burden on patients when they are taking antiretrovirals	
vi) Causes treatment delay	

4a) Do you put suspected SNPTB HIV-positive patients on a one week course of antibiotics before the anti-TB treatment?

a) Always	b) Sometimes	c) Never
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4b) If your answer to this questions was “sometimes” or “never”, please tick one or more of the following reasons why you do not always do this:

i) Fear of loss to follow-up of the patient	
ii) Some need more than one week especially when they show signs of improvement	
iii) Fear of introducing drug resistance	
iv) Other (please specify)	

5a) Before an HIV-positive patient is diagnosed of SNPTB do you do a chest X-ray?

a) Always	b) Sometimes	c) Never
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5b) If your answer to this question was “sometimes” or “never”, please tick one or more of the following reasons why you do not always do this:

i) Clinical history is usually obvious	
ii) Patient may be pregnant	
iii) Other (please specify)	

6a) Do you make the SNPTB diagnosis in HIV-positive patients only when the chest X-ray shows the features of TB?

a) Always	b) Sometimes	c) Never
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6b) If your answer to this questions was “sometimes” or “never”, please tick one or more of the following reasons why you do not always do this:

i) Chest X-ray can be normal in the presence of TB	
ii) Clinical history is often obvious	
iii) Other causes would have been excluded	
vi) Other (please specify)	

Use of other possible methods in confirming a diagnosis of SNPTB

1) Below are other methods that you may use to rule out other respiratory conditions and to confirm a diagnosis of SNPTB. Please tick the appropriate box to indicate how often you use these methods.

a) Sputum for microscopy, culture and sensitivity for bacteria and fungus (MCS)

a) Always	b) Sometimes	c) Never
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b) Fluorescent Microscopy for bacilli in the sputum if light microscope result is negative

a) Always	b) Sometimes	c) Never
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c) Sputum culture for the bacilli for every case which is a suspect of SNPTB

a) Always	b) Sometimes	c) Never
-----------	--------------	----------

d) Blood cultures

a) Always	b) Sometimes	c) Never
-----------	--------------	----------

e) Chest X-ray

a) Always	b) Sometimes	c) Never
-----------	--------------	----------

f) Bronchoscope for biopsy and aspiration

a) Always	b) Sometimes	c) Never
-----------	--------------	----------

g) PCR for mycobacteraemia

a) Always	b) Sometimes	c) Never
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Please complete the following section if you find it difficult to comply with the guidelines:

1) Are you aware of the Botswana National Tuberculosis recommended guidelines in the diagnosis of SNPTB?

a) Yes	b) No
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2) Do you have a copy of the guidelines?

a) Yes	b) No
--------	-------

3) Are the guidelines easy to use in clinical practice?

a) Yes	b) No
--------	-------

4) Are the recommended guidelines relevant in helping clinicians to make a diagnosis of SNPTB?

a) Yes	b) No
--------	-------

5) Is it possible for all cases of suspected PTB to submit sputum?

a) Yes	b) No
--------	-------

6) Do patients come on consecutive days with their sputum as the guidelines dictates that they need one spot and at least one early morning specimen?

a) Yes	b) No
--------	-------

7) Do you think the turnover of results has an effect to the flow of your management when it comes to SNPTB cases?

a) Yes	b) No
--------	-------

8) Do you trust the accuracy of the laboratory results?

a) Yes	b) No
--------	-------

9) Are you afraid that you will lose SNPTB patients during the period when you are trying to confirm their diagnosis?

a) Yes	b) No
--------	-------

10) Is there a Lung Disease specialist at your hospital who reviews your non-specific Chest-X rays before you start a patient on SNPTB treatment?

a) Yes	b) No
--------	-------

11) Does the Botswana TB National Program review and provide feedback and recommendations on your clinical practice on the diagnosis of SNPTB in HIV-positive patients?

a) Yes	b) No
--------	-------

12) Do you think feedback will not help in working out the best way to manage SNPTB in HIV-positive patients in Botswana?

a) Yes	b) No
--------	-------

Thank you! Kealeboga! Tatenda!

Appendix C: Consent Form (Statement concerning participation in a Research Study)

Name of Study: **Clinical diagnosis of smear negative Pulmonary Tuberculosis (SNPTB) in HIV-positive patients at Athlone Hospital in Botswana**

Dear Clinician

You are being invited to participate in the above named research study. In particular, the researcher is interested in how the clinicians comply with the Botswana National Tuberculosis guidelines especially when making a diagnosis of SNPTB, use of other methods to rule out other respiratory conditions and confirming the diagnosis. In addition, the researcher will be interested in establishing the reasons which make the clinicians not to comply with the guidelines.

This research will require about 30 minutes of your time. During this time, you will be completing a questionnaire which will be collected when you are through. There are no anticipated risks related to this research. By participating in this research, you may benefit the researcher in completing his masters degree and as well as advising the Botswana National Tuberculosis Program in making improvements to the management of SNPTB patients who are HIV-positive.

Several steps will be taken to protect your anonymity and identity. The questionnaire will **NOT** contain any mention of your name, and any identifying information. The records will be kept in a locked filing cabinet and only the researcher will have access to them till he is through with data analysis. Your participation in this research is completely voluntary.

The results from this study will be communicated to you and the University of Limpopo and the Ministry of Health (Botswana). At no time, however, will your name be used or any identifying information revealed. I have read the above information regarding this research study and consent to participate in this study.

_____ (Signature) _____ (Date)

Statement by the Researcher

I have provided information regarding this Study. I agree to answer any future questions concerning the Study as best as I am able. I will adhere to the approved protocol.

Name of Researcher

Signature

Date

Appendix D: MEDUNSA Research & Ethics Committee

CLEARANCE CERTIFICATE

MEETING: 02/2010

PROJECT NUMBER: MREC/H/24/2010: PG

PROJECT :

Title: Clinical diagnosis of smear negative Pulmonary Tuberculosis in HIV-positive patients at Athlone Hospital in Botswana

Researcher: Dr TA Tafuma
Supervisor: Ms R Burnett
Department: Public Health
School: Health Care Sciences
Degree: Master of Public Health

DECISION OF THE COMMITTEE:

MREC approved the project.

DATE: 03 March 2010

PROF N EBRAHIM
DEPUTY CHAIRPERSON MREC

Note:

Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.

The budget for the research will be considered separately from the protocol. PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.

Appendix E Permission Letter (Ministry of Health)

Research Ethics Committee

Ministry of Health

Bag 0038, Gaborone

Botswana

15 January 2010

Dear Sir/Madam

Ref: Application for permission to carry out a research entitled: Clinical diagnosis of smear negative Pulmonary Tuberculosis (SNPTB) in HIV-positive patients at Athlone Hospital in Botswana

I am requesting your permission to allow me to carry out the above named research. The purpose of this research is to allow me to complete my Masters in Public Health at the University of Limpopo. This study has been approved by the Medunsa Research Ethics Committee of the University (see attached clearance certificate).

Files of the patients who completed their tuberculosis treatment after they were diagnosed of smear negative pulmonary tuberculosis at Athlone hospital will be audited. In addition a self administered questionnaire will be given to the doctors at hospital to complete. They will answer questions highlighting factors they encounter in complying with the recommended guidelines.

The findings from this study will be submitted to the University of Limpopo and your office. Recommendations will be made depending on the findings. The hospital files and completed questionnaires will be held with care to protect their confidentiality.

Your assistance will be greatly appreciated.

Yours faithfully

Dr Taurayi Adriano Tafuma



Approval:

Telephone: (267) 3632000
FAX (267) 353100
TELEGRAMS: RABONGAKA
TELEX: 2818 CARE BD



MINISTRY OF HEALTH
PRIVATE BAG 0038
GABORONE

REPUBLIC OF BOTSWANA

REFERENCE No: PPME 13/18/ 1 PS Vol V (104)

27 April 2010

Health Research and Development Division

Notification of IRB Review: New application

Dr Taurayi Adriano Tafuma
P/Bag 00480
Suite 73
Gaborone

Protocol title:	Clinical Diagnosis of Smear Negative Pulmonary Tuberculosis in HIV Positive Patients at Athlone hospital in Botswana
HRDD Protocol number:	HRU 00615
Sponsor:	Self Sponsored.
HRDD Review Date:	April 27, 2010
HRDD Review Type:	HRDD reviewed
HRDD Review Determination:	Approved
Risk Determination:	Minimal risk

Dear Dr Tafuma

Thank you for submitting an application for the above referenced protocol to Health Research and Development Division (HRDD) for review and approval. We have noted that all HRDD concerns had been addressed satisfactorily.

Permission is therefore granted to conduct the above mentioned study. This approval is valid for a period of 1 year effective 27 April, 2010.

This approval includes the following:

1. Application form
2. Protocol
3. Clearance certificate from Limpopo university
4. Curriculum vitae
5. A questionnaire
6. Consent form

The permit does not however give you authority to collect data from selected sites without prior approval from the management. Consent from the identified individuals should be obtained at all times.

The research should be conducted as outlined in the approved proposal. Any changes to the approved proposal must be submitted to the Health Research and Development Division in the Ministry of Health for consideration and approval.

Furthermore, you are requested to submit at least one hard copy and an electronic copy of the report to the Health Research and Development Division, Ministry of Health within 3 months of completion of the study. Copies should also be submitted to all other relevant authorities.

If you have any questions please do not hesitate to contact Mr. P. Khulumani at pkhulumani@gov.bw, Tel +267-3914467 or Mary Kasule at mkasule@gov.bw or marykasule@gmail.com Tel +267-3632466

Continuing review

In order to continue work on this study (including data analysis) beyond the expiry date, submit a Continuing Review Application Form for approval at least three (3) months prior to the protocol's expiration date. The Continuing Review Form can be obtained from the Health Research and Development Division office (HRDD), office No. 9A.10 or Ministry of Health website: www.moh.gov.bw or can be requested via e-mail from Mr. Kgomoitso Motlhanka, e-mail address: kgmmotlhanka@gov.bw. As a courtesy, the HRDD will send you a reminder e-mail about eight (8) weeks before the lapse date, but failure to receive it does not affect your responsibility to submit a timely Continuing Review Form.

Amendments

During the approval period, if you propose any change to the protocol such as its funding source, recruiting materials, or consent documents, you must seek HRDD approval before implementing it. Please summarize the proposed change and the rationale for it in the amendment form available from Health Research and Development Division office (HRDD), office No. 9A.10 or Ministry of Health website: www.moh.gov.bw or can be requested via e-mail from Mr. Kgomoitso Motlhanka, e-mail address: kmotlhanka@gov.bw. In addition submit three copies of an updated version of your original protocol application showing all proposed changes in bold or "track changes".

Reporting

Other events which must be reported promptly in writing to the HRDC include:

- Suspension or termination of the protocol by you or the grantor
- Unexpected problems involving risk to subjects or others
- Adverse events, including unanticipated or anticipated but severe physical harm to subjects.

Thank you for your cooperation and your commitment to the protection of human subjects in research.

Yours sincerely


P. Khulumani

For Permanent Secretary



TELEPHONE:3914467
FAX:3914697
KOPANYOHOUSE
PLOT: 5131
STATION ROAD
GABORONE



HEALTH RESEARCH UNIT
PRIVATE BAG 0038
GABORONE
BOTSWANA

REPUBLIC OF BOTSWANA
MINISTRY OF HEALTH

REFERENCE No: PPME 13/18/1/IV (92)

15 July 2010

Dr Taurayi Adriano Tafuma
Bokamoso Private Hospital
Private Bag 00205
Gaborone

Dear Dr Tafuma

AMENDMENT: CLINICAL DIAGNOSIS OF SMEAR NEGATIVE PULMONARY TUBERCULOSIS IN HIV POSITIVE PATIENTS AT ATHLONE HOSPITAL IN BOTSWANA

Reference is made to the above mentioned protocol amendment application submitted to the Health Research and Development Committee (HRDC) in the Ministry of Health for review and approval. HRDC have reviewed and approved the amendment protocol on the 14 July 2010.

Therefore permission is granted to use the TB files kept at the clinics within Athlone Hospital catchment area for participants who initiated their TB treatment at Athlone Hospital.

Please note that the amendment will expire together with the initial permit.

Amendments

During the approval period, if you propose any change to the protocol such as its funding source, recruiting materials, or consent documents, you must seek HRDC approval before implementing it.

Please summarize the proposed change and the rationale for it in the amendment form available from the Health Research Division Office (HRDD), Office No. 9A 11 or Ministry of Health website: www.moh.gov.bw or can be requested via e-mail from Mr. Kgomoiso Motlhanka, e-mail address: kgmmotlhanka@gov.bw. In addition submit three copies of an updated version of your original protocol application showing all proposed changes in bold or "track changes".

Reporting

Other events which must be reported promptly in writing to the HRDC include:

- Suspension or termination of the protocol by you or the grantor

Appendix F Letter to the Hospital

Hospital Superintendent and District Public Health Specialist

Athlone District Hospital

P. Bag 20, Lobatse

Botswana

15 January 2010

Dear Sir/Madam

Ref: Application for permission to carry out a research entitled: Clinical diagnosis of smear negative Pulmonary Tuberculosis (SNPTB) in HIV-positive patients at Athlone Hospital in Botswana

I am requesting your permission to allow me to carry out the above named research. The purpose of this research is to allow me to complete my Masters in Public Health at the University of Limpopo. This study has been approved by the Medunsa Research Ethics Committee of the University (see attached clearance certificate). The Ministry of Health (Botswana) has offered permission for this study (see attached permission letter).

I chose your hospital because I have been part of the clinicians for the period of 2006-2009 and I believe you can provide me with all the information I will be looking for. I hope to work with your Tuberculosis Coordinator who will be verifying the data collected. The study intends to review the clinical files of all the patients who were diagnosed of smear negative pulmonary tuberculosis from 2006-2009. In addition, a self administered questionnaire will be issued to your doctors for completion and will highlight the factors encountered in complying with the recommended guidelines.

The findings from this study will be submitted to the University of Limpopo, Ministry of Health and your office. Recommendations will be made depending on the findings. The hospital files and completed questionnaires will be held with care to protect their confidentiality.

Your assistance will be greatly appreciated.

Yours faithfully

Dr Taurayi Adriano Tafuma

A square image containing a handwritten signature in black ink on a light grey background. The signature is stylized and appears to be the name 'Dr Taurayi Adriano Tafuma'.

Approval

Tel. No. 5330333
Fax No. 5330719



Athlone Hospital
Private Bag 20
LOBATSE
BOTSWANA

REPUBLIC OF BOTSWANA
MINISTRY OF HEALTH

REF: AH 10/7 I

13th July 2010

Dr T.A. Tafuma
Bokamoso Private Hospital
Private Bag 00205
Gaborone

Dear Sir

PERMISSION TO UNDERTAKE RESEARCH STUDIES – DR. T.A TAFUMA

Following your application and approval by the Ministry of Health, permission is hereby granted for you to undertake your studies at our Hospital for the duration of two weeks.

Kindly see the CMO so that appropriate support and allocation can be provided to you.

Wish you all best in your activities.

Yours faithfully


J.A.F Kasambala
/for **Hospital Superintendent**

CHIEF MEDICAL OFFICER
REGISTRY UNIT
ATHLONE HOSPITAL
2010 -07- 13
TEL. 5330333
P/BAG 20
LOBATSE

Tel. No. 5330333
Fax No. 5330719
Reference: AH



REPUBLIC OF BOTSWANA

Athlone Hospital
Private Bag 20
LOBATSE
BOTSWANA

REFERENCE NO: AH 12/5 I (5)

23 August 2010

Dr. T. A. Tafuma

Dear Doctor,

RE: APPLICATION FOR PERMISSION TO CARRY OUT A RESEARCH ENTITLED; CLINICAL DIAGNOSIS OF SMEAR NEGATIVE PULMONARY TUBERCULOSIS (SNPTB) IN HIV POSITIVE PATIENTS AT ATHLONE HOSPITAL IN BOTSWANA

I would like to acknowledge receipt of your letter dated 11th August 2010 requesting to carry out a study on the above mentioned topic.

I am pleased to inform you that permission is granted to carry out the study at Athlone hospital and accessing files at the clinics. The criteria to give you permission is based on the fact that you have followed all necessary procedures to get permission and it is hoped that you will observe all protocol as required by the Ministry of Health Research Unit.

Please see relevant authorities whenever you get to respective units
Thank you

Sincerely,

T. J. Mokgatle
HEAD OF DHMT - LOBATSE



