AN EVALUATION OF TUBERCULOSIS DIAGNOSTIC METHODS AT PIETERSBURG HOSPITAL IN LIMPOPO PROVINCE, SOUTH AFRICA.

by

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DEDICATION

I dedicate this mini-dissertation to my late beloved mother (Germina Matome Lamola) and to my father (Hamilton Padima Makgato). Their belief in my academic prowess and their nurturing during my childhood development has formed my firm foundation to excel academically.

DECLARATION

I declare that AN EVALUATION OF TUBERCULOSIS DIAGNOSTIC METHODS AT PIETERSBURG HOSPITAL IN LIMPOPO PROVINCE, SOUTH AFRICA is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work had not been submitted for any other degree at any institution.

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Dr Innocent Maroslyn Lamola Date:12 October 2022

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ABSTRACT

Background: The World Health Organization (WHO) has embarked on a mission to reduce tuberculosis (TB) prevalence by 90% and completely eradicate TB infection by the years 2035 and 2050 respectively (Barberis, Bragazzi, Galluzzo, *et al.*, 2017). The attainment of these two major goals will depend largely on the successful preventative measures, diagnosis, and treatment for both latent and undiagnosed active TB infections.

Aim: We evaluated the nature of the various diagnostic modalities employed in the diagnosis of tuberculosis in adult patients notified as having TB at Pietersburg Hospital from January to December 2020.

Methods: A retrospective descriptive, observational quantitative study based on secondary data was conducted at a tertiary hospital. The study included 135 cases of TB patients aged 12 years and above, that were notified to the infection prevention and control unit (IPCU) over a 12-month period. The data was captured using an appropriately designed data collection tool and analysed using STATA 14 (College Station, Texas 77845 USA) software.

Results: A total of 135 of notified individuals with TB were analysed. The data set comprised of 57% (77/135) females and 43% (58/135) with a mean age of 37 years with an interquartile range of 29 to 45 years. Over half of the cases were notified as pulmonary TB (57.7%), while the remaining cases were notified as various forms of extra pulmonary TB. In this study, the commonest form of TB diagnostic method was imaging (92%) followed by sputum tests (55.6%), TB blood culture (52.5%), and urine LAM (36.3%), special fluid analysis (25.2%) and lastly tissue histology (3%). The commonest co-morbidity identified was HIV (92%). The urine LAM (100%) and TB blood culture (50.5%) were performed mainly in HIV positive patients.

Conclusion: In this study, TB was mostly diagnosed using various imaging techniques more than microbiological tests such as sputum, urine LAM, TB blood

culture, special fluid analysis or tissue histology. Furthermore, HIV emerged as the commonest co-morbid condition.

Key concepts: WHO, TB, HIV, diagnostic methods

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LIST OF ACRONYMS AND ABREVIATIONS

AIDS: Acquired Immunodeficiency Syndrome

BSI: Bloodstream Infection

CRS: Composite Reference Standard

DNA: Deoxyribonucleic Acid

DST: Drug susceptibility testing

DOTS: Directly observed therapy

EPTB: Extrapulmonary tuberculosis

GXP: Gene Xpert

HIV: Human Immunodeficiency Virus

IGRAs: Interferon-gamma release assays

IPCU: Infection Prevention and Control unit

LTBI: Latent tuberculosis infection

MRS: Microbiological Reference Standard

NAAT: Nucleic Acid Amplification Test

U-LAM: Urine lipoarabinomannan

PCR: Polymerase chain reaction

PTB: Pulmonary tuberculosis

NMC: Notifiable medical condition

SLE: Systemic Lupus Erythromatosus

TST: Tuberculin skin test

WHO: World Health Organization

Xpert MTB/RIF: Xpert Mycobacterium tuberculosis and resistance to rifampicin

DEFINITIONS OF CONCEPTS

Disseminated TB: is defined as the presence of two or more non-contiguous sites resulting from haematogenous and dissemination of Mycobacterium tuberculosis, occurring as a result of progressive primary infection, reactivation of a latent focus with subsequent spread or rarely through iatrogenic origin (ref)

Extrapulmonary Tuberculosis: TB infections elsewhere in the body outside the lung parenchyma, resulting in a clinically apparent disease state (Aaron, Saadoun, Calatroni, *et al.*, 2004). In this study, refers to patients any form of tuberculosis other than pulmonary tuberculosis.

Gene Xpert (GXP): refers to a polymerase chain reaction (PCR) test used to diagnose tuberculosis infection on various bodily fluids or media such as sputum, cerebrospinal fluid, pleural fluid et cetera. In this study, it refers to the test method used for the diagnosis of both pulmonary tuberculosis or extrapulmonary tuberculosis.

Inpatients: refers to hospitalised patients receiving medical care in the hospital (*Dorland's Illustrated Medical Dictionary*, 2012). In this study, inpatients are tuberculosis patients that are receiving treatment in a hospital ward.

Active tuberculosis: refers to TB infection resulting in a clinically apparent disease state (Drain, Losina, Coleman, *et al.*, 2014). In this study, it refers to ill patients in whom tuberculosis has been diagnosed.

Pulmonary tuberculosis: refers to infection of the lungs by *Mycobacterium tuberculosis* (*Dorland's Illustrated Medical Dictionary*, 2012). In this study, it refers to those diagnosed with TB on sputum, chest X-ray or Computed tomography of the chest (CT Chest).

Primary TB: refers to pulmonary tuberculosis when a person is first infected, usually seen in children or immunocompromised adults. It is often asymptomatic, with simply a positive result on a tuberculin test (Dorland's Illustrated Medical Dictionary, 32nd ed, 2012).

Notifiable medical condition (NMC): are diseases that are of public health importance because they pose significant public health risks that can result in disease outbreaks or disease epidemics with high case fatality rates both nationally and internationally (Ashbrook, 2014). In this study, this refers to all forms of diagnosed active TB.

TB reactivation: also known as post-primary tuberculosis or secondary tuberculosis refers to pulmonary tuberculosis that is typical of a fresh infection but in which the person has had an earlier, probably subclinical attack, it is distinguished by caseation and cavitation with healing that results in fibrosis (Dorland's Illustrated Medical Dictionary, 32nd ed, 2012).

CHAPTER 1: INTRODUCTION

1.1 RESEARCH BACKGROUND

Tuberculosis (TB) is an airborne disease that is transmitted through the inhalation of respiratory droplet containing *mycobacterium tuberculosis* bacilli resulting in lung infection (pulmonary TB) or infection of virtually any other organ in the body through haematogenous spread (Fogel, 2015). The latter manifestation of TB infection is referred to as extra-pulmonary tuberculosis -EPTB (Shah, Hanrahan, Wang, *et al.*, 2016). Tuberculosis may occur as a primary infection or reactivation of a latent TB infection. Primary TB occurs when a person is first infected, usually seen in children or immunocompromised adults. It is often asymptomatic, with simply a positive result on a tuberculin test (Dorland's Illustrated Medical Dictionary, 32nd ed, 2012).

In contrast to primary TB, reactivation TB refers to tuberculosis that is typical of a fresh infection but in which the person has had an earlier, probably subclinical, attack, it is distinguished by caseation and cavitation with healing that results in fibrosis (Dorland's Illustrated Medical Dictionary, 32nd ed, 2012). Pulmonary Tuberculosis (PTB) is the most common form of the disease, may present with a productive cough with blood-stained sputum, shortness of breath, and pleuritic chest pains associated with constitutional symptoms namely, unintentional weight loss, drenching night sweats, and fever (Cruz-Knight & Blake-Gumbs, 2013). Patients with EPTB usually present with signs and symptoms in keeping with the organ that is the focus of TB infection (Mehta, Raj, Singh *et al.*, 2012). For example, TB meningitis may result in chronic headache associated with neck stiffness due to irritation of the meninges while those with TB arthritis may present with a painful and swollen knee joint (Kohli, Schiller, Dendukuri *et al.*, 2018).

The inhalation of TB bacilli through respiratory droplets from person to person may

result in either phagocytosis and successful killing of the bacilli through the T cell mediated immune response, containment of the TB bacilli by macrophages resulting in latent TB infection (LTBI) or the development of active TB (Fogel, 2015; de Martino, Galli & Chiappin, 2014). The World Health Organization (WHO) has reported that approximately one third of the world's population is infected with LTBI (Barberis, Bragazzi, Galluzzo, *et al.*, 2017). Studies show that 80% of these LTBI are found in just 22 low to middle-income countries (Fogel, 2015). Furthermore, a significant proportion of these cases is found in the Sub-Saharan Africa, which already has the world's highest incidence of HIV co-infection (Glaziou, Floyd & Raviglione, 2018).

LTBI is often more challenging to diagnose in patient populations in the low to middle-income countries due to the Bacilli Calmette-Guerin (BCG) vaccine that is still being administered as part of the essential childhood immunisation (Fogel, 2015). The affordable Tuberculin Skin Test (TST) is fraught with interpretation difficulties in those who have received a BCG vaccine necessitating the use of interferon-gamma release assays (IGRAs) test which is more expensive, and indeed beyond the reach of many public health systems (Thillai, Pollock, Pareek, *et al.*, 2014).Nonetheless, specific cut-off values for the interpretation of TST have been validated more especially in the paediatric age groups (Seddon, Paton, Nademi, *et al.*, 2016). As a result, more concerted effort is directed towards the diagnosis and treatment of active TB as opposed to LTBI.

TB is the world's second leading infectious cause of mortality, behind only HIV/AIDS. (Barberis *et al.*, 2017). This is despite the discovery of effective and efficacious anti-tuberculous drugs and later the advent of the TB vaccine known as the BCG vaccine (Fogel, 2015). The complexity of the treatment regimen, treatment associated adverse reactions and the required duration of the anti-TB regimen poses significant challenge to treatment compliance (Keshavjee & Farmer, 2012). The treatment duration varies

from 6 months to 24 months depending on the drug susceptibility (Keshavjee & Farmer, 2012). Alarmingly, South Africa's prevalence of TB dominates the Sub-Saharan region. The WHO Global Tuberculosis Report (2020) lists South Africa in the global top 20 countries of high TB prevalence by absolute number.

Poor treatment compliance has resulted in the emergence of multidrug resistant TB (MDR-TB) and extensive drug resistant TB (XDR-TB) (Keshavjee & Farmer, 2012). The WHO has driven the implementation of treatment protocols such as directly observed therapy (DOTS) and DOTS-PLUS in order to curb the increasing incidence and prevalence of these forms of drug resistant TB (Fogel, 2015). The BCG vaccine has been shown to have a limited efficacy in the prevention of TB in adults though it does protect from severe PTB and the occurrence of EPTB especially TB meningitis and miliary TB (Barreto, Pereira & Ferreira, 2006).

The WHO has embarked on a mission to reduce TB prevalence by 90% and completely eradicate TB infection by the years 2035 and 2050 respectively (Barberis *et al.*, 2017). The attainment of these two major goals will depend largely on the successful diagnosis and treatment of both latent and undiagnosed active TB infections. The WHO estimates that approximately 30% of active TB cases go undiagnosed. There is no such comparative data at Pietersburg Hospital as there has not been such a study conducted locally.

Thus, the use of reliable, user-friendly, and cost-effective diagnostic modalities with acceptable sensitivity and specificity values will be paramount in this fight against TB. This research project seeks to evaluate the diagnostic modalities that are in full use at Pietersburg Hospital in the diagnosis of active TB in adult patients. The findings of this study will serve to inform the local efforts aimed at advancing the global fight against TB.

The following table shows some of the key TB diagnostic modalities that shall form the basis of this research.

TABLE 1 Technologies reviewed by WHO for TB case detection

Year	Method	Technology reviewed by WHO
2007	Culture (growth-based)	Commercial liquid culture and rapid speciation strip tests
2010	Microscopy	LED microscopy
2010	NAAT	Xpert MTB/RIF
2016	Antigen detection test	Urine LAM rapid test
2016	NAAT	Loop-mediated amplification test (LAMP)

Figure 1.1: Illustration of TB diagnostics (source "Pai, Nicol and Boehme,2016")

1.2. RESEARCH QUESTION

What diagnostic modalities were used in the diagnosis of tuberculosis of the lungs, extra-pulmonary sites (central nervous system, bones, joints, abdomen, kidneys etc) or disseminated TB at Pietersburg Hospital, over a 12-month period from January to December 2020?

1.3. PURPOSE OF THE STUDY

1.3.1. Aim

To evaluate the nature of the various diagnostic modalities employed in the diagnosis of tuberculosis in adult patients admitted to Pietersburg Hospital from January to December 2020.

1.3.2. Objectives

1.3.2.1. To measure the frequency of use of various diagnostic tests in patients diagnosed with TB at Pietersburg Hospital in Limpopo Province, from January to December 2020.

1.3.2.2. To describe the quarterly TB infection trends by age and sex at Pietersburg Hospital in Limpopo Province, from January to December 2020.

1.3.2.3. To determine the frequency of various TB diagnostic modalities in patients with HIV co-infection and in patients with Diabetes Mellitus at Pietersburg Hospital in Limpopo Province, from January to December 2020.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Literature review will focus on the evidence relating to the usage of various diagnostic methods for TB infection. These diagnostic methods or tests can be performed on a variety of bodily fluids or tissues ranging from sputum, blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid to histological diagnosis on excised tissue. Research articles citing all TB diagnostic modalities, including those testing methods that are not available at Pietersburg hospital will be reviewed. This shall be preceded by a review of the history and epidemiology of TB including the predisposing risk factors and/ or medical co-morbidities that may render some TB diagnostic tests ineffective.

The literature search was conducted using PubMed and Google scholar as the main search engines. The following key words were used in order to track down relevant literature; “TB diagnosis”, “History of Tuberculosis” “TB diagnostic methods”, “TB diagnostic modalities”. The relevant articles that were published within the last five years were prioritised. However, articles that constituted salient publications based on their high citations were also included, even if they were published more than five years ago.

2.2. The history and epidemiology of tuberculosis

Tuberculosis is a devastatingly contagious and infective disease that has afflicted human kind for millennia (Barberis *et al.*, 2017). It is caused by a bacterium known as the *Mycobacterium tuberculosis,* which was first isolated by a German Pathologist, Robert Koch on the 24th of March 1882 (Barberis *et al.*, 2017; Fogel, 2015). Robert

Koch would go on to receive a Nobel Prize in Medicine in 1905 for this monumental discovery (Gradmann, 2001). This bacterium belongs to the genus *Mycobacterium,* which is postulated to have had its genesis more than 150 million ago (Barberis *et al.*, 2017). Tuberculosis is arguably the most studied disease entity to date. It has been a subject of intense medical scientific enquiry for centuries (Barberis *et al.*, 2017). This has led to the wealth of knowledge that we now have of this disease albeit a lot remains unknown.

This disease has enjoyed a myriad of names prior to the more scientific name of tuberculosis coined by Johann Lukas Schönlein in the mid-19th century (Barberis *et al.*, 2017) This was largely driven by the poor understanding of this disease. The English and French commonly referred to this disease as “King’s evil” during the Middle Ages (Barberis *et al.*, 2017). This was later followed by the names of “consumption” and “phthisis” in the 17th and 18th centuries (Barberis *et al.*, 2017; Fogel, 2015). It was later referred to as the “the robber of youth” owing to its high mortality amongst the Western Europe youth in the late 1800s (Barberis *et al.*, 2017). In the same time period, the term “white plague” was coined as a description of the profound anaemia that the victims of Tuberculosis often displayed (Daniel, 2006).

It is a disease that claims a global presence albeit more prevalent in some regions than others owing to the environmental factors required for its dissemination (Barberis *et al.*, 2017). Tuberculosis has been characterised by a high mortality owing to the lack of effective treatment in the early centuries (Barberis *et al.*, 2017). For example, the mortality was recorded to be about 900 in every 100000 inhabitants per year in Western Europe during the 18th century (Barberis *et al.*, 2017).

The WHO estimates that approximately one third of the world population is latently

infected with tuberculosis albeit only a relatively small proportion (5 to 15%) will go on

to develop active disease (Fogel, 2015; Glaziou *et al.*, 2018; Vynnycky & Fine, 2000). Notwithstanding the discovery of effective anti-tuberculosis drugs in the early 1940s, tuberculosis related mortality remains high, second only to HIV/AIDS. Tuberculosis has claimed over 15 million lives worldwide in 2018 alone. TB is the world's second leading infectious cause of mortality after HIV/AIDS infection (Glaziou *et al.*, 2018).

2.3 Risk factors for acquisition of tuberculosis

Tuberculosis has proven itself not to be merely a medical quagmire but a socioeconomic conundrum as well (Barberis *et al.*, 2017). Its prevalence and dissemination are driven by a plethora of socioeconomic determinants, and in turn worsens the economic standing of its victims. Barberis et al. (2017) lament the poor socio-economic factors such poor sanitation, overcrowded and poorly ventilated living spaces, deprived working conditions, alcohol abuse, use of illicit drugs, incarceration, under-nutrition, and other risk factors as having aided the spreading of tuberculosis. Studies that were done by the WHO show that people suffering from TB lose more than 50% of their income during the duration of their illness(Barberis *et al.*, 2017).

Getahum et al. (2015) demonstrated that patients infected with HIV have a 20-fold

chance of contracting TB. Globally, HIV contributed to 9% of the new TB cases in the year 2000. This contrasts with the 31% of such cases associated with HIV infection in Sub-Saharan Africa, which is home to the world's highest prevalence of HIV (Keshinro & Diul, 2006). TB has emerged as the world's number one infectious cause of mortality in 2006. Furthermore, medical conditions such as malnutrition, protein imbalance, chronic kidney disease and haemodialysis have been shown to increase the risk of contracting TB (Glaziou *et al.*, 2018; Marais *et al.*, 2013).

In a longitudinal study involving 163 countries, Goldhaber-Fiebert et al. (2018) demonstrated that the high prevalence of diabetes mellitus led to an increase in the incidence of TB. It was further determined that diabetes mellitus (DM) led to a threefold increase chance of contracting TB (Dabhi, Thangakunam, Gupta *et al.*, 2020). TB infection in patients with Diabetes Mellitus was also associated with a more severe TB infection illustrated by high rates of cavitary smear positive disease (Restrepo, 2018). Furthermore, patients with DM had a higher chance of having a pulmonary TB as opposed to the extrapulmonary form of this disease. Finally, TB proved more difficult to treat requiring longer duration of standard TB therapy with a higher incidence of treatment related adverse events and higher mortality (Restrepo, 2018). Some of the risk factors that are associated with DM, such as older age and obesity, exacerbate this.

2.4. Diagnostic methods of tuberculosis

The WHO has embarked on a grand mission of eradicating the scourge of TB worldwide. The organisation aims to achieve a worldwide reduction of TB prevalence by 90% in 2035 (Barberis *et al.*, 2017). Furthermore, a total eradication of active TB infection by the year 2050 is planned (Barberis *et al.*, 2017). These two highly ambitious goals can only be achieved if highly reliable and sensitive TB diagnostic tests are made available for the successful identification and treatment of all the active and latent TB infections. There are various diagnostic modalities for the diagnosis of both the pulmonary and extra-pulmonary TB. These diagnostic tests can be performed on a variety of bodily fluids or tissues ranging from sputum, blood, cerebrospinal fluid,

pleural fluid, peritoneal fluid, pericardial fluid to histological diagnosis on excised tissue.

2.4.1. Sputum based methods

Sputum based TB diagnostic modalities play an essential role in the microbiological diagnosis of pulmonary TB (Pai, Nicol & Boehme, 2016). PTB constitutes a greater proportion of all tuberculosis cases. In one large Cochrane review looking at 16,213 specimens from 66 studies, pulmonary TB made up 85% of the total cases (Horne, Kohli, Zifodya, *et al.*, 2019). In contrast to EPTB, PTB turns to be more common in HIV negative patients or HIV positive patient with preserved CD4 count of more than 200 cells per cubic metre (Aaron *et al.*, 2004).

Sputum microscopy and culture are amongst the first TB diagnostic methods to be espoused by the WHO in its TB diagnostic algorithm (Lange & Mori, 2010). Sputum microscopy is used to diagnose TB through the detection of the acid-fast bacilli (AFB) albeit with a low sensitivity of 44% or less (Lange & Mori, 2010). Furthermore, the detection of AFBs could be due to the presence of other infective agents other than *Mycobacterium tuberculosis* such as *Nocardia, Actinomyces, Rhodococcus, Legionella micdadei or cysts of Cryptosporidium species* or even *non-tuberculous mycobacterium* (Jae, Kim, Sei *et al.*, 2008). Thus, sputum culture and the nucleic acid amplification test (NAAT) are essential in the identification of the mycobacterial tuberculosis species in this instance (Lange & Mori, 2010).

The current WHO consolidated guidelines on tuberculosis (2021 update) recommend the use of sputum NAAT as first line diagnosis diagnostic modality. NAAT encompasses the Xpert MTB/RIF and the Xpert MTB/RIF Ultra assays. Furthermore, the WHO recommends that the NAATs be used as first line tests for detection of rifampicin drug susceptibility.

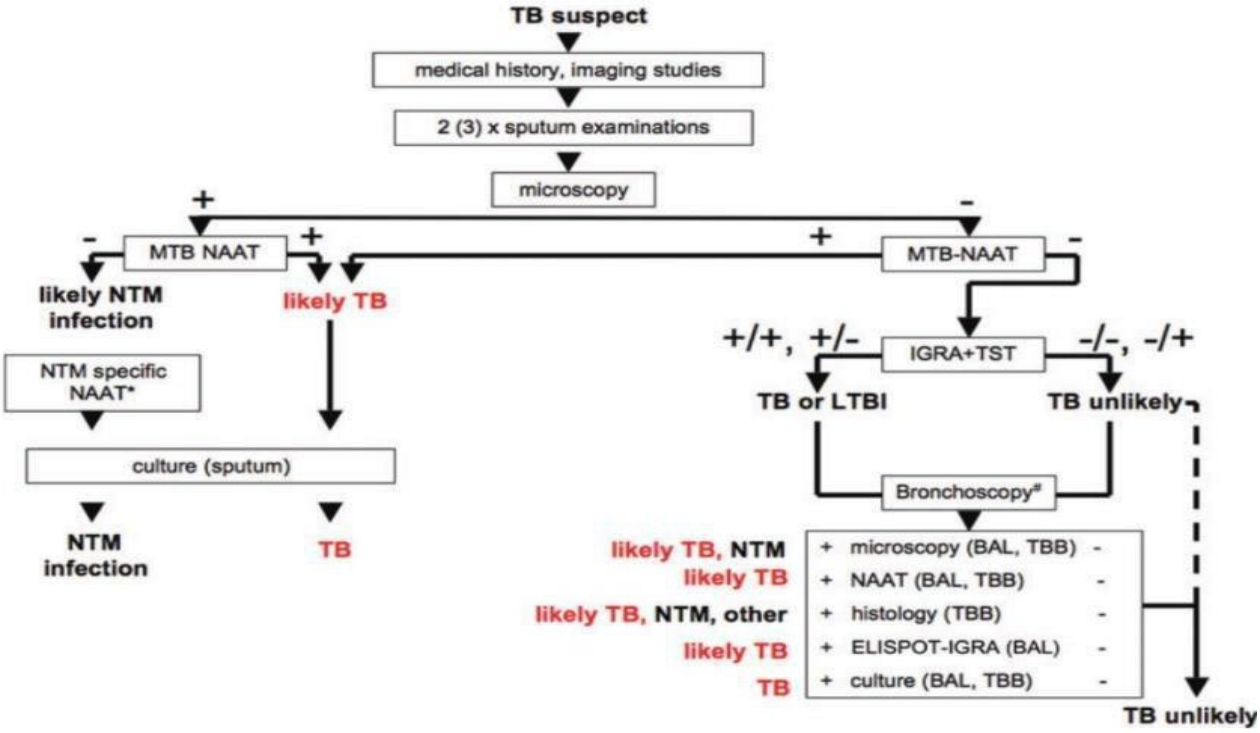


Figure 1 Flow diagram for the diagnosis of tuberculosis in clinical practice. *NTM NAAT may be helpful, when available. ≠ In accordance with WHO recommendations (WHO. Treatment of tuberculosis. Guidelines for national programmes. Geneva; 2003), clinical response to antibiotic therapy may be considered before further investigations; however, in countries of low TB incidence immediate further diagnosis with bronchoscopy can be indicated at this stage to better rule out other diseases. BAL, bronchoalveolar lavage; IGRA, interferon-γ release assay; MTB, Mycobacterium tuberculosis; NAAT, nucleic acid amplification test; NTM, non-tuberculous Mycobacteria; TB, tuberculosis; TBB, tubercle bacilli; TST, tuberculin skin test; WHO, World Health Organisation.

Figure 2.1: WHO TB diagnostic algorithm (source “Lange & Mori, 2010)

Sputum collection is less invasive and easier to collect (Drain *et al.*, 2014). There are various diagnostic tests that may be performed on sputum specimen in order to make a TB diagnosis (Horne *et al.*, 2019). In a Cochrane review of 27 studies involving 9557 subjects ‘respiratory specimens, the Xpert Mycobacterium tuberculosis deoxyribonucleic acid and resistance to rifampicin (Xpert® MTB/RIF) had a pooled sensitivity of 89% (95% CI 85%-92%), and a pooled specificity of 99% (95% CI 98-99%) (Steingart, Schiller, Horne *et al.*, 2014).

However, according to the data published in the WHO consolidated guidelines on TB (2021 upate) reveal sensitivity of 85% and specificity of 98% for sputum Xpert/Rif in the HIV negative patients. This sensitivity drops to 81% in the HIV positive patients and drops further to 67% in smear negative patients with the specificity remaining at 98% across the board as shown on table 2.1 below. The Xpert/Rif Ultra offers higher sensitivities and specificities as shown in table 2.2 below.

Table 2.1A: The diagnostic accuracy of Xpert MTB/RIF for pulmonary TB in adults, as compared with MRS

Patient population	Test accuracy	Studies (participants)	Certainty of evidence	2.5% prevalence	10% prevalence	30% prevalence
Adults PTB, MRS	Se: 0.85	70 (10 409)	High	TP: 21 / FN: 4	TP: 85 / FN: 15	TP: 255 / FN: 45
	Sp: 0.98	70 (26 828)	High	TN: 965 / FP: 10	TN: 891 / FP: 9	TN: 693 / FP: 7
Adults PTB, SS–, MRS	Se: 0.67	45 (2315)	High	TP: 17 / FN: 8	TP: 67 / FN: 33	TP: 201 / FN: 99
	Sp: 0.98	45 (16 647)	High	TN: 956 / FP: 19	TN: 882 / FP: 18	TN: 686 / FP: 14
Adults PTB, HIV+, MRS	Se: 0.81	14 (1159)	High	TP: 20 / FN: 5	TP: 81 / FN: 19	TP: 243 / FN: 57
	Sp: 0.98	14 (3505)	High	TN: 956 / FP: 19	TN: 882 / FP: 18	TN: 686 / FP: 14
Adults PTB, previous TB, MRS	Se: 0.86	14 (2197)	Low	TP: 22 / FN: 3	TP: 86 / FN: 14	TP: 258 / FN: 42
	Sp: 0.95	14 (2998)	Moderate	TN: 924 / FP: 51	TN: 853 / FP: 47	TN: 664 / FP: 36

FN: false negative; FP: false positive; HIV+: human immunodeficiency virus positive; MRS: microbiological reference standard; PICO: population, intervention, comparator and outcomes; PTB: pulmonary tuberculosis; Se: sensitivity; Sp: specificity; SS–: sputum smear negative; TB: tuberculosis; TN: true negative; TP: true positive.

Source: WHO Consolidated guidelines on TB, Module 3: Diagnosis, Rapid diagnostics for detection of TB, 2021 update.

Table 2.1B: The diagnostic accuracy of Xpert Ultra for pulmonary TB, as compared with MRS

Patient population	Test accuracy	Studies (participants)	Certainty of evidence	2.5% prevalence	10% prevalence	30% prevalence
Adults PTB, MRS	Se: 0.90	6 (960)	High	TP: 22 / FN: 3	TP: 90 / FN: 10	TP: 269 / FN: 31
	Sp: 0.96	6 (1694)	High	TN: 932 / FP: 43	TN: 860 / FP: 40	TN: 669 / FP: 31
Adults PTB, SS–, MRS	Se: 0.77	6 (378)	High	TP: 19 / FN: 6	TP: 77 / FN: 23	TP: 231 / FN: 69
	Sp: 0.96	6 (1671)	High	TN: 932 / FP: 43	TN: 860 / FP: 40	TN: 669 / FP: 31
Adults PTB, HIV+, MRS	Se: 0.88	2 (149)	Low	TP: 22 / FN: 3	TP: 88 / FN: 12	TP: 265 / FN: 35
	Sp: 0.95	2 (430)	High	TN: 923 / FP: 52	TN: 852 / FP: 48	TN: 663 / FP: 37
Adults PTB, prior TB, MRS	Se: 0.84	4 (127)	Low	TP: 21 / FN: 4	TP: 84 / FN: 16	TP: 251 / FN: 49
	Sp: 0.86	4 (475)	Low	TN: 842 / FP: 133	TN: 778 / FP: 122	TN: 605 / FP: 95

FN: false negative; FP: false positive; HIV+: human immunodeficiency virus positive; MRS: microbiological reference standard; PICO: population, intervention, comparator and outcomes; PTB: pulmonary tuberculosis; Se: sensitivity; Sp: specificity; SS–: sputum smear negative; TB: tuberculosis; TN: true negative; TP: true positive.

Source: WHO Consolidated guidelines on TB, Module 3: Diagnosis, Rapid diagnostics for detection of TB, 2021 update.

2.4.2. Blood-based methods

Blood-based TB diagnostic methods can be used to diagnose latent TB infection (LTBI) or active TB referred to as *Mycobacterium Tuberculosis* bloodstream infection (BSI). LTBI is diagnosed with an IGRA and has a sensitivity and specificity that ranges from 81% to 88% (Diel, Loddenkemper & Nienhaus, 2012). TB BSI can be diagnosed with a TB blood culture in patients that are HIV positive (Barr, Lewis, Feasey *et al.*, 2020).

Studies have also been conducted to evaluate the sensitivity of TB blood serology. These studies revealed poor yield leading to non-adoption of this diagnostic modality in clinical medicine. Peripheral blood based mononuclear cell and bone marrow aspirate TB PCR for diagnosis of disseminated TB has been used with varying successes (Mehta *et al.*, 2012). More recently, the WHO consolidated TB guidelines (2021 update) recommends the use of blood based NAATs namely the serum Xpert MTB/Rif with a sensitivity of 56% and specificity of 94% in HIV positive subjects.

2.4.3. Urine-based methods

There are at least four diagnostic modalities that have been studied in the quest to find a less invasive method of establishing a pulmonary TB diagnosis. These are the urine microscopy, culture, DNA PCR and of recent urinary lipoarabinomannan (Drain *et al.*, 2014). However, two large retrospective reviews demonstrated that the urine microscopy and culture yielded a 2% in sensitivity in HIV negative patients thereby making these diagnostic modalities unfavourable. This is contrast to the 77% yield demonstrated in an HIV co-infected cohort particularly amongst those with low CD4

counts and useful in HIV burdened areas (Shah *et al.*, 2016).In a Cochrane review by Bjerrum, Schiller, Dendukuri et al. (2019) of 8 studies involving 3449 HIV positive participants, lateral flow Urine LAM had a pooled sensitivity of 42% (95% CI 31%-55%) and a pooled specificity of 91% (95% CI 85% -95%). The pooled sensitivity increases to 62% (95% CI 41%-83%) whilst the pooled specificity reduces to 84% (95% CI 48%- 96%) among inpatients (Bjerrum et al, 2019). The urine LAM sensitivity and specificity demonstrates and inverse proportionality with the degree of immunosuppression measured by CD4 counts as shown in table 2.2 below.

Table 2.2: AlereLAM pooled sensitivity and specificity for TB diagnosis, by study population

Type of analysis	Symptomatic participants				Unselected participants			
	Studies (total participants)	Participants with TB	Pooled sensitivity (95% CrI)	Pooled specificity (95% CrI)	Studies (total participants)	Participants with TB	Pooled sensitivity (95% CrI)	Pooled specificity (95% CrI)
Overall accuracy	8 studies (3449)	1277 (37%)	42% (31–55%)	91% (85–95%)	7 studies (3365)	432 (13%)	35% (22–50%)	95% (89–98%)
By setting								
Inpatient	6 studies (2253)	868 (39%)	52% (40–64%)	87% (78–93%)	3 studies (537)	159 (30%)	62% (41–83%)	84% (48–96%)
Outpatient	4 studies (1196)	409 (34%)	29% (17–47%)	96% (91–99%)	6 studies (2828)	273 (10%)	31% (18–47%)	95% (87–99%)
By CD4 cell count								
CD4 >200	3 studies (738)	163 (22%)	16% (8–31%)	94% (81–97%)	1 study[a] (156)	11 (7%)	Not applicable	Not applicable
CD4 ≤200	4 studies (1825)	722 (40%)	45% (31–61%)	89% (77–94%)	2 studies (706)	82 (12%)	26% (9–56%)	96% (87–98%)
CD4 >100	4 studies (1519)	425 (28%)	17% (10–27%)	95% (89–98%)	4 studies (952)	115 (12%)	20% (10–35%)	98% (95–99%)
CD4 ≤100	4 studies (1239)	512 (41%)	54% (38–69%)	88% (77–94%)	3 studies (417)	130 (31%)	47% (40–64%)	90% (77–96%)
CD4 101–200	4 studies (586)	210 (36%)	24% (14–38%)	90% (77–96%)	1 study[b] (103)	13 (13%)	Not applicable	Not applicable
By CD4 and setting								
CD4 ≤200 inpatient	2 studies (1009)	348 (34%)	54% (34–73%)	80% (58–91%)	1 study[c] (54)	14 (26%)	Not applicable	Not applicable

Source: WHO Consolidated guidelines on TB, Module 3: Diagnosis, Rapid diagnostics for detection of TB, 2021 update.

2.4.4. NAATs for the diagnosis of extrapulmonary TB

The Xpert MTB/RIF demonstrates relatively lower sensitivity and specificity in the diagnosis of extrapulmonary TB. The sensitivity of this test varies from a relative high of 89% on lymph node aspiration to a low of 50% on pleural fluid. Conversely the specificity varies from a relative high of 97% on CSF to a low of 79% on lymph node biopsy.

Table 2.3: The diagnostic accuracy of Xpert MTB/RIF for extrapulmonary TB in adults, as compared with MRS and CRS

Patient population	Test accuracy	Studies (persons)	Certainty of evidence	2.5% prevalence	10% prevalence	20% prevalence
Adults CSF, MRS	Se: 0.70	28 (521)	Moderate	TP: 18 / FN: 7	TP: 70 / FN: 30	TP: 141 / FN: 59
	Sp: 0.97	28 (2582)	High	TN: 944 / FP: 31	TN: 871 / FP: 29	TN: 774 / FP: 26
Adults CSF, CRS	Se: 0.41	12 (774)	Low	TP: 10 / FN: 15	TP: 41 / FN:59	TP: 81 / FN: 119
	Sp: 0.99	12 (1123)	Moderate	TN: 970 / FP: 5	TN: 896 / FP: 4	TN: 796 / FP: 4
Adults LNA, MRS	Se: 0.89	14 (627)	Moderate	TP: 22 / FN: 3	TP: 89 / FN:11	TP: 177 / FN: 23
	Sp: 0.86	14 (961)	Very low	TN: 839 / FP: 136	TN: 774 / FP:126	TN: 688 / FP:112
Adults LNA, CRS	Se: 0.81	4 (377)	Low	TP: 20 / FN: 5	TP: 81 / FN: 19	TP: 162 / FN: 38
	Sp: 0.96	4 (302)	Low	TN: 935 / FP: 40	TN: 863 / FP: 37	TN: 767 / FP:33
Adults LNB, MRS	Se: 0.82	11 (220)	Low	TP: 21 / FN: 4	TP: 82 / FN: 18	TP: 164 / FN: 36
	Sp: 0.79	11 (566)	Very low	TN: 773 / FP: 202	TN: 714 / FP:186	TN: 634 / FP:166
Adults, pleural fluid, MRS	Se: 0.50	24 (589)	Very low	TP: 12 / FN: 13	TP: 50 / FN: 50	TP: 99 / FN: 101
	Sp: 0.99	24 (2337)	High	TN: 962 / FP: 13	TN: 888 / FP: 12	TN: 790 / FP: 10
Adults, pleural fluid, CRS	Se: 0.19	10 (616)	Moderate	TP: 5 / FN: 20	TP: 19 / FN: 81	TP: 39 / FN: 161
	Sp: 0.99	10 (408)	High	TN: 964 / FP: 11	TN: 890 / FP: 10	TN: 791 / FP: 9
Adults, peritoneal fluid, MRS	Se: 0.59	13 (94)	Low	TP: 15 / FN: 10	TP: 59 / FN: 41	TP: 118 / FN: 82
	Sp: 0.97	13 (486)	High	TN: 949 / FP: 26	TN: 876 / FP: 24	TN: 778 / FP: 22
Adults, pericardial fluid, MRS	Se: 0.60	5 (57)	Very low	TP: 15 / FN: 10	TP: 60 / FN:40	TP: 121 / FN: 79
	Sp: 0.88	5 (124)	Low	TN: 856 / FP: 119	TN: 790 / FP:110	TN: 702 / FP: 98
Adults, pericardial fluid, CRS	Se: 0.66	2 (60)	Very low	TP: 16 / FN: 9	TP: 66 / FN: 34	TP: 132 / FN: 68
	Sp: 0.96	2 (17)	Very low	TN: 936 / FP:39	TN: 864 / FP: 36	TN: 768 / FP: 32
Adults, urine, MRS	Se: 0.85	9 (72)	Low	TP: 21 / FN: 4	TP: 85 / FN: 15	TP: 169 / FN: 31
	Sp: 0.97	9 (871)	Moderate	TN: 949 / FP: 26	TN: 876 / FP: 24	TN: 778 / FP: 22
Adults, synovial fluid, MRS	Se: 0.97	6 (110)	Moderate	TP: 24 / FN: 1	TP: 97 / FN: 3	TP: 194 / FN: 6
	Sp: 0.94	6 (361)	Very low	TN: 914 / FP: 61	TN: 843 / FP: 57	TN: 750 / FP: 50
Adults, synovial fluid, CRS	Se: 0.88	2 (161)	Low	TP: 22 / FN: 3	TP: 88 / FN: 12	TP: 177 / FN: 23
	Sp: 0.98	2 (44)	Very low	TN: 955 / FP: 20	TN: 881 / FP: 19	TN: 783 / FP: 17
Adults HIV+, blood, MRS	Se: 0.56	1 (9)	Very low	TP: 14 / FN: 11	TP: 56 / FN: 44	TP: 112 / FN: 88
	Sp: 0.94	1 (65)	Very low	TN: 917 / FP: 58	TN: 846 / FP: 54	TN: 752 / FP: 48

CRS: composite reference standard; CSF: cerebrospinal fluid; FN: false negative; FP: false positive; HIV+: human immunodeficiency virus positive; LNA: lymph node aspirate; LNB: lymph node biopsy; MRS: microbiological reference standard; PICO: population, intervention, comparator and outcomes; Se: sensitivity; Sp: specificity; TB: tuberculosis; TN: true negative; TP: true positive.

Source: WHO Consolidated guidelines on TB, Module 3: Diagnosis, Rapid diagnostics for detection of TB, 2021 update.

2.4.5 Imaging diagnostic modalities

There are various imaging modalities that play a crucial role in both the screening and diagnosis of PTB and EPTB (Skoura, Zumla & Bomanji, 2015). These imaging diagnostic modalities play a crucial role in cases where the microbiological investigations fail to confirm the diagnosis of TB in patients with clinical signs and symptoms in keeping with TB infection (Davies & Pai, 2008). The most commonly used imaging modality is a plain chest radiograph, which can reveal features of both primary and post pulmonary TB (Skoura *et al.*, 2015).

The primary pulmonary TB can be demonstrated by the presence one or more of the following features; lung consolidation, pleural effusion, hilar lymphadenopathy or miliary pattern (Skoura *et al.*, 2015). The computed tomography (CT) of the chest is the preferred method of imaging to demonstrate the presence of TB associated lymphadenopathy and parenchymal bronchiectatic changes (Hahm, Park, Jeon *et al.*, 2010).

Abdominal ultrasonography is essential in the diagnosis of abdominal TB which mostly presents as intra-abdominal lymphadenopathy in 55 -66% of the patients (Skoura, Zumla & Bomanji, 2015). Furthermore hepatic, or splenic increased echogenicity on ultrasound would be diagnostic of hepatic and splenic TB(Rodriguez-Takeuchi, Renjifo & Medina, 2019). These features are better defined on a CT abdomen as innumerable small deposits diagnostic on abdominal viscera diagnostic of generalised milliary TB(Skoura *et al.*, 2015). CT abdomen may also reveal hepatic tuberculomas as slightly enhancing peripheral rim (Skoura *et al.*, 2015).

A magnetic resonance imaging (MRI) is considered superior to a CT scan in the

diagnosis of TB of the central nervous system (Skoura *et al.*, 2015). A nuclear medicine study known as the Positron emission tomography/computed tomography with the use of 18F-fluorodeoxyglucose (18F-FDG PET/CT) can differentiate TB from malignant lesions as well as evaluate the response to TB treatment (Malherbe, Chen, Dupont *et al.*, 2020).

2.5. Current search for a quintessential TB diagnostic methods

The WHO estimates that approximately 37% of active TB cases remain undiagnosed contributing to the high morbidity and mortality globally (Glaziou *et al.*, 2018; Pai *et al.*, 2016). This is partly due to the misapplication of the TB diagnostic modalities. However, co-morbid conditions such as HIV infection, diabetes mellitus, chronic kidney disease, and chronic haemodialysis render some of the TB diagnostic modalities less sensitive in the diagnosis of TB (Pisoni, Aros, Ruggenenti *et al.*, 2002). This has led to the ongoing innovation to find robust TB diagnostic tests that can be used to diagnose TB in patients that are immunocompromised. There remains a great deal of uncertainty regarding the extent to which clinicians follow the WHO validated TB diagnostic algorithm when diagnosing TB. Thus, this study shall seek to describe the TB diagnostic methods used by the clinicians at Pietersburg Hospital.

CHAPTER 3: RESEARCH METHODOLOGY

3.1. Introduction

This chapter enunciates the full account of the research methodology that was employed in this research undertaking, as well conferring reasons for adopting such a research method. It begins with giving an elaboration on the research method, research design, and the study population. This is followed by the discussion of the sampling method, data collection process, and the data handling in the way of statistical analysis. Lastly, the validity of the study and the ethical considerations in the conduct of the study are also discussed.

3.2. Research Method

A census method was used to collect data for this statistical analysis. In a census method data about all the members of the study population is collected for statistical analysis (Joubert & Ehrlich, 2007). The researcher used secondary data of the TB notification records of the Infection prevention and control unit (IPCU) at Pietersburg Hospital.

Pietersburg Hospital is part of the Pietersburg Mankweng Hospital Complex, and it provides tertiary health care services in the rural Limpopo Province of South Africa. It is the major referral centre for all the health care facilities in the province. It is situated in the city of Polokwane within the Capricorn district of the Limpopo Province.

It is a relatively large hospital consisting of more than 700 commissioned beds; however, just over 500 of those beds are in active use. It has the following clinical departments and clinical units, Paediatrics, Obstetrics and Gynaecology, Internal

Medicine, Nephrology, Cardiology, Neurology, Neurosurgery, Orthopaedics, General Surgery, Radiation Oncology, Medical Oncology and Psychiatry.

The IPCU at this hospital comprises of the nursing personnel that is tasked with notification of Notifiable Medical Conditions (NMC), including tuberculosis, as per South Africa's National Institute for Communicable Diseases (NICD). Thus, the meticulous records of patients diagnosed with NMCs are kept at IPCU.

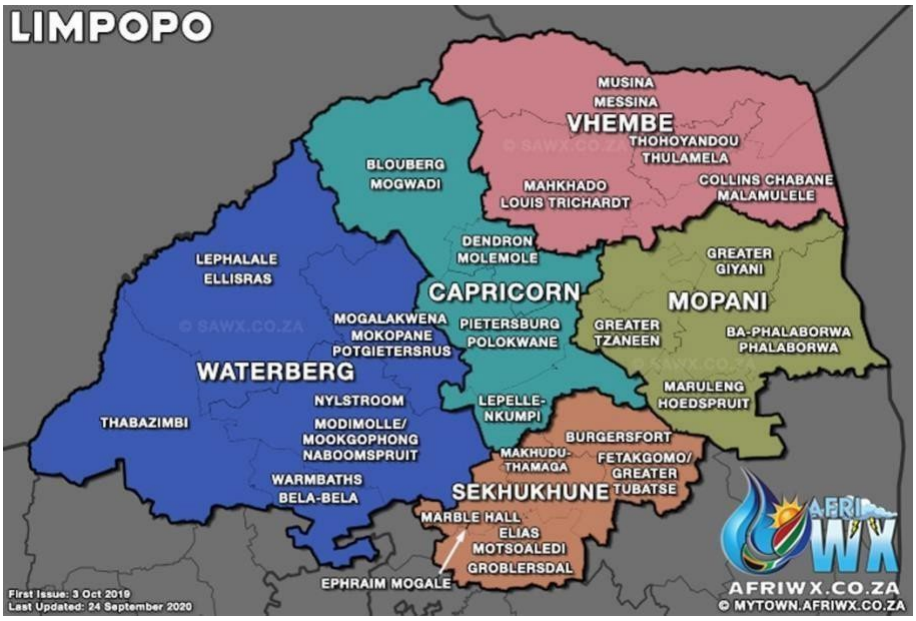


Figure 3.1: The map of study site, Limpopo province

3.3. Research design

A retrospective descriptive study of the patients aged 12 years or older diagnosed with tuberculosis at Pietersburg Hospital, Limpopo Province, South Africa, was undertaken. A quantitative analysis of these patients' collected data was performed.

3.4. Study Population

The study population consisted of all patients aged 12 years or older who were diagnosed with tuberculosis, and notified at the ICPU at Pietersburg Hospital over a 12-month period from 01 January 2020 to 31 December 2020.

3.5. Sampling technique and Sample size

This was a census study wherein all the inpatients admitted to medical, surgical, and gynaecological wards wherein a subsequent diagnosis of active pulmonary tuberculosis (PTB) or extra-pulmonary tuberculosis (EPTB) was made and met the inclusion criteria as set out below, were included in the study. Thus, a whole population (as opposed to a sample) of adult patients that have been diagnosed with all forms of tuberculosis at Pietersburg Hospital from January 2020 to December 2020 were studied through a descriptive retrospective study.

Inclusion criteria

- All the inpatients over the age of 12 years diagnosed with PTB and/or EPTB at Pietersburg Hospital and recorded by the ICPU staff from 01 January 2020 to 31 December 2022.

Exclusion criteria

- All TB inpatients and outpatients under the age of 12 years.

3.6. Data collection

A retrospective secondary data collection from the hospital's ICPU record book wherein all inpatient TB diagnoses are captured and recorded on a specifically designed data collection sheet. The data collection tool contained all the relevant variables meant to achieve the aim and objectives of this research undertaking. The data collection sheet comprised of the following two sections;

Section A: Demographic data

- Age
- Sex
- Weight

Section B: Clinical data

- Clinical site of TB diagnosis
- Diagnostic method
- HIV status
- CD4 count
- HAART
- Other co-morbidities
- Date of TB notification
- Previous TB infection

The study data was then subsequently electronically captured on a spreadsheet with

the use of Microsoft Excel 2016 software (Microsoft Corporation, Redmond, Washington, USA) for analysis and interpretation.

3.7. Data analysis

The study data was captured using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA) which was password encrypted. The captured data was then imported into STATA 14 (College Station, Texas 77845 USA) software. The cleaning of the data was done using Stata software whereby removal of duplicates and cases that did not meet the inclusion criteria. This was accomplished by maintaining a separate log of patients that were stored securely at the IPCU in order to identify any duplicates that appeared on the data collection sheets.

The categorical data was described in detail and displayed using bar graphs, pie charts, frequencies, percentages, and fractions. The continuous variables were described using measures of central tendencies such as mean, median, standard deviation, ranges et cetera. The author has also made use of visual data display diagrams such as line graphs, histogram, and a scatter plot.

3.8. Internal and external validity of the study

The quality and the relevance of the collected data has a significant bearing on the validity of the study. The data collection tool used in this study had not been used

before in another setting. Nonetheless, it was subjected to critical academic scrutiny by the staff members of the department of Internal medicine. Furthermore, inputs were also made by the other structures of the University research committees. Thus, at the time of collecting data, the data collection sheet had been well designed to strengthen and ensure the validity of the study.

3.9. Ethical considerations

The conduct of this study, including data collection, occurred following the approval by five structures of the University of Limpopo, Limpopo department of Health and the Pietersburg Hospital management. These structures are: Faculty of Health Sciences committee, senor degree committee, University of Limpopo Turfloop research ethics committee (TREC), Limpopo provincial research committee and the Polokwane Mankweng Hospital complex.

Furthermore, the patients were anonymised through the creation of unique identification codes during the data collection. The TB records were kept safe in a locker at the IPCU for the duration of the study.

3.10. Conclusion

The focus of chapter three has been to detail the steps undertook to conduct this study. To that end, a description of the research method, research design, data collection and data analysis has been elaborated on. Furthermore, an account of the ethical considerations and the validity of the study has been provided.

CHAPTER 4: PRESENTATION/INTERPRETATION/DISCUSSION OF FINDINGS

4.1. Introduction

This chapter shall systematically outline the steps undertook by the researcher in processing the collected data. A discussion of the data handling using STATA 14 software will also be outlined (College Station, Texas 77845 USA). A display of the study results in diagrammatic form using flow charts, tables, graphs, and pie charts is made. Furthermore, the researcher will discuss the findings as they relate to each study objectives and the overarching aim of the study. In addition, significant additional findings will also be discussed in this chapter.

4.2. Data Management and Analysis

The study data was captured from the TB notification records book at the IPCU and transcribed onto the data collection sheet per individual patient. These data were then further captured electronically onto the excel spreadsheet using the Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Furthermore, the captured data was then imported into the STATA 14 software for statistical analysis (College Station, Texas 77845 USA).

4.3. Research Results

There were 136 cases of TB cases for patients aged 12 year and older recorded in the TB notification book at the IPCU from 1 st of January 2020 to 31st of December 2020. All these cases were captured and one had to be removed as it was a duplicate. All the remaining 135 cases had all the necessary details as per the data collection sheet and met the inclusion criteria. Figure 4.1 shows the data collection algorithm as followed in this study.

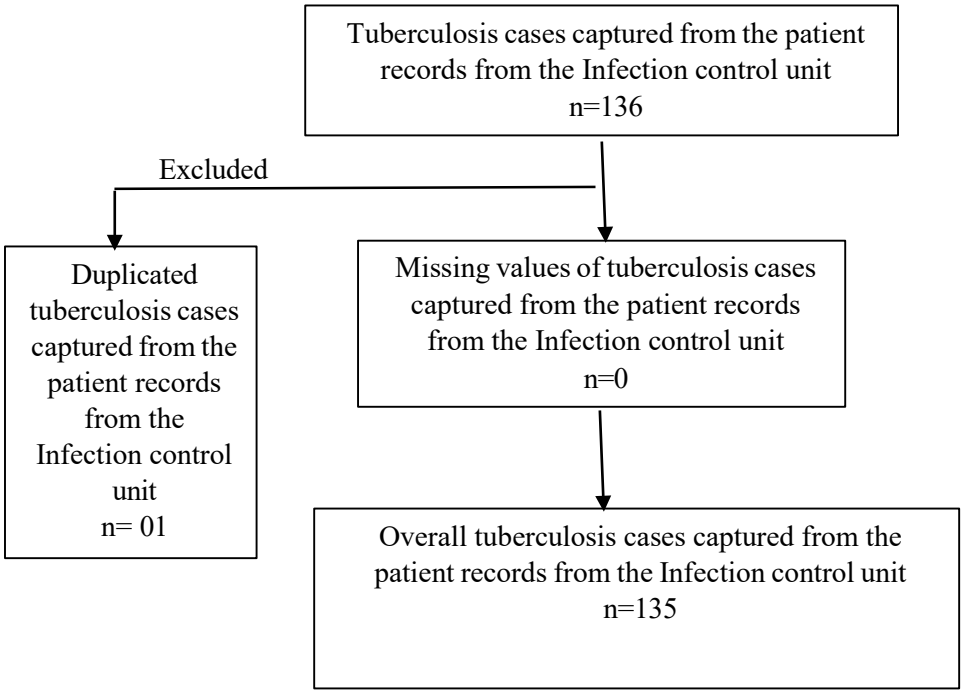


Figure 4.1: Flow chart showing extraction of tuberculosis cases at Pietersburg Hospital from January 2020 to December 2020, Limpopo Province of South Africa.

Thus, the study had 135 individuals of whom, 77/135 (57%) were female and 43% 58/135 (43%) were male. The median age of the study patients was 37 years with an interquartile range of 29 to 45 years as shown on table 4.1.

Table 4.1. Baseline characteristics of the TB cases captured from the IPCU at Pietersburg Hospital from January 2020 to December 2020 (N=135).

Demographic variables	Number of cases	Proportion% (n/N)
Sex		
Female	77	57
Male	58	43
Age category in years		
12-24	18	13.3
25-34	38	28.2
35-44	43	31.9
45-54	16	11.9
55-64	11	8.2
≥65	9	6.7
clinical site of the diagnosis		
Extra-pulmonary TB	57	42.2
Pulmonary TB	78	57.8
HIV Status		
Positive	98	72.6
Negative	34	25.2
Unknown	3	2.2
CD4 count		
<50	43	31.9
≥50	51	37.8
Unknown	5	3.7
Not Applicable	36	26.7
Previous TB infection		
Yes	6	4.4
No	128	94.8
Unknown	1	0.7
Total	**135**	**100**

Pulmonary TB was the commonest clinical site of TB diagnosis at 57.8% (78/135), followed by TB meningitis at 11/135 (8.1%) as shown on figure 4.2A. Pleural TB made up 10/135 (7.4%) followed by miliary TB at 9/135 (6.6%) and TB lymphadenitis at 8/135 (5.9%). The rest of the cases were made up of TB spine, disseminated TB, TB joint, pericardial TB, TB skin (lupus vulgaris & erythema induratum), TB abdomen and perianal TB, which accounted for 13.3% (18 cases) and less than 3% individually.

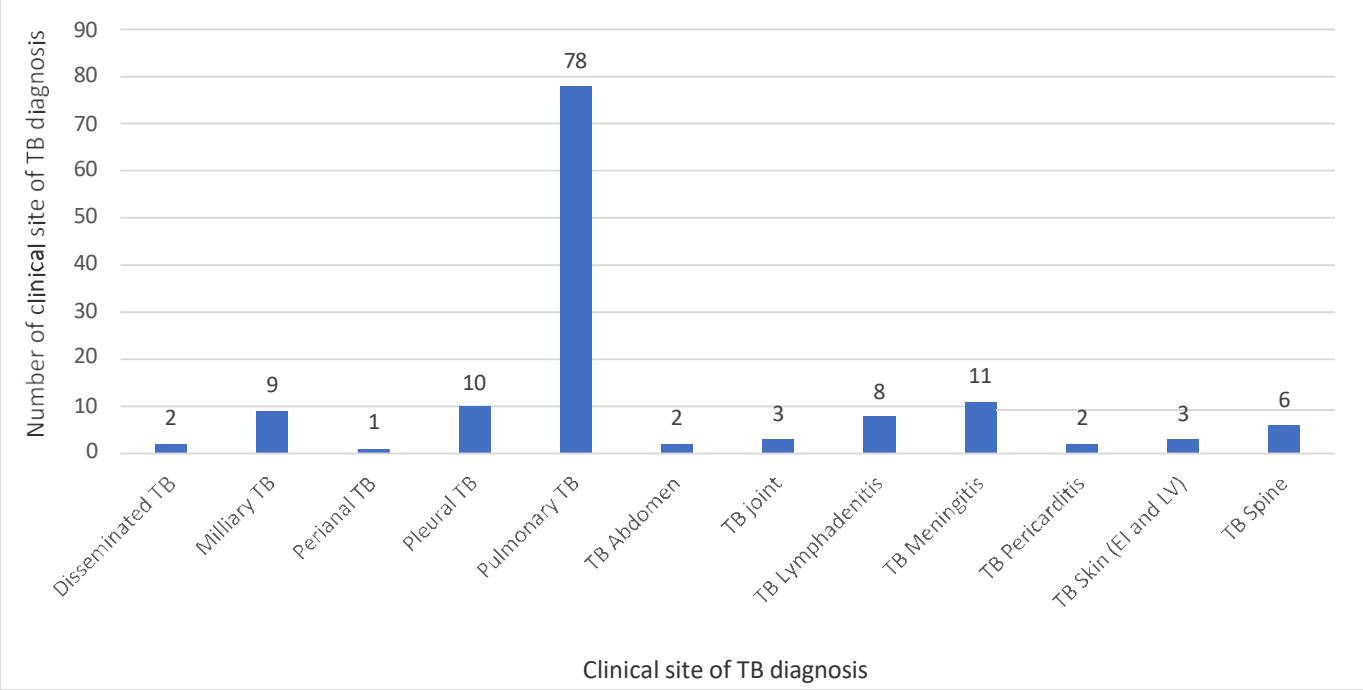


Figure 4.2A: The clinical site of TB diagnosis in patients diagnosed with TB at Pietersburg Hospital in Limpopo Province, from January to December 2020.

Table 4.2A: Breakdown of test outcomes in patients with definitive TB

Sputum test name	Number of definitive TB cases by Sputum	Proportion (%) of definitive TB cases by Sputum
Sputum AFB		
Positive	16	50.0
Negative	10	31.3
Not done	6	18.8
Sputum GXP		
Positive	28	87.5
Positive-Rif resistant	1	3.1
Negative	2	6.3
Not done	1	3.1
Sputum TB Culture		
Positive	8	25.0
Mycobacterium Semiae positive	1	3.1
Negative	4	12.5
Not done	19	59.4
Overall	**32**	**100.0**

Various imaging techniques were used in 123/135 (91%) of the individuals to aid in the diagnosis of TB as shown on figure 4.2B. These consisted of 105/135 (77.7%) chest x-rays of which 94/105 (89.5%) were deemed suggestive of TB by the treating clinicians. Furthermore, there were 8/135 (5.9%) patients in whom CT Brain imaging were done, and deemed suggestive of TB meningitis by the treating clinicians, and five MRI spine imaging deemed diagnostic of TB spine by the treating team. The remainder of the imaging techniques done were one abdominal ultrasound, two echocardiograms and two thoracolumbar spine X-rays which were all deemed diagnostic of TB of the relevant clinical site by the treating clinicians.

A total of 78 pulmonary tuberculosis were recorded, consisting of pulmonary TB (97.4%, 76/78 (97.4%), Pulmonary TB- Rifampicin mono-resistant 1/78 (1.3%), and Pulmonary TB-MDR 1/78 (1.3%) as shown on table 2B below. There were 16 PTB cases that had multiple sputa tests being positive while only two had triple sputa tests being positive.

Table 4.2B: Breakdown of test outcomes in patients with presumptive PTB on CXR

Test name	Number of presumptive TB cases by Sputum	Proportion (%) of presumptive TB cases by Sputum
Sputum AFB		
Negative	5	10.9
Not done	41	89.1
Sputum GXP		
Negative	25	54.3
Not done	21	45.7
Sputum TB Culture		
Negative	6	13.0
Not done	40	87.0
Overall	**46**	**100.0**

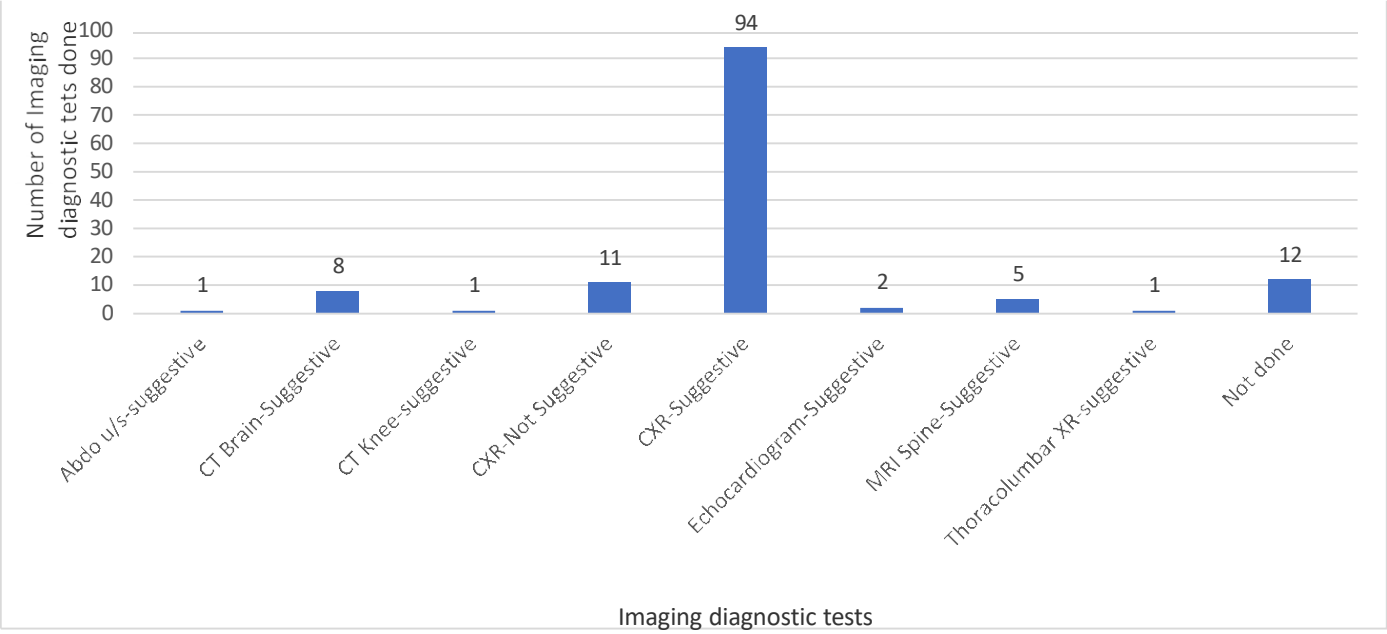


Figure 4.2 B: Imaging diagnostic modalities

Of the sputa tests that were conducted, 41/135 (30.3%) of the individuals had AFB done of which17/41(41.5%) were positive, 75/135 (55.6%) of the patients had GXP done with 32/75 (42.7%) being positive and 24 sputa TB cultures were conducted with a 10/24 (41.7%) positive yield including one case of *Mycobacterium seminae*. Furthermore, all but one case had a rifampicin sensitive TB as per the GXP as shown on figure 4.2C.

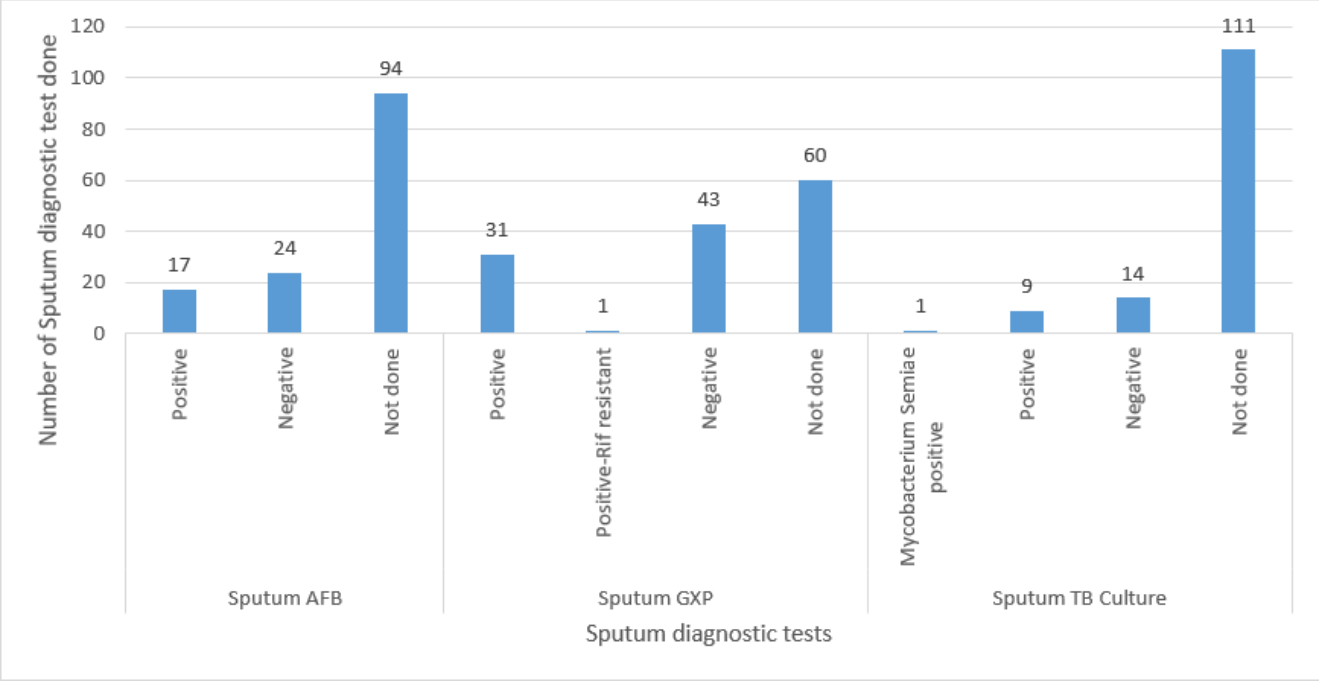


Figure 4.2 C: Sputum based diagnostic tests

The Urine LAM test was conducted in only 36.3% (49/135) of the patients with 85.7% (42/49) of them yielding a positive result as shown on figure 4.2D. There were 52.5% (63/135) TB blood cultures done of which 11.1% (7/63) were positive for *Mycobacterium tuberculosis*, 4 6.3% (4/63) positive for *Mycobacterium avium complex*, 0.7% (1/63) positive for *Mycobacterium seminae*, one was contaminated and the remaining 50 tested negative for TB. Other tests that were done ranged from CSF analysis, ascitic fluid, pleural fluid, pericardial fluid, joint aspirate, soft tissue biopsies to pus swab as shown on figure 4.2E.

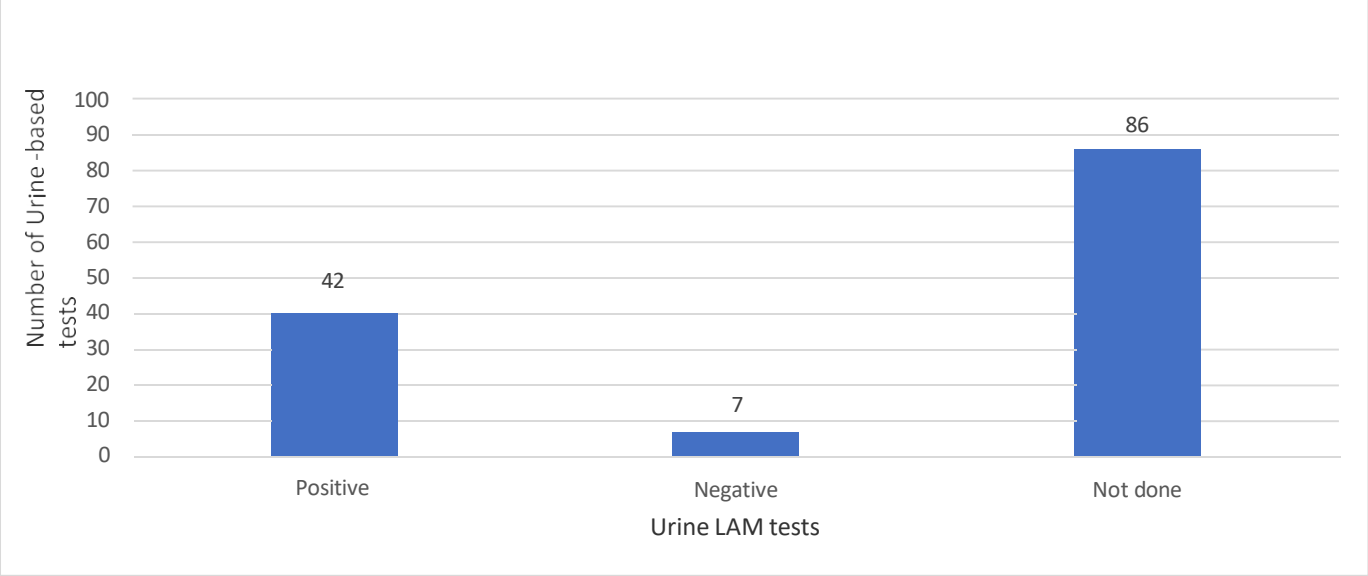


Figure 4.2D: Urine LAM test results

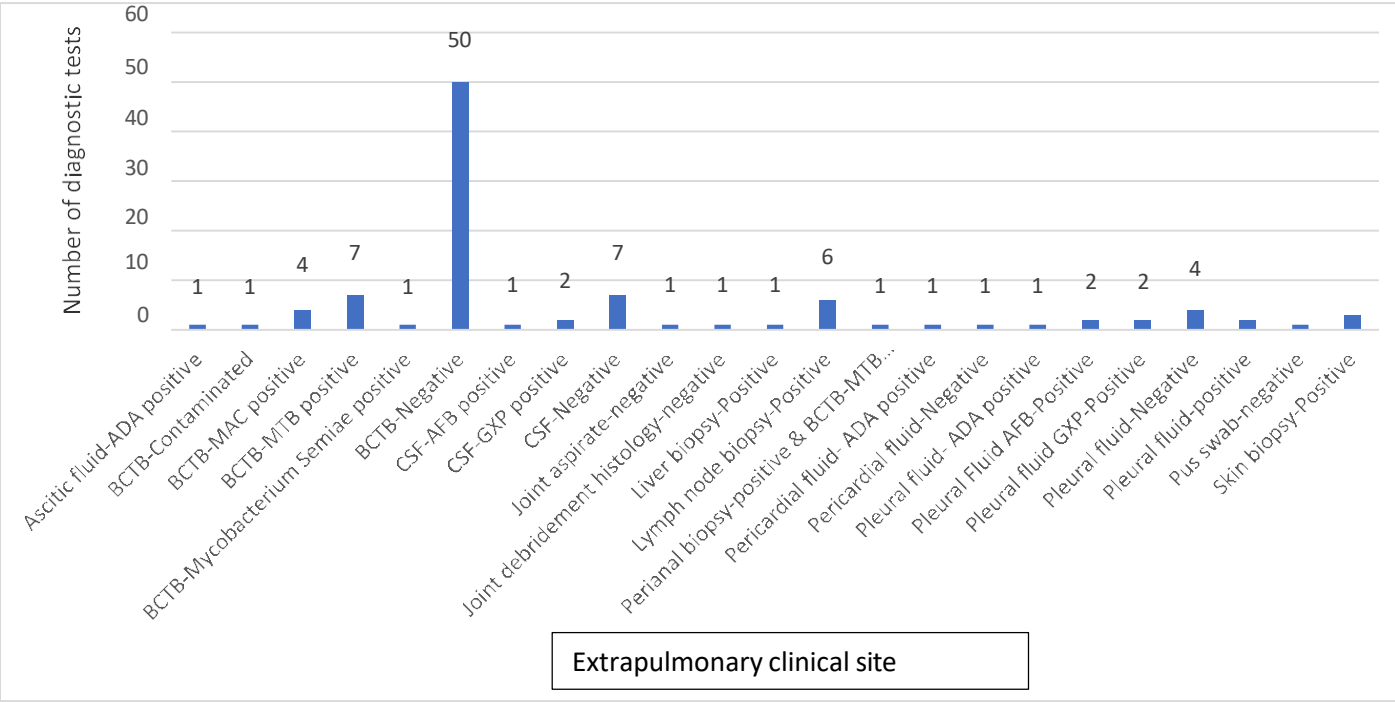
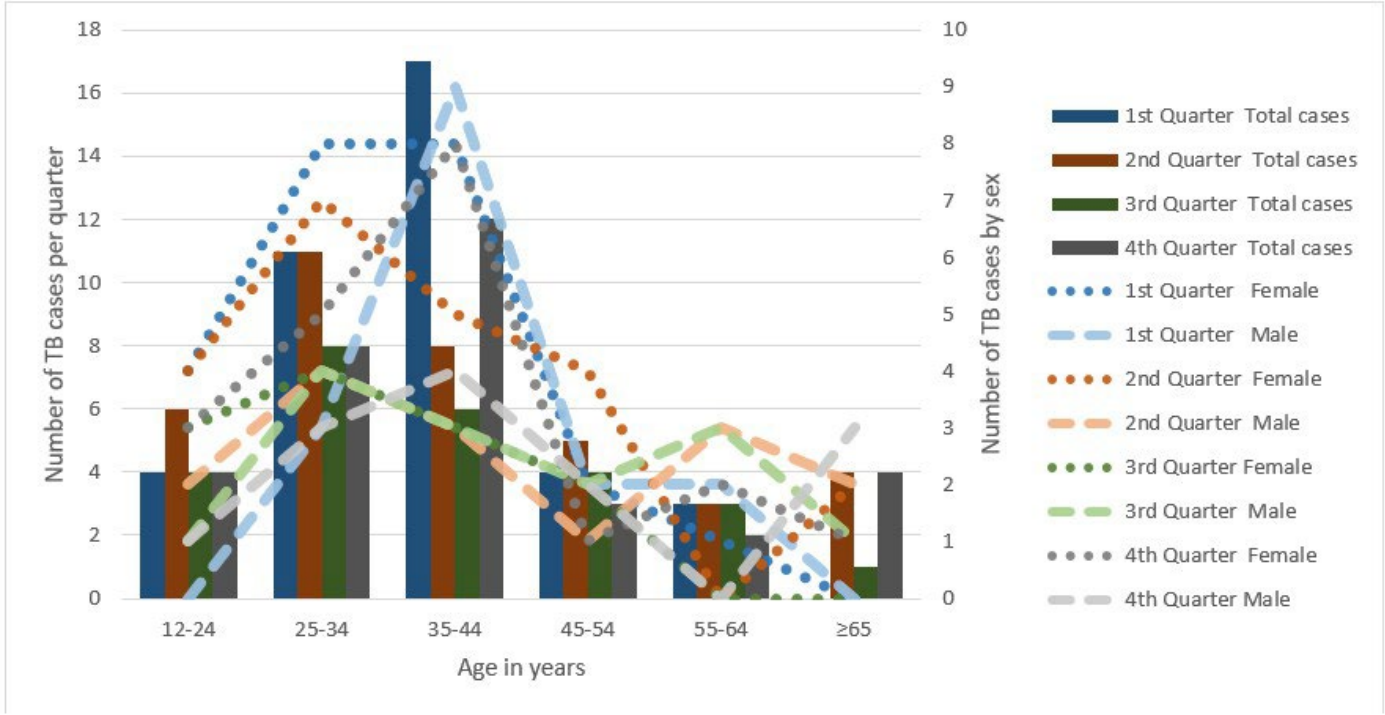


Figure 4.2 E: Blood based and other diagnostic tests; adenine deaminase (ADA), acid fast bacilli (AFB) TB blood culture (BCTB), cerebrospinal fluid (CSF), MAC (*Mycobacterium avium*

More than half (56.3%) of the reported patient cases for both male and female across all the age groups occurred in the first and second quarters of the year, as shown on figure 4.3. There was a high number of TB cases in patients aged between 35 and 44 years, and fewer cases in those aged above 65 years. The data demonstrated a high number of cases amongst females aged 12-54 years both in the first and second quarters of the year, and showing a decline in reported cases in the third and fourth quarters across the various female age groups. The trend amongst male patients mirrors that of female patients by also showing a sharp increase in reported TB cases in the first and second quarter followed by a decline in the third and fourth quarters across the age groups.



Of the 135 tuberculosis cases captured from the patient records from the Infection control unit, 72.6% (98/135) were found to be HIV Positive, while 3.0% (4/135) were diagnosed with Diabetes Mellitus. Furthermore, 46% (45/98) of these HIV positive patients had a CD4 cell count of below 50, as shown on figure 4.4. Various imaging techniques were conducted in 94.7% (90/98) of the individuals. Of the 80 CXRs that were performed in these HIV positive patients, 90% (72/80) had results in keeping with active pulmonary TB as per the treating team. The remainder of the imaging techniques consisted of CT brain imaging, one echocardiogram, MRI spine and thoracolumbar X-rays, which were also deemed suggestive of TB of the relevant clinical site by the treating clinicians.

Of the 98 HIV co-infected individuals, of those that had sputa tests 31/98 (31.6%) had sputum AFB performed with 13/31(41.1%) positive, culture sent in 19/98 (19.4%) with 6/19 (31.6%) positive and 56/98 (57.1%) sent for GXP with 24/56(42%) being positive. patients had GXP test done which showed positive results in 42.8% (24/56) as shown on table 4.2. Of the 47/98(47.9%) urine LAM tests that were done in HIV co-infected individuals, 40/47 (85.1%) were positive. Furthermore, of the 51% (50/98) TB blood cultures that were done in HIV positive individuals 37/50 (74%) were negative for TB. The remainder of the TB blood cultures grew *Mycobacterium tuberculosis* 7/50(14%), *Mycobacterium avium complex* 4/50 (8%) and *Mycobacterium seminae* 1/50 (2%). Of note is that all the four patients that grew *Mycobacterium avium complex* and as a the one who grew *Mycobacterium seminae* on TB blood culture had CD4 cell counts below 50 cubic millimetre.

Table 4.3: The frequency of use of various diagnostic tests in patients diagnosed with TB and HIV co-infection at Pietersburg Hospital in Limpopo Province, from January to December 2020.

Diagnostic methods	Number of tests	%
HIV coinfection patients		
Imaging		
CT Brain-Suggestive	7	7.1
CXR-Not Suggestive	8	8.2
CXR-Suggestive	72	73.5
Echocardiogram-Suggestive	1	1.0
MRI Spine-Suggestive	1	1.0
Thoracolumbar XR-suggestive	1	1.0
Not done	8	
Sputum		
Sputum AFB		

Positive	13	13.3
Negative	18	18.4
Not done	67	68.4
Sputum GXP		
Positive	23	23.5
Positive-Rif resistant	1	1.0
Negative	32	32.7
Not done	42	42.9
Sputum TB culture		
Positive	5	5.1
Mycobacterium Seminae positive	1	1.0
Negative	13	13.3
Not done	79	80.6
Urine LAM		
Positive	40	40.8
Negative	7	7.1
Not done	51	52.0
Other tests		
Ascitic fluid-ADA positive	1	1.0
BCTB-Contaminated	1	1.0
BCTB-MAC positive	4	4.1
BCTB-MTB positive	7	7.1
BCTB-Mycobacterium Semiae positive	1	1.0
BCTB-Negative	36	36.7
CSF-AFB positive	1	1.0
CSF-GXP positive	1	1.0
CSF-Negative	7	7.1
Lymph node biopsy-Positive	4	4.1
Pericardial fluid-Negative	1	1.0
Pleural fluid-negative	1	1.0
Pleural Fluid AFB-Positive	2	2.0
Pleural fluid-GXP positive	1	1.0
Pleural fluid-positive	2	2.0
Pus swab-negative	1	1.0
Skin biopsy-Positive	1	1.0
BCTB-negative	1	1.0
None	25	25.5
Total tests done for HIV co-infection patients	**98**	**100**

There were only four TB patients that had diabetes mellitus as a co-morbid disease as shown on table 4.3. Two of these patients were diagnosed with pulmonary TB on CXR, one diagnosed with abdominal TB on abdominal sonar and one diagnosed with TB Meningitis on both CT brain imaging and CSF analysis. There was no sputum-based test or urine LAM done for any of these four patients. Two of the patients had blood TB culture done which was negative for TB.

Table 4.3. The frequency of use of various diagnostic tests in patients diagnosed with TB and Diabetes Mellitus at Pietersburg Hospital in Limpopo Province, from January to December 2020.

Diagnostic methods on *Diabetes Mellitus patients*	Number of tests	%
Imaging		
Abdo u/s-suggestive	1	25.0
CT Brain-Suggestive	1	25.0
CXR-Suggestive	2	50.0
Sputum		
sputumafb		
Not done	4	100.0
sputumgxp		
Not done	4	100.0
sputumtbculture		
Not done	4	100.0
urinelam		
Not done	4	100.0
Other tests		
BCTB-Negative	2	50.0
CSF-GXP positive	1	25.0
Pleural fluid-Negative	1	25.0
Total for Diabetes Mellitus patients	**4**	**100.0**

Additional findings

There were other organisms that were cultured on the sputa of the patients that were suspected to have TB, as shown on figure 4.4. These organisms include *Staphylococcus aureus* (3%), *Streptococcus agalactiae* (3%), *Pseudomonas pneumoniae* (3*%), Klebsiella pneumoniae* (9%), *Acinetobacter baumanii pneumonia* (3%),

Haemophilus influenza (3%) and *Enterobacter cloaca* (3%). A significant proportion of the patients (11%) tested positive for SARS-CoV-2 on PCR whilst some were diagnosed with *Pneumocystis jirovici pneumonia* (14%) based on the high B-D-glucan and the clinical picture. Additionally, 3 % of the patient thought to have pleural TB were found to have metastatic adenocarcinoma with malignant pleural effusion.

The following co-morbid conditions (other than HIV and diabetes mellitus were found in patients diagnosed with TB; pregnancy (6%), SLE (3%), necrotising fasciitis secondary to *Pantoea species* (3%) and cryptococcal meningitis (3%).

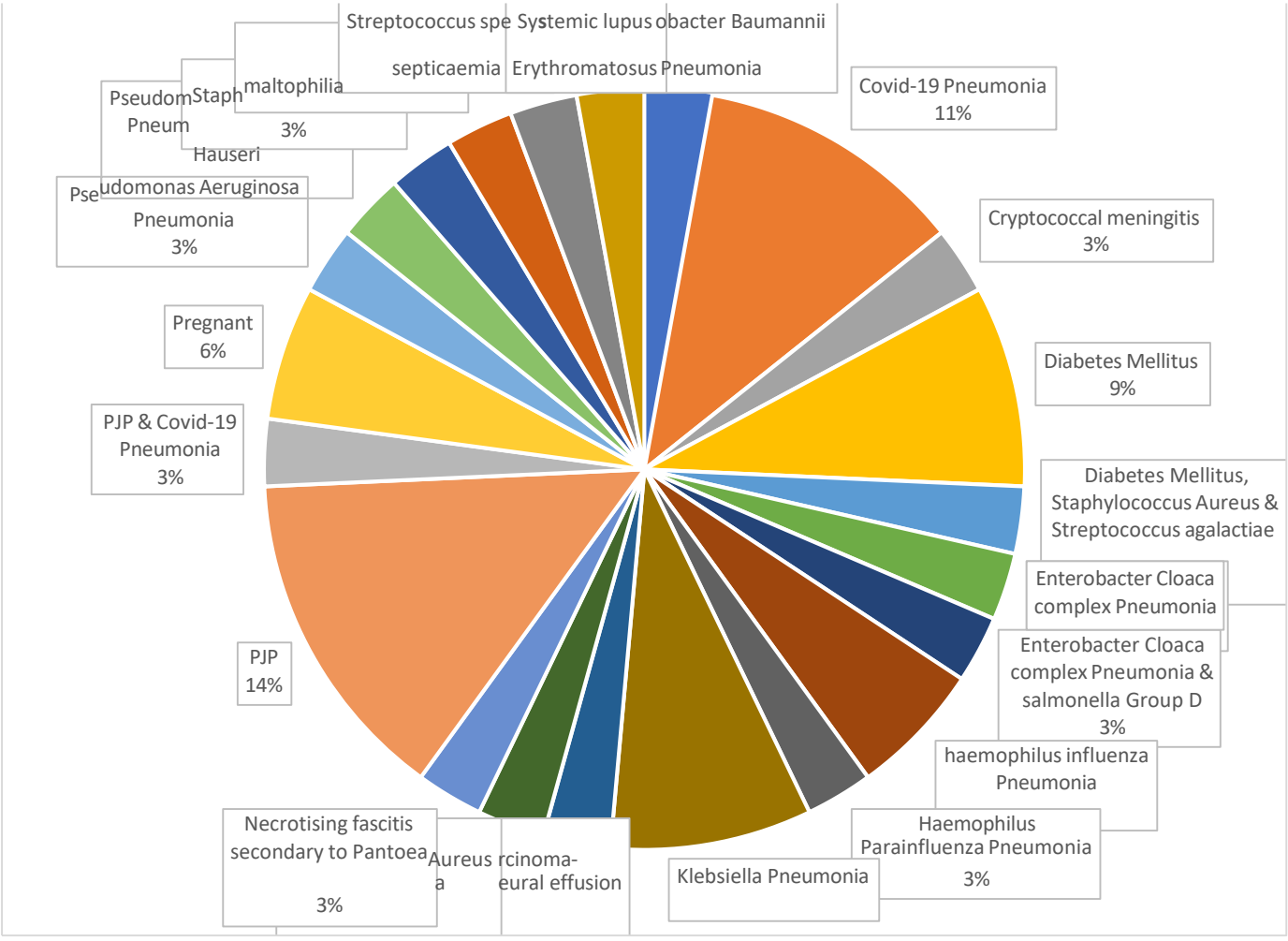


Figure 4.5:The other co-morbidities in patients diagnosed with TB at Pietersburg Hospital in Limpopo Province, from January to December 2020

4.4. Overview and Discussion of Research Findings

4.4.1. Diagnostic tests used in patients diagnosed with TB

In our study, PTB comprised 57% (77/135) of the total number of TB cases whilst the remainder of the cases were of EPTB. This is in contrast with international studies wherein PTB makes up 85% of the TB cases ((Horne, Kohli, Zifodya *et al.*, 2019). Most of the TB diagnoses in our study were made mostly using various imaging modalities (92%), followed by sputum-based methods at 75/135 (55.6%), TB blood culture at 63/135 (52.5%), urine lam at 49/135 (36.3%) whilst the remainder of the TB diagnoses were made on microbiological and biochemical analysis of various fluids (pleural, ascitic, knee aspirate and pericardial as well as tissue histology (lymph nodes and skin biopsies).

Thus, a significant number of the cases were of presumptive TB due to lack of definitive microbiological (GXP, AFB or TB culture on various media) or histological confirmation on various media. In terms of pulmonary TB, 32/78 (41%) had definitive TB as shown on table 4.2A. Of those that had definitive PTB, 16 had more than one positive test for TB whilst only two had all the three sputa tests for TB positive.

This study shows that there was an underutilisation of sputum collection as a primary mode of diagnosing both PTB and EPTB and thus it represents a significant deviation from the WHO TB diagnostic algorithm. The WHO TB diagnostic algorithm advocates for the use of sputum collection for smear, GXP and TB culture in the first instance for patients suspected to have PTB or EPTB (Lange & Mori, 2010).

4.4.2. Quarterly TB infection trends by age and sex

The seasonality of TB trends is a well-established concept in medical literature (Kirolos, Thindwa, Khundi, *et al.*, 2021). However, such seasonality seems to vary widely from one geographical region to another owing to a myriad of postulated reasons (Ballif, Zürcher, Reid *et al.*, 2018). One prominent theory relates to the weather patterns wherein it is postulated that cold weather promotes prolonged indoor dwelling with limited ventilation together with reduced exposure to sunlight. This is believed to foster an environment that is favourable for the acquisition and transmission of TB.

Another theory relates to the festivities that often mark the celebrations of the end of the year (Azeez, Obaromi, Odeyemi *et al.*, 2016). It is believed that the overcrowding at various celebratory events as well as some behaviours displayed at such events such as excess alcohol intake, liberal sexual conduct, poor health seeking behaviour et cetera (Azeez *et al.*, 2016). These behaviours may lead to promotion of the risk factors that are favourable for TB acquisition and transmission.

In our study, the peak of TB notification took place in the 1st and 2nd quarters across both sexes and all the age groups, which gives credence to both theories outlined above. It is likely that the 1st quarter cases represent patients that would most likely contracted TB during the last month of the year whilst the 2nd quarter cases reflect the winter months in the Limpopo province of South Africa. Conversely, the decline in TB notification during the 3rd and 4th quarters could reflect the beneficial effects of mandatory face-mask wearing that was mandated by the South African government

from the latter part of 2020 in response to the coronavirus 2019 pandemic (Driessche, Mahlobo, Venter *et al.*, 2021).

4.4.3. TB diagnostic modalities used in patients with HIV co-infection or diabetes mellitus

The prevalence of HIV in our study of patients diagnosed with TB was 72.5% (98/135) which mirrors the 73% that is reported in literature. The most common diagnostic tests that were done in our study was imaging at 94.7% (90/98), followed by, sputum tests at 57.1% (56/98), TB blood culture at 51% (50/98), urine LAM at 47.9% (47/98), and then the various fluid analysis and tissue biopsies. This reflects the relatively high prevalence of extrapulmonary TB amongst our HIV positive patients in our study. Furthermore, the mean CD4 cell counts of these HIV positive patients was very low at 118.4 cells with an interquartile range of 22 to 142 cell and thus rendering these patients severely immunosuppressed. As a result, these patients had high prevalence of pauci-bacillary EPTB rendering other diagnostic tests save for the urine LAM and TB blood culture very ineffective in diagnosing TB in these patients (Degtyareva, Heysell, Matin *et al.*, 2021).

4.4.4. Additional findings

In the additional findings of this study, the relative high number of PJP cases (14%) stands out. Once again, this reflects the low mean CD4 cell counts of the patients enrolled in the study. PJP is an AIDS defining illness that occurs in HIV patients with profound immunocompromise. Furthermore, 11% of the patients that underwent investigations for PTB were found to have covid-19 Pneumonia as well as an additional 3%, which were diagnosed with both covid-19 pneumonia and PJP. This suggests that clinicians should consider simultaneous coinfection of TB and other

respiratory infections in their HIV-positive patients.

Unsurprisingly, a plethora of other pathogenic organisms known to cause respiratory infections were cultured in some patients that were thought to have TB. These organisms were *Staphylococcus aureus* (3%), *Streptococcus agalactiae* (3%), *Pseudomonas pneumoniae* (3%), *Klebsiella pneumoniae* (9%), *Acinetobacter baumanii pneumonia* (3%), *Haemophilus influenza* (3%) and *Enterobacter cloaca* (3%). Furthermore, 3% of the patient that had an exudative pleural effusion thought to be due to pleural TB were instead found to have metastatic pleural effusion from malignant adenocarcinoma.

Lastly, two other co-morbid conditions other than HIV and diabetes mellitus were reported in the patients diagnosed with TB. These co-morbidities were pregnancy (6%) and SLE (3%) both of which are relative states of immunosuppression. Thus, these co-morbidities render the patients more likely to contract TB than a person without these co-morbidities (Getahum *et al,2015;* Miele, Bamrah Morris & Tepper, 2020).

4.5. Conclusion

This chapter gave a detailed account on the findings obtained from the data analysis of the TB cases of the IPCU at Pietersburg Hospital. The next chapter will discuss the salient findings of this study as per the study objectives. It will also compare these findings with the published literature.

CHAPTER 5: SUMMARY, RECOMMENDATIONS, CONCLUSION

5.1. Introduction

In this chapter, a summary of the study results will be given by outlining the pertinent study findings. This will also cover the extent to which the study managed to achieve the study aim and objectives as set out in the study protocol. Recommendation arising from the study findings will also be shared in this chapter. Finally, a carefully synthesised conclusion derived from the study finding will then complete this chapter.

5.2. Research design method

This study was an observational retrospective study of descriptive nature based on secondary data collected from the ICPU at Pietersburg hospital. It included all the patients aged 12 years and above who were diagnosed with various forms of TB and notified at the ICPU from 1st January 2020 to 31st December 2020. It was a census study that included all the 135 patients that met inclusion criteria during the study period. The data was captured from the ICPU TB notification record book using a data collection tool. This was then followed by transcription of the captured data onto the password protected Microsoft excel spreadsheet (Microsoft Corporation, Redmond, Washington, USA). This data was then imported into STATA 14 software (College Station, Texas 77845 USA) for statistical analysis.

5.3. Summary and interpretation of the research findings

In this study of 135 notified cases of TB in patients aged 12 years and above, more than half of the cases (57.3%) were PTB and the remainder were various forms of extrapulmonary TB (42.7%). In terms of diagnosis of TB, most cases were diagnosed using various imaging techniques (92%) followed by sputum tests (55.6%), TB blood culture (52.5%), urine LAM (36.3%), whilst fluid analysis and tissue biopsies accounted for the remainder of the cases.

The TB case notifications demonstrated the same trend as documented in literature of seasonality. HIV was the most common co-morbid condition at 92% followed by pregnancy at 6%, diabetes mellitus at 4% and SLE at 3%. Diagnostic modalities used in the HIV patients mirrored those of the general study population except that urine LAM and TB blood cultures were used almost exclusively in this patient sub-population. These findings were most likely due to very mean low CD4 cell count in the HIV positive patients in this study.

Additional findings related to the reported cases of PJP (14%), covid-19 pneumonia, PJP & covid-19 co-infection (3%) and other cases of pathogenic respiratory organisms such as *Staphylococcus aureus* (3%), *Streptococcus agalactiae* (3%), *Pseudomonas pneumoniae* (3%), *Klebsiella pneumoniae* (9%), *Acinetobacter baumanii pneumonia* (3%), *Haemophilus influenza* (3%) and *Enterobacter cloaca* (3%).

5.4. Conclusion

In this study, PTB was the commonest form of TB diagnosed with the remainder of the cases made up of various forms of extrapulmonary TB. TB was mostly diagnosed using various imaging techniques, followed by sputum tests, TB blood culture, urine

lam, analysis of various sterile fluids (*viz.* pleural, ascitic, knee aspirate and pericardial) and lastly tissue histology. This is in contrast with the WHO TB diagnostic algorithm whereby microbiological diagnostic methods based on sputum are the preferred mode of diagnosis (Lange & Mori, 2010).

The TB quarterly trends in our study showed peaks in the first two quarters of the year. This trend was observed across both sexes and across the studied age groups. The most common co-morbid condition in this study was HIV co-infection at 72.5 % which mirrors that of 73% as reported in literature. Pregnancy, diabetes mellitus, SLE and made the remainder of the co-morbid conditions at less than 6% each. The HIV positive patients were more likely to have positive urine lam tests and positive TB blood culture.

5.5. Recommendations

- When investigating a patient for TB or EPTB, microbiological confirmation should always be prioritised with sputum being the preferred first line media where possible.
- It is important to follow up on TB culture taken on various media (sputum, blood etc) to rule out non-tuberculous mycobacteria.
- Knowing the HIV status of the patient in whom TB is suspected will aid the choice of diagnostic tests.
- A positive urine LAM test should always be followed up with a TB blood culture in order to rule out false positive TB results.
- Screening for common co-morbid conditions such as diabetes mellitus that can induce immunosuppression in patients suspected to have TB is strongly advised.

5.6. Contributions of the study

This study has assisted in giving clearer picture of the various forms of TB diagnosed at Pietersburg Hospital. It has also given the objective evidence of the various diagnostic methods that are commonly employed by the clinicians at the institution and how these fare with the recommended WHO TB diagnostic algorithm. Thus, it has highlighted a gap that could be remedied in order to align the clinical practice at the institution with the recommendations of the WHO.

Furthermore, the study has highlighted the extent to which HIV is a burden in patients in whom TB was diagnosed or suspected.

5.7. Limitations of the study

This was a retrospective study based on secondary data. As a result, it is fraught with several limitations. The following limitations relate to its retrospective nature; the inability to request additional TB tests (that were missed) on the patient, the lack of access to the treating clinicians to raise clarity seeking questions and the inability to repeat tests that would have otherwise yielded a different result.

The reliance on the secondary data also deprived the investigator the opportunity to seek other useful test parameters such as HIV viral loads that were not documented in the TB notification records. The exclusion of patients diagnosed with TB but not notified at the ICPU may have also been a limitation. The covid-19 pandemic was at its peak during the duration of the study period. This may have also affected the health seeking behaviour of many patients including those that may suffered from TB given the prevailing nationwide lockdown at that time. Thus, the data may not reflect the true TB burden as captured by the IPCU.

Furthermore, the nature of the study site as a tertiary hospital in Limpopo limits the generalisability of the study findings. Pietersburg Hospital is relatively well resourced in terms of medical equipment such as specialised imaging machines but also in terms of human resources. Its human resource consists of a sizable complement of specialists across various departments who supervise diagnostic investigations and management of the individual patients.

5.8. Concluding remarks

In conclusion, this study highlights the importance of availability of various TB diagnostic tools that are in clinical use. Even so, it reinforces the need to follow the WHO TB diagnostic algorithm in employing these various diagnostic methods in various clinical settings. It also highlights, the importance of screening patients suspected to have TB or medical co-morbid conditions that could render these patients more susceptible to the contraction and transmission of TB.

Furthermore, the study sheds some light in those patients with HIV and those with advanced HIV disease as evidenced by a low CD4 cell count. It highlights, the appropriate TB diagnostic tests that are likely to yield positive results in those that are HIV co-infected. Finally, the study reinforces the need to have periodic studies of this nature to continuously assess the utilisation of various clinical tools to the benefit of the patients.

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ANNEXURE 1: TREC ethics approval

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NAME OF STUDENT: **DR MI LAMOLA**
STUDENT NUMBER: **202065765**
DEPARTMENT: **INTERNAL MEDICINE**
SCHOOL: **MEDICINE**
QUALIFICATION: **MMED**

Dear Student

FACULTY APPROVAL OF PROPOSAL (PROPOSAL NO. FHDC2021/7)

I have pleasure in informing you that your MMED proposal served at the Faculty Higher Degrees Meeting on the 13 OCTOBER 2021 and your title was approved as follows:

Approved Title: "An Evaluation of Tuberculosis Diagnostic Methods at Pietersburg Hospital in Limpopo Province, South Africa".

Note the following:

Ethical Clearance	Tick One
Requires no ethical clearance Proceed with the study	
Requires ethical clearance (TREC) (apply online) Proceed with the study only after receipt of ethical clearance certificate	√

Yours faithfully

Prof T.M Mothiba
Chairperson

CC: **Supervisor: Dr P.M Mangena**
Co- Supervisor: Dr M.J Nchabeleng

Finding solutions for Africa

ANNEXURE 2: Limpopo Department of Health approval letter

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

Department of Health

Ref : LP_2022-02-019
Enquires : Ms PF Mahlokwane
Tel : 015-293 6028
Email : Phoebe.Mahlokwane@dhsd.limpopo.gov.za

INNOCENT MAROSLYN LAMOLA

PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES

Your Study Topic as indicated below;

AN EVALUATION OF TUBERCULOSIS DIAGNOSTIC METHODS AT PIETERSBURG HOSPITAL IN LIMPOPO PROVINCE, SOUTH AFRICA.

1. Permission to conduct research study as per your research proposal is hereby Granted.
2. Kindly note the following:
   a. Present this letter of permission to the institution supervisor/s a week before the study is conducted.
   b. This approval is Only for Pietersburg Hospital
   c. In the course of your study, there should be no action that disrupts the routine services, or incur any cost on the Department.
   d. After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   e. The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   f. The approval is only valid for a 1-year period.
   g. If the proposal has been amended, a new approval should be sought from the Department of Health
   h. Kindly note that, the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated

Head of Department 14/03/2022 **Date**

pp

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Website: http/www.limpopo.gov.za

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ANNEXURE 3: Pietersburg Hospital approval letter

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF HEALTH
PIETERSBURG/MANKWENG RESEARCH ETHICS COMMITTEE (PMREC)

ENQUIRIES: DR MA POOPEDI **DATE: 27 June 2022**

MANAGER: CLINICAL RESEARCH

ananiaspoopedi@gmail.com

REFERENCE	:	**PMREC 26 May UL 2022/C**
DATE	:	**27 June 2022**
RESEARCHER	:	**Dr MI Lamola**
		(PRINCIPAL INVESTIGATOR)
RESEARCH	:	**Post-graduate Research**
DEPARTMENT	:	**Internal Medicine (University of Limpopo)**

Protocol Title : An evaluation of tuberculosis diagnostic methods at Pietersburg hospital in Limpopo province, South Africa.

Candidate: Dr MI Lamola

Approval status: Approved.

SIGNED:

Prof TAB Mashego, PhD
Chairperson: Pietersburg/Mankweng Complex Research Ethics Committee
School of Medicine
University of Limpopo
REC 300408-006

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ANNEXURE 4: DATA COLLECTION SHEET

Identification code:				
Demographic information:				
Age				
Sex	Male		Female	
Weight				
Clinical information:				
Clinical site of TB diagnosis				
Diagnostic method	Imaging done	Suggestive	Not suggestive	Not done
	Sputum AFB	Positive	Negative	Not done
	Sputum GXP	Positive	Negative	Not done
	Sputum TB culture	Positive	Negative	Not done
	Urine LAM	Positive	Negative	Not done
	Other	Positive	Negative	
HIV	Positive	Negative	Unknown	
CD4+ count	< 50	>50	Unknown	N/A
HAART	Yes	No	Unknown	N/A
Other co-morbidities	Diabetes Mellitus	Other		
Date of TB notification	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
Previous TB infection	Yes	No	Unknown	

findings. *Radiographics*. 39(7):2023–2037.
Seddon, J.A., Paton, J., Nademi, Z., Keane, D., Williams, B., Williams, A., Welch, S.B., Liebeschutz, S., et al. 2016. The impact of BCG vaccination on tuberculin skin test responses in children is age dependent: Evidence to be considered when screening children for tuberculosis infection. *Thorax*. 71(10):932–939.
Skoura, E., Zumla, A. & Bomanji, J. 2015. Imaging in tuberculosis. *International Journal of Infectious Diseases*. 32:87–93.