# Concurrence of active Mycobacterium Tuberculosis and aspergilloma in patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital from 2000-2016

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**Declaration**

I, Gloria Anibea Asiedu, declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the field of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

28th day of January 2021 in Johannesburg

**Dedication**

For all those who have died from complications arising from Pulmonary Tuberculosis

**Presentations arising from research project**

**Nil**

## Publications arising from research project

**Nil**

**Abstract**

Introduction:

South Africa has a high prevalence of pulmonary tuberculosis (PTB). Clinicians are often faced with a myriad of complications that arise following PTB, one of which is life-threatening or recurrent haemoptysis as a result of aspergilloma, sometimes requiring lobectomy.

Methods:

We reviewed the demographics, and the concurrence of aspergilloma and active tuberculosis in patients undergoing lobectomies for suspected aspergilloma at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) between 2000 and 2016.

Results:

There were 121 patients in this study, of which 62.8% were male, 49% above 42 years and 47% between ages of 25 to 42 years. There was a history of previous pulmonary tuberculosis in 66.9% of the cohort, and of these, 28% had evidence of previous positive smear microscopy, 18% were culture positive, and 1.65% of patients in the cohort had positive sputum Xpert MTB/RIF test. Patients with active PTB on histology formed 30% of the cohort, and 70% had sequelae of PTB. Multivariate analysis showed a history of previous TB and HIV positivity or unknown HIV status to be risk factors for active TB. More than half the cohort, (54%) were HIV positive, which is more than four times the national prevalence (13%). The median $CD_4$+ count was 250 cell/$mm^3$. *Aspergillus* species was found histologically in 81% of cases. There was no positive sputum culture of *Aspergillus* species.

Conclusions:

Although the sample size is small, this study was able to show that a history of previous PTB, and HIV positivity are risk factors for the development of active TB in this cohort. We were unable to determine a statistically significant association

between active TB and aspergilloma and therefore cannot recommend empiric TB treatment for patients with massive haemoptysis. There is a need for further investigation into this subject matter.

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**Nomenclature**

| | |
|---|---|
| ABPA | Allergic broncho-pulmonary aspergillosis |
| AFB | Acid fast bacilli |
| ART | Anti -retroviral therapy |
| ARV | Anti- retroviral |
| CCPA | Chronic cavitatory pulmonary aspergillosis |
| CD | Cluster of Differentiation |
| CDW | Central Data Warehouse |
| CMJAH | Charlotte Maxeke Johannesburg Academic Hospital |
| CPA | Chronic Pulmonary aspergillosis |
| CNA | Chronic necrotising aspergillosis |
| CFA | Chronic fibrosing aspergillosis |
| CT scan | Computer tomography scan |
| CXR | Chest X-ray |
| DNA | Deoxyribonucleic Acid |
| DOTS | Directly Observed Treatment Short Course |
| EPTB | Extra pulmonary tuberculosis |
| GXP | Xpert MTB/Rif® |
| HAART | Highly active antiretroviral therapy |
| HIV | Human immune deficiency syndrome |
| IgG | Immunoglobulin G |
| IPA | Invasive pulmonary aspergillosis |
| IRIS | Immune reconstitution inflammatory syndrome |
| LAM | Lipoarabinomannan |
| LRA | Logistic regression analysis |
| MTB | Mycobacterium tuberculosis |
| MDR- TB | Multi drug resistant TB |
| NHLS | National Health Laboratory Service |
| NIOH | National Institute of Occupational Health |

| | |
|---|---|
| OR | Odds ratio |
| PCR | Polymerase chain reaction |
| PTB | Pulmonary tuberculosis |
| SAIA | Sub-acute invasive aspergillosis |
| SDG's | Sustainable development goals |
| TB | Tuberculosis |

# 1 Contents

# 1 CHAPTER 1

## 1.1 Introduction and literature review

### 1.1.1 Introduction

South Africa has for years featured among the top ten countries in global statistics for the high prevalence of Human Immuno-deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) and tuberculosis (TB), with TB being the number one cause of death for most of the last decade (McLaren et al., 2018). The country is committed to changing this by attempting to achieve targets set out by the WHO End TB Strategy campaign and the Sustainable Development Goals (SDG's) in order to reduce TB mortality rates (Loveday et al., 2019). TB mortality rates fell substantially between 2006 and 2016 with deaths from TB decreasing from 76 881 to 29 399 and all-cause mortality decreasing due to TB from 13% to 6% (Loveday et al., 2019). Great strides have also been made in ensuring that the scourge of HIV/AIDS is contained with a massive rollout of ARVs since 2004, which in turn has increased the focus on TB screening and brought new people into the health care system (McLaren et al., 2018). While there has been substantial research and increasing knowledge about these two diseases in the South African setting, there is insufficient data on the burden of chronic pulmonary aspergillosis (CPA) which often occurs often as sequelae of cavitating PTB. CPA is an uncommon respiratory disease which complicates other pulmonary disorders. The most common form of it is chronic cavitatory pulmonary aspergillosis (CCPA), usually found in non- immune compromised patients. Other manifestations include, aspergilloma, aspergillus nodule and chronic fibrosing aspergilloma (CFA). Furthermore, there is a paucity of data addressing the association of CPA with active TB in Sub Sahara Africa, hence the need for a study such as this one to bridge this gap in knowledge.

### 1.1.2 Justification for study

In a country like South Africa with such a high prevalence of PTB and its sequelae, it is not unusual to find patients with and without a history of previous PTB being placed on empiric TB treatment when they present with symptoms or radiographical changes common to both pulmonary aspergillosis and PTB. The diagnosis of PTB

can prove to be challenging in the initial stages when patients have active haemoptysis, and as a result, empiric TB therapy is often initiated and is continued even though the results of several investigations, prove to be negative for TB. Empiric TB treatment is commonly used in patients with haemoptysis as a consequence of a Rasmussen aneurysm, which is a pseudoaneurysm that arises from the pulmonary circulation as a result of chronic inflammation often in a contiguous lung cavity due to active or previous PTB (Chatterjee et al., 2015). It can lead to life-threatening haemoptysis when it ruptures. This complication accounts for less than 10% of all causes of haemoptysis (Rajamannar et al., 2017). GeneXpert MTB/RIF® (Xpert) was found to have the lowest sensitivity (28%; 95% CI 12–49) when tested on blood-stained sputum compared with salivary or mucoid sputum in a study done in Uganda, a country with a high burden of PTB (Meyer et al., 2017). TB treatment has the potential for life-threatening complications, including drug induced liver injury, and if used empirically and inappropriately, may put patients at unnecessary risk. Side effects of TB therapy such as skin rashes, peripheral neuropathy, nausea and vomiting negatively affect patients' quality of life. It is therefore essential that such a study be conducted to ascertain if there is any evidence to support empiric TB treatment in patients who present with haemoptysis and suspected CPA.

## 1.2 Literature review

### 1.2.1 Historical Perspective

In 1882, Robert Koch identified the 'tubercle bacillus' as the causative agent in tuberculosis; however, it a disease that existed for centuries prior. It was previously known as the 'white plague', 'white death', 'consumption' and 'pthisis.' It became known as 'tuberculosis' for the first time in the nineteenth century when Bayle introduced the term. In 1891 Fowler advocated for the use of the term 'pulmonary tuberculosis' instead of 'consumption' and 'phthisis'.

It is believed that the arrival of TB in South Africa occurred during the 16$^{th}$ century when settlers from Europe arrived to establish a colony at the Cape. Prior to this, there are differing views about its existence in the native people of South Africa. By

the late 19th century, however, TB was established in native Africans. This is documented in a report written by Dr Gregory, Cape Colony Medical officer of Health in 1895 where he stated, 'of all the diseases attacking the natives and coloureds of Cape Colony, TB is by far the most important" (Oswald, 1946)

One of the factors that lead to the spread of TB amongst native South Africans was the increased interaction between Europeans and natives as a result of increased trade and missionary activity (Oswald, 1946). The discovery of diamonds in Kimberly in 1867 and gold in the Witwatersrand in 1886 and between 1891 and 1911 meant that the urban population increased dramatically, which previously was not the case as prior to this the South African economy had been dominated by agriculture. The increase in the urbanised population was dominated by the arrival of large numbers of unskilled African and coloured workers to these mines (Kanabus, 2018).

Tuberculosis reached epidemic proportions in South Africa between 1895-1910. TB mortality rates reached 8 per thousand in 1906 in Cape Town and up to 15/1000 in Beaufort West, which was one of so called 'resort towns' chosen by European immigrants with TB because its climate was believed to be favourable for the treatment of 'consumption' (Kanabus, 2018).

Aspergillosis was first recognised as a fatal disease in Edinburgh, U.K in 1847. The antifungal drug amphotericin B was first used in 1957 to treat a patient with aspergilloma complicating pulmonary tuberculosis (Denning et al., 2016). Pulmonary aspergilloma caused by *A. fumigatus* the most widespread form of aspergillosis, develops in pre-existing pulmonary cavities, most frequently tuberculous caverns (Pohl et al., 2013). The reason for this is that *Aspergillus* species can readily colonise and proliferate in cavities as phagocytosis is hindered in lung cavities (Moodley et al., 2014).

Over the years, many different classifications have been used to classify chronic aspergillosis with the first classification occurring in 1959.

### 1.2.2 Definitions

Chronic pulmonary aspergillosis (CPA) is an uncommon, chronic disease that complicates various lung diseases. It is caused by *Aspergillus* species, a mould that usually occurs in the environment. It has many different presentations depending on the immune status of the host. In a review of CPA written by Kousha et al (Kousha et al., 2011), pulmonary aspergillosis is classified into four groups which are aspergilloma, chronic necrotising aspergillosis (CNA), allergic bronchopulmonary aspergillosis (ABPA) and invasive pulmonary aspergillosis (IPA).

The common term mycetoma is used to describe soft tissue infections caused by Aspergillus species, and should not be used to describe an intra cavitatory fungal mycelial body, which should be referred to as pulmonary aspergilloma (Pohl et al., 2013)

The American Society of Infectious Diseases classified aspergillosis into chronic, invasive and allergic forms (Walsh et al., 2008).

Chronic pulmonary aspergillosis is classified slightly differently in the European Respiratory Society (ERS)/ European Society for Clinical Microbiology and Infectious Diseases (ESCMID) clinical guidelines (Denning et al., 2016). They used the terms chronic cavitatory pulmonary aspergillosis (CCPA), chronic fibrosing aspergillosis (CFA), sub-acute invasive pulmonary aspergillosis (SAIA), simple aspergilloma and aspergillus nodule to classify the disease spectrum of CPA. This is the most recent classification of CPA available.

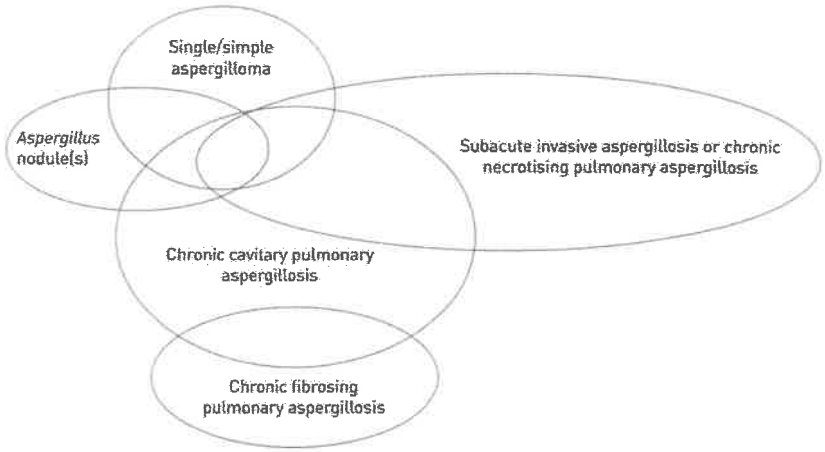


**Figure 1.1: Illustration of different forms of CPA and the overlap often seen**

Figure courtesy of Denning et al.: Chronic pulmonary aspergillosis: rationale and clinical guidelines for diagnosis and management, Eur Respir J. 2016 Jan;47(1):45-68.

In an earlier article written by Denning et al (Denning et al., 2011), CPA is classified as simple aspergilloma, chronic fibrosing aspergillosis and chronic cavitatory aspergillosis with or without aspergilloma, with the invasive forms of the disease not falling under the umbrella term of CPA but being seen as a separate entity.

The differences in classification of CPA over the years has led to confusion over the correct management of the disease.

PTB is spread by inhalation of aerosols coughed by those with active disease, with successful transmission being detected by an antigen specific T-cell response in exposed contacts. It is estimated that one in every ten adults that develop this response will develop active disease (Barry et al., 2009).

PTB is one of the most prevalent infections in the world that shows little sign of abating. The current epidemic is being fuelled by the worldwide HIV epidemic and the increasing incidence of resistance to first line treatment against Mycobacterium tuberculosis (Ahmad, 2011). Other factors that contribute to its spread are, overcrowding, poor detection and cure rates of disease, and increasing migration of people from countries with high TB incidence rates to those of low rates.

PTB is a significant cause of chronic respiratory complaints in low and middle income countries, with previous PTB being the main predictor of chronic bronchitis in the South African Demographic and Health survey of 1996 (Meghji et al., 2016).

Post TB structural lung damage is common and in a study conducted by Meghji et al. (Meghji et al., 2016), the most common changes found on pulmonary CT scans of patients with sequelae of pulmonary TB, included evidence of cavitation, bronchiectasis and fibrosis with a more diverse range of abnormalities, including nodules, consolidation and pleural thickening being detected on CT scan compared with chest x-rays (CXR). In the same study, the most consistent sequelae of pleural disease noted on both CXR and CT scan was differing degrees of pleural thickening.

### 1.2.3 Laboratory tests for aspergillosis and PTB

#### 1.2.3.1. Laboratory tests for aspergillosis

According to ERS/ESCMID guidelines of 2016, CPA is diagnosed when there is consistent appearance on imaging, direct evidence of infection or immunological response to *Aspergillus* species, and exclusion of other alternative diagnoses such as chronic cavitatory histoplasmosis, paracoccidiodomycosis, coccidiodomycosis, mycobacterial and non-mycobacterial infections, necrotising lung cancer and with the condition being present for three months. Patients are also not usually immunocompromised by cancer, HIV infection or immunosuppressive agents.

The same guidelines also state that in the presence of a fungal ball, *Aspergillus* serology (*Aspergillus* IgG or precipitins) should be positive to confirm *Aspergillus* as the cause of the fungal ball. The finding of *Aspergillus* antibodies is a key diagnostic feature of CPA. The presence of anti-*Aspergillus* antibodies differentiates between infected and colonised patients with a positive predictive value of 100% for detecting infection (Denning et al., 2016)

Numerous cavities with a fungal ball require either *Aspergillus* IgG or precipitins to be positive or DNA PCR of *Aspergillus* in respiratory tract fluid or excision biopsy showing fungal hyphae on microscopy.

Fungal hyphae seen to be invading tissue on microscopy are diagnostic of sub-acute (SAIA) or acute invasive aspergillosis.

The presence of *Aspergillus* species in sputum is not diagnostic of CPA due to the ubiquitous nature of the fungus; however, its presence in bronchoscopic fluid is more indicative of infection. DNA PCR testing is more sensitive than culture (Denning et al., 2016).

Galactomannan assay tests the presence of the polysaccharide galactomannan present in *Aspergillus* cell walls and is available in some centres. Galactomannan assay sensitivity is greater with broncho-alveolar lavage fluid (77%) than in serum (66.7%) (Denning et al., 2016). It may be falsely positive in those taking piperacillin or tazobactam and falsely negative in those on corticosteroids or where another *Aspergillus* species is involved (Ofori et al., 2016).

#### 1.2.3.2 Laboratory tests for PTB

The gold standard for diagnosis of active PTB is culture. In many resource-limited countries, the most available form of diagnosis is smear microscopy (Hermans et al., 2017). The overall sensitivity of smear microscopy is 60% compared with culture, with HIV positive patients having lower rates of sputum smear positivity due to lower rates of cavitatory disease and overall bacillary burden (Cudahy and Shenoi, 2016). Studies have shown that fluorescent light microscopy using auramine staining is 10% more sensitive than standard microscopy using Ziehl Nielsen staining with similar specificity.

Traditionally tuberculosis was cultured on solid culture media such as Louwenstein Jensen; however, newer liquid media systems being used have resulted in a 10% increase in sensitivity and a shorter time to result positivity. An example of liquid media is the Bactec® system. There is a 3% false positivity rate with liquid media culture (Cudahy and Shenoi, 2016).

PCR based tests such as the Xpert MTB/RIF® have sensitivity in sputum samples of 89% compared with culture which has a specificity of 99%, whereas sputum microscopy has a sensitivity of 65%. Xpert MTB/RIF® is used to simultaneously test

for rifampicin resistance. A limitation of this diagnostic tool is the requirement of expensive infrastructure and highly trained staff. It is also limited by its ability to detect DNA of non-viable MTB organisms and is therefore not to be used to monitor treatment response. In South Africa, Xpert MTB/Rif® testing was incorporated into the National TB programme in 2011 and since then has become the first line test for TB. Since its implementation, the rate of empirical treatment in HIV positive patients has declined from 42% to 27% and from 23% to 11% in HIV negative patients (Hermans et al., 2017). The test has also led to an increase in bacteriologically confirmed TB (Hermans et al., 2017). Xpert MTB/Rif® was not available to all patients in this study since we included patients treated from 2000 onwards, before the inception of the test.

Histopathology with Ziehl Nielsen staining and culture is considered the diagnosis of choice for extra pulmonary tuberculosis (EPTB). Identification of necrotising granulomatous inflammation with or without caseation in endemic countries is considered and treated as TB (Purohit and Mustafa, 2015). A study based on autopsy findings conducted in India defined TB on histopathological analysis as including the presence of four characteristics namely, necrotising granulomatous inflammation, acid-fast bacilli, or a history of previous TB with response to therapy in the absence of caseous necrosis or acid-fast bacilli and lastly the exclusion of other causes of granulomatous inflammation (Gupta et al., 2016). Tuberculosis can be characterised by the presence of necrotic and non- necrotic granuloma and often both types of lesions are present in infected tissues (Patterson et al., 2019)

Non- tuberculous mycobacteria can have similar histopathological features as *Mycobacterium tuberculosis,* and the distinction must be made between them as treatment varies for both.

### 1.2.4 Epidemiology

According to the World Health Organisation Global TB report released in 2018, 322000 people fell ill with TB in South Africa in 2017 with 78000 TB related deaths, 56000 of the 78000 people were HIV positive (WHO, 2018).

There is no available epidemiological data on aspergillosis in sub-Saharan Africa; however, it is estimated that globally 1.2 million people have CPA as a sequel to PTB with South-East Asia, Western Pacific and Africa most greatly affected.

### 1.2.5 Treatment of chronic aspergillosis and tuberculosis

According to the South African National TB guidelines published in 2017, treatment of pulmonary rifampicin sensitive TB comprises of a two- month intensive phase of co-formulated drugs of rifampicin, ethambutol, isoniazid and pyrazinamide followed by a continuation phase of a two-drug regimen of isoniazid and rifampicin. Extrapulmonary TB is treated for six months unless the initial diagnosis is TB meningitis or pericarditis or TB spine, for which the recommended duration of treatment is nine months in total. TB treatment in South Africa follows the DOTS programme.

The treatment of choice for patients with aspergilloma with haemoptysis is surgical resection (Lee et al., 2004). Surgical resection can either be a lobectomy or pneumonectomy or lobectomy with segmentectomy (Moodley et al., 2014) In patients with poor functional or respiratory reserve with recurrent haemoptysis as a result of aspergilloma, bronchial artery embolisation is recommended. (Lee et al. 2004). In this group of patients, a review conducted by Moodley et al. recommended cavernostomy with thoracoplasty. Bronchial artery embolisation is also used as a bridging modality to stabilise patients before surgical intervention.

All the patients in this study underwent lobectomy or pneumonectomy.

There is no consensus regarding the timing of surgical intervention with some members of the surgical fraternity preferring early surgical intervention despite symptoms and others favouring intervention once complications arise (Moodley et al.,

2014). The risks associated with surgery include broncho-pleural fistula, infection and bleeding (Pohl et al., 2013). Surgery carries mortality and morbidity rates of 4-8% and up to 25%, respectively. In addition, the need for surgery is often not clear cut because patients often have multiple co-morbidities and diffuse lung pathology with limited pulmonary reserve making them high risk anaesthetic patients (Moodley et al., 2014).

Medical treatment of chronic pulmonary aspergillosis is difficult even with newer agents available. According to ERS/ESCMID guidelines of 2015, oral triazole therapy has been shown to reduce the likelihood of severe life-threatening haemoptysis and is now the standard of care for CCPA. Long term itraconozole has been shown to stabilise the general condition of CFPA with limited effect on breathlessness. It has also been shown to be of benefit in allergic bronchopulmonary aspergillosis (ABPA) by improving symptoms, reducing IgE titres and facilitating, the weaning of corticosteroids in ABPA (Kousha et al., 2011). The mainstay of therapy for ABPA, however, is corticosteroid therapy.

Voriconazole is superior to amphotericin B in terms of improved survival, clinical response and side effect profile, in the treatment of invasive forms of aspergillosis (Herbrecht et al., 2002).

### 1.3 Study Aim and Objectives

This study aims to describe and review the characteristics of those patients who underwent lobectomies for the indication of possible aspergilloma at Charlotte Maxeke Johannesburg Academic Hospital, a quaternary level hospital servicing Johannesburg and other parts of Gauteng.

The primary objective was to describe the cohort of patients who underwent lobectomies during the sixteen years between January 2000 and December 2016 at Charlotte Maxeke Johannesburg Academic Hospital. The secondary objective was to identify and quantify the relationship between risk factors and histological findings of active, and sequelae of previous PTB and to determine if there's evidence that

patients with massive or recurrent haemoptysis due to CPA, who require lobectomies, have active PTB at the time of surgery.

# 2. Methodology

## 2.1 Research Question

Is there evidence that patients with massive or recurrent haemoptysis due to CPA, who require lobectomies, have active PTB?

## 2.2 Research Design

This study was a retrospective descriptive study, and pathology records of patients were studied to retrieve the necessary information using a data collection sheet.

## 2.3 Materials and Methods

Patients who underwent lobectomies had their samples analysed by pathologists based at the National Institute of Occupational Health (NIOH). The histological results were kept in paper files at the NIOH offices. These results were assessed and patients whose surgery were carried out in private hospitals and whose samples were initially analysed at private laboratories were excluded. Patients whose diagnosis of *Aspergillus* infection was made based on samples other than lung lobectomies or pneumonectomies, were excluded. Samples taken from people less than 18 years were excluded.

## 2.4 Study population

There were 121 patients in the cohort, referred from different areas in Gauteng to the cardio-thoracic department at CMJAH for lobectomies or pneumonectomies

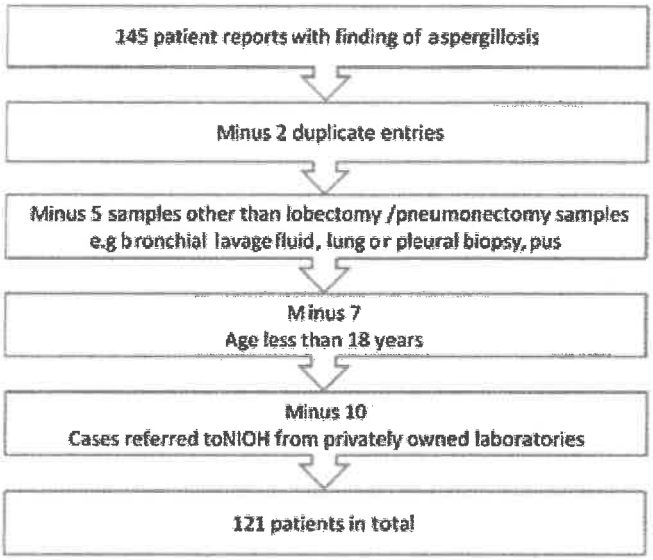


**Figure 2.1: Sample selection process**

## 2.5 Data Collection

All printed copies of histological results in paper files dating from 1st January 2000 until 31st December 2016, were read, and only the results of lobectomy and pneumonectomy samples were selected. Demographic data was gathered from the laboratory request form attached to each printed result copy and was entered onto a data collection sheet (Appendix 1). Information regarding the histological diagnosis of TB was obtained by reading through each report and grouping the data according to whether or not there were features of active or sequelae of TB or combinations of the above as described by the pathologist. We defined active PTB as the presence of necrotising or non- necrotising granulomatous inflammation with or without the presence of acid-fast bacilli on Ziehl Nielsen staining. Sequelae of PTB was defined as the presence of any degree of fibrosis with or without necrosis without granulomatous inflammation as well as the presence of other sequelae of PTB such as fibrotic scars, cavitation, bronchiectasis, end stage lung disease. Patients with no evidence of TB on histology were also grouped under sequelae of TB to allow for statistical analysis due to the small component this group formed of the entire cohort.

Data concerning evidence of TB in various other samples such as sputum and blood cultures processed by the National Health Laboratory Service (NHLS) for each patient over the sixteen- year period, was obtained with permission from the Central Data Warehouse (CDW) database of the NHLS. This data was captured by a data-capturer at the laboratory and was made available on a Microsoft Excel® spreadsheet for further analysis. 75% of the data extracted from the spreadsheet were within a year of the date of surgery.

Any positive culture, sputum smear microscopy or Xpert MTB/Rif® result, was recorded as evidence of active TB even if there was evidence of only sequelae of TB on the histological sample; similarly, samples with evidence of only sequelae of TB and no other evidence of TB in any other samples were recorded as sequelae of TB.

### 2.6 Data Analysis

Data were double entered into a Microsoft Office Excel® spreadsheet and imported to Stata version 15® for analysis. Descriptive statistics such as frequencies, means and standard deviations were used to analyse data as appropriate. Bar graphs and pie charts were used to illustrate descriptive data as necessary. Chi-squared test and Fischer's exact test were applied to determine the relationship between the type of TB diagnosis (sequelae of PTB/active TB) and explanatory variables.

Crude odds ratios (OR) and 95% confidence intervals were calculated using univariate and multiple logistic regression analysis (LRA) to estimate the association between the type of TB and explanatory variables. Missing values were automatically excluded in each LRA model. To obtain the odds ratios, the type of TB and one explanatory variable were included in the initial LRA model. This was followed by the addition of another potential explanatory variable in a stepwise manner starting with the most statistically significant from the univariate analysis. Each time a new potential variable was added to the model if the effect estimated between the type of TB and the initial explanatory variable in the model changed by more than 5%, the additional variable was retained in the final multiple LRA's; otherwise, the variable was removed, and a different one was added. The most stringent multiple LRA models were reported, those values having a p-value $< 0.05$.

## CHAPTER 3

# 3. Results

## 3.1 General Characteristics of the Study Population

This study consisted of 121 patients, with 80% of the lobectomies taking place between 2009 and 2016. Most of the patients were male (62.8%), and there was even representation of the patients in the 25 to 42 year age group, forming 47% of the cohort, with 49% being above the age of 42 years. Younger patients in the 21 to 24 year age group constituted 4% of the sample. The mean age was 42 years.

Of those patients who had HIV results available (75%), 54.5% of were positive, and 20.6% were negative. A mean $CD_4$+ count of 250 cells/mm$^3$ (SD 282 cells/mm$^3$) was found in those known to be HIV positive.

## 3.2 Tuberculosis

### 3.2.1 Sputum and blood

81 (66%) of patients had a history of previous PTB as documented on laboratory request forms. There was no record of any test performed for TB for 40 (33%) patients, within a year of the date of surgery in this study and 38 (31.4%) had at least one positive test for TB. Only 1 (0.8%) patient had negative results for TB bactec®, Xpert MTB/RIF®, culture and auramine tests. In this cohort, only 26 (21%) of patients had data on Xpert MTB/RIF® results, of which 2 (7.6%) had a positive Xpert MTB/RIF® result. 74 sputum auramine staining tests were performed, of which 34 (45.9%) were positive.13 patients had TB bactec results, of which 1 (7.69%) was positive. Results of TB culture of various samples other than blood were positive in 22 (18.1%) of cases, but there were no records available for 66 (56.2%) of patients and 30 (24.7%) had negative TB culture results.

**Table 1: TB results of the study participants (n= 121)**

| | Positive result (n) | % |
|---|---|---|
| History of previous TB (n=121) | 81 | 66.94 |
| Gene Xpert (n=26) | 2 | 7.69 |
| TB bactec® (n=13) | 1 | 7.69 |
| Auramine (n=74) | 34 | 45.9 |
| TB culture (n=52) | 22 | 42.3 |

### 3.2.2 Histology

A diagnosis of active TB was made on histological analysis based on the presence of necrotising and/ or non- necrotising granuloma in over 30% of the cohort. Those with the classical finding of necrotising granulomatous inflammation constituted 24% of the total sample whilst 7.43% had features of active TB with non-necrotising granulomas present on histology. Data on Ziehl Nielsen stain results on lobectomy samples were available for 43 of the patients, of which 2.4% were positive.

In the cohort, 80.9% had evidence of TB sequelae on histology with the categories of active TB and sequelae of TB not being mutually exclusive. Sequelae of PTB was characterised histologically either by the presence of fibrosis with necrosis (18.1%) and without necrosis (62.8%).

**Table 2: Histology characteristics of TB among the study participants (n = 121)**

| Characteristics | n | % |
|---|---|---|
| ***Histology*** | | |
| ***Active TB, Necrotising granuloma*** | | |
| Nil | 90 | 74.38 |
| Yes | 31 | 25.62 |
| ***Active TB, Non-necrotising granuloma*** | | |
| Nil | 112 | 92.56 |
| Yes | 9 | 7.43 |
| ***Ziehl Nielsen stain*** | | |
| Unknown | 78 | 64.46 |
| Negative | 40 | 33.06 |
| Positive | 3 | 2.48 |
| ***Sequelae of TB, fibrosis with necrosis*** | | |
| Nil | 99 | 80.82 |
| Yes | 22 | 18.18 |
| ***Sequelae of TB, fibrosis without necrosis*** | | |
| Nil | 45 | 37.19 |
| Yes | 76 | 62.81 |

***Table 3: The association between the type of TB diagnosis and related variables***

| | Type of TB | | |
|---|---|---|---|
| | **Sequelae** | **Active TB** | **P value** |
| **Gender** | | | |
| Male | **44 (36.36)** | **32 (26.45)** | **0.032** |
| Female | **17 (14.05)** | **28 (23.41)** | |
| **Age** | | | |
| 21-24 years | **2 (1.65)** | **3 (2.48)** | 0.693 |
| 25- 42 years | **27 (22.31)** | **30 (24.79)** | |
| >42 years | **32 (26.45)** | **27 (22.31)** | |
| **HIV status** | | | |
| positive | **38 (62.30)** | **28 (46.67)** | 0.219 |
| negative | **10 (16.39)** | **15 (25.00)** | |
| unknown | **13 (21.31)** | **17 (28.33)** | |
| **Cd4+ count** | | | |
| 0-179 | **22 (43.14)** | **26 (57.78)** | 0.344 |
| 180- 412 | **14 (27.45)** | **10 (22.22)** | |
| >412 | **15 (29.41)** | **9 (20.00)** | |

***Note: figures in parentheses are percentages; *P-value of the Chi-squared test and Fischer's exact test***

### 3.3 Aspergillosis

The diagnosis of aspergillosis was made on histology in 81.8% of cases, based on the presence of fungal hyphae in the tissue analysed. There were no results relating

to Aspergillus species on sputum microscopy and culture in the cohort. Normal respiratory flora was cultured in 10.7% of cases. In 4.1% of cases, additional organisms such as Bilharzia and *Pseudomonas aeruginosa* were identified histologically. Data regarding *Aspergillus* serology or precipitins for the patients were not included in the study. The cavities in lobectomy samples in this cohort were bronchiectactic in nature in over 95% of patients

**Table 4: Characteristics of aspergilloma among the study participants (n= 121)**

| Characteristics | N | % |
|---|---|---|
| ***Type of cavity*** | | |
| Bronchiectatic | 116 | 95.87 |
| Other[1] | 5 | 4.13 |
| ***Sputum (microscopy and culture)*** | | |
| Nil | 116 | 89.26 |
| Normal flora | 13 | 10.74 |
| ***Presence of fungal hyphae (Histology)*** | | |
| No | 17 | 14.05 |
| Yes | 99 | 81.82 |
| Other[2] | 5 | 4.13 |

Other[1] = no cavity found in sample or no mention of bronchiectasis in pathology report

Nil = no record of *Aspergillus* species cultured on sputum MC&S

Other[2] = 1 case Enterobacter cloacae, 1 case of *Pseudomonas aeruginosa*, 2 cases of *Candida albicans, 1 Bilharzia* ova

Fungal hyphae suggestive of *Aspergillus* species were found in 42.1% of patients with sequelae of PTB as opposed to 39.6% of those with active TB. Fungal hyphae were not identified histologically in 8.2% and 9.9% of those with PTB sequelae and active TB respectively. The association between type of TB (active or sequelae) and presence of aspergilloma, i.e the presence of fungal hyphae on histology was not statistically significant (p-value 0.6).

There was a statistically significant association found between gender and type of PTB (p-value 0.032), using the chi-square test. Other variables such as age and a positive result of various tests for TB were not significantly associated with the type of TB.

Univariate analysis of the data revealed that patients with active TB were more likely to have a history of previous TB than those with only PTB sequelae, (OR 2.23 CI 0.87- 5.70, p-value 0.095). Patients with active TB also were also more likely to be HIV positive compared with those without active TB, (OR 3.95 CI 0.86- 11.8, p-value 0.084), which is statistically not significant. Variables such as age, gender and $CD_4$+ counts, proved not to be statistically significant in determining the outcome of active TB, using univariate analysis.

Multiple logistic regression revealed that those with active TB were more likely to have a history of previous TB (OR 2.6 CI 1.03 – 5.96, p-value 0.05), which is statistically significant. The odds of HIV positive patients having active TB were higher than those who were HIV negative, (OR 4.68 CI 1.04 – 20.96, p-value 0.044). Multiple logistic regression, similar to univariate analysis, also showed that age, gender and CD4+ count, were not statistically significant in the development of active TB.

**Table 5: Univariate analyses of the type of TB and risk factors/potential confounders**

| Risk factor/potential confounder | OR | 95% CI | P-Value |
|---|---|---|---|
| ***History of previous TB*** | | | |
| No | 1 | 1 | 1 |
| Yes | 2.23 | 0.87 – 5.70 | 0.095 |
| ***HIV status*** | | | |
| Negative | 1 | 1 | 1 |
| Positive | 3.19 | 0.86 – 11.88 | 0.084 |
| ***Sex*** | | | |
| Female | 1 | 1 | 1 |
| Male | 0.88 | 0.39 – 2.00 | 0.759 |
| ***Age group*** | | | |
| 21 – 24 years | 1 | 1 | 1 |
| 25 – 42 years | 0.54 | 0.08 – 3.52 | 0.561 |
| > 42 years | 0.56 | 0.09 – 3.65 | 0.543 |
| ***CD4+ count*** | | | |
| 0 – 179 | 1 | 1 | 1 |
| 180 – 412.5 | 0.73 | 0.24 – 2.22 | 0.583 |
| > 412.5 | 0.71 | 0.29 – 1.74 | 0.458 |

**Table 6: Multiple regression analyses of the type of TB and risk factors/potential confounders**

| Risk factor/potential confounder | OR | 95% CI | P-Value* |
|---|---|---|---|
| ***History of previous TB*** | | | |
| No | 1 | 1 | 1 |
| Yes | **2.60** | **1.03 – 5.96** | **0.050** |
| ***HIV status*** | | | |
| Negative | 1 | 1 | 1 |
| Positive | **4.68** | **1.04 – 20.96** | **0.044** |
| ***Sex*** | | | |
| Female | 1 | 1 | 1 |
| Male | 0.91 | 0.37 – 2.20 | 0.828 |
| ***Age group*** | | | |
| 21 – 24 years | 1 | 1 | 1 |
| 25 – 42 years | 0.59 | 0.07 – 4.47 | 0.610 |
| > 42 years | 0.47 | 0.06 – 3.63 | 0.468 |
| **CD4 count** | | | |
| 0 – 174 | 1 | 1 | 1 |
| 175 – 412.5 | 0.87 | 0.20 – 3.70 | 0.850 |
| > 412.5 | 1.63 | 0.41 – 6.44 | 0.483 |

*: Model adjusted for history of previous TB, HIV status, sex, age group and CD4 count.

# 4. Discussion

## 4.1 General Characteristics of the population

This study was a retrospective analysis of the cohort of patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic hospital from 2000 to 2016.

In this cohort, 62.8% were male, and 37.2% were female, which is reflective of global statistics. A meta-analysis based on TB prevalence surveys found that TB prevalence is significantly higher, up to double the prevalence in males than in females in low and middle -income settings (Horton, 2018).

According to the World Health TB report published in 2018, South Africa had a total incidence rate of TB of 268/1000 cases in those older than 14 years, with 167/1000 of those being male. The same report showed that of the 277999 new and relapsed cases of TB that were notified in South Africa, 56% were male, and 37% were female, a statistic similar to that found in this study.

Possible reasons for the discrepancy in TB prevalence between the two gender groups were explored in a report by Rhines (Rhines, 2013) and included this being a reflection of real epidemiological differences, a difference in rates of transmission and rates of progression from latent TB to active TB between the different sexes, reporting bias or the presence of confounding factors such as smoking which are linked to TB and which is more common in males. In a review article about the epidemiology of TB in South Africa by Perumal et al (Perumal, 2017), the authors argue that the male to female TB prevalence ratios are lower and demonstrate a female predominance in countries with a high HIV burden than previously thought. This differs from the findings in this study and many other earlier studies. The authors argue that the reason for female underrepresentation in previous meta-analyses include the exclusion of studies from high HIV prevalent countries, among other reasons. The explanations given for the increase in TB prevalence in young women include the rise in both smoking and alcohol abuse in this population, the widespread use of intramuscular progesterone contraceptives, which is believed to reduce the protective effect of oestrogen against TB. Other reasons mentioned in the article

include the high prevalence of anaemia of chronic disease in HIV positive females which negates the protective effect of iron deficiency commonly found in females of reproductive age (Perumal et al., 2018).

In our study 49% of the total sample size was above the age of 42 years with less than 5% belonging to the age group 21 -24 year group, 47% were in the age group of 25-42 years. In the 2018 World Health TB report, the highest prevalence of notified TB cases in South Africa occurred in the age groups of 25-34 years and 35 to 44 years, with the lowest occurring in those in the age group of 5-14 years. The statistics are alarming as the greatest impact of TB is on the economically active proportion of the population.

Multivariate analysis in this study with a much smaller sample size, however, showed age and sex were not statistically significant variables or risk factors for the development of active TB. A study conducted looking at the prevalence of active TB in diabetic patients in a high TB prevalence area like Khayelitsha, Cape Town found no statistically significant interactions between age, gender among other factors, similar to our study (Berkowitz et al., 2018).

Another important finding in this study was the prevalence of HIV positive patients, with 54.5% being HIV positive and 20.6% being negative. In South Africa, as in many sub-Saharan countries, the dual epidemics of TB and HIV has been well documented. According to UNAIDS, 7.7 million people were living with HIV in South Africa in 2018, with a prevalence of HIV of about 20.4% among the general population, which is the highest in the world (UNAIDS, 2018). This country also has the highest prevalence of HIV and TB co-infection worldwide (UNAIDS, 2019). It is well known that co-infection confers a reciprocal advantage for both pathogens. One of the factors implicated in the dual epidemic of HIV and TB include inadequate diagnosis and treatment of latent TB in resource-limited settings, most of which have a high burden of active TB (Letang et al., 2020). Other reasons mentioned in the same article include the slow pace of TB drug development, the prevalence of multidrug-resistant TB (MDR- TB), challenges in the treatment of active disease such as adverse interactions between HAART and TB drugs and the risk of TB immune reconstitution inflammatory syndrome (IRIS) on initiation of therapy.

Multiple regression analysis in our study showed that an unknown HIV status was statistically significant in the development of active TB (OR 7.90 CI 1.01- 62.4 p-value 0.050) as was HIV positivity (OR 4.36 CI 1.04- 20.9 p-value 0.04). This finding in those whose status was unknown was thought to have been influenced by the sample size of this study and the relatively large proportion of this subgroup or the possibility that a significant proportion of those whose HIV status was not known were in fact positive.

In South African prison inmates, a study done by Jordan et al (Jordan et al., 2019), found that the odds of TB for the screened population was significantly higher among persons known to have HIV (OR 1.4, CI 1.1–1.9; p-value 0.008) than persons who did not or who had an unknown HIV status. It is concerning to note that 24% of our patients had an unknown HIV status due to the implications it has on therapy and mortality. A retrospective study conducted in Ethiopia over 5 years between 2012 and 2017 looking at treatment outcomes of 1249 patients with TB, concluded that unknown HIV status was associated with unsuccessful treatment outcomes among other risk factors (Abebe et al., 2019). A similar South African retrospective study also found an increased risk of mortality from TB in males and females with unknown HIV status (Heunis et al., 2017).

The mean $CD_4$+ count in our cohort was 250 cells/mm$^3$ with standard deviation of 282 cells/mm$^3$. Multivariate analysis showed that $CD_4$+ count was statistically insignificant as a confounding variable for active TB, $CD_4$+ counts between 179 and 412 cell/mm$^3$ (OR 0.87 CI 0.2 – 3.7 p-value 0.85) and counts above 412 cells/mm$^3$ (OR 1.63 CI 0.41 – 6.44 p-value 0.483). Numerous studies have identified a low $CD_4$+ as a risk factor for the development of active TB. There have been comparatively fewer trials looking at TB in patients with higher $CD_4$+ counts. One such study conducted in South Africa looking at incident TB in HIV positive patients with high $CD_4$+ counts, i.e. above 350 cells/mm$^3$ found that a high $CD_4$+ count was not a risk factor for TB but rather having a low BMI was (Kufa et al., 2016). A study conducted in rural Tanzania by Said et al. also looking at the incidence of TB in HIV positive patients with $CD_4$+ counts above 250 cells/mm$^3$ to identify potential risk

factors also did not find a high $CD_4$+ count to be a risk factor but only the history of previous TB similar to what was found in our study.

### 4.2 Tuberculosis

In our study, 66% of patients had a history of previous TB. Evidence of this was found in non- histological samples whereby, 28% (34) had positive auramine staining, and 18% had positive TB culture. It was surprising to find that although the majority of these samples were collected between 2009 and 2016 when PCR tests were already widely used, 22% of our cohort had a record of the test being done and only 7.69% of these had a positive Xpert MTB/RIF® (GXP) result. The fact that less than 10% of patients had positive Xpert MTB/RIF® is likely reflective of a small sample size or other sampling error. Further data analysis revealed that patients with a history of previous TB were 2.6 times more likely to have active TB than non-active TB, with a p-value of 0.05. A study conducted in 16 South African prisons in 2015 revealed a similar finding of previous TB being associated with an increased risk (OR 4.3, CI 2.5–7.3 p-value < 0.0001) of TB in both HIV positive inmates and HIV negative patients.

Analysis of the histological data revealed a vastly greater percentage with sequelae of TB compared with patients who had active PTB at the time of lobectomy, in keeping with the finding of a large proportion of the cohort having a history of previous TB. This finding is contrary to what has been described in the literature and is also surprising, in light of the proportion of HIV positive patients in our study. A review article detailing the pathogenesis of HIV and TB co-infection noted that HIV infection is the leading risk factor for the development of active TB, increasing 2 to 5 fold above baseline in the early and the chronic phase of infection with HIV (Bell and Noursadeghi, 2018). Other confounders such as socio-economic factors, lifestyle habits, occupation and co-morbidities of our patients that may have influenced this result in our study were outside the scope of this research topic and would have to be studied in further studies.

### 4.3 Aspergilloma

In patients who had lobectomies for suspected aspergilloma, 81.8% had fungal hyphae suggestive *of Aspergillus* species identified on histological analysis, 95% of which existed within bronchiectatic lung cavities. There was no evidence of aspergilloma in 18% of cases. Fungal hyphae suggestive of *Aspergillus* species was found in 42% of patients with sequelae of PTB as opposed to 39% of those with active PTB, this showed a statistically insignificant association between the type of TB and the presence of fungal hyphae on histology (p-value 0.6). Studies addressing TB sequelae versus active PTB and aspergillosis are minimal, a potential subject of investigation in the future.

### 4.4 Recommendations

Based on the results from this study, the following recommendations are made:

- Improved awareness of methods of diagnosis of aspergilloma by clinicians.
- There needs to be improved linkage of care, so that all patients have confirmed results at the time of lobectomy, and importantly, all lobectomy specimens are sent for TB culture and sensitivity testing.
- At the time of this study, pathology reports of the patients in our study were kept manually in paper files at the NIOH premises, which poses a risk to the safekeeping of the data. There were no pathology records found of patients who underwent lobectomies from mid- 2002 to 2004; the reason for this was not apparent. Updating and collating pathology results from different sources like NIOH and NHLS and forensic department onto a single electronic platform, i.e. NHLS labtrack/Trackcare would allow for improved data collection and research.
- Surgeons and other clinicians who are responsible for taking samples for histo-pathology are to be re-informed about the importance of including as much known detail regarding patients' conditions on the laboratory request form as possible to aid in accurate diagnosis and interpretation of results and data collection for research purposes.

### 4.5 Limitations

1. The sample size of 121 is much smaller than initially anticipated, this could have been due to some lobectomy samples being processed and analysed by the NHLS histology laboratory at CMJAH, as routinely done for other surgical samples instead of the NIOH. During data collection, for unknown reasons, no lobectomy histological results were found in paper files from June 2002 until December 2004. This likely affected the power of the study.

2. The different ways in which histological results were reported, based on the individual pathologist's preference, sometimes lead to ambiguity regarding the spectrum of PTB being described, which made data capturing and therefore data interpretation challenging.

3. Improved linkage of care within the laboratory domain is recommended, specifically a unified patient identifier.

4. Missing data especially pertaining to TB diagnosis in other samples, HIV status and $CD_4$+ counts, made it difficult to depict the actual nature of my cohort.

5. Data regarding the use of TB therapy in the period preceding the date of surgery was not collected and it is therefore not known the degree to which this could have confounded histological and other findings.

## CHAPTER 5

### 6. Conclusion

This study confirmed that TB is more common in males than in females and more common in HIV positive patients than HIV negative patients and therefore health policies and interventions should target these population groups to curb the complications that arise from both TB and HIV if left untreated. Our results also showed that patients whose HIV status was unknown, of which there was a significant proportion, also had an increased risk of developing active PTB. It is vital that the 'test and treat' guideline or policy currently in place for the treatment of HIV in South Africa be enforced at all levels of health care.

It was discouraging to note that 50% of our study participants who were HIV positive had $CD_4$+ counts of less than 180 cells/$mm^3$ around the time of surgery. We, unfortunately, didn't collect data regarding ART usage or WHO clinical stage in this population and cannot draw any meaningful conclusions about this. Low $CD_4$+ counts were not associated with an increased risk of active PTB in this study, this we felt might have been due to a small sample size and would suggest that further studies be done to determine if this association is accurate.

Age and gender were not significant risk factors for the presence of pulmonary aspergilloma in patients undergoing lobectomies in this cohort; however, a history of previous PTB is associated with an increased risk of developing aspergilloma. This requires that clinicians and healthcare workers diagnose and treat patients early and appropriately and monitor response to therapy in order to detect and act expeditiously when complications arise.

There was no statistically significant association between the type of PTB, i.e. active versus sequelae of TB and the presence of aspergilloma at time of lobectomy, although our sample size was small. In patients who present with massive haemoptysis due to suspected aspergilloma requiring surgery, the decision about TB therapy will need to be individualised. This is a question worth investigating further in the future.

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# 7 Appendices

## 7.1 Data collection form

## 7.2 Wits Human Research Ethics Committee Approval Letter (original)

## 7.3 Permission from Head of NIOH to conduct study

## 7.4 Permission letter from NHLS (CDW) to collect data

## 7.5 Research proposal

## 7.6 Change of title of research

## 7.7 Wits Human Research Ethics Committee Approval Letter (amended)

# Data Collection Appendix 2

| Study No. | Age (Years) | Gender (M/F) | | | Date of Sample/Surgery | HIV Status | | | CD4 Count (cells /mm³) | TB | | | | | | | | | | | | | Aspergilloma | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | Histology | | | | | | | | | Histology | |
| | | | | | | | | | | | | | | Evidence of Cavitation | | Active TB | | | | Non- Active TB | | Concurrent active, Non-active and Sequelae of TB,no TB | Sputum | | |
| | | | | | | | | | | | | | | | | Granuloma | | Ziehl Neelsen stain | | | | | | | |
| | | Male | Female | Unknown | | Positive | Negative | Unknown | | Patient History of Previous TB | Gene Xpert | TB bactec | Auramine | TB Culture | Bronchiectatic | Other | Necrotising Granuloma | Non- Necrotising Granuloma | Positive or Negative | Unknown | Fibrosis with Necrosis | Fibrosis without Necrosis | | | Fungi Hyphae | Other |

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

**HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)**

03 September 2018

**Dr Gloria Anibea Asiedu**
National Institute of Communicable Diseases

Sent by email to: 1351580@students.wits.ac.za

Dear Dr Gloria Anibea Asiedu

**Re:** **Protocol Ref no: M170313**
**Protocol Title:** Concurrence of Active Mycobacterium Tuberculosis and Aspergilloma in those who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital from 2000-2016.
**Principal Investigator:** Dr Gloria Anibea Asiedu
**Protocol Amendment: Title Change**

This letter serves to confirm that the Chairperson of the Human Research Ethics Committee (Medical) has noted the amendment for the above mentioned protocol, as detailed in your letter, dated 07 August 2018.

The following documents were received:

- Summary Letter
- Clearance Certificate
- Approval letter from faculty registrar

UNIVERSITY OF THE WITWATERSRAND
OFFICE OF THE DEPUTY VICE CHANCELLOR RESEARCH
03 SEP 2018
RESEARCH OFFICE HREC (Medical)
JOHANNESBURG

Thank you for keeping us informed and updated.

Yours Sincerely,

.....
**Mr Joshua Ndlangamandla**
**Administrative Officer**
**Human Research Ethics Committee (Medical)**

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6 March 2017

**RE: MMed Research Project Dr Gloria Asiedu**

I hereby grant permission for Dr Gloria Asiedu to utilise the National Institute for Occupational Health (NIOH) database at the Pathology division of National Institute for Occupational Health for the purpose of her MMed research project titled "Concurrence of active Mycobacterium Tuberculosis and Aspergilloma in Patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital from 2000-2016"

Yours Sincerely,

DR N. VORAJEE

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**23 August 2018**

**Applicant:** Dr Gloria Asiedu
**Institution:** NICD/CMJAH
**Department:** Medical Microbiology
**Email:** gloriasiedu@yahoo.com
**Cell:** 073 373 8366

**Re: Approval to access National Health Laboratory Service (NHLS) Data**

Your application to undertake a research project "**Concurrence of active Mycobacterium Tuberculosis and Aspergilloma in Patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital from 2000-2016**" using data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available to you to conduct the proposed study as outlined in the submitted application.

Please note that final approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. Any data related queries may be directed to NHLS Corporate Data Warehouse, contact number: 011 386 6074 email: zarina.sabat@nhls.ac.za

**Dr Babatyi Malope-Kgokong**
**National Manager: Academic Affairs and Research**

**Concurrence of active Mycobacterium Tuberculosis and Aspergilloma in Patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital from 2000-2016**


| | |
|---|---|
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## INTRODUCTION:

South Africa has a significant tuberculosis (TB) burden. The World Health Organisation (WHO) gives an estimated incidence of 450000 cases of active TB in 2013, the third highest incidence in the world after India and China (WHO 2013) . WHO has also estimated that a third of the world's population is latently infected with Mycobacterium tuberculosis, and that 5-10% will develop active TB in their lifetime (WHO 2010).

Tuberculosis is an important contributor to chronic respiratory disease (CRD) in resource limited settings like SA. This is attributed to granuloma formation, tissue necrosis and aberrant healing processes (Meghji, Simpson et al. 2016). In SA, the PCR based test (Xpert MTB/Rif) used on different types of samples has become the primary method of active TB diagnosis since its introduction in 2011. However the gold standard for diagnosis remains culture.

The diagnosis of latent TB infection (LTBI) is made by a positive tuberculin skin test in a patient with no overt clinical signs of TB disease. The test consists of an intradermal injection of M. tuberculosis antigens or purified protein derivative (PPD) which when read 48-72 hours later, produces an induration of 5-10mm. Immunity based interferon gamma (IFN γ) release assays (IGRAs) are more recent and specific ways of testing for latent TB (Ahmad 2011). The histo-pathological hallmark of TB is caseating granulomatous inflammation. These lesions consist of amorphous eosinophilic debris surrounded by a margin of palisading epithelioid cells and Langhans' giant cells with multiple nuclei. There is also an outer margin of lymphocytes and plasma cells with varying amounts of fibroblasts. Granulomas may or may not contain acid fast bacilli. Granulomatous inflammation can either be necrotising or non-necrotising. The granulomas of TB are typically necrotising, but can be non- necrotising. Fibrotic lesions are seen mostly in latent or inactive disease and are composed almost entirely of fibroblasts, with a minimal number of macrophages (Barry, Boshoff et al. 2009). Other causes of granulomatous lung diseases include, fungal infections like cryptococcus and histoplasma and non-infectious diseases like sarcoidosis, Wegerner's granulomatosis, Churg -Strauss syndrome and aspiration pneumonia.

Common sequelae of pulmonary TB as detected on chest X-ray and CT scan imaging are cavitation, bronchiectasis and fibrosis with features of smaller airways disease, i.e. mosaicism and emphysema being more easily detected on CT scan (Meghji, Simpson et al. 2016). The prevalence of cavitation is higher in re-treatment patients and those treated for multi-drug resistant (MDR) TB than in those with fully drug susceptible TB (Meghji, Simpson et al. 2016).

Aspergillus species are common saprophytes in the environment which cause a wide spectrum of disease. Aspergillus fumigatus is mainly responsible for pulmonary disease. The clinical syndromes associated with Aspergillus fumigatus are; *Aspergilloma*, also known as mycetoma or 'fungus ball' which is a conglomeration of mucus, fungal hyphae, inflammatory cells and altered blood components usually found in a cavity in the lung. *Invasive pulmonary aspergillosis (IPA)* is a severe disease found mainly in critically ill or severely immune-compromised individuals as

well as patients with chronic pulmonary obstructive disease. *Chronic necrotising aspergillosis (CNA)* is another form of the disease which is locally invasive and is seen in those with mild immune deficiencies or chronic lung disease. Aspergilloma and *allergic bronchopulmonary aspergillosis (ABPA)* are the two non- invasive forms of Aspergillosis, with the latter being most commonly found in patients with asthma and cystic fibrosis. Globally, it is estimated that 1.2 million people have Chronic Pulmonary Aspergilloma (CPA) as a sequel of TB, with Africa, Western Pacific and South East Asia being most affected (Denning, Pleuvry et al. 2011). Aspergillomas commonly colonise a cavity in the lung, usually in the upper lobes. In a study of 544 patients with pulmonary cavities secondary to TB, 11% had radiological evidence of Aspergilloma (1970). Other causes of lung cavities include bronchiectasis, sarcoidosis, cysts, cavitatory tumours and bullae (Moodley, Pillay et al. 2014).

According to the European Society for Clinical Microbiology and Infectious Diseases which collaborated with the European Respiratory Society to release new guidelines for the management of CPA in 2011, the diagnosis of pulmonary Aspergillosis needs radiological imaging consistent with aspergilloma, direct serological, microbiological, histological or cyto-pathological evidence of Aspergillus species, exclusion of other diagnoses and at least a 3 month duration of symptoms before the criteria are met (Denning, Cadranel et al. 2016). The classical pathological appearance of Aspergillus is that of septate hyphae with acute angle branching. In aspergillomas, the fungal organisms do not invade the surrounding parenchyma or evoke a granulomatous response.

The clinical presentation of Aspergilloma varies with most patients being asymptomatic to a productive cough containing mucous, pus or blood, fevers and chest pain (Lee, Lee et al. 2004). Haemoptysis is reported to be the most common symptom in most case series with an incidence rate of 80% (Brik, Salem et al. 2008). Massive haemoptysis is potentially a life threatening consequence of both PTB and Aspergilloma and it cannot be predicted with certainty who will progress to massive haemoptysis. It is reported that up to 30% of those with minor haemoptysis can go on to develop life threatening haemoptysis (Chen, Jiang et al. 2012). The most common radiographic sign of aspergilloma is that of an intra-cavitatory mass which changes

position with movement with an air crescent, or Monod sign, seen in two-thirds of cases (Walusimbi, Semitala et al. 2016).

Definitive treatment for aspergilloma is surgical intervention especially if recurrent or severe haemoptysis is the main presentation (Pohl, Jugheli et al. 2013). Anatomical resection i.e. lobectomy or pneumonectomy, both usually reserved for large simple or complex aspergilloma remain the gold standard (Moodley, Pillay et al. 2014).

Medical treatment including systemic and intra-cavitatory antifungal therapy have shown inconsistent success (Kousha, Tadi et al. 2011). Data guiding the medical management of Aspergilloma is often based on uncontrolled trials and case reports (Walsh, Anaissie et al. 2008). Itraconozole is the preferred antifungal of choice, however, due to its slow effect, treatment regimens may be required to extend for 6 months or even longer.

The diagnosis and treatment of Aspergillosis is challenging in resource limited settings where regular access to healthcare, a high TB burden and the overlap of symptomatology between aspergilloma and TB, coupled with the lack of consensus on the therapeutic approach exist. (Pohl, Jugheli et al. 2013).

In a lot of developing countries, haemoptysis is equated to TB without consideration for other diagnoses. In cases where sputum for acid fast bacilli (AFB) or molecular testin, such as GeneXpert, is negative or when patients fail to improve on standard therapy, patients may be initiated on treatment for smear negative TB, especially if radiology or serology is unavailable (Ofori, Steinmetz et al. 2016). The associated adverse consequences of empiric TB treatment in this group of patients include, drug toxicity, long treatment duration and the possibility of generating multidrug resistant TB (Walusimbi, Semitala et al. 2016). Other risks of empiric TB treatment include potential drug interactions with anti- retroviral therapy (Cummings and O'Donnell 2015).

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) is a quaternary level hospital in Johannesburg, in South Africa's Gauteng province, servicing many surrounding clinics and hospitals as well as other secondary and tertiary level hospitals outside of the province. The Cardiothoracic Surgical department at CMJAH is one of only two such departments in the public health sector in Gauteng Province and therefore services a large number of patients.

The aim of this study is to review and describe the database of histological samples from lobectomies done for the indication of Aspergilloma and to assess if the incidence of concurrent active TB justifies the current common practice of initiating empiric TB treatment for sputum smear negative patients who present with haemoptysis and Aspergilloma.

## OBJECTIVES

1. To describe patients who had lobectomies for Aspergilloma.
2. To compare the proportion, characteristics and risk factors of patients with concurrent active, non-active and sequelae of previous TB, who had lobectomies for Aspergilloma.

## METHODOLOGY

This study is a retrospective record review from 31st December 2016 to 1st January 2000 or until a total of 250 records of patients. Patients who had lobectomies at the cardiothoracic department at CMJAH, for clinical or radiological suspicion of Aspergilloma will be studied. The analysis will include histological findings of those samples, demographics of the patients, as well as other laboratory investigations and results related to the diagnosis of TB, such as sputum, blood and other samples sent for GeneXpert, auramine or Ziehl Nielsen staining or culture. The histological results will be obtained by manually examining the files kept at the NIOH premises, of the reports of lobectomy samples received during the time period mentioned above, as well as information available on the National Health Laboratory Services (NHLS) Labtrack platform, using details like patient name and initials, hospital number and

date of birth. Information not available on Labtrack will be obtained from the NHLS DISA lab system, the system previously in use prior to the introduction of Labtrack. Application will be made to Clinical Laboratory Warehouse (CDW) for any other missing data.

Ethical approval will be sought from the Human Research Ethics Committee (HREC) at Wits University.

Consent to use the database of histological samples mentioned earlier, will be obtained from National Institutes for Occupational Health, Dr Vorajee who is the head of the pathology department.

## INCLUSION AND EXCLUSION CRITERIA

Any patient with histological features in keeping with aspergilloma.

Indication for lobectomy described as aspergilloma (or equivalent) or suspected aspergilloma.

Patients younger than 18 years of age will be excluded.

## FUNDING

The costs of stationary, printing and internet data costs which will be covered by the principal investigator.

## DATA ANALYSIS AND STATISTICS

Patient demographics will be summarised using descriptive statistics. The three groups of patients will be compared using the chi squared test for categorical variables and Kruskal Wallis test for continuous variables. Logistic regression models to estimate relative risks or odds ratios and 95% confidence intervals (CI) will be used to describe the association between patient characteristics and risk factors such as age, gender, HIV status, previous history of TB. Assistance from the statisticians at the Wits post graduate office will be enlisted should it be become necessary.

## ETHICS APPROVAL

Ethics approval will be sought from Human Research Ethics Committee at Wits University. Consent has been obtained from Dr Vorajee, the head of the pathology department of the NIOH.

## TIMING

See Appendix 1

## DATA COLLECTION SHEET

See Appendix 2

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07 August 2018
Person No: 1351580
TAA

Dr GA Asiedu
Po Box 3796
Randburg
2125
South Africa

Dear Dr Asiedu

**Master of Medicine: Change of title of research**

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Medicine** has been approved:

| | |
|---|---|
| From: | **Concurrence of active mycobacterium tuberculosis and aspergilloma in patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital** |
| To: | **Concurrence of active Mycobacterium Tuberculosis and Aspergilloma in Patients who underwent lobectomies at Charlotte Maxeke Johannesburg Academic Hospital from 2000-2016** |

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences