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Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: a post mortem study

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Undiagnosed TB in Adults Dying at Home from Natural Causes in a High TB Burden Setting: A Post Mortem Study

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfillment of the requirement for the degree of Master of Medicine in the branch Anatomical Pathology

Johannesburg, 2016

Declaration

I, Tanvier Omar, hereby declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch Anatomical Pathology, in the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



Tanvier Omar

13th March 2016

Dedication

This research report is dedicated to the deceased and their families, who gave of themselves and their time whilst in mourning to make these findings a reality.

PUBLICATIONS

1 Omar T, Variava E, Moroe E, Billioux A, Chaisson RE, Lebina L, Martinson N. Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: a post mortem study. ***International Journal of Tuberculosis and Lung Disease*** November 2015,19(11): 1320-1325.

ABSTRACT

Background: A high proportion of deaths in Africa occur at home where cause of death (CoD) is often unknown. We ascertained undiagnosed pulmonary TB in adults dying at home in whom there was no apparent CoD by doing limited autopsies.

Methods: Mortuaries in Matlosana, South Africa identified eligible adults without an ante-mortem diagnosis and/or no recent hospital admission. A questionnaire was administered to next-of-kin. Bilateral lung core biopsies, and modified broncho-alveolar lavages (BAL) were performed. Biopsies were examined histologically and submitted with BAL aspirates for mycobacterial culture and Xpert® MTB/RIF. HIV testing was not performed.

Results: 162 families were approached to participate. 28 Refused; 49 were not eligible for the study and included 29 deceased who were on, or had recently stopped TB treatment; 85 were included. All were Black and 53% were men. The median age was 57 years (IQR: 44-66), and median symptom duration 63 days (IQR: 14-112). Laboratory evidence of TB was found in 27(31.8%); 21 were Xpert positive, 23 were MGIT positive, and 14 had histological evidence consistent with TB.

Conclusion: In this high HIV prevalence setting, almost one third of home deaths had evidence of undiagnosed TB, suggesting TB-related mortality is under-ascertained and under-reported, with grave implications for TB control in high TB burden settings.

Acknowledgements

I would like to acknowledge my supervisors, Professor Mario Altini and Dr. Y Perner for their knowledge, practical guidance, valuable suggestions, criticism and moral support.

Thanks are also due to all my co-authors on the published paper on which this report is based. In particular, I would like to thank Professor Neil Martinson whose vision, persistence and patience breathed life into, and sustained this project.

The Department of Anatomical Pathology, University of the Witwatersrand, and the National Health Laboratory Service, provided infrastructural support, time and resources necessary to complete this research.

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NOMENCLATURE/ABBREVIATIONS IN ALPHABETICAL ORDER:

BAL – Broncho-alveolar lavage

CoD – Cause of death

DNF – Death notification form

DoE – Department of education

HIV – Human immune-deficiency virus

MGIT – Mycobacterial Growth Indicator Tube

TB – Tuberculosis

PREFACE:

This research report is based on an article accepted for publication in the ***International Journal of Tuberculosis and Lung Disease.*** 19(11):000–000 Q 2015 The Union http://dx.doi.org/10.5588/ijtld.15.0222. Impact factor: 2,756.

Omar T, Variava E, Moroe E, Billioux A, Chaisson RE, Lebina L, Martinson N. Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: a post mortem study.

This study formed part of a larger parent study undertaken by the Perinatal HIV Research Unit (PHRU) into active case finding of TB in households of index TB cases, looking for HIV and TB. Collaborators include researchers at the Klerksdorp/Tshepong Hospital Complex, Health Economics and Epidemiology Research Office (HE^2RO), Respiratory and Meningeal pathogens research Unit University of Witwatersrand, and Department of Clinical Microbiology and Infectious Diseases, National Health Laboratory Service. Dr. N Martinson is the principal investigator of the larger parent study, whilst the student is the lead scientist in this part of the study.

Contributors to this study:

TO, EV and NM planned the study. EV and LL trained and supervised the professional nurse, EM who administered the questionnaire, performed the limited post mortems, and collected the specimens. TO was responsible for all histopathological aspects of the study. She collected and correlated all laboratory data, prepared the data files, performed the data analysis, statistical evaluation,

drawing up and interpretation of the results, and did the photomicrography. AB assisted in collecting data. The manuscript was written and prepared by TO and was critically revised by all the co-authors.

Funding:

The parent study was funded by USAID. This study received a R100 000 grant from Kwazulu Natal Research Institute for Tuberculosis and HIV (KRITH).

Formatting and style:

In accordance with faculty guidelines, this research report has been reformatted as the journal format requires a particularly shortened version and imposes strict limits on the overall length, use of colour photomicrography, and size and number of Tables. In addition, the style has been adapted to show continuity of style across all research reports submitted to the Faculty. Accordingly, this research report differs from the publication in the following aspects:

- It has been divided into separate chapters with headings and subheadings
- A chapter on Aims and objectives has been added - Chapter 2
- A literature review has been included – Chapter 3
- The Methods have been expanded – Chapter 4
- Table 4 has been split into two (Tables 4 and 5) for ease of interpretation
- Additional Figures have been added – Figures 3, 5a, 5b, 6a, 6b, 7a and 7b

STATEMENT BY SUPERVISORS AND HEAD OF DEPARTMENT:

The Faculty of Health Sciences of the University of Witwatersrand provides inter alia the following guidelines to students submitting research reports:

The report may be presented in either the standard research report format or on the basis of a published paper. In either case, the quality and presentation should follow the recognised criteria as indicated in the style guide for the Faculty.
If submission of the research report is by published paper:

- A single paper is acceptable
- The student must be the first author of the published paper
- A letter must accompany the report, signed by all co-authors, stating the role played by the candidate in the writing of the paper, and how much of the work in the study was performed by the candidate. (Annexure A)
- The paper must have been accepted by a DoE accredited journal (***International Journal of Tuberculosis and Lung Disease -*** monthly ISSN 1027-3719) (Annexure B). The paper must have been published or accepted for publication after the date of registration of the candidate for the degree.
- A School assessor group must have approved the research protocol in the usual manner. (Annexure C)
- The research protocol must have received ethics clearance by the Human Ethics Research Committee (Medical) of the university. (Annexure D)
- The research must have been supported by an appropriate supervisor

- In Press articles will be accepted but must be accompanied by a letter from the Journal stating that the article is to be published. (Annexure E)
- The examiners must be furnished with the reviewers' comments from the Journal. (Annexure F)
- The final bound version submitted for examination must show continuity of style across all research report submissions to the Faculty.
- All sections may be expanded to the students and supervisors discretion. This is particularly true of the of the Introduction and Methods sections as journal formats require particularly shortened versions of these.
- A clear research question must be developed.
- The scope of the project should be limited and the report does not have to make a unique contribution to the literature.

  (Extracted from the post-graduate information booklet issued by the Health Sciences Faculty of the University of the Witwatersrand, 2015, pp36-39)

We certify that the candidate has fulfilled all of the above criteria and are satisfied that the research report is of sufficiently high standard and can be accepted in partial fulfillment of the requirements for the degree of Master of Medicine (in the branch of Anatomical Pathology).

M Altini	Y Perner	M Hale
(Supervisor)	(Supervisor)	(Head of Department)

CHAPTER 1

1.0 Introduction and literature review

Tuberculosis (TB) is a leading infectious cause of death worldwide and remains a massive global health challenge, causing 1.5 million deaths in the 9 million people estimated to have developed TB in 2013[1]. Globally, significant progress has been made in reducing annual TB incidence and TB mortality[2] but to achieve Millennium Development Goal 6 targets[3] – "reduce the global TB burden (prevalence and death) by 50% compared to 1990 levels by 2015"[4] - novel strategies to curb transmission need to be urgently implemented, especially for infectious individuals.

South Africa (S.A) has a particularly severe TB burden. In 2013, the annual TB incidence was 860 per 100,000, and TB has been reported as the leading cause of death (CoD) on death notification forms (DNF) for almost a decade. TB was recorded as the CoD on 40,542 (8.8%) death notification forms (DNFs) in 2011[5]. Mortality reports show that at least one quarter of deaths in South Africa and 49% in less-resourced Zambia, occur at home, where CoD is probably unknown[5,6]. Moreover, a recurrent theme in South African mortality reports is that one-quarter of all DNFs have an ill-defined CoD not allowing disease categorization and coding.

Deaths in individuals recently diagnosed and started on TB treatment follow a well-reported pattern. In sub-Saharan African countries, “high, early” mortality in the pre-ART era was described, with about one quarter of TB patients dying during TB treatment, most in the days or weeks following TB diagnosis or hospital admission[7-9]. A similar pattern of early mortality was reported in the pre-HIV era in developed settings[10-12]. This pattern of early mortality suggests that late presentation with TB is frequent; some who are diagnosed and initiated on TB treatment have advanced TB and die despite therapy; and others die prior to presenting to health systems for diagnosis and treatment.

In a setting where TB is the leading CoD, a better understanding of the presence of TB in those dying at home with an unknown CoD is important to plan efforts to prevent transmission and deaths associated with TB disease. Information on TB prevalence in home deaths, especially in high HIV and TB incidence settings, will contribute to these strategies.

We conducted a limited autopsy study to provide data on laboratory confirmed pulmonary TB in adults dying at home without a clear or apparent classifiable CoD.

For many years TB has been a significant cause of death in hospitalized patients in the developing world. This is particularly relevant in a high HIV prevalence setting where over 40-50% of hospital deaths could be attributed to TB [13, 14] . Like elsewhere in sub-Saharan Africa, the HIV epidemic has fueled the TB epidemic in

S.A; HIV increases the relative risk for tuberculosis 20 fold and just over a quarter of global HIV-associated TB cases occur in South Africa [1]. Approximately half the medical admissions to Chris Hani Baragwanath hospital (CHBH), a 3000 bed tertiary hospital serving an urban population of 3 million people in Johannesburg, are HIV positive. Of these admissions, over 1/3 have a discharge diagnosis of tuberculosis [8]. It is estimated that 65% of incident TB cases in South Africa in 2011 were HIV co-infected [15] and in 2005, 80% of adult TB inpatients at CHBH were reported to be HIV co-infected [8]. In addition, up to a quarter of people starting antiretroviral treatment have undiagnosed culture positive TB [16]. This double disease burden of TB in the context of a high HIV prevalence country poses a significant public health challenge in South Africa. Whilst internationally, progress is being made in reducing prevalence and mortality due to TB, this is not as apparent in South Africa [1].

Due to absent or inadequate death registration processes in developing settings, only a third of deaths have medically certified causes [6]. South Africa is not exceptional in this regard. Death certificates analyzed by Statistics South Africa show that about a quarter of all deaths in this country occur at home where cause of death is often unknown [15].

There is little information on TB prevalence in home deaths in high prevalence settings, including South Africa. The technical and cost implications of post mortem studies in developing settings contribute to the dearth of published data. Indeed, no published articles documenting post mortem findings in those dying at

home in a high TB burden setting were found. However, it is probable that a significant number of deaths in this high HIV prevalence and high TB incidence setting may have TB.

The gold standard for determining cause of death is the post mortem. Several post mortem studies have highlighted the discrepancy between ante mortem clinical diagnosis and post mortem findings, which can be as high as 50%. A study by Wong et al. demonstrated that 49% of post mortem diagnoses, and a third of TB infections found at post mortem were not considered during the clinical management of the patient [14].

This paucity of post mortem data has resulted in the development and use of verbal autopsies (VA) in resource constrained settings. VA, whist lacking in specificity, are able to provide valuable, if crude, information on cause of death. As an example, a population based verbal autopsy study in rural Kwazulu –Natal attributed 50% of the cause specific mortality rates over a 10-year period to HIV/TB [17], though without differentiating between these two endemic diseases. To provide more data on the prevalence of TB in people at home, this study assessed the prevalence of pulmonary TB - which in many instances is infectious – in adults dying at home.

CHAPTER 2

2.0 Aims and Objectives

2.1 Overall Aims:

2.1.1 To determine the prevalence of laboratory confirmed pulmonary TB in adults dying at home without a clear or apparent classifiable CoD.

2.1.2 To describe the pathology found on post mortem lung needle core biopsies in adults dying at home without a clear or apparent classifiable CoD.

2.2 Primary Objectives:

2.2.1 To describe the patient demographics of adults dying at home without a clear or apparent classifiable CoD.

2.2.2 To assess the prevalence of pulmonary TB using the following diagnostic modalities:

- Histology with Ziehl Neelsen (ZN) staining of lung tissue
- MGIT culture of lung tissue
- MGIT culture of broncho-alveolar lavage (BAL) fluid
- Xpert® MTB/RIF of lung tissue
- Xpert® MTB/RIF of bronchiolar alveolar lavage fluid (BAL)
- Smear microscopy of lung tissue and BAL fluid

2.2.3 To describe other infectious and non-infectious pulmonary pathologies identified histologically.

2.3 Secondary Objective:

2.3.1 To compare the performance of histology against mycobacterial culture and Xpert® MTB/RIF of lung tissue and bronchiolar alveolar lavage material in the diagnosis of mycobacterial pneumonia.

CHAPTER 3

3.0 Materials and methods

3.1 Study design

We conducted a cross-sectional descriptive survey in Matlosana, a health sub-district with a population of ~450,000 in four urban areas with limited farming communities in the Dr Kenneth Kaunda health district, North West Province, South Africa[18]. HIV prevalence in this province was 13.3% in 2012, and that in pregnant women is almost 30%[19, 20]; the annual TB incidence for the health district in which Matlosana is located was 937/100,000 in 2012 [21]. This study was part of a larger effort assessing rates of detection of active and latent TB in various populations, and their household contacts. In this study, using two minimally invasive sampling methods, we assessed the presence of TB in lungs of adults who died at home from natural causes (from illness and not from external forces such as assault, road injuries, suicide, poisoning, post-operative complications, etc.), and whose CoD under routine circumstances would have been unclassifiable.

3.2 Recruitment:

After approaching the undertakers' trade association in Matlosana, North West Province, seven private mortuaries agreed to identify potential study subjects and provide study staff with contact details of the next of kin of adults who may have fulfilled the eligibility criteria - adults dying at home of natural causes, with no clear ante-mortem clinical diagnosis and no recent (within the prior 2 months) hospital

admission. Next of kin identified in this manner were approached at their homes and invited to consent for limited post mortem sampling of the lungs of their deceased relative. Thereafter, the next of kin were interviewed using a structured questionnaire to confirm eligibility of the deceased to be included in the study (Annexure G). If eligible, the presence of any ante-mortem symptoms, their duration, and prior medical and smoking history was ascertained.

3.3 Collection of specimens – Post mortem lung core biopsies and BAL:

After confirming eligibility, a trained study nurse performed the post mortem lung biopsies in the private mortuary, wearing appropriate personal protective equipment including gloves, disposable gown, boots, N95 respirator, and protective eyewear. Firstly, multiple bilateral Tru-cut® lung core biopsies[22, 23] were performed using a 14-gauge needle through a single 1cm incision in each axilla to minimize disfigurement. An approximate total length of 6cm of lung tissue was obtained from each lung. Secondly, a modified bronchiolar-alveolar lavage (BAL) was performed through a horizontal, midline 1cm incision into the trachea at the base of the neck anteriorly. A suction tube was inserted and passed into a main bronchus. Twenty milliliters of sterile saline was instilled into the suction tube, then re-aspirated after gentle rocking of the body.

3.4 Testing of specimens:

Biopsy material was submitted for histological evaluation, Xpert® MTB/RIF polymerase chain reaction (Cepheid, Sunnyvale California) and automated Mycobacterial Growth Indicator Tube (MGIT) liquid mycobacterial culture (Becton-Dickinson, Franklin Lakes, New Jersey). Histology specimens were fixed in 10% buffered formalin. All biopsies had sections stained with hematoxilin and eosin (H & E), and with Ziehl Neelsen (ZN) and Grocott's stains for mycobacterial and fungal identification, respectively. Further special stains, including immunoperoxidase stains were performed as directed by the findings on histology.

Biopsy material and aspirated fluid was submitted for MGIT culture and Xpert® MTB/RIF in sterile containers; fluid was also subjected to auramine smear microscopy. Post mortem HIV testing was not performed. Four laboratory modalities were used to ascertain the presence of pulmonary TB on each study subject: auramine smear microscopy, histological evaluation of lung biopsies with ZN staining, MGIT culture and Xpert® MTB/RIF.

Two study definitions of pulmonary TB were used. The first was the diagnosis of TB on any one of the four tests; a second, more conservative definition required at least two diagnostic modalities to be positive.

3.5 Data Collection:

Data derived from the questionnaire, and results from the diagnostic tests performed were captured on an Excel spreadsheet. It included the following information:

3.5.1 Demographic data obtained from family interview e.g. age at death, sex, symptoms of TB and their duration, co-morbidities, etc.

3.5.2 Histological diagnosis including findings on special stains.

3.5.3 Microbiological results: lung core biopsies

- Auramine smear microscopy results
- Xpert® MTB/RIF results
- MGIT culture results

3.5.4 Microbiological results: broncho-alveolar lavage (BAL) Fluid:

- Auramine smear microscopy results
- Xpert® MTB/RIF results
- MGIT culture results

3.6 Eligibility Criteria:

After interviewing the next of kin, an eligibility assessment was made based on the following criteria:

3.6.1 Inclusion Criteria -

- No apparent ante mortem obvious/expected cause of death.
- Adults 18 years or older dying at home or in emergency rooms prior to investigations.

- Consent obtained from adult next of kin.
- Not on TB treatment or recently diagnosed with TB.

3.6.2 Exclusion Criteria -

- Any subject whose death occurred while on TB treatment.
- Death likely to be as a result of injury or surgery.
- History of admission to hospital in the two months prior to death

3.7 Statistical analysis

3.7.1 Sample Size:

Based on available funding, 85 pulmonary post mortem core biopsies were performed and submitted for investigations. These have been analyzed.

3.7.2 Data Analysis:

TB disease diagnosed by any diagnostic modality, and TB diagnosed by 2 or more modalities has been calculated.

Histologically diagnosed TB has been compared to TB diagnosed by smear microscopy, culture and Xpert® MTB/RIF of both BAL material and biopsy material.

Statistical analysis of data has been performed using simple descriptive statistical methods including reporting of medians with their inter-quartile ranges (IQR) for continuous variables such as age, and proportions with their 95% confidence intervals (CI) for dichotomous variables such as gender. Comparisons have been made between those subjects with pulmonary TB and those without, using the

Fisher's Exact and Wilcoxon-Mann-Whitney tests because there were few observations.

3.8 Ethics

Approvals were obtained from Human Research Ethics Committee (Medical) of the University of Witwatersrand (see ethics clearance certificate –Annexure D) and from the chief executive officer at the Klerksdorp-Tshepong Hospital Complex in Matlosana, North West Province.

CHAPTER 4

4.0 Results

4.1 Sample Size:

From January to August 2012, 162 potentially eligible deceased's next-of-kin were identified by six of the seven mortuaries, and approached to consent; 28 (17.2%) refused participation. Upon further questioning, 49 of the remaining 134 were not eligible for the study: 29 were either on, or had been on TB treatment at the time of death, 12 had a recent admission to hospital, 3 had a current cancer diagnosis, 2 had heart ailments, 1 subject was decomposed, 1 had a prior stab wound, and relatives of another were not available. Eighty-five decedents were therefore included.

4.2 Clinical Characteristics:

There was an approximately equal gender distribution, and median age at death was 57 years (IQR: 44-66) (Table 4.1). 35/85 (41.18%) participants had no significant prior medical history. Symptoms prior to death, as reported by next-of-kin, were present in the deceased for a median of 63 days (IQR: 14-112). Cough was the leading symptom by severity, followed by dyspnoea and chest pain (Table 4.1).

Twelve subjects had symptoms for one day or less, four of whom were diagnosed with TB by study procedures. More than half of the deceased reportedly smoked, just over a third had a history of hypertension, and 40% were bedridden prior to

death. Less common histories included epilepsy - 5/85 (5.9%), asthma and diabetes - 4/85 each (4.7%), cardiomyopathy and pneumonia - 2/85 each (2.4%). According to next of kin 14/85 (16.5%) participants were HIV positive, 12/85 (14.12%) were HIV negative, and in 59/85 (69.41%) the person being interviewed did not know the HIV status of the deceased**.**

Those diagnosed as having pulmonary TB by the study were perceived to have cough (p=0.0735) more frequently than their peers without TB (Table 4.1). A history of shortness of breath (p=0.0546) was more frequent in participants without TB than in those diagnosed with pulmonary TB (Table 4.1). The median time from death to autopsy was five days (IQR: 3-8)

Table 4.1: Characteristics of adults who died at home without an apparent cause of death - as reported by the next of kin			
	ALL N=85	**TB n=27**	**No TB n=58**
Male	46 (54.1%)	15 (55.6%)	31 (53.4%)
Median Age (years)	57 (IQR: 44-66)	49 (IQR: 42-64)	59 (IQR: 51-68)
HIV-infection (as reported)	14 (16.5%)	4 (14.8%)	10(17.2%)
Smokers	49 (57.6%)	17 (63.0%)	32 (55.1%)
Median number of cigarettes per day	12 (10-14)	12 (IQR:10-14)	12 (IQR: 10-13)
Median duration of any symptom/s prior to death (days)	63 (IQR: 14-112)	68 (IQR: 30-126)	39 (IQR: 9.3-92.8)
Symptoms prior to death by severity:			
Cough	58 (68.2%)	22 (81.5%)	36 (62.0%)
Shortness of breath	52 (61.1%)	12 (44.4%)	40 (69.0%)
Chest pain	37 (43.5%)	10 (37.0%)	27 (46.6%)
Bedridden	34 (40.0%)	13 (48.2%)	21 (36.2%)
Co-morbid conditions:			
Hypertension	31 (36.5%)	7 (25.9%)	24 (41.4%)
Diabetes	4 (4.7%)	1 (3.7%)	3 (5.1%)

4.3 Core Biopsies:

4.3.1 Histological Findings -

Two of 85 core biopsies did not include lung tissue; one was liver with evidence of hepatitis and steatosis, and the other connective tissue without pathology. Fifty of the remaining 83 (60.2%; 95%CI: 49.7-70.7) lung specimens evaluated histologically showed no specific pathological changes (Table 4.2 / Figure 4.1).

Histological features consistent with mycobacterial pneumonia (Figure 4.2) was the lead finding in 15/83 (18.1% 95%CI: 11.3-27.7), corroborated by the presence of acid-fast bacilli (AFB) on ZN staining in 13/15 (86.7%) (Table 4.2 / Figure 4.4), and by Xpert® MTB/RIF or MGIT culture in 14/15 cases (93.3%). *Mycobacterium gordonae* was cultured in one case. Histology identified a single case of TB not detected by other tests.

Acute suppurative pneumonia consistent with a bacterial etiology was the second leading histological diagnosis in 10/83 (12.0%) (Figure 4.5), followed by interstitial pneumonitis in 9/83 (10.8%). Three lungs with interstitial pneumonitis had evidence of TB on another diagnostic test.

Three of the 83 biopsies had histological evidence of *Pneumocystis jirovecii* pneumonia (Figure 4.6a), highlighted by Grocott's staining (Figure 4.6b); 2 of these had coexistent non-tuberculous mycobacteria diagnosed on MGIT culture (*M.avium* complex and *M. scrofulaceum*).

One deceased had a small cell neuroendocrine carcinoma (Figure 4.7) associated with acute suppurative pneumonia.

Table 4.2: Summary of histological Findings in lung core biopsies∞.	N=83 (%)
No specific pathological changes	50 (60.2)
Granulomatous inflammation consistent with mycobacterial pneumonia*	15 (18.1)
ZN positive	13 (15.7)
Pneumocystis pneumonia	3 (3.6)
Bacterial pneumonia	10 (12.1)
Interstitial pneumonitis	9 (10.8)
Small cell neuro-endocrine carcinoma	1 (1.2)
*One case had *M. gordonae*	
Cases may have had more than one histological diagnosis	

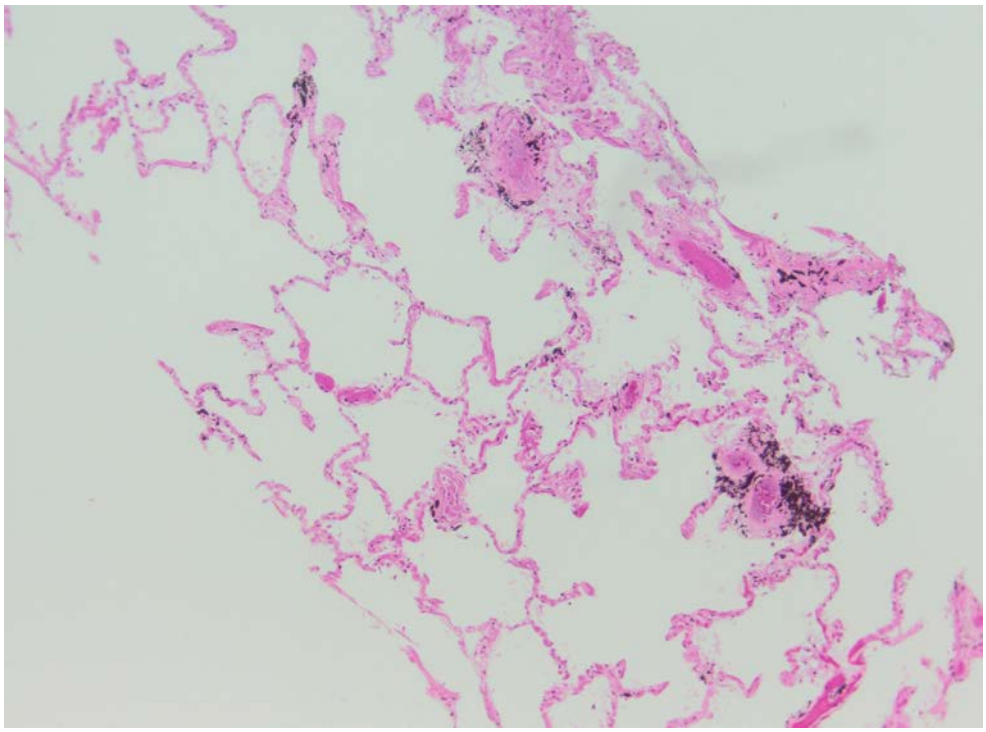


Figure 4.1. Lung core biopsy demonstrating a normal microscopic architecture; H&E x10

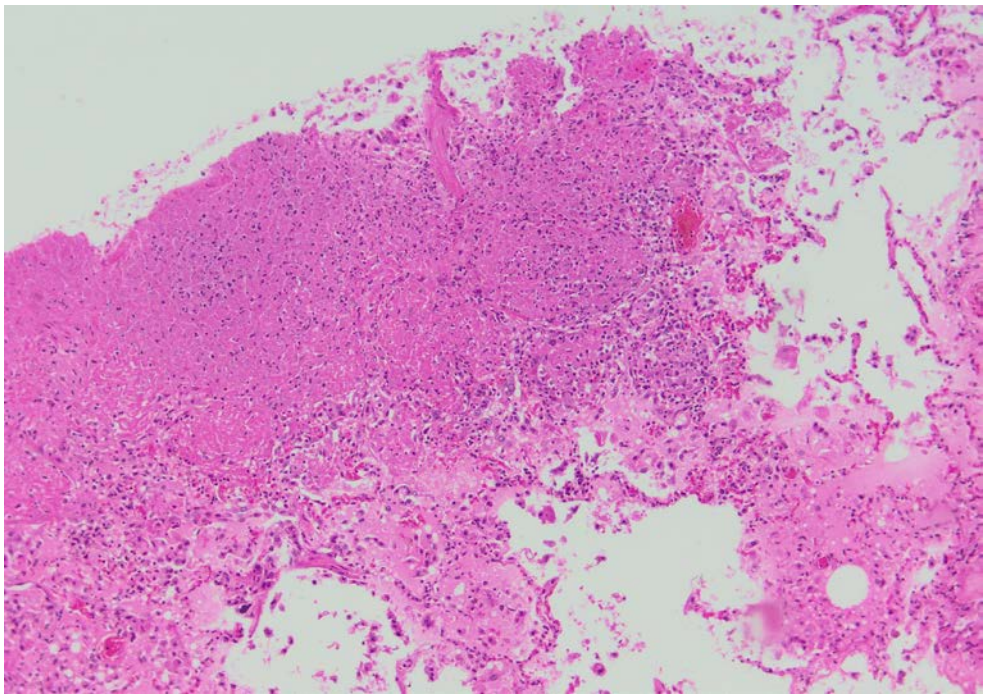


Figure 4.2. Lung core biopsy showing necrotizing granulomatous inflammation in a case of TB bronchopneumonia; H&E x10

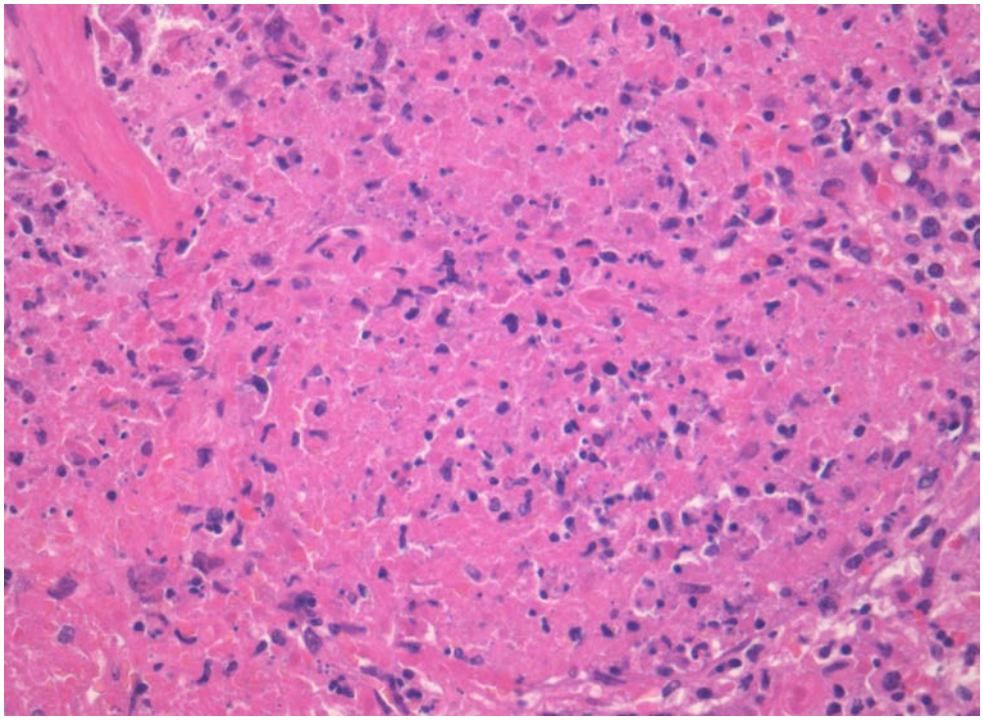


Figure 4.3. Granuloma demonstrating epitheloid histiocytes around areas of central necrosis; H&E x40

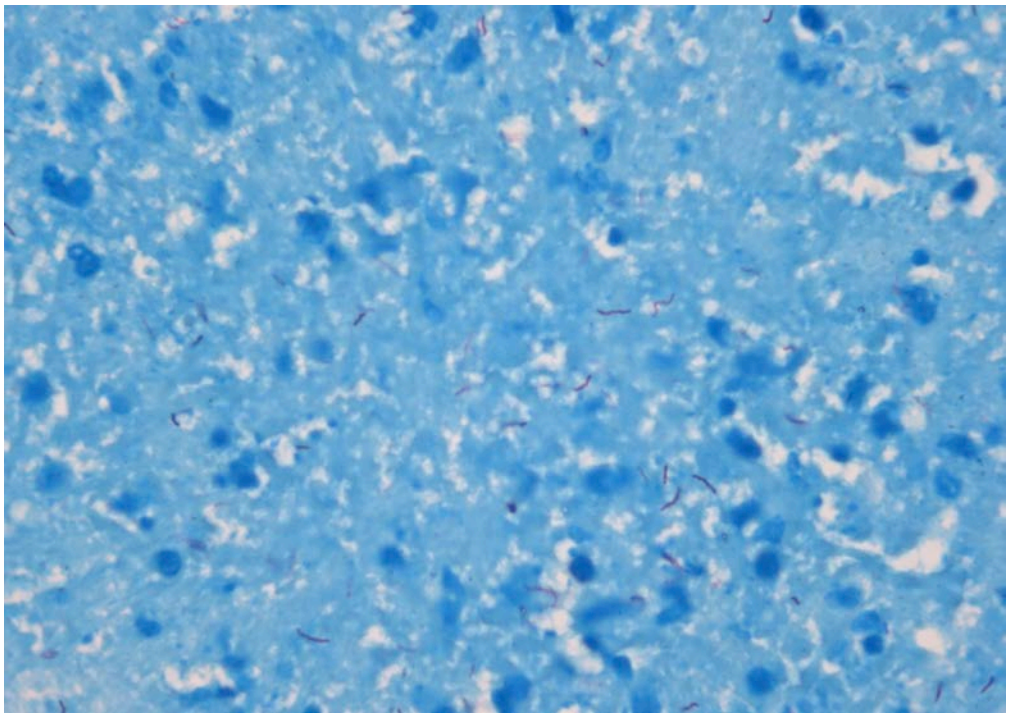


Figure 4.4 Numerous acid-fast bacilli, Ziehl Neelsen stain x100

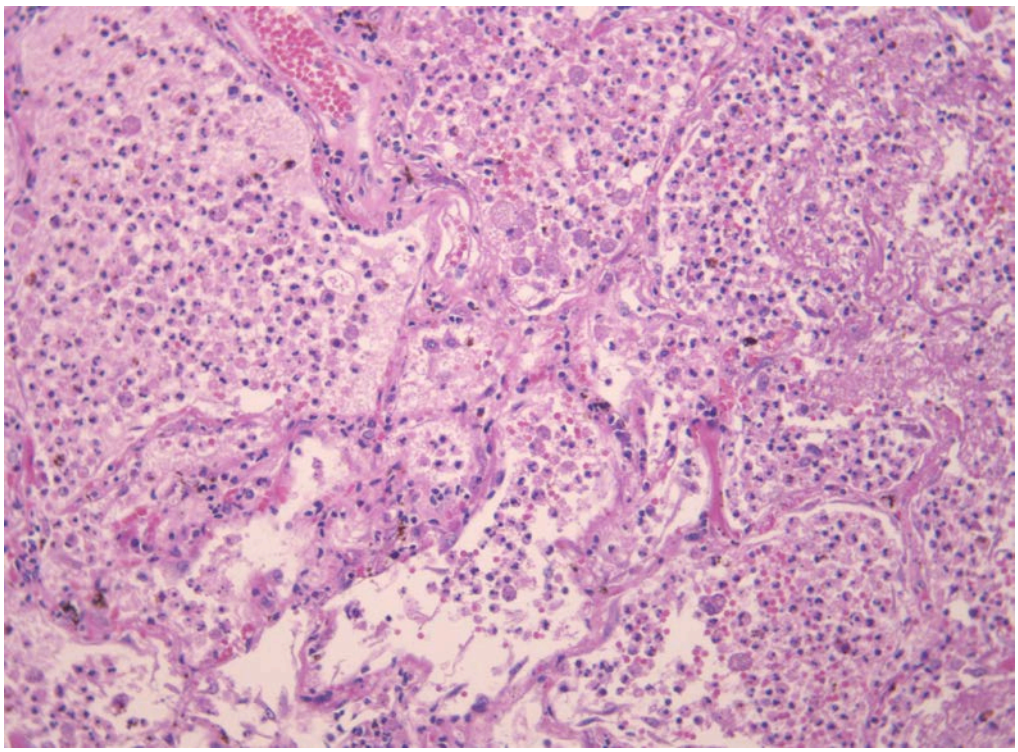


Figure 4.5a: Acute bacterial pneumonia-suppurative inflammation in alveoli; H&E x20

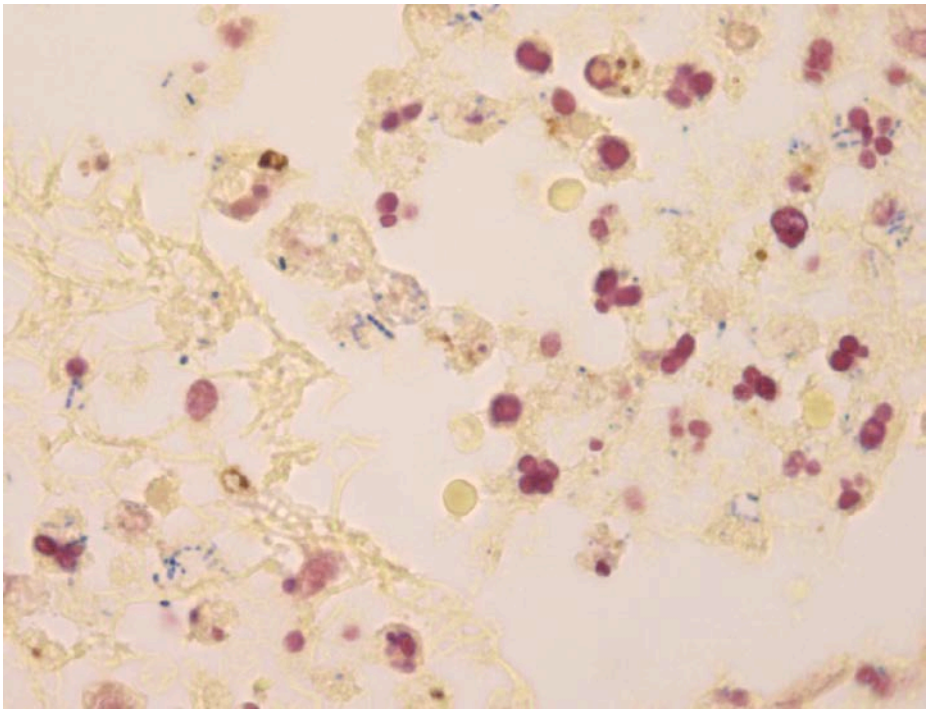


Figure 4.5b: Brown & Hopps stain demonstrates Gram-positive streptococci; x40

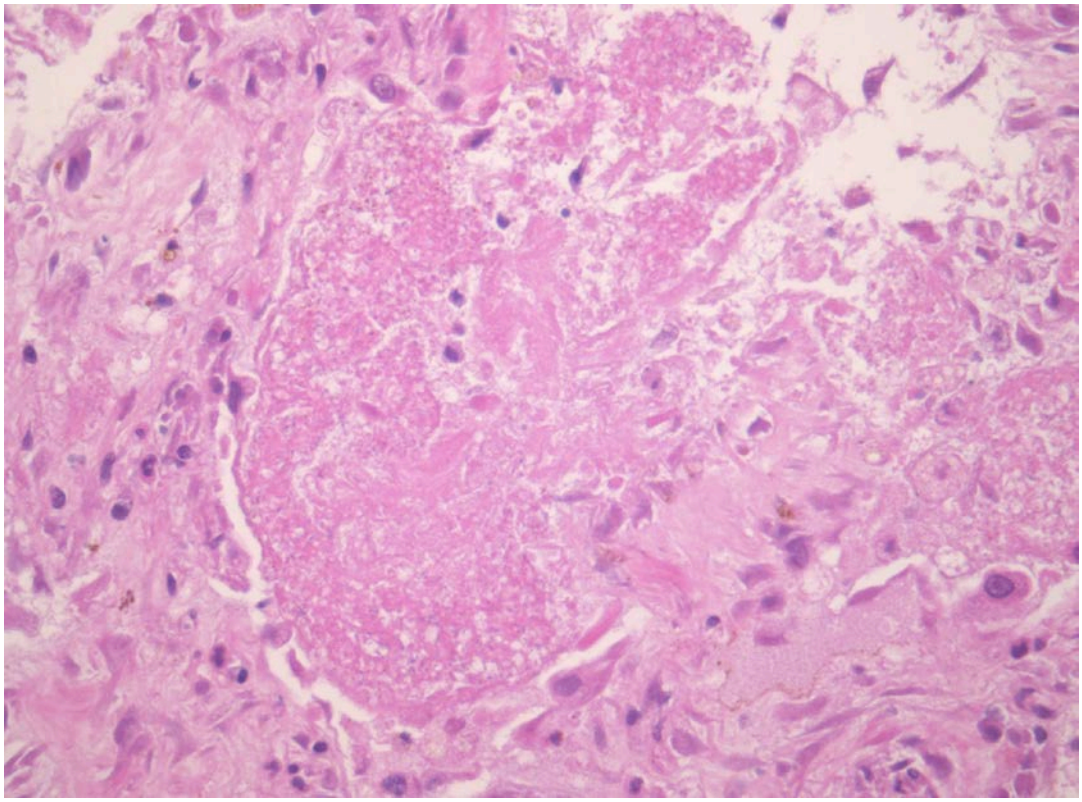


Figure 4.6a: Distended alveolus with frothy, eosinophilic exudate – *Pneumocystis Jirovecii* Pneumonia; H&E x 40.

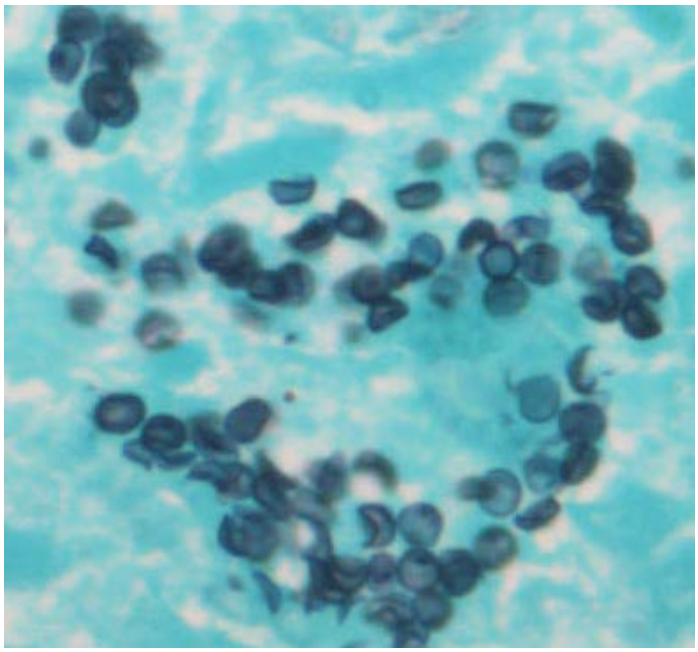


Figure 4.6b: Grocott's stain illustrating cup- and saucer shaped fungi in the exudate; x 40

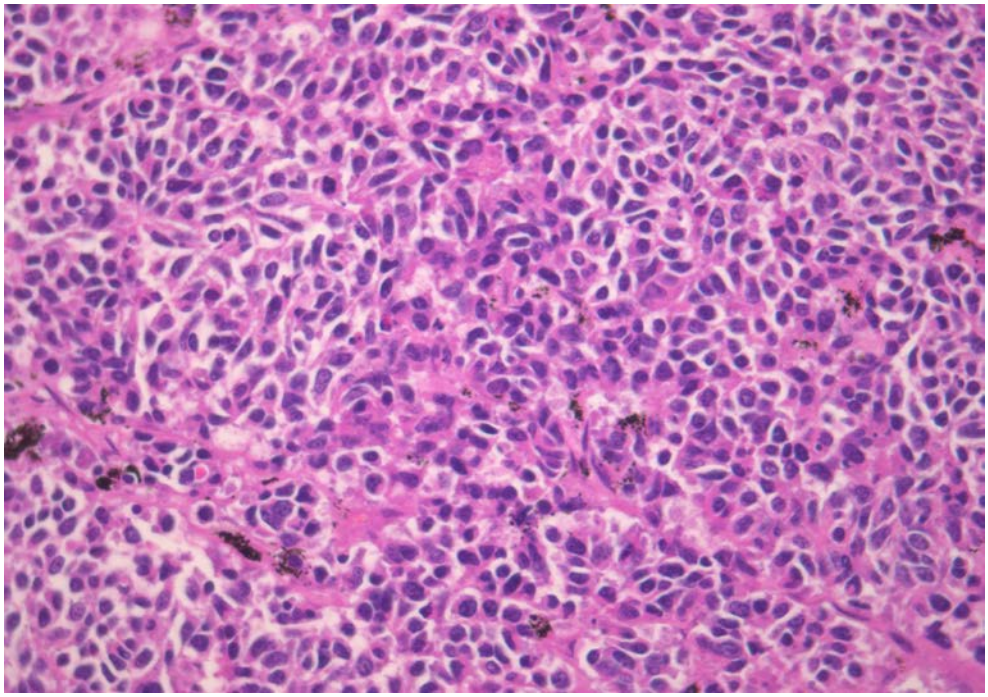


Figure 4.7a: Small moulded tumour cells of neuroendocrine carcinoma; H& E x 40

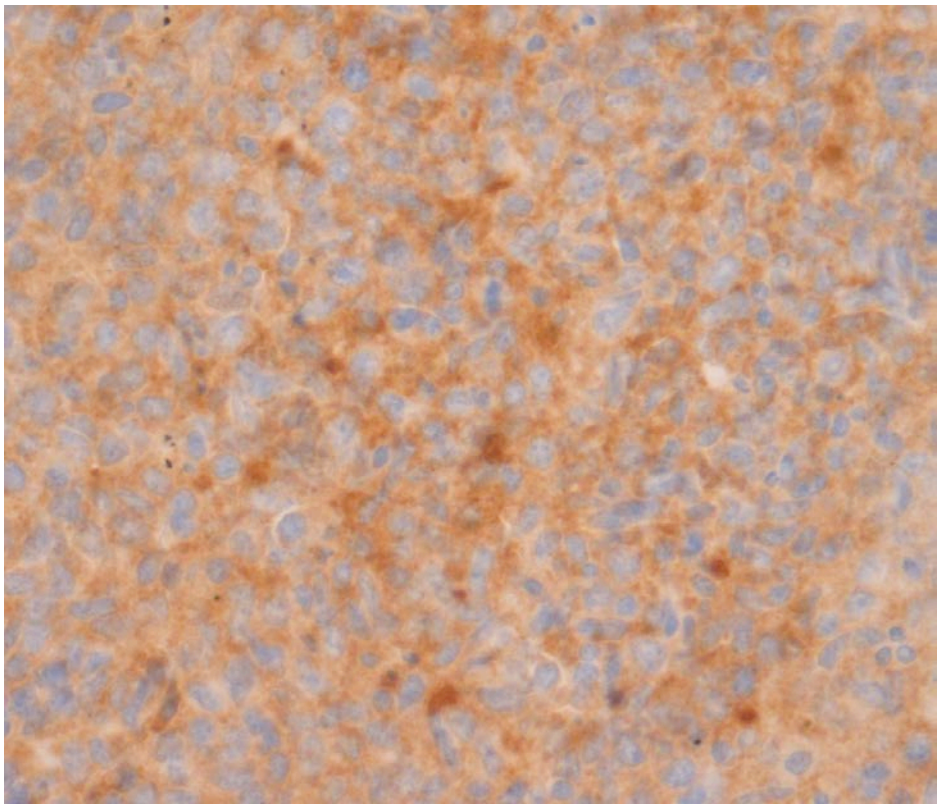


Figure 4.7b: Synaptophysin positivity confirming a small cell neuroendocrine carcinoma. Immunoperoxidase stain x40.

4.3.2 TB Diagnosis:

TB was diagnosed on core biopsies in 20/85 (23.5%; 95%CI 15.8-33.6) cases (Table 4. 3). We were able to isolate *M. tuberculosis* from biopsy specimens in 17/85 cases (20%) using either Xpert® MTB/RIF (n=13), or MGIT culture (n=17). The 17 positive MGIT cultures included all 13 of the cases identified by Xpert® MTB/RIF, indicating a diagnostic concordance of 76.5% (Table 4.4).

Histology diagnosed 14/27(51.85%) of the total TB cases, demonstrating a poorer diagnostic sensitivity than tissue and BAL MGIT culture -17/27 (62.96%), and a significantly poorer performance than BAL Xpert® MTB/RIF – 20/27 (74.07%).

ZN staining of the 14 histology sections with granulomas showed a surprisingly high yield (86.67%), and was more effective at demonstrating acid- fast bacilli (48.15%) than auramine staining of lavage fluid (33.33%) or tissues (25.93%) (Table 4.3) for the 27 cases of TB diagnosed. These latter 2 diagnostic modalities proved to be the least sensitive, but were corroborated by positivity of two or more other diagnostic modalities in every instance.

TB diagnosed on at least one laboratory test	27 (31.8%)
Table 4.3:Post mortem TB diagnosis by diagnostic modality N=85	
TB diagnosed on two or more laboratory tests	18 (21.2%)
Biopsy Specimens with TB*	**n=20**
Histology suggestive of TB	14
Acid fast bacilli on auramine-stained tissue smears	7
Xpert MTB/Rif Pos	13
MGIT positive for M. tuberculosis	17
BAL Specimens with TB	**n=22**
Acid fast bacilli on auramine-stained smears of fluid	9
Xpert MTB/Rif Positive for M. tuberculosis	20
MGIT positive for M. tuberculosis	17

Table 4.4: Diagnostic concordance between post mortem Xpert® MTB/RIF and Mycobacterial Growth Indicator Tube (MGIT) culture in tissue samples				
		Tissue Xpert		
Tissue		+	_	
MGIT	+	13	4	**17**
	_	0	68	**68**
		13	**72**	**85**

4.4 Broncho-alveolar lavage specimens:

M. tuberculosis was identified in aspirated lung fluid in 22/85 decedents (25.9%; 95%CI: 17.8% to 36.1%): 20 on Xpert® MTB/RIF (23.5%) and 17 on MGIT culture (20%), with a diagnostic concordance between Xpert and culture of 15 (68.2%) (Table 4.5). Three non-tuberculous mycobacterial infections were identified on MGIT culture of BAL fluid. Nine BAL samples were positive for acid-fast bacilli on fluid smear microscopy (10.6%).

Table 4.5: Diagnostic concordance between post mortem Xpert® MTB/RIF and Mycobacterial Growth Indicator Tube (MGIT) culture in bronchiolar alveolar lavage (BAL) fluid				
		BAL Xpert		
		+	_	
BAL MGIT	+	15	2	**17**
	_	5	63	**68**
		20	**65**	**85**

4.5 HIV infection:

Of the 14 decedents (16.5%) reported by next-of-kin as being HIV-infected, 6 (42.86%) had evidence of a mycobacterial infection, 4 with *M. tuberculosis* and 2 with non-tuberculous mycobacteria, represented by one case each of *Mycobacterium avium* complex and *Mycobacterium Gordonae.* *Pneumocystis jirovecii* pneumonia was diagnosed in 2 cadavers; one had co-infection with *Mycobacterium avium* complex. 3 decedents had interstitial pneumonias and\2 bacterial pneumonia histologically. The single case of malignancy (small cell neuroendocrine carcinoma) was diagnosed in an HIV infected individual.

4.6 Overall Prevalence of TB

Overall, 27 decedents (31.8%; 95%CI: 22.8-42.3) had at least one positive diagnostic test for TB; using the more conservative definition, 18 (21.2%; 95%CI: 13.8-31.0), had at least two tests positive for pulmonary TB (**Table 3**). Overall, Xpert® MTB/RIF of either biopsy or lung aspirate was positive for *M. tuberculosis* in 21 cases (24.7%) and MGIT was positive in 23 cases (27.1%). Histological evidence of mycobacterial infection was noted in 13 of the 23 TB cases identified by MGIT (56.5%), and 13 of the 21 cases identified by Xpert® MTB/RIF (61.9%). Histology “missed” pulmonary TB in 13/27 cases (48.1%) identified by either MGIT culture or Xpert® MTB/RIF

Drug resistant tuberculosis was not identified by any MGIT cultures or Xpert® MTB/RIF tests.

When ranked by diagnostic yield, Xpert® MTB/RIF testing of BAL fluid identified the highest number of TB cases - 20/27(74.07%), followed by both BAL and tissue

MGIT culture at 17/27 (62.96%), histology - 14/27(51.85%), and lastly tissue Xpert® MTB/RIF 13/27(48.15%).

4.7 Non-TB Mycobacterial Infections:

Three non-TB mycobacterial infections were identified on MGIT culture - one case each of *M gordonae, M scrofulaceum* and *M avium intracellulare* . 2/3 cases showed no pulmonary pathology on histological examination and ZN staining.

CHAPTER 5

5.0 Discussion

5.1 Post mortem TB prevalence

This study shows that 31.8% of all home deaths in which the cause of death under normal conditions would not be able to be categorized, had infectious TB. We are unaware of another study describing TB prevalence in adults dying at home from unknown causes. Further emphasizing the burden of TB in this community, 21.6% of those screened and not included in this study were either receiving TB treatment or had recently been treated for TB. Although the confidence intervals on our point estimates are wide, our data has clear implications for the measurement of targets included in the WHO Post-2015 Global TB Strategy which sets a 95% reduction in TB deaths by 2035 compared to 2015[24]. This data suggests untreated, infectious, pulmonary TB is present in a high proportion of people dying at home without an apparent cause of death. This supposition is supported by several autopsy studies, which demonstrate the enormous burden of TB in adult hospital deaths in high HIV burden settings[22, 25-29], a large proportion of whom were not diagnosed ante-mortem. [13]. Moreover, forensic post mortem studies from South Africa and India found TB in 11% and 5.1% of decedents respectively[30, 31].

This study is not directly comparable to these studies, or to verbal autopsy (VA) studies, which provide only crude estimates of cause of disease and death but are often the only source of community-based mortality data in sub-Saharan

Africa[32, 33]. In Ethiopia, a VA tool reported pulmonary TB in 36% of all deaths, but did not report the proportions who were already on TB treatment[34]. In Limpopo Province in the pre-ART era, of 409 deaths assessed by VA, few (3.2%) were considered to be due to undiagnosed TB[35], and a recent study from Zambia, attributed 5% of all deaths to TB[6].

The study methodology was acceptable to a high proportion of the next of kin, as the majority consented to limited autopsy compared to a prior study in Soweto, where few next of kin consented for full autopsy to be done[36].

5.2 Comparison of diagnostic modalities

Culture and Xpert® MTB/RIF proved diagnostically superior to histology and smear microscopy. The excellent diagnostic yield from Xpert® MTB/RIF in this study is consistent with previous findings. It has demonstrated high sensitivity and specificity for TB diagnosis and was endorsed by the WHO in 2010 as a first line diagnostic test in HIV associated TB and suspected multi-drug resistant (MDR) TB [37, 38].

Xpert® MTB/RIF had a greater diagnostic yield to culture in BAL fluid, yet MGIT was apparently superior for tissue diagnosis of TB. The poorer performance of BAL culture when compared to Xpert® MTB/RIF of BAL fluid may result from the time delay in obtaining samples for culture (median time to post mortem was 5 days) causing bacterial contamination of samples, and a consequent poorer yield. A reason for Xpert® MTB/RIF's poorer performance on tissue samples is not

apparent as a prior report suggests that the high Xpert sensitivity is maintained in non-sputum samples[38]. The poor sensitivity of smear microscopy observed in this study follows a well-established trend, particularly in HIV positive individuals where there is reduced shedding of mycobacteria in sputum[37, 39]. It remains, however, the first line TB diagnostic in resource constraint settings despite its limitations.

The mediocre performance of histology is partially explained by possible sampling of non-diagnostic lung, which nonetheless harbored bacilli detectable by alternative diagnostics. Whilst histology detected only a single TB case not picked up by other diagnostics, it proved essential in confirming the presence of non-tuberculous pulmonary pathology. The first of the two inadequate histology specimens occurred early in the study, and may be related to nurse inexperience. Neither showed evidence of TB by another diagnostic modality and did not contribute to the histology "misses".

5.3 Consequences of findings

This study illustrates the fatal consequence of delaying TB diagnosis and treatment. Barriers and delays in seeking care for TB have been reported in many settings[40-42] and it is worrying that a high proportion of adults with TB apparently did not access health care prior to death, as suggested by next-of kin interviews, despite more than half of those with TB having symptoms for at least two months. This group of undiagnosed, likely infectious but untreated adults should be included in strategies both to interrupt TB transmission and prevent mortality.

5.4 Study limitations:

Like most autopsy studies, this one has limitations. We recruited a small sample at a single study site, and relied on six private mortuaries to identify potentially eligible decedents. This may introduce a selection bias. Next of kin interviews, shortly after the death of a loved-one, may have been influenced by both recall and social desirability bias. The deceased may not have disclosed HIV or TB disease status to family members, resulting in over estimation of undiagnosed TB. We did not confirm HIV sero-status as many next of kin refused consent for HIV testing. In addition, the research laboratory was unwilling to perform an invalidated test on post-mortem samples. This precludes evaluation of home deaths due to dual infection.

Core biopsies may miss focal pulmonary lesions due to the limited sampling[25], thereby resulting in underestimation of TB. Sampling lung only tissue rather than multiple organs may reduce the detection of TB and precludes evaluation of disseminated TB in home deaths. While there is data validating Xpert® MTB/RIF test characteristics in tissue[43, 44], there is none validating its use in post mortem specimens. Anecdotally, it appears that at least some of the deceased had contact with emergency rooms but we did not explore this. Finally, the stated CoD on the DNF's were not collated or analyzed.

CHAPTER 6

6.0 Conclusion:

This study shows that almost one third of all home deaths in whom the cause of death under normal conditions would not be able to be categorized, had infectious TB. Undiagnosed infectious TB disease in adults dying at home without an apparent CoD are not TB transmission “dead ends”, particularly as MGIT culture of BAL fluid identified the presence of viable bacilli in high proportion, and many decedents needed close care, including bathing and feeding, in their final days. These findings need to be considered when developing plans to comply with the Global TB Strategy, which sets a 95% reduction in TB deaths by 2035 compared to 2015. Moreover, to ensure more reliable statistics on CoD, greater efforts should be made to ensure that DNF’s are completed for people dying at home.

6.1 Suggestions for future research:

We propose autopsy studies such as ours are repeated either as full post mortems, or with sampling of additional organs, to determine TB prevalence in home deaths more accurately. Studies to understand health seeking behavior and failure to obtain help for serious symptoms earlier in the disease process and to better understand reservoirs of undiagnosed TB are urgently needed. Our results call for a better understanding of reservoirs of TB infection, an urgent assessment of reasons for delayed or non-attendance at health fascilities by TB patients, and for more research into the benefits of population based screening and treatment for TB.

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Annexure A: Letter from co-authors

We, the undersigned, are co-authors with Dr Tanvier Omar on the paper: Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: a post mortem study. It has been accepted for publication in the *International Journal of Tuberculosis and Lung Disease*.

We give permission for Dr. Omar, the first author on the publication, to submit a research report based on the article to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfillment of the requirement for the degree of Master of Medicine in the branch Anatomical Pathology

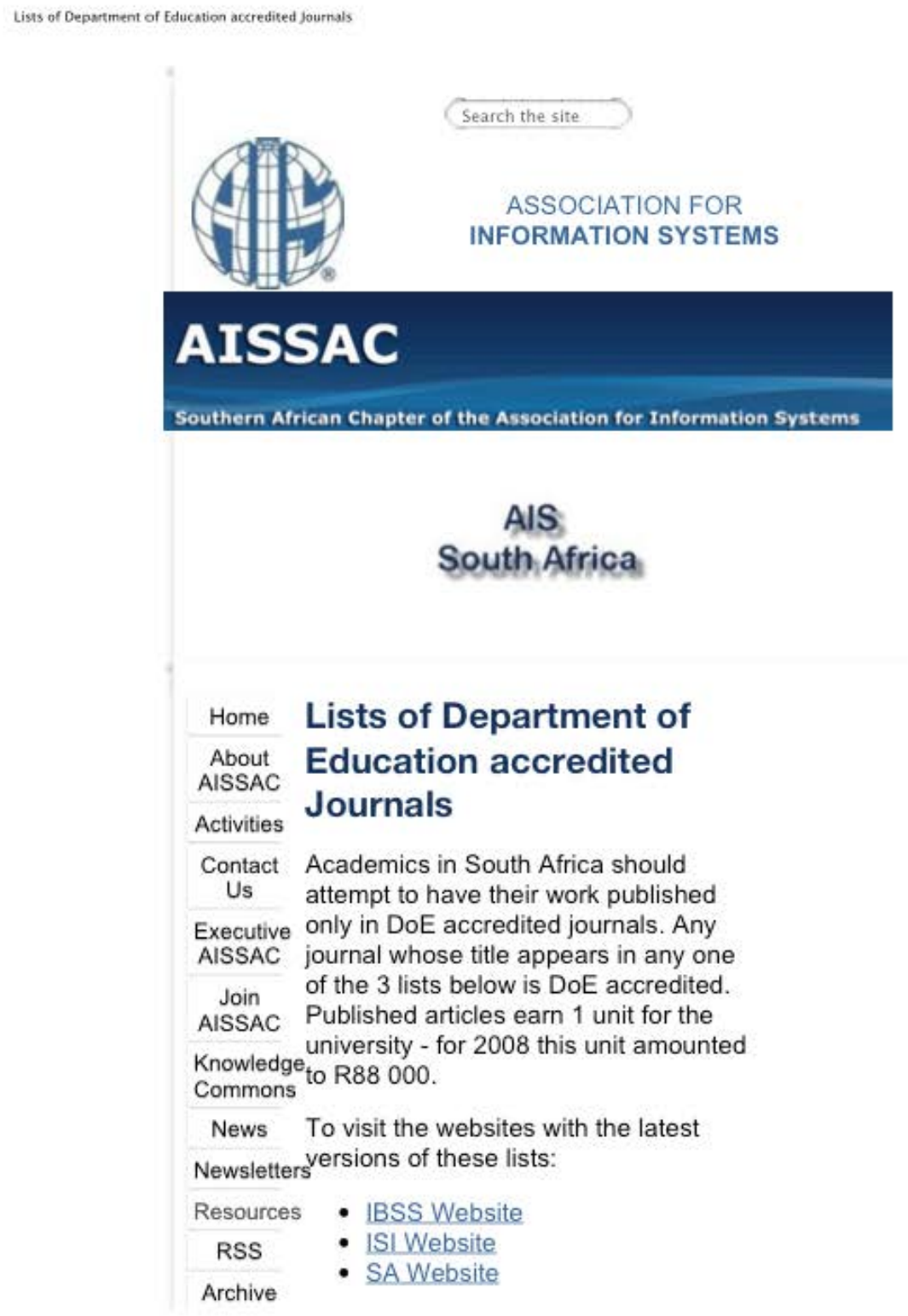
Dr Omar, together with Drs Variava and Martinson, planned the study. Dr Chaisson provided strategic direction. Drs Variava and Lebina trained and supervised the nurse, Mr Moroe. Dr Omar was responsible for all histopathological analyses in the study. She collected and correlated laboratory data, prepared the data files, performed the data analysis, and statistical evaluation, collated and interpreted the results, and did the photomicrography. Dr Billioux assisted with data capture and analysis. The manuscript was written and prepared by the first author, and was critically revised by all other co-authors.

Variava E | Moroe E | Billioux A

Lebina L | Martinson N | Chaisson RE

22nd September 2015

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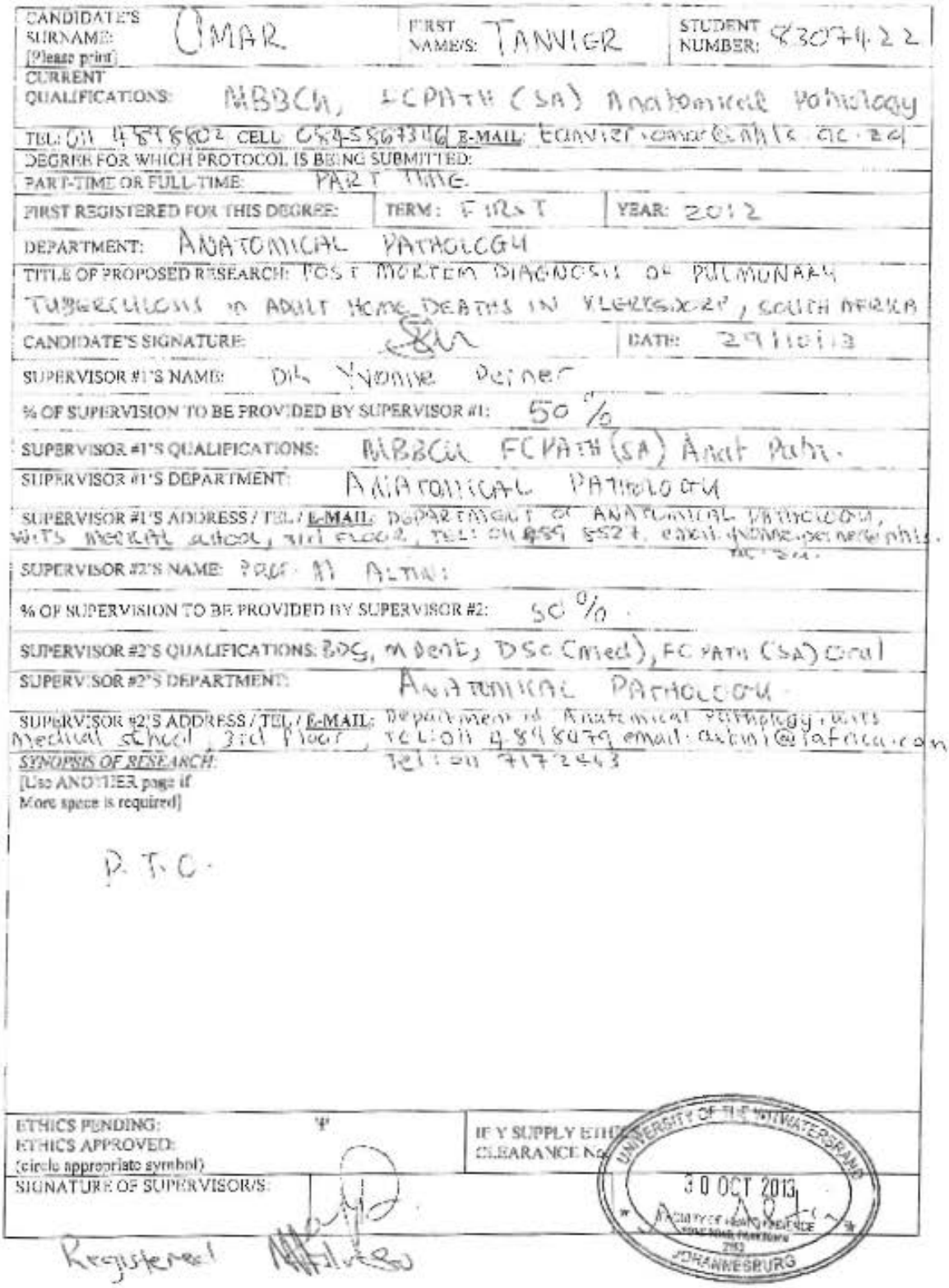
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TITLE OF PROPOSED RESEARCH: POST MORTEM DIAGNOSIS OF PULMONARY TUBERCULOSIS IN ADULT HOME DEATHS IN KLERKSDORP, SOUTH AFRICA
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SUPERVISOR #1'S NAME: Dr Yvonne Reiner
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30 October 2015
Person No: 8307422
TAA

Dr T Omar
P O Box 53633
Troyville
2139
South Africa

Dear Dr Omar

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:	**Post mortem diagnosis of pulmonary tuberculosis in adult home deaths in Klerksdorp, South Africa**
To:	**Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: a post mortem study**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Annexure D: Ethics Clearance Certificate

R14/49 Dr Tanvier Omar

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M131060

NAME: (Principal Investigator)	Dr Tanvier Omar
DEPARTMENT:	Division of Anatomical Pathology
PROJECT TITLE:	Undiagnosed TB in Adults Dying at Home from Natural Causes in a High TB Burden Setting: A Post Mortem Study
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Changed Title
SUPERVISOR:	Prof Mario Altini
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	15/11/2013 (Initial Approval)23/09/2015(Recertified)

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

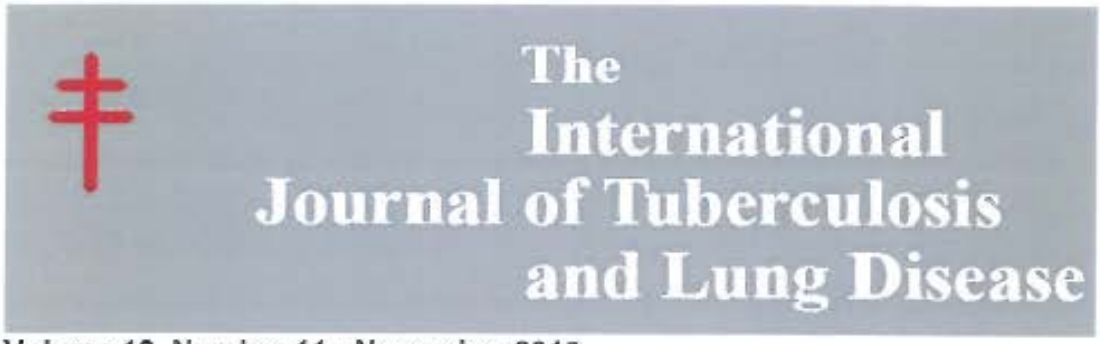
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Annexure E: Proof of acceptance by journal



The International Journal of Tuberculosis and Lung Disease

Volume 19, Number 11 - November 2015

Now free access: May 2015

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Annexure F: Journal reviewers Comments - Manuscript ID IJTLD-03-15-0222

Editor's Comments:

1) It would be helpful to have a better sense of concordance between Xpert and MGIT. It is clear that at least in the modified BAL, you had some patients with positive Xpert and negative MGIT. Given that these patients were not on treatment, it would be useful to hear your comments as to why this might be, and it would be useful to understand whether these patients had pathological abnormalities consistent with TB or not, especially given your stated limitation of Xpert not being validated for post-mortem tissue.

The performance of Xpert and MGIT on both tissue and BAL fluid has been compared and correlations with histological findings commented on – see Results section paragraphs 2,3,5 and 7. A 2x2 table (table 4) has been added to illustrate concordance. The differences in TB pickup between Xpert and MGIT culture are unclear.

2) You note that you did not assess CoD and then note some previous VA results. It is not clear that VA has any validated ability to detect TB as a cause of death. Full post-mortem exam seems likely the only validated approach to establishing CoD. Consider referencing instead of VA (or in addition to), data on use of full post-mortem exam for TB diagnosis.

The limitations of VA and the reason for comparison in this case have been stated. Studies on full post mortems have already been referenced (see ref 16, 20-23) but direct comparisons are not possible, as these studies do not investigate home deaths from unknown causes. Two additional forensic autopsy studies with arguably more comparable sample populations have been referenced. The need for further full post mortem studies has been stated.

3) In the methods, I found it slightly confusing to understand your more narrow definition of TB. When you state that at least 2 laboratory diagnostic tests were required to be positive, does that include histology and smear or just Xpert and culture? You could revise by stating that you did 4 laboratory tests on each patient (list them - smear, histo, culture, xpert), and then state that the first definition required any one of those to be positive and the second required two to be positive.

The definition has been clarified as per the editor's recommendations. See paragraph 3 methods section.

4) Why was HIV testing not done?

Many next of kin refused consent for HIV testing on their relatives despite consenting to the autopsy. In addition, the research laboratory was unwilling to perform an invalidated test on post-mortem samples. This has been included in the statement on limitations.

5) In the methods, you state that patients on TB treatment currently or previously on treatment and those with an established cause of death were not eligible, you go on to say that "therefore" patients who were recently hospitalized were not eligible. It is not clear to me how recent hospitalization as a criterion for ineligibility follows from the previous statement about TB treatment and known causes of death (many patients are hospitalized and still die without a known cause of death).

A hospital admission would raise the probability of a known cause of death. We wanted to ensure that no deceased with a known cause of death was included. Since this study did not have the resources to follow up hospital records to determine eligibility, this category of decedents was excluded. The description of eligibility has been refined in the text.

6) You state in the discussion that undiagnosed, infectious TB is present in at least "some" who die at home. This strikes me as a bit conservative. I defer to you, but it seems to me that your data find that the number who are dying of undiagnosed, untreated TB at home may be / appears to be very high.

Agree. The scale of the problem is clarified in the discussion section: “our data has clear implications …… as it suggests that untreated, infectious, pulmonary TB is present in a high proportion of people dying at home without an apparent cause of death.”

Reviewer 1 – Comments:

1. Use "deceased" instead of "cadaver"

The term cadaver has been replaced throughout the text.

2. Discuss the two biopsy misses, was there any lesson to be learned here? Were they within the histological misses (the 9/27 cases identified by culture or Xpert or were they excluded)?

A description of the two inadequate histologies is provided in the results section and contribution to diagnostic misses is discussed (paragraph 4 of discussion).

3. What is the national average of TB incidence?

“In 2013,the annual TB incidence was 860 per 100,000 …” see paragraph 2 of introduction.

4. Is Matlosana an urban or rural setting?

Four urban areas with limited farming communities. This has been added to methods paragraph 1.

5. What is the HIV prevalence in Matlosana?

Specific figures for the Matlosana sub-district are not available. Overall HIV prevalence in the North West Province, where Matlosana is located was 13.3% in 2012 and that in pregnant women close to 30%. See methods, paragraph 1.

6. What were the CoD listed for the cases?

The stated CoD on the DNF were not collated or analyzed. This has been listed as a limitation.

7. Add a figure showing an example of a positive and negative needle biopsy histology.

This has been done. See Figure 1 – 3 images.

Reviewer: 2 – Comments:

1. Abstract - add HIV mention - see below.

This has been done.

2. Add HIV to keywords list.

This has been done.

3. Methods - I suggest a couple of clarifications.

3.1. The Abstract states the scenario of how these cadavers are selected more clearly, i.e. died home + no premortem diagnosis + no recent hospital admission. Please put this info here.

Eligibility criteria have been clarified. Please see methods section paragraph 2.

3.2. HIV. No autopsy HIV tests were done - should be stated. Thus HIV+ epi data is that reported by relatives. Correct? Clarify - in Abstract and also in Table 1

No post mortem HIV testing was performed. This has been included in the abstract, methods section and limitations. The table has been appropriately labeled.

Annexure G: Questionnaire administered to next-of-kin

Perinatal HIV Research Unit (PHRU) — **Massive Case Finding Study (POS-1)**

Massive Case Finding 067 | Plt 20 (020) | Visit 1 (010)

Index ID: 7 _ _ _ _ 0 1

Post Mortem

Date of sample taken: _ _ dd | _ _ mm | 2 0 1 _ yyyy

1.Initials of deceased *(first/last)*	_ _
2.Date of death *(dd/mm/yyyy)*	_ _ _ _ 2 0 1 _
3.Age at death in years *(must be >18 yrs)*	_ _
4.Died from trauma? *(Motor vehicle accident, stabbing, gunshot, etc.)*	☐ Y ☐ N → **If yes, do NOT continue!!!**
5.Which family member did you interview?	☐ *1=sibling 2=spouse 3=cousin/uncle/aunt 4=other*
6.Is this person HIV infected?	☐ Y ☐ N ☐ Unsure
7.Does this person have TB?	☐ Y ☐ N ☐ Maybe
7.1.If yes, on TB treatment?	☐ Y ☐ N ☐ Unsure
8.Deceased had a recent admission to hospital? *(in past 6 months)*	☐ Y ☐ N ☐ Unsure
8.1.If yes, was admission TB related?	☐ Y ☐ N ☐ Unsure
8.1.1.If yes, specify diagnosis:	☐ *1=PTB 2=TB Meningitis 3=TB other organ*
8.2.If yes, was admission HIV related?	☐ Y ☐ N ☐ Unsure
8.2.1.If yes, specify diagnosis?	☐ *1=TB 2=ARV 3=TCrypto 4=Bacterial infection 5=toxoplasmosis 6=Candidal infection 7=other*
9.Is there a recent confirmed diagnosis from a doctor or hospital that is likely to have resulted in death?	☐ Y ☐ N
9.1.If yes to Q9, specify _____	

Do not proceed unless one of the following : Cancer or its treatment, Long term steroid therapy, Gastrectomy or small bowel bypass surgery, chronic disability, diabetes, dialysis, silicosis (check with Drs.V or L or M if unsure).

10.Duration of symptoms preceeding death? *(in days)*	☐☐☐ *(365 = 1 year, 730 = 2 years, 999 = > 3 years)*
11.Reported symptoms in order of severity to patient *(list three most important)*.	*01=cough 02=loss of weight/wasting 03=chest pain 04=shortness of breath 05=unable to walk/bedridden 06=diarrhoea for at least one month 07=severe rash or ulcers 08=blindness 09=memory/cognitive deterioration 10=other* — 1 ☐☐ 2 ☐☐ 3 ☐☐
12.Did the deceased have diabetes?	☐ Y ☐ N ☐ Unsure
12.1.If yes to Q12, the deceased was taking what treatment for diabetes?	☐ Pills ☐ Injection ☐ Don't know

Staff Initials / Date _____

Annexure H: Published article

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Undiagnosed TB in adults dying at home from natural causes in a high TB burden setting: a post-mortem study

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SUMMARY

BACKGROUND: A high proportion of deaths in Africa occur at home, where cause of death (CoD) is often unknown. We ascertained undiagnosed pulmonary tuberculosis (TB) by performing limited autopsies in adults dying at home in whom there was no apparent CoD.

METHODS: Mortuaries in Matlosana, South Africa, identified potentially eligible adults with no ante-mortem diagnosis and/or no recent hospital admission. A questionnaire was administered to family members. Bilateral lung core biopsies and modified bronchoalveolar lavage (BAL) were performed. The biopsies were examined histologically and submitted with BAL aspirates for mycobacterial culture and Xpert® MTB/RIF testing. Human immunodeficiency virus (HIV) testing was not performed.

RESULTS: Of 162 families approached, 28 refused and 29 of the deceased were on or had recently stopped anti-tuberculosis treatment; 85 were included. All were Black and 53% were men. The median age was 57 years (interquartile range [IQR] 44–66) and median symptom duration (mainly cough) was 63 days (IQR 14–112). Laboratory evidence of TB was found in 27 (31.8%); 21 were Xpert-positive, 23 were MGIT™-positive and 14 had histological evidence consistent with active TB.

CONCLUSION: In this high HIV prevalence setting, a quarter of the home deaths had evidence of undiagnosed, likely infectious TB, suggesting that TB-related mortality is under-ascertained and under-reported, with serious implications for TB control in high TB burden settings.

KEY WORDS: autopsy; tuberculosis; home deaths; case ascertainment; HIV

TUBERCULOSIS (TB) is a leading cause of death due to infection worldwide, causing 1.5 million deaths in the 9 million people estimated to have developed TB in 2013.[1] Globally, significant progress has been made in reducing annual TB incidence and TB mortality;[2] however, to achieve the Millennium Development Goal 6 targets[3] of reduction in the global TB burden (prevalence and death) by 50% compared to 1990 levels by 2015,[4] novel strategies to curb transmission urgently need to be implemented, particularly among infectious individuals.

In 2013, the annual TB incidence in South Africa, a country with a particularly high TB burden, was 860 per 100 000 population, and TB has been reported as the leading cause of death (CoD) on death notification forms (DNFs) for almost a decade. TB was recorded as the CoD on 40 542 (8.8%) DNFs in 2011.[5] Mortality reports show that at least one quarter of deaths in South Africa, and 49% in less-resourced Zambia, occur at home, where CoD is probably unknown.[5,6] Moreover, a recurrent theme in South African mortality reports is that one quarter of all DNFs have an ill-defined CoD not allowing disease categorisation and coding.

Deaths in individuals recently diagnosed and started on anti-tuberculosis treatment follow a well-reported pattern. In sub-Saharan African countries, 'high, early' mortality in the pre-antiretroviral treatment (ART) era was described, with about one quarter of TB patients dying during anti-tuberculosis treatment, mostly in the days or weeks following TB diagnosis or hospital admission.[7–9] A similar pattern of early mortality was reported in the pre-human immunodeficiency virus (HIV) era in industrialised settings.[10–12] This pattern of early mortality suggests that late presentation with TB is frequent: some who

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are diagnosed and started on anti-tuberculosis treatment have advanced TB and die despite treatment; others die prior to presenting to health systems for diagnosis and treatment.

In a setting where TB is the leading CoD, a better understanding of the presence of TB in those dying at home with an unknown CoD is important to plan efforts to prevent transmission and deaths associated with TB disease. Information on TB prevalence in home deaths—particularly in high HIV and TB incidence settings—will contribute to these strategies. We conducted a limited autopsy study to provide data on laboratory-confirmed pulmonary TB in adults dying at home without a clear or apparent classifiable CoD.

METHODS

The study site was the Matlosana health sub-district (population ~750 000), which covers four urban areas with limited farming communities in North West Province, South Africa. In 2012, HIV prevalence in the province was 13.3% and nearly 30% among pregnant women;[13,14] the annual TB incidence for the health district in which Matlosana is located was 937/100 000 in 2012.[15] This study was part of a larger effort to assess rates of detection of active TB and latent tuberculous infection in various populations and their household contacts. Using two methods of sampling, we assessed the presence of TB in lungs of adults who died at home from natural causes (from illness and not from external forces such as assault, road injuries, suicide, poisoning, post-operative complications, etc.), whose CoD under routine circumstances would have been unclassifiable. The undertakers' trade association in Matlosana were contacted; seven private mortuaries agreed to identify potential study subjects and provide study staff with contact details of the next of kin of adults who fulfilled the eligibility criteria. The next of kin thus identified were then contacted and asked for consent to limited post-mortem sampling of the lungs of their deceased relative. They were then administered a structured questionnaire to confirm the eligibility of the deceased to be included in the study. If eligible, the presence of ante-mortem symptoms, their duration, and prior medical and smoking history were ascertained.

To be eligible for the study, the deceased had to be aged ⩾18 years, have no apparent pre-mortem cause of death, not be on anti-tuberculosis treatment or have been recently diagnosed with TB. Any potential subject whose death occurred while on anti-tuberculosis treatment or was likely to be due to injury or surgery was excluded. In addition, to ensure that all subjects with a potentially identifiable premortem CoD were excluded, a history of hospitalisation in the 2 months prior to death was used as an exclusion criterion. After confirming eligibility, a trained nurse performed a post mortem in the private mortuary, wearing appropriate personal protective equipment, including gloves, disposable gown, boots, N95 respirator and protective eyewear. First, multiple bilateral Tru-cut® (CareFusion Corporation, San Diego, CA, USA) lung core biopsies were performed[16,17] using a 14-gauge needle through a single 1 cm incision in each axilla to minimize disfigurement. An approximate total length of 6 cm of lung tissue was obtained from each lung. Second, a modified bronchoalveolar lavage (BAL) was performed through a horizontal, midline 1 cm anterior incision into the trachea at the base of the neck. A suction tube was inserted into the trachea and passed into a main bronchus. Twenty ml of sterile saline was instilled into the suction tube, then re-aspirated after gently rocking the body.

The biopsy material was submitted for histological evaluation using Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) and automated MGIT™ liquid mycobacterial culture (Mycobacteria Growth Indicator Tube; BD, Franklin Lakes, NJ, USA). Histology specimens were fixed in 10% buffered formalin. All biopsies were stained with haematoxilin and eosin (H&E) and with Ziehl-Neelsen (ZN) and Grocott's stains for mycobacterial and fungal identification, respectively (Figure). Further special stains were performed as directed by microscopy. Biopsy material and aspirated fluid was submitted for MGIT culture and Xpert testing in sterile containers; fluid was also subjected to auramine smear microscopy. Post-mortem HIV testing was not performed.

Four laboratory modalities were used to ascertain the presence of pulmonary TB on each study subject: auramine smear microscopy, histological evaluation of lung biopsies with ZN staining, MGIT culture and Xpert testing. Two study definitions of pulmonary TB were used: the first was the diagnosis of TB on any one of the four tests; a second, more conservative, definition required at least two modalities to be positive.

The following descriptive statistics are reported: medians with their interquartile ranges (IQRs) for continuous variables, and proportions with their 95% confidence intervals (CIs) for dichotomous variables. Comparisons were made between those subjects with pulmonary TB and those without using the Fisher's exact and Wilcoxon Mann-Whitney tests, as there were relatively few observations.

Ethics approval was obtained from the institutional review boards of the University of Witwatersrand, Johannesburg, South Africa, and the Johns Hopkins University, Baltimore, MD, USA, and from the chief executive officer at the Klerksdorp-Tshepong Hospital Complex in Matlosana.

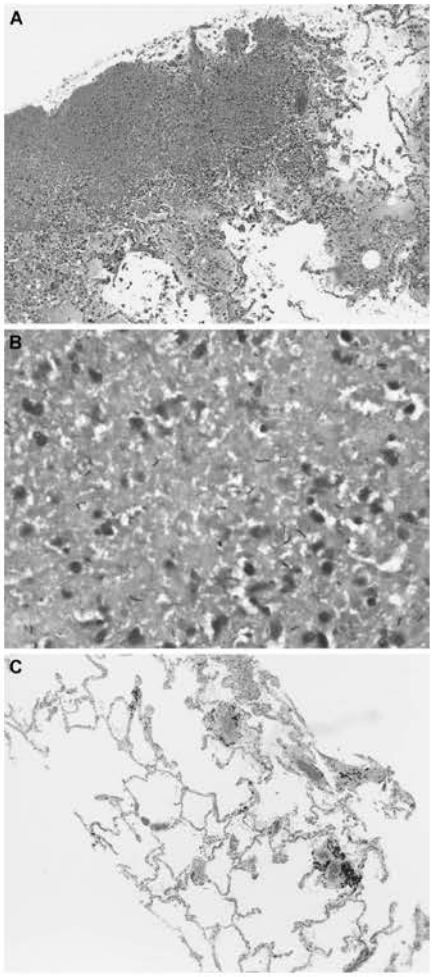


Figure Specimens of lung showing necrotising granulomatous inflammation in a tuberculosis bronchopneumonia case: **A)** H&E x10; **B)** Ziehl-Neelsen stain demonstrating numerous acid-fast bacilli, x100; **C)** no pathological changes, H&E x10. H&E = haematoxilin and eosin. This image can be viewed online in colour at http://www.ingentaconnect.com/content/iuatld/ijtld/2015/00000019/00000011/art000....

RESULTS

From January to August 2012, 162 potentially eligible deceased's next-of-kin were identified by six of the seven mortuaries, and contacted for consent; 28 (17.2%) refused participation. Upon further questioning, 49 were not eligible for the study: 29 were either on, or had been on anti-tuberculosis treatment at the time of death, 12 had a recent admission to hospital, 3 had a current cancer diagnosis, 2 had heart ailments, 1 subject was decomposed, 1 had a prior stab wound and close relatives of another were not available. A final 85 decedents were therefore included. There was an approximately equal sex distribution, and median age at death was 57 years (IQR 44–66) (Table 1). Symptoms prior to death as reported by the next of kin were present in the deceased for a median of 63 days (IQR 14–112); cough was the leading symptom in terms of severity, followed by dyspnoea and chest pain. Twelve had symptoms for ⩽1 day, four of whom were diagnosed with TB by the study procedures. More than half of the deceased reportedly smoked, almost a third had a history of hypertension and 40% were bedridden prior to death. Those diagnosed as having pulmonary TB by the study were perceived to have cough ($P = 0.0001$) and shortness of breath more frequently ($P = 0.0546$) than those without TB. The median time from death to autopsy was 5 days (IQR 3–8).

Core biopsies

Two of 85 core biopsies did not include lung tissue; one was liver with evidence of hepatitis and steatosis, and the other was connective tissue without pathology. Fifty of the remaining 83 (60.2%, 95%CI 49.7–70.7) lung specimens evaluated histologically showed no specific pathological changes (Table 2). Histological features consistent with mycobacterial pneumonia were the lead finding in 15/83 (18.1%, 95%CI 11.3–27.7): this was confirmed by the presence of acid-fast bacilli (AFB) on ZN staining in 13/15 (86.7%) (Table 3), and by Xpert or MGIT culture in 14/15 cases (93.3%). *Mycobacterium gordonae* was cultured in one case. One TB case not detected by other tests was identified on histological examination.

TB was diagnosed on core biopsies in 20/85 cases (23.5%, 95%CI 15.8–33.6). We were able to isolate *M. tuberculosis* from biopsy specimens in 17/85 cases (20%) using Xpert ($n = 13$) or MGIT culture ($n = 17$). The 17 positive MGIT cultures included all 13 of the cases identified by Xpert, indicating a diagnostic concordance of 76.5% (Table 4A).

Acute suppurative pneumonia consistent with a bacterial aetiology was the second leading histological diagnosis in 10/83 (12.0%), followed by interstitial pneumonitis in 9/83 (10.8%). Three lungs with interstitial pneumonitis had evidence of TB on another diagnostic test. Three of the 83 biopsies had histological evidence of *Pneumocystis jiroveci* pneumonia, highlighted by Grocott's staining; two of these had coexistent non-tuberculous mycobacteria (NTM) diagnosed on MGIT culture (*M. avium* complex and *M. scrofulaceum*). One deceased had a small cell neuroendocrine carcinoma associated with acute suppurative pneumonia.

Table 1 Characteristics of adults who died at home without any apparent cause of death, as reported by the next of kin

	All ($n = 85$) n (%)	TB ($n = 27$) n (%)	No TB ($n = 58$) n (%)
Male	46 (54.1)	15 (55.6)	31 (53.4)
Age, years, median [IQR]	57 [44–66]	49 [42–64]	59 [51–68]
HIV infection (as reported)	14 (16.5)	7 (25.9)	7 (12.1)
Smokers	49 (57.6)	17 (63.0)	32 (55.1)
Number of cigarettes per day, median [IQR]	12 [10–14]	12 [10–14]	12 [10–13]
Duration of any symptom/s prior to death, days, median, [IQR]	63 [14–112]	68 [30–126]	39 [9.3–92.8]
Symptoms prior to death by severity			
Cough	58 (68.2)	22 (81.5)	21 (36.2)
Shortness of breath	52 (61.1)	12 (44.4)	40 (69.0)
Chest pain	37 (43.5)	10 (37.0)	27 (46.6)
Bedridden	34 (40.0)	13 (38.2)	21 (36.2)
Comorbid conditions			
Hypertension	24 (28)	22 (31.8)	16 (22.4)
Diabetes	4 (4.7)	1 (3.7)	3 (5.1)

TB = tuberculosis; IQR = interquartile range; HIV = human immunodeficiency virus.

Bronchoalveolar lavage specimens

M. tuberculosis was identified in aspirated fluid in 22/85 decedents (25.9%, 95%CI 17.8–36.1): 20 on Xpert (23.5%) and 17 on MGIT culture (20%), with a diagnostic concordance between Xpert and culture of 15 (68.2%) (Table 4B). Three NTM infections were identified on MGIT culture of BAL fluid. Nine BAL samples were positive for AFB on fluid smear microscopy (10.6%).

HIV infection

Of the 11 decedents (12.9%) reported by their next of kin as being HIV-infected, four (36.4%) had evidence of a mycobacterial infection, 2 with *M. tuberculosis* and 2 with NTM. *P. jiroveci* pneumonia was diagnosed in two of the deceased; one had co-infection with *M. avium* complex. Another 3 had interstitial pneumonias and 2 bacterial pneumonias on histology. The single case of malignancy (small cell neuroendocrine carcinoma) was diagnosed in an HIV-infected individual.

Overall prevalence of TB

Overall, 27 decedents (31.8%, 95%CI 22.8–42.3) had at least one positive diagnostic test for TB; using the more conservative definition, 18 (21.2%, 95%CI 13.8–31.0) had at least two tests positive for pulmonary TB (Table 3). Overall, Xpert testing of either biopsy or lung aspirate was positive for *M. tuberculosis* in 21 cases (24.7%) and 23 cases (27.1%) were MGIT-positive. Histological evidence of mycobacterial infection was noted in 13/23 TB cases identified using MGIT (56.5%) and 13/21 cases identified using Xpert MTB/Rif (61.9%). Histological testing 'missed' pulmonary TB in 13/27 cases (48.1%) identified by either MGIT culture or Xpert. Drug-resistant TB was not identified by MGIT culture or Xpert testing.

Table 2 Summary of histological findings in lung core biopsies ($n = 83$)*

	n (%)
No specific pathological changes	50 (60.2)
Granulomatous inflammation consistent with mycobacterial pneumonia†	15 (18.1)
ZN-positive	13 (15.7)
Pneumocystis pneumonia‡	3 (3.6)
Bacterial pneumonia	10 (12.1)
Interstitial pneumonitis§	9 (10.8)
Small cell neuro-endocrine carcinoma	1 (1.2)

* Cases may have had more than one histological diagnosis.
† One case had *M. gordonae*.
‡ One co-infected with *M. avium* complex.
§ One co-infected with *M. scrofulaceum*.
ZN = Ziehl-Neelsen.

DISCUSSION

In this study, 31.8% of all home deaths with uncategorisable CoD under normal conditions had infectious TB. We are unaware of any other study describing TB prevalence in adults dying at home from unknown causes. Further emphasizing the burden of TB in this community, 21.6% of those screened and

Table 3 Post-mortem TB diagnosis by diagnostic modality ($n = 85$)

	n (%)
TB diagnosed on at least one laboratory test	27 (31.8)
TB diagnosed on two or more laboratory tests	18 (21.2)
Biopsy specimens with TB ($n = 20$)*	
Histology suggestive of TB	14
AFB on auramine-stained tissue smears	7
Xpert-positive	13
MGIT positive for *M. tuberculosis*	17
BAL specimens with TB ($n = 22$)	
AFB on auramine-stained smears of fluid	9
Xpert positive for *M. tuberculosis*	20
MGIT positive for *M. tuberculosis*	17

TB = tuberculosis; AFB = acid-fast bacilli; Xpert = Xpert® MTB/RIF; MGIT = Mycobacteria Growth Indicator Tube; BAL = bronchoalveolar lavage.

Table 4 Diagnostic concordance between post mortem Xpert® MTB/RIF and MGIT™ culture in A) tissue samples and B) BAL fluid

	Tissue Xpert		
A)	+	−	Total
Tissue MGIT			
+	13	4	17
−	0	68	68
Total	13	72	85
B)		BAL Xpert	
BAL MGIT			
+	15	2	17
−	5	63	68
Total	20	65	85

MGIT = Mycobacteria Growth Indicator Tube; BAL = bronchoalveolar lavage; + = positive; − = negative.

not included were either receiving anti-tuberculosis treatment or had recently been treated for TB. Although the CIs on our point estimates are wide, our data have clear implications for the measurement of targets included in the World Health Organization Post-2015 Global TB Strategy which sets a 95% reduction in TB deaths by 2035 compared to 2015.[18] Our data suggest that untreated, infectious, pulmonary TB is present in a high proportion of people dying at home without an apparent CoD. This supposition is supported by several autopsy studies demonstrating the enormous burden of TB in adult hospital deaths in high HIV burden settings,[16,19–23] a large proportion of whom were not diagnosed ante-mortem.

Our methodology was acceptable to a high proportion of the next of kin, as the majority consented to a limited autopsy compared to a previous study in Soweto, where few next of kin consented to a full autopsy.[24]

Forensic post-mortem studies from South Africa and India found TB in respectively 11% and 5.1% of decedents.[25,26] Our study is not directly comparable to these studies or to verbal autopsy (VA) studies, which provide crude estimates of cause of disease and CoD, but are often the only source of community mortality data in sub-Saharan Africa.[27,28] In Ethiopia, a VA tool reported pulmonary TB in 36% of all deaths; however, it did not report the proportions who were already on anti-tuberculosis treatment.[29] In the pre-antiretroviral therapy era, of 409 deaths assessed by VA in Limpopo Province, few (3.2%) were considered to be due to undiagnosed TB,[30] and a recent study from Zambia attributed 5% of all deaths to TB.[6] We propose that autopsy studies such as ours be repeated either as full post mortems or with sampling of additional organs to determine TB prevalence in home deaths more accurately.

Culture and Xpert proved diagnostically superior to histology and smear microscopy. The poorer performance of histology may be explained in part by the possible sampling of non-diagnostic lungs, which nonetheless harboured bacilli detectable by alternative diagnostics. The first of the two inadequate histology specimens occurred early in the study, and may be related to nurse inexperience. Neither showed evidence of TB by another diagnostic modality and neither contributed to the histology 'misses'.

We would like to underline the fatal consequences of delayed TB diagnosis and treatment. Barriers and delays in seeking care for TB have been reported in many settings,[31–33] and it is worrying that a high proportion of adults with TB apparently did not access health care prior to death, although more than half of those with TB experienced symptoms for at least 2 months. This group of undiagnosed, likely infectious but untreated adults should be included in strategies to prevent both TB transmission and mortality.

Like most autopsy studies, ours has limitations. We recruited a small sample at a single study site and relied on six private mortuaries to identify potentially eligible decedents. Next-of-kin interviews shortly after the death of a loved one may have been influenced by both recall and social desirability bias as the deceased may not have disclosed HIV or TB disease status, resulting in an overestimation of undiagnosed TB. We did not confirm HIV serostatus postmortem, as many next of kin refused consent for HIV testing. In addition, the research laboratory was unwilling to perform an invalidated test on post-mortem samples. Post-mortem lung biopsies miss focal pulmonary lesions due to the limited sampling,[19] thereby resulting in an underestimation of TB. While there are data validating Xpert test characteristics in tissue,[34,35] there are none to validate its use in post-mortem specimens. Anecdotally, it appears that at least some of the deceased had contact with emergency rooms but we did not explore this. Finally, the stated CoD on the DNFs were not collated or analysed.

Undiagnosed infectious TB disease in adults dying at home without an apparent CoD are not TB transmission 'dead-ends', particularly as MGIT culture of BAL fluid identified the presence of viable bacilli in a high proportion, and many decedents needed close care, including bathing and feeding, in their final days. Studies to confirm our findings, to understand health-seeking behaviour and failure to obtain help for serious symptoms and to better understand these reservoirs of undiagnosed TB, are therefore urgently needed.

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Conflicts of interest: PHRU was contracted by Becton Dickinson (Sparks, MD, USA) to conduct clinical research. No other conflicts of interest are declared.

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Annexure I – Turnitin Similarity Report

