A Description Of The Profile Of The Patients And Outcomes Of Fiber-optic Bronchoscopies, Performed At A Tertiary Care Hospital In KwaZulu Natal, South Africa, From January To December 2011

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DEDICATION

“Everything is created twice, first in the mind and then in reality” – Robin S Sharma

This work is dedicated to those who believed in my dreams as if it were their own; my mother, father and sister.

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ABSTRACT

Background

Tuberculosis (TB), pneumonia and human immunodeficiency virus (HIV) were the three leading causes of natural deaths in South Africa in 2013 and 11.9% of all deaths in KwaZulu Natal were attributed to TB. In 2013, there was an estimated 5.26 million people infected with HIV in South Africa. HIV infected individuals have an increased risk of respiratory tract infections including smear negative TB. Lung cancer is the most common type of cancer in the world. However, due to infrequent updates of the cancer registry in South Africa, current prevalence is unknown. Bronchoscopy is a useful tool for the diagnosis of broncho-respiratory pathology.

Aims and Objectives

This study describes the patient profile and outcomes of bronchoscopy in a tertiary centre in KwaZulu Natal in 2011. Specific objectives were to describe bronchoscopy indications, microbiological, cellular and histological findings and prevalence of TB amongst smear negative patients by broncho-alveolar lavage (BAL). In addition, the common types of lung cancer diagnosed on biopsy during bronchoscopy were to be described.

Methods

A retrospective review of consecutive bronchoscopies performed by the pulmonologist at a tertiary hospital in western KwaZulu Natal, between 1 January and 31 December 2011 was performed. A total of 107 patients met the inclusion criteria. Data was collected from clinical records, laboratory and radiology computerised record systems and entered on an Excel workbook using Microsoft Office 2010® software. Data was analysed using Epi-Info Version 3.5.4® and Stata/IC 13.0®. The demographic, bronchoscopy and chest CT scan findings were summarised with descriptive summary measures and expressed as means ± standard deviation (SD) and/or medians with the range and interquartile range for quantitative variables. Percentages, frequencies and proportions were used to describe categorical variables.

Results

The median age of patients was 55 ± 14.4 (Interquartile range (IQR) 43 - 63) years and 68 (63.6%) patients were male. Twenty-eight (26.2%) patients were HIV infected with a median cluster of differentiation 4 count of 254 ±164 (IQR 126 – 366.5) cells per cubic millimetre. Nine patients were on antiretroviral therapy.

The commonest indications for bronchoscopy were investigation of a lung mass (35.8%), non-resolving lower respiratory tract infection (15%) and suspected TB (15%). Microbiological findings on BAL samples included gram positive and negative bacteria (14%) and fungi (20%). TB microscopy, polymerase chain reaction and culture revealed *mycobacterium tuberculosis* on 22.2% of all BAL samples. Two patients with *mycobacterium tuberculosis* on BAL samples were

HIV infected. The prevalence of TB on smear negative patients was 11.1%. Cytological analysis of BAL samples detected pathology on eight (13.1%) patients and two (3.3%) of these patients had lung cancer. Malignant (52.9%) (squamous cell carcinoma and adenocarcinoma) and benign (11.1%) (pneumonia and interstitial fibrosis) pathology was found on histology. Squamous cell carcinoma (37%) was the commonest lung cancer detected.

Bronchoscopy was helpful in determining broncho-respiratory pathology in 38 (35.5%) patients. The commonest diagnosis was lower respiratory tract infection in 7 of 15 (46.7%) patients referred with diffuse pulmonary infiltrates. Bronchoscopy also assisted with the diagnosis of lung cancer in 20 of 43 (46.5%) patients referred with suspected lung mass. Overall the procedure complication rate was 3.7%.

Conclusion

Bronchoscopy may be a useful tool in diagnosing and decreasing the morbidity associated with respiratory illness in South Africa as the diagnostic yield was greatest for lower respiratory tract infections. Samples collected during BAL had a relatively low diagnostic yield for TB. Prompt referral of smear negative TB suspects is recommended to assist with the microbiological diagnosis of TB and direct therapy thereof.

Cytological examination of BAL samples was associated with a low yield of lung cancer and biopsy samples were more useful for this purpose. SCC was the

commonest histological subtype of lung cancer in this cohort. Bronchoscopy was a relatively safe procedure in determining the aetiology of broncho-respiratory pathology.

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CHAPTER 1

INTRODUCTION

In 2012, lower respiratory tract infection (LRTI) was the leading cause of death in low income countries, accounting for 91 deaths per 100 000 population.(1) In South Africa, tuberculosis (TB) and pneumonia were the leading causes of natural deaths in 2013, with 11.9% of all deaths in KwaZulu Natal attributed to TB.(2) Although TB and pneumonia are potentially curable, the diagnosis remains a challenge.(3) Time delays in obtaining and analysing sputa amongst smear negative and resistant TB cases may contribute to this high mortality rate.(4)

Bronchoscopy is a useful tool in achieving microbiological diagnoses in LRTI.(5, 6) In particular, broncho-alveolar samples collected during bronchoscopy have a higher sensitivity in detecting acid fast bacilli (AFB) in smear negative pulmonary TB than sputa samples.(7) Although the use of bronchoscopy in the diagnosis of smear negative TB has been well described, detection rates have not been documented in high burden human immunodeficiency virus (HIV) and TB syndemic areas, like KwaZulu Natal.(8, 9) The current use of broncho-alveolar lavage (BAL) in a high TB/HIV prevalence area is explored in this study.

Mortality due to chronic diseases has been increasing globally over the past decade.(1) One such chronic disease is lung cancer.(1) Lung cancer is the most

common cancer diagnosed worldwide,(10) with a projected increase in disease burden by 2030.(11) The true burden of disease in South Africa is largely unknown due to lack of updates of the national cancer registry.(12)

The risk of developing lung cancer is higher amongst HIV infected individuals than the general population.(13) Considering the burden of HIV infection in South Africa, determining the prevalence of lung cancer is a vital initial step in establishing links between the two chronic diseases. Despite advances in radiology, biopsy of a suspicious lung mass remains the only method for diagnosis of lung cancer.(14) Bronchoscopy provides a means to obtain bronchial tissue samples and therefore is an essential tool in the diagnosis of lung cancer.

The two most common histological subtypes of lung cancer are squamous cell carcinoma (SCC) and adenocarcinoma.(15) Adenocarcinoma is the most common lung cancer in western countries(15) whilst in the east, histological subtypes differ in different regions.(16, 17) This may be due to different ethnicities, cultures and social habits in different regions in the world. In South Africa, individual studies from Johannesburg and Bloemfontein report SCC as the most common histological subtype of lung cancer.(18, 19) In Cape Town, however, lung adenocarcinoma is more common than SCC.(20) Regional bronchoscopy studies are therefore important to increase knowledge of lung cancer trends in the different settings in South Africa. This study describes the most common histological subtypes diagnosed during bronchoscopy at this institution.

Due to the lack of information with respect to the outcomes of bronchoscopy in KwaZulu Natal; a review of bronchoscopy outcomes between 1 January and 31 December 2011, at a tertiary hospital in Pietermaritzburg was undertaken. This hospital serves 4.5 million individuals in western KwaZulu Natal. The objectives of this study were to describe bronchoscopy indications, microbiological, cellular and histological findings and prevalence of TB amongst smear negative patients by BAL. In addition, the common types of lung cancer diagnosed on biopsy during bronchoscopy were to be described.

CHAPTER 2

LITERATURE REVIEW

Tuberculosis (TB) and pneumonia were the two leading causes of natural deaths in 2010 in South Africa.(2) In KwaZulu Natal, TB accounted for 12% of all deaths.(2) The diagnosis of TB was particularly challenging prior to the introduction of the GeneXpert® rapid mycobacterium tuberculosis/ rifampicin (MTB/RIF) assay in South Africa. Prior to this, there was a significant delay in time to diagnosis of TB especially smear negative and drug resistant TB.(4) These delays may have directly contributed to mortality as well as community and nosocomial transmission of TB.(4) Furthermore, obtaining sputum may be a challenge in patients who are unable to expectorate sputum.(21) The use of chest radiographs may be associated with under-diagnosis of TB in these patients.(21)

There is a higher sensitivity of acid fast bacilli on BAL samples than sputum samples. Broncho-alveolar lavage (BAL) samples analysed for acid fast bacilli (AFB) has proven to have a higher sensitivity than sputum samples for *mycobacterium tuberculosis* amongst smear negative patients.(7) Due to the high rates of TB in KwaZulu Natal, a review of bronchoscopies was performed to determine the outcomes of bronchoscopy in this high respiratory tract infection burden setting.

2.1. Introduction to Bronchoscopy

Bronchoscopy is an essential tool that is widely used for the diagnosis and treatment of broncho-pulmonary diseases.(5, 6) The rigid bronchoscope was first introduced by Dr Gustav Killian in 1897.(6) This invention was succeeded by the first prototype flexible fiber-optic bronchoscope, developed by Machida and presented by Shigeto Ikeda in 1966.(6) This was the beginning of the revolution of the world of bronchoscopy.(6) Today both rigid and flexible bronchoscopes are used depending on the indication for the bronchoscopy.(5)

There are two types of bronchoscopes; namely rigid and flexible.(5) A flexible bronchoscope comprises of three bundles of optical fibers, two of which carry light to the distal end and one which projects the image from the distal end to the eyepiece.(5) There is an instrument or suction channel at the proximal end which allows secretions to be suctioned and samples to be obtained.(5) The distal end can be manoeuvred within the airway for better visualisation by means of a lever at the proximal end of the bronchoscope.(5) Flexible bronchoscopy is usually performed under local anaesthetic.(5)

Rigid bronchoscopes are available in different sizes and have a halogen light source.(22) Telescopes of 0^0, 30^0 and 90^0 can be placed down the rigid barrel; to improve visualisation of the tracheo-bronchial tree.(22) Instruments to assist with biopsy and suction devices may also be placed down the barrel.(22) Rigid bronchoscopy is usually performed under general anaesthesia.(5, 22)

2.2. The Bronchoscopy Procedure

Prior to bronchoscopy, patient informed consent must be obtained and the full blood count and clotting profile must be checked to ensure that there will be no risk of massive haemorrhage during biopsy.(23) Intravenous access is mandatory in all patients.(23) An electrocardiograph is only required if the patient has a history of cardiac disease.(23) Bronchodilators may be used if the patient has bronchoconstriction.(5, 23, 24) Ideally patients should be "*nil per os*" for a minimum of four hours before bronchoscopy. (5, 23, 24)

Bronchoscopy is usually performed with the patient in the 45^0 recumbent position.(5, 22) Lignocaine is the local anaesthetic agent of choice to ensure patient comfort and should be limited to eight milligrams per kilogram (mg/kg) in the adult patient.(5, 23) The route of administration of lignocaine is a matter of preference.(5) In a survey amongst respiratory physicians in the United Kingdom lignocaine spray was preferred over trans-tracheal lignocaine injection and nebulised lignocaine, as a local anaesthetic agent.(25) Supplemental oxygen may be used to achieve an oxygen saturation of at least 90% prior to the commencement of bronchoscopy.(5)

The bronchoscope is introduced via the nose or mouth, into the glottis and tracheo-bronchial tree.(5, 22, 24) The bronchial anatomy down to the sub-segmental level is inspected for the presence of endo-bronchial lesions and mucosal abnormalities.(5, 22, 24) Lavage and tissue samples may also be obtained during bronchoscopy.(5, 22, 24) Sampling techniques include bronchial

washings, BAL, bronchial brushings, endo-bronchial biopsy, trans-bronchial lung biopsy, trans-bronchial fine needle aspiration and endo-bronchial ultrasound assisted trans-bronchial needle aspiration.(5, 22)

2.3. Sampling Techniques

2.3.1. Bronchial Washings

This is a lavage of the bronchus that requires instillation of between five to 30 millilitres (ml) of saline into the bronchus to clear secretions or mucus in that area.(24, 26) This fluid is then aspirated and used for cytological and microbiological analysis.(24) The sample may contain normal respiratory flora.(24) This process is commonly used with other sampling techniques.(24)

2.3.2. Broncho-alveolar Lavage

Like bronchial washings, during BAL, saline is instilled into the bronchial tree.(24) It is lavaged into two to three different regions to reach a higher representation of the entire lung.(24, 27) The bronchoscope is wedged at the level of the third to fourth bronchi, and between 20 ml and 180 ml of saline is instilled.(23, 24, 27) This is gently aspirated to prevent bronchial collapse.(23, 24) BAL is helpful in the diagnosis and aetiology of many conditions including; interstitial lung disease, pneumocystis jirovecii pneumonia (PCP) and pulmonary fibrosis.(24) BAL samples of immune-compromised patients, should routinely be sent for AFB and TB culture.(23)

2.3.3. Bronchial Brushings

When a lesion is identified at bronchoscopy a brush is passed through the instrument channel and advanced three centimetres (cm) out of the distal end.(24) The brush is then moved back and forth and brushed over the lesion.(23, 24) Thereafter, it is retracted into its sheath and pulled back through the channel.(23, 24) This sample is used for either cytological or microbiological analysis.(24) There are several factors that may influence the diagnostic yield of this technique.(24) These include repeated brushings as well as using brushes with different diameters and types of bristles for different lesions.(24) Bronchial brushing increases the diagnostic yield of lung malignancies in particular.(23)

2.3.4. Endo-bronchial Biopsy

At bronchoscopy when a lesion is identified, a biopsy is performed under direct vision.(24) A biopsy forceps is inserted through the operating channel of the bronchoscope in a closed position.(23, 24) After the forceps has reached the distal end of the bronchoscope, it is opened, anchored onto the lesion and a biopsy is taken.(23, 24) The forceps is removed and the tissue obtained is sent for histological analysis.(23) This process may be repeated up to five times.(23, 24)

2.3.5. Trans-bronchial Lung Biopsy

After complete endo-bronchial inspection of all lung segments, the bronchoscope is advanced through to the narrowest airway, until it cannot be advanced any further.(24) A biopsy forceps is inserted into the channel and advanced past the

distal end of the bronchoscope to the lesion at the periphery of the lung.(24) Endo-bronchial ultrasound or a fluoroscopy unit may be activated to allow visualisation of the forceps as it enters the distal segments of the lung.(24) The forceps is retracted one centimetre to avoid a biopsy of the pleura whilst the patient inspires.(23) When the patient expires, the forceps is opened, advanced and closed.(23) The biopsy is performed and the sample is sent for histological analysis.(24) This biopsied area is observed for post-biopsy bleeding using the bronchoscope.(24) This procedure may be repeated up to six times to increase the diagnostic yield.(23, 24)

2.3.6. Trans-bronchial Needle Aspiration

This sampling technique is used when a lesion in the mediastinum lies adjacent to the tracheo-bronchial tree, in the airway wall or lung parenchyma.(24) The bronchoscope is used to locate the lesion and a trans-bronchial needle at the end of a catheter, is passed through the bronchoscope to the distal end.(22) The catheter and needle tip are moved back and forth to shear cells from the lesion.(22) The catheter is then removed and smears of cells may be prepared for cytological evaluation.(22) There are different techniques that may be employed to ensure an adequate sample is obtained.(22)

2.3.7. Endo-bronchial Ultrasound Assisted Trans-bronchial Needle Aspiration

The addition of endo-bronchial ultrasound to conventional trans-bronchial needle aspiration has assisted with minimising complications.(24) This technique is used in the evaluation of mediastinal lymphadenopathy, intrapulmonary tumours and lung cancer staging.(24) The endo-bronchial ultrasound assists by identifying lesions within the airway and enlarged lymph nodes.(24) The bronchoscope is then placed in the area of pathology with ultrasound guidance, and the trans-bronchial needle aspiration is performed in the conventional manner.(24) This is a relatively recent tool in aiding diagnostic bronchoscopy and is currently unavailable at Greys Hospital but is available at Inkosi Albert Luthuli Central Hospital.

2.4. Complications of Bronchoscopy

Bronchoscopy is a relatively safe procedure, however, the risk to benefit ratio should be individualised for each patient.(24) Complications described in a university hospital in Columbus, where 4 273 consecutive flexible fiberoptic bronchoscopies were reviewed, included respiratory failure (0.2%), vomiting (0.1%), pneumothorax (0.16%), laryngospasm (0.6%), pulmonary haemorrhage (0.12%) and bronchospasm (0.02%).(28) A larger, multicentre study conducted in 19 centres in Italy, reviewed complications in 20 980 bronchoscopies done over a year.(29) The overall complication rate was 1.08% with a mortality rate of 0.02%.(29) The most common complications included haemoptysis of greater than 50 ml (0.26%), haemoptysis under 50 ml (0.19%), hypoxia (0.12%) and pneumothorax (0.1%).(29) Complications were mainly associated with trans-bronchial needle aspiration.(29)

2.5. Indications for Bronchoscopy

The indications for flexible and rigid bronchoscopies differ. Rigid bronchoscopy is performed mainly for therapeutic procedures such as the removal of foreign bodies, tumour ablation, insertion of stents and staging of tumours prior to surgical resection by thoracic surgeons.(5)

Flexible bronchoscopies are used for investigation and diagnosis of respiratory pathology such as haemoptysis, persistent cough, recurrent lower respiratory tract infections (LRTI), suspected lung malignancy and interstitial lung disease.(5) There are regional differences regarding the most common indications for bronchoscopy.(16) In 1989, the American College of Chest Physicians (ACCP) conducted a survey in North America which found that suspected lung cancer was the most common indication in 96.4% of bronchoscopies.(15) This differs with other studies in the Middle East where 35.9% to 51.6% of bronchoscopies were requested for suspected respiratory infection.(16, 17, 30)

In a study conducted in Cape Town between 1976 and 1980 on 705 patients, suspected malignancy (41%) and TB (31%) were the most common indications for bronchoscopy, however, this study was conducted in a pre-human immunodeficiency virus (HIV) era.(31) HIV infected patients are more at risk of pneumonia and smear negative TB.(3) It is uncertain if this risk of lower respiratory tract infection amongst the HIV infected population has altered the indications for bronchoscopy in South Africa.

2.6. Tuberculosis

2.6.1. The Burden of Tuberculosis

Tuberculosis is an airborne infection caused by *mycobacterium tuberculosis*.(32) The characteristic symptoms of the disease are that of persistent cough or fever of two weeks or more, drenching night sweats and unexplained loss of weight.(21, 32) Other symptoms include chest pain, breathlessness and wheezes.(21) An individual is regarded as a TB “suspect” if a cough is present for two or more weeks.(21) The absence of a cough, however, does not exclude TB and a high index of suspicion must be maintained especially in HIV infected individuals.(21)

According to the 2012 Global TB report, there were an estimated 8.7 million incident cases of TB in the world with 0.4 to 0.6 million cases in South Africa.(33) South Africa has the highest incidence (1 180 per 100 000 population) and prevalence (1 250 per 100 000 population) of TB in the world.(33) In addition, South Africa also has the leading HIV prevalence amongst patients with TB, in the world.(33)

Globally, there were 1.4 million reported deaths from TB in 2011 (990 000 HIV uninfected and 430 000 HIV infected individuals).(33) Over 95% of TB deaths occurred in low-middle income countries.(33) In South Africa the leading cause of natural deaths from 2011 to 2013 was TB (10.7% in 2011, 9.9% in 2012 and 8.8% in 2013).(2)

The burden of TB in South Africa is exacerbated by its drug resistant strains.(34) Multi-drug resistant TB (MDR TB) is defined as *mycobacterium tuberculosis* that is resistant, *in vitro*, to both rifampicin and isoniazid.(21, 35) Extreme drug resistant TB (XDR TB) is *mycobacterium tuberculosis* that is resistant, *in vitro*, to rifampicin, isoniazid, any one of the fluoro-quinolones and any one of the second line injectable drugs (capreomycin, kanamycin or amikacin).(21, 35) Between 2004 and 2010, there were 45 196 laboratory confirmed cases of MDR TB and 3 128 laboratory confirmed cases of XDR TB in South Africa.(34) KwaZulu Natal had the highest number of both MDR TB (11 393 confirmed cases) and XDR TB (1 499 confirmed cases) diagnoses.(34)

2.6.2. Diagnosis of Pulmonary Tuberculosis

The screening tools for the diagnosis of TB in South Africa has recently been revised. The South African 2009 TB guidelines suggested that TB diagnosis be made on the collection of two sputum specimens on consecutive days for TB smear microscopy.(36) The first sample needed to be a spot sputum sample, whilst the second sample needed to be an early morning specimen.(36) If the patient was HIV infected then a third sample would be required for TB smear and culture.(36) The aim for sputum turnaround time was 48 hours.(36) This guideline has been revised due to the advent of GeneXpert ®.

Current TB guidelines in South Africa recommend that TB is diagnosed by GeneXpert® performed on one sputum sample.(21) If this is positive and the TB is sensitive to rifampicin, TB treatment is commenced, microscopy is performed and

the patient is treated according to the results.(21) If the GeneXpert® is positive and the TB is resistant to rifampicin then treatment for MDR TB is commenced, culture and drug susceptibility testing are performed and the patient is further managed according to the results.(21) If GeneXpert® is negative and the patient is HIV infected then culture and drug susceptibility testing are performed and the patient is further investigated for TB with a chest radiograph whilst commenced on empiric antibiotics.(21) If the GeneXpert® is negative and the patient is HIV uninfected then empiric antibiotics are commenced.(21) (Appendix 2)

a. GeneXpert® and pulmonary Tuberculosis

The GeneXpert® assay uses deoxyribonucleic acid (DNA) sequences amplified in a real time polymerase chain reaction (PCR) assay to detect *mycobacterium tuberculosis* and determine sensitivity of the strain to rifampicin.(37) In 95% of rifampicin resistance strains, there is a mutation located within the 81bp core region of the ribonucleic acid polymerase beta subunit gene.(37) The core region is flanked by *mycobacterium tuberculosis* complex specific DNA sequences.(37) This allows the simultaneous diagnosis of TB and rifampicin resistance by targeting one amplicon generated with PCR.(37) The assay is able to detect 131 colony forming units (cfu) per ml of sputum, which is much higher than that of microscopy and culture.(37) The test can be performed in two hours which has improved time to detection of TB infection and rifampicin resistance.(37) GeneXpert® can be performed on gastric lavage samples for the diagnosis of TB, but there is no clear guideline that suggests BAL samples may be used. (Appendix 1)

In a multicentre trial, in Durban and Cape Town, the sensitivity of GeneXpert® for pulmonary TB, amongst HIV infected individuals with culture positive TB was 93.9% as compared with 98.4% in HIV uninfected individuals with culture positive TB (P = 0.02).(4) In the same study, the sensitivity and specificity of GeneXpert® on sputum smear negative samples was 86% and 90% respectively(4). When the sensitivities and specificities for rifampicin resistance in Cape Town were assessed it was 93% (15 of 16 patients) and 100% (126 of 126 patients) respectively, whereas in Durban both sensitivity (3 of 3 patients) and specificity (38 of 38 patients) was 100%.(4) A subsequent meta-analysis of 15 studies using GeneXpert® on sputa for TB detection found a pooled sensitivity of 90.4% and pooled specificity of 98.4%.(37) The pooled sensitivities of smear negative and smear positive disease in the same cohorts were 75.0% and 98.7% respectively.(37)

b. Sputum for acid fast bacilli (smear positive Tuberculosis)

Acid fast bacilli examination on sputa is widely performed in South Africa in regions without access to GeneXpert®, in all cases where GeneXpert® is positive and for monitoring of response to TB treatment.(21) TB “suspects” are required to produce two sputa samples, the first is a “spot” or immediate sample and the second is an early morning sample.(21) These are analysed to detect AFB using either Ziehl Neelsen (Carbol Fuchsin) staining or fluorescent auramine staining.(21) For the detection of pulmonary TB, these stains may be performed on sputa or BAL samples.(21) The sensitivity and specificity for AFB on sputa in HIV

infected individuals is 53.3% (95%CI 38-68%) and 89.5% (95%CI 79.7-95%) respectively.(38)

Acid fast bacilli detection on induced sputum may be considered in patients unable to produce sputum.(39) In a prospective study in Bangladesh, conducted on 52 smear negative patients, suspected of having TB, the sensitivity of AFB-smears in samples from induced sputum and BAL were 74% and 58% respectively.(39) Fishers Exact test performed on induced sputum and BAL groups showed they were in agreement in 90% cases (p=0.0001).(39)

Another study conducted in Brasil between 1996 and 1998, demonstrated no significant differences in the yields of AFB smears or cultures whether obtained via sputum induction or BAL.(40) In the study, among 207 HIV-seronegative patients, the AFB smear and mycobacterial culture results from specimens obtained by sputum induction and BAL were in agreement in 97% (202 of 207) (kappa test = 0.92) and 90% (186 of 207) (kappa test =0.78), respectively.(40)

c. Tuberculosis culture

A more sensitive test to detect TB is culture of *mycobacterium tuberculosis*.(21) However; this method may take between four to six weeks to provide a result and is prone to contamination.(21) Samples that may be sent for TB culture include sputa and BAL samples.(21) Cultures should routinely be requested for all TB “suspects” who have a positive GeneXpert® and rifampicin resistance, a negative

GeneXpert® and HIV infection, two negative sputa smears for AFB and HIV infection, TB “suspects” who were previously treated for TB and those with MDR TB or XDR TB contacts.(21)

A liquid culture medium is usually used in conjunction with a solid medium as a “backup” system.(21, 41, 42) The solid medium uses coagulated egg (e.g. Lowenstein-Jensen) or agar (Middlebrook 7H10) as a base.(21, 42) For the liquid medium technique, a semi-automated radiometric system with radiation technology (e.g. BACTEC 460) may be used.(21) Alternatively, an automated non radiometric system, using fluorometric technology (e.g. mycobacteria growth indicator tube) may be used.(21)

d. Drug susceptibility testing for pulmonary Tuberculosis

This test determines if the *mycobacterium tuberculosis* organism is sensitive or resistant to individual drugs namely; rifampicin, isoniazid, pyrazinamide, ethambutol, moxifloxacin, ofloxacin, streptomycin, kanamycin, capreomycin, amikacin and ethionamide.(21) The test is indicated in patients who have taken TB treatment previously and high risk TB “suspects” with MDR TB and XDR TB contacts.(21) This test may be done by direct or indirect means.(21) Direct tests are performed on samples that are rich in bacilli, whilst for indirect approaches, cultures have to be grown and tested in the exponential phase of growth.(21) The process is time consuming and therefore results may take between four to six weeks.(21)

e. Chest radiographs and Tuberculosis

Although radiographs are the quickest and easiest way to determine and confirm the presence of lung pathology, they cannot make a microbiological diagnosis of TB.(21) Chest radiographs often lead to under or over-diagnosis of TB.(21) They are indicated in patients who cannot produce sputum and HIV infected patients with a negative GeneXpert®.(21) Chest radiograph findings that may be compatible with TB include cavities, ill-defined infiltrates, multiple nodular infiltrates and infiltrates with mediastinal enlargement.(43)

2.6.3. The Challenge of Tuberculosis Diagnosis in HIV Infected Individuals

The rates of TB detection are much lower in HIV infected individuals.(37) A meta-analysis of seven studies compared the sensitivity of sputum microscopy and GeneXpert®, with culture as the gold standard, in HIV infected patients.(37) The median sensitivity of smear microscopy was 52.8%, whilst that of GeneXpert® was 84.0%.(37) A median increment of 30% was noted using GeneXpert® in all seven studies.(37) The overall sensitivity of GeneXpert® was between 58.3% and 91.7%.(37) When the sensitivity of sputum microscopy and GeneXpert® were compared at an antiretroviral clinic in South Africa, the overall sensitivity of the GeneXpert® for culture positive TB was 73.3% (specificity 99.2%) compared to 28% (specificity 100%) using smear microscopy.(44) Similarly in an HIV infected cohort in Tanzania, the specificity of GeneXpert® for culture positive TB was 98% (95%CI=89.4-100%).(45)

Other studies from developing countries, Cape Town, South Africa; Kampala, Uganda; Jharkhand, India and suggest that in HIV infected patients with smear negative TB, analysis of BAL samples offer better detection rates.(7, 8, 23, 46) In a cohort of HIV infected patients with smear negative TB and sputa culture as the gold standard test, BAL microscopy had a sensitivity of between 10% and 30% whilst BAL culture had a sensitivity of between 52% and 95%.(23) When PCR was performed on these BAL samples, the resultant sensitivity was 85.7% and specificity was 90.9%.(23) Various other studies have also indicated that the use of bronchoscopy with BAL reduces the time to diagnosis and increases the sensitivity of pulmonary TB diagnosis in HIV infected smear negative patients.(8, 9)

A study conducted on 496 patients, between 2007 and 2010 in two primary care clinics in Cape Town, South Africa demonstrated that amongst smear negative patients, GeneXpert® was able to detect 58% more TB on BAL samples compared to microscopy smears.(47) GeneXpert® on BAL samples proved to be a rapid diagnostic tool and reduced rates of empirical TB treatment.(47) The use of bronchoscopy and GeneXpert® as a diagnostic tool for TB may therefore improve the case detection rates in South Africa.(47)

2.7. Lung Cancer

2.7.1. Introduction and Burden of Disease

Lung cancer is the most common type of cancer in the world.(48) In 2012, there were an estimated 1.8 million new cases diagnosed globally.(48) At least 58% of these new diagnoses occurred in less developed countries.(48) Globally the highest incidence of lung cancer for men occurred in central Europe, eastern Europe and eastern Asia, whilst for women it occurred in North America, northern Europe and eastern Asia.(48)

In 2000, lung cancer was the leading cause of fatal cancers in South Africa with the highest rates amongst males and females in the Western Cape.(49) The South African national cancer registry was last updated in 2006.(12) At the time, lung cancer was the fifth most common cancer diagnosed in males with an age standardised incidence rate of 8.75 per 100 000 world standard population.(12) Lung cancer amongst females was 3.52 per 100 000 age standardised incidence rate per world standard population.(12) The cancer registry has not been updated since 2006 and lung cancer trends in South Africa have not been evaluated in the past eight years.(12) As a result, all cancers now need to be notified on a cancer registration form and submitted to the National Cancer Register.

A study done in 24 villages in Mpumalanga/Bushbuckridge Local Municipality, South Africa, in 2011, found that cancer was amongst the top five causes of death in patients over 15 years (14 of 286 patients in the age group 15-49 years, 7 of 108 patients in the age group 50-64 years and 21 of 162 patients in the age group >= 65).(50) The type of cancer, however, was not indicated in this study.(50)

A study investigating cancer projections to 2030, indicates that lung cancer is likely to remain a global epidemic.(11) The study extrapolated data from the global cancer report and analysed the human development index, life expectancy, education, gross domestic product per year and global cancer trends from 1988 to 2002.(11) In very high human development index areas (North America, Australia, New Zealand, Britain and western Europe) and high human development index areas (South America, western Europe, northern Europe and southern Europe), female breast, lung, colorectal and prostate cancer will account for nearly half of the overall cancer burden (1 031 million persons).(11) In medium human development index areas (Asia, South Africa and other African countries) lung and female breast cancers will be the most common cancers (773 000 persons and 500 000 persons respectively).(11, 51)

Lung cancer is divided into 2 histological subtypes namely; non-small cell carcinoma (adenocarcinoma, squamous cell carcinoma (SCC) and large cell carcinoma) and small cell carcinoma (oat cell).(52, 53) Peripheral lung cancers tend to be adenocarcinoma, whilst central cancers are more likely to be SCC(54).

2.7.2. Risk Factors for Lung Cancer

a. Modifiable risk factors

i. Tobacco smoking and lung cancer

Tobacco smoking is a well-recognised risk factor for lung cancer.(55) Tobacco contains at least 20 substances that are carcinogenic.(56) In addition to the

carcinogens in tobacco, lung cancer risk depends on the duration of smoking, frequency of smoking, age of smoking initiation and the type of tobacco used.(56)

An autopsy study, conducted from 1998 to 2004 in 5 340 South Africans adults, directly linked 58% of lung cancer deaths to smoking.(55) Another study describing smoking related mortality in South Africans aged 35 to 74 years, found that smoking prevalence was highest in the Coloured population.(57) Lung cancer mortality, however, was highest in African, male smokers and White, female smokers.(57) The reasons for this were unclear.(57)

In an attempt to regulate tobacco product use in South Africa, the Tobacco Products Control Act (amended as the Tobacco Products Control Amendment Act, Act Number 63 of 2008) was introduced.(58) Under this act, regulations were introduced regarding the production, marketing, advertising, selling and smoking of tobacco in public places.(58) It is unclear how these laws may have influenced trends in lung cancer in South Africa.

There are many unanswered questions about the recently popular electronic cigarettes.(59) The impact of electronic cigarettes on lung cancer trends are undetermined; however, they are still classified as tobacco products.(59) The risk of nicotine vapour and other harmful substances in these electronic cigarettes is still to be evaluated.(59)

ii. Occupational carcinogens and lung cancer

Occupational exposure to carcinogens is a major contributing factor to the development of lung cancer.(56) Common carcinogens include arsenic, asbestos, beryllium, cadmium, chromium, diesel, nickel and silica.(56, 60) This makes people working at gold mines, refineries, construction industries, shipbuilding and motor-vehicle industries, painters and with wood at risk.(56)

The prevalence of lung cancer for every occupation at risk is unavailable in South Africa; however, individual studies in mine workers have been performed. A South African study that focused on lung cancer in amphibole miners who used two types of asbestos rocks; amosite and crocidolite, found that crocidolite was more carcinogenic.(61) In the cohort of 7 317 patients, 1 225 (17%) patients died with 56 deaths attributable to lung cancer.(61)

The link between silica dust, silicosis and lung cancer is well described, however, it is unclear whether silica dust exposure in the absence of silicosis increases the risk of lung cancer.(62, 63) Confounding factors like smoking make it difficult to assess the true exposure-response relationship between lung cancer and silica.(62, 63)

iii. Environmental pollution and lung cancer

Environmental pollution, in the form of motor vehicle exhaust fumes, heating systems, wood fired ovens, cooking with seed oils, industrial emissions and radon gas, are risk factors for lung cancer.(56) An estimated 20% of South Africans use coal or biomass fuels for cooking or heating.(64) This exposure was estimated to have caused 2 489 deaths in South Africans in 2000.(64) It is unclear as to how many of these affected individuals developed lung cancer.(64)

Smoking may pose a synergistic effect with environmental pollution in the development of lung cancer.(56) Radon gas in particular has shown to account for between 10% and 20% of all lung cancers.(56) There have been further associations between the exposure to radon gas and mutations in p53 genes predisposing exposed individuals to lung cancer.(56)

b. Non modifiable risk factors

i. Gender and lung cancer

A review of lung cancer trends in South Africa between 1995 and 2006 demonstrates that the mortality rate in females increased at an annual rate of 0.19 per 100 000 persons (p= 0.043).(65) The reason for this finding was uncertain and in the absence of South African data post 2006, it is unknown if the mortality rate in South African females continues to increase.

Although, overall, the rates of lung cancer are higher in men than women, women are more susceptible to disease.(56, 66) The pathogenesis for this is incompletely understood but hormonal factors are postulated.(56, 66) Oestrogens may act as lung tumour promoters and have been associated with lung adenocarcinoma in particular.(66)

ii. Age and lung cancer

There are more incident cases of lung cancer reported after the age of 65 years.(56) This is in keeping with the time that it takes for the effect of smoking on the lung to be evident.(56) Patients who are younger than 50 years of age at the time of the diagnosis tend to have a better prognosis.(67)

iii. Ethnic differences and lung cancer

Ethnic differences have been observed with higher mortality rates in African Americans and New Zealand Māoris.(56) Chinese women also have a higher incidence of lung cancer, however, it is currently uncertain if this is due to a genetic predisposition or due to widespread use of wood fired ovens and cooking with seed oil.(56)

The last national cancer registry does not categorise lung cancer prevalence by race.(12) A South African study evaluating smoking related mortality in different races demonstrated that Coloured males had the highest relative risk of

developing lung cancer.(57) The Coloured male group was followed by a group of Other races, Whites and lastly Blacks.(57) The relative risk of lung cancer amongst female smokers was highest in the group of Other races followed by White, African and Coloured smokers.(57) Confounding factors such as; smoking in each ethnic group, may have contributed to the difference in results in males and females.(57)

iv. Lung disease and lung cancer

Lung disease such as; sarcoidosis, chronic obstructive pulmonary disease (COPD) and silicosis, may cause inflammation of the lung which predisposes to lung cancer.(56) There is a strong link between smoking and the development of COPD.(56) This poses a higher risk of lung cancer with 8.8% of cases leading to lung cancer within 10 years.(56)

There is conflicting evidence regarding an association between TB and lung cancer.(68) Experimental evidence suggests that *mycobacterium tuberculosis* is capable of DNA damage which has been associated with inflammation related carcinogenesis.(68) *Mycobacterium tuberculosis* may also enhance bcl-2 oncogene synthesis which increases anti-apoptotic activity.(68) It is hypothesised that the combination of DNA damage, apoptosis inhibition and chronic inflammation of TB may enhance carcinogenesis.(68)

A review of 37 case control and four cohort studies (published between January 1966 and January 2009) on MEDLINE, PubMed, EMBASE and China National Knowledge infrastructure databases show an increased lung cancer risk associated with TB.(69) This association was not related to the confounding effects of tobacco smoking or the timing of TB diagnosis.(69) The association was related to adenocarcinoma only (relative risk = 1.6; 95%CI 1.2-2.1).(69)

Another review of the PubMed database up to June 2008 found that the co-existence of TB and lung cancer was merely a co-incidence.(68) Since the studies used in this review were retrospective and prone to recall bias, the association between TB and lung cancer remains an area of potential research.(68)

The risk of developing lung cancer is higher in HIV infected individuals than the general population.(13) Lung adenocarcinoma is the most frequently diagnosed lung cancer in HIV infected individuals.(13) Confounding factors such as tobacco smoking and chronic immunosuppression limit the conclusions in terms of an association between lung cancer and HIV.(13) It is unclear if HIV has oncogenic potential.(13) *In vitro* studies suggest that HIV produces a trans-activator of transcription gene, which can increase the expression of proto-oncogenes and down regulate tumour suppressor gene p53, in lung adenocarcinoma lines.(13) This hypothesis is yet to be proven.(13) HIV infected individuals may also have an increased susceptibility to carcinogens present in tobacco smoke, however, the evidence for this is also conflicting.(13) There is no clear relationship between the degree of immunosuppression and lung cancer risk.(13)

In the pre-antiretroviral therapy (ART) era in the United States of America, lung cancer was the most commonly diagnosed non-acquired immunodeficiency syndrome defining malignancy.(13) A study from South Africa in 2000, reported no increase in prevalence of lung cancer amongst HIV infected patients.(70) More recent South African studies evaluating lung cancer prevalence in HIV infected patients are unavailable.

v. Family history and lung cancer

There is a definite association between the risk of lung cancer and a family history of lung cancer in a first degree relative.(71) This may be attributed to environmental or genetic factors.(71) Studies have observed a significant association between the presence of a family history of lung cancer and the histological subtype of the predisposed patients.(71) SCC incidence tends to be higher when there is a positive family history.(71)

vi. Molecular genetics and lung cancer

Chromosomal aberrations and fractional allele loss are higher in smokers than non-smokers.(72) Point aberrations occur in different areas in the two groups.(72) The 6q region of the chromosome has been identified as a susceptible locus for lung cancer in family linkage studies.(72) Several oncogenes have been associated with adenocarcinoma.(72) These oncogenes cause point mutations but are not specific for lung adenocarcinoma.(72)

Mutations cause an overexpression of epidermal growth factor receptor (EGFR) tyrosine kinase which accelerates tumour cell proliferation.(72) There is an inverse relationship between EGFR mutations and tobacco smoke exposure, such that these mutations are not detected in patients with lung cancer and a 75 pack year smoking history.(72) The Kirsten rat sarcoma (KRAS) viral oncogene homolog is another member of the EGRF group but is found mainly in patients who smoked and have adenocarcinoma.(72) KRAS mutations are usually glutamine-to-tyrosine transversion.(72) Unlike KRAS, v-erb-b2 avian erythroblastic leukaemia viral oncogene homolog 2 (*ERBB2)* oncogenes occur with adenocarcinoma in patients who have never smoked.(72) Furthermore, *ERBB2* mutations are not detected in tumours containing either EGRF or KRAS mutations.(72)

Mutations of p53, a tumour suppressor gene, has shown to be present in between 40% and 60% of non-small cell lung carcinoma.(72) Such mutations are more common in smokers than non-smokers.(72) Tobacco associated lung cancer is characterised by transversion mutations (substitution of a purine for a pyrimidine).(72) Lung cancer in people who have never smoked is associated with transition mutations (purine for purine or pyrimidine for pyrimidine).(72)

Biomarkers like serum amyloid A have been used to detect lung cancer but do not further indicate the histological subtype of the cancer.(73) Other biomarkers namely; haptoglobin alpha, serum amyloid protein, apolipoprotein A1 and as mentioned EGFR detect adenocarcinoma, whilst dihydrodiol dehydrogenase non-

specifically suggests non-small cell lung cancer.(73) EGRF mutations are almost exclusively found in adenocarcinoma, but may be present in SCC also.(73)

2.7.3. Diagnosis of Lung Cancer

a. Chest radiograph

The indications for a chest radiograph include symptoms or signs of lung cancer namely; haemoptysis, cough, shoulder or chest pain, dyspnoea, unintentional weight loss, hoarseness of voice, finger clubbing, cervical or supraclavicular lymphadenopathy or features suggestive of metastases from a lung cancer, which persists beyond three weeks.(52) The detection of pulmonary nodules on traditional chest radiograph alone is challenging, but the use of grey-scale inversion on the radiograph may improve nodule detection and eliminate false positive nodule findings.(74)

b. Sputum cytology

According to the National Institute for Health and Clinical Excellence (NICE) and ACCP guidelines, cytological analysis of sputum is rarely diagnostic.(52, 75) The recommendation is that it should only be performed on patients with central lung masses or when patients are unwilling to undergo bronchoscopy or other invasive tests.(76) This recommendation has been supported by studies that demonstrate the sensitivity of sputum cytology to be as low as 0.2%.(77) Factors affecting the yield of cytology vary depending on the site of the lung mass, the number of samples analysed and the presence or absence of co-existing respiratory

infection.(76) Since a negative finding does not exclude lung cancer, patients may need further investigations.(76) This is therefore, not a cost effective screening tool.(76)

c. Chest computed tomography scan for lung cancer screening and management of pulmonary nodules

Routine screening of smokers and high risk individuals for lung cancer remains controversial. The National Lung Screening Trial conducted in the United States of America, showed that screening for lung cancer with low dose chest computed tomography (CT) scan rather than chest radiograph reduced mortality from lung cancer.(78) Low dose chest CT scan, as a lung cancer screening test, proved superior with a sensitivity of 93.8% compared to 73.5% on chest radiography.(78) The specificity of low dose chest CT scan was, however, less than chest radiography (73.4% compared to 91.3%).(78) These findings have been similar to other large studies of low dose chest CT screening for lung cancer namely; the International Early Lung Cancer Action Program(79) and a Dutch Belgian lung cancer screening trial.(78-80)

Screening by low dose chest CT scan has raised several concerns such as the cost effectiveness of this tool, the large number of false positives on chest CT scan (98% in the National Lung Screening Trial), the radiation exposure delivered by repeat scans and the management of pulmonary nodules detected on the scans.(81) Further investigation of the National Lung Screening Trial demonstrates that over-diagnosis of lung cancer is also a major concern in low dose chest CT

screening.(82) In this trial, the probability of over-diagnosis of any lung cancer detected by screening was 22.5% (95%CI 9.7-34.3%) whilst the probability of over-diagnosis of broncho-alveolar carcinoma was 78.9% (95%CI 62.2-93.5%).(82)

Current recommendations by the NICE guidelines is that routine screening of smokers is not yet advocated.(52) Annual low dose chest CT screening is, however, recommended in the ACCP guidelines to patients between 55 and 74 years old with a 30 pack year smoking history and greater.(75)

When nodules are detected on a chest CT scan, they are classified as benign, malignant or indeterminate.(81) Features suggesting benign nodules include fatty plaques, calcification within the nodule or polygonal shaped nodules.(81) These features are in keeping with hamarto-chondroma, post-infectious granulomas and lymph nodes.(81)

After benign disease is excluded, features of malignant disease are assessed. Predictors of malignant disease include; solid or semi-solid nodules measuring more than 10 mm, nodule/s with speckled outlines and with air bronchograms or pleural retraction, nodule/s in the upper lobe and a low nodule count (less than or equal to four).(81, 83, 84)

If the nature of the nodule does not meet the criteria for benign or malignant disease, it is deemed indeterminate.(81) Such nodules should be followed up by repeat chest CT scan in two years.(81) It is generally accepted that solid nodules that remain unchanged in size for two years are benign.(81) Any change in size of 25% or more is deemed significant.(81)

d. Bronchoscopy and lung cancer

Bronchoscopy is beneficial as it provides a means for tissue diagnosis which determines the histological subtype of the lung cancer.(14) When bronchoscopy was compared to chest CT scan for the detection of endo-bronchial tumours, it was found to be superior with bronchoscopy detecting 37% more malignancies than chest CT scan.(85)

Chest CT scan is helpful prior to the bronchoscopy as it directs the bronchoscopist toward the mass and allows a biopsy method to be chosen.(24) Bronchoscopy has a high positive predictive value of between 70% and 90% for obtaining a tissue diagnosis of central lung lesions (less than four cm from the lobar bronchus) with poorly defined margins.(14) Peripheral lesions towards the outer third of the lung, are associated with lower yields of cancer detection during bronchoscopy(86) and a chest CT or ultrasound guided trans-thoracic biopsy may be considered instead.(24) Other studies, however, suggest that the sensitivity for detecting small, peripheral lung cancers on bronchoscopy varies from 38% to 86% depending on the size of the mass.(87)

Detection of lung cancer using biopsy during bronchoscopy ranges between 57% and 94%.(16, 30, 88-90) Rates of detection of lung cancer may be improved by direct visualisation of the tumour and multiple biopsies of the same lesion.(91) Rapid on site evaluation of trans-bronchial needle aspirates obtained using bronchoscopy maybe helpful in ensuring that all samples obtained are suitable to improve sensitivity for malignancy detection.(91) However, this is time consuming for the cyto-pathologist and may not be feasible in resource limited settings.(91)

The use of advanced bronchoscopy, namely auto-fluorescent bronchoscopy, endo-bronchial ultrasound and virtual bronchoscopic navigation, further improve the diagnostic yield of the biopsy.(24) Navigational bronchoscopy has facilitated easier diagnosis of small, peripheral lesions.(87) Electromagnetic navigation may increase bronchoscopy diagnostic yields of lung cancer from 69% to 74%.(87) When electromagnetic navigation assisted bronchoscopy is combined with endo-bronchial ultrasound, the sensitivity is greater than when either modality is used alone.(87)

Virtual bronchoscopic navigation generates virtual images of the bronchial path to the peripheral lesion.(87) As the virtual images are similar to the bronchoscopy images, the bronchoscope may be advanced to the lesion using the virtual images to navigate the bronchial tree.(87) The diagnostic yield for small, peripheral lung lesions is further increased when virtual bronchoscopic navigation is combined with endo-bronchial ultrasound.(87)

2.8. Summary

Although the use of bronchoscopy in the diagnosis of TB and lung cancer is well defined, little is known about the outcomes of bronchoscopy in South Africa. This is important as one of the main indications for bronchoscopy is to diagnose LRTI(31) which is also the leading cause of mortality in South Africa.(2) Whilst bronchoscopy is not the panacea to the diagnosis of TB and LRTI infections, this technology offers better detection rates amongst smear negative TB suspects.(23) Such a tool in KwaZulu Natal, where TB accounts for 12% of all deaths, is meaningful when appropriately applied.(2) Furthermore, since smear negative TB is more common amongst HIV infected individuals, and KwaZulu Natal has the highest rates of TB and HIV co-infection in South Africa,(33) this study evaluated the outcomes of bronchoscopy as well as the prevalence of smear negative TB detected by bronchoscopy at this site in western KwaZulu Natal.

The other main indication for bronchoscopy is the diagnosis of lung cancer.(31) Lung cancer is emerging as the most common cancer associated with mortality worldwide, and especially in high income countries.(1) The burden of lung cancer in South Africa is unknown due to infrequent updates of lung cancer registers. In KwaZulu Natal, the prevalence of lung cancer is not known. This study investigated the prevalence of lung cancer and the frequencies of histological subtypes at this public sector hospital in western KwaZulu Natal.

CHAPTER 3

STUDY OBJECTIVES

3.1. Aim of the Study

To describe the outcomes of bronchoscopies undertaken at a tertiary hospital in KwaZulu Natal in 2011

3.2. Specific Objectives

3.2.1. To list the indications for bronchoscopy at Greys Hospital

3.2.2. To describe the microbiological, cellular and histological findings at bronchoscopy

3.2.3. To determine the prevalence of tuberculosis diagnosed on broncho-alveolar lavage amongst smear negative patients

3.2.4. To determine the distribution of the histological subtypes of lung cancer at Greys Hospital

CHAPTER 4

METHODOLOGY

4.1. Study Design

This is a hospital based, retrospective study, of consecutive patients who had a bronchoscopy at Greys Hospital, department of Internal Medicine, between 1 January and 31 December 2011.

4.2. Study Population

Greys Hospital is a tertiary care hospital located in Pietermaritzburg and provides services to 4.5 million individuals in western KwaZulu Natal.(92) The study population included patients older than 18 years, referred to the pulmonologist at Greys Hospital, for further investigation and management of lung diseases. The study participants were both in and out-patients.

4.3. Inclusion Criteria

All patients for whom a fiber-optic bronchoscopy was performed, between 1 January and 31 December 2011.

4.4. Exclusion Criteria

4.4.1. All patients who did not have a bronchoscopy due to contra-indications

4.4.2. Failed bronchoscopy

4.4.3. All patients under the age of 18 years

4.4.4. All bronchoscopies not conducted in the bronchoscopy suite at Greys Hospital

4.4.5. Repeat bronchoscopies

4.5. Sample Size

This was a convenience sample. A total of 129 bronchoscopy procedures were requested between 1 January 2011 and 31 December 2011. Of this, 22 patients were excluded due to failure to meet the inclusion criteria. Reasons for exclusion included: 12 bronchoscopies were not undertaken in the bronchoscopy suite, two patients were underage and two procedures were cancelled for unclear reasons. A total of six bronchoscopies were repeated hence these were included only once. The reasons for repeat biopsy requests were a high international normalised ratio (INR) result (one case, INR=1.92), an absent INR result (one case), technical difficulty at first bronchoscopy attempt (three cases) and a repeat bronchial lavage for a non-resolving lower respiratory tract infection (LRTI) where no microbiology result was obtained (one case). A total of 107 patients met the inclusion criteria of the study and were analysed.

4.6. Data Collection Methods and Tools

A review of all bronchoscopies performed on adult patients in the bronchoscopy suite of Greys Hospital between 1 January and 31 December 2011 was conducted. All the bronchoscopies were performed by the pulmonologist at Greys Hospital, Dr K.T. Naidoo.

Bronchoscopy request forms stored in the bronchoscopy suite were reviewed to identify the patients who were eligible for inclusion in the study. Patient's medical records were reviewed to determine if the inclusion and exclusion criteria were met. A total of 129 were reviewed and 107 patients were included in the study. All data was extracted from the clinical records and entered on to the case report form (Appendix 3) by the principal investigator.

The bronchoscopy reports, in-patient and out-patient hospital clinical records were traced and reviewed to capture missing data. Variables that were recorded included age, gender, race, referring institution, outcomes and complications of bronchoscopy, indication/s for bronchoscopy, human immunodeficiency virus (HIV) status, history of antiretroviral therapy (ART) use, previous or current lung irritant exposure and the use of protective respiratory equipment, first degree relatives with a history of lung malignancy, smoking status, haemoglobin (Hb), international normalised ratio (INR) and oxygen saturations.

Indications for bronchoscopy were obtained from a pre-selected list on the bronchoscopy request forms. There were no definitions for "non-resolving lower

respiratory tract infection (LRTI)" or "diffuse pulmonary infiltrates." No clear distinction could be made between "non-resolving LRTI" and "suspected TB" and overlap may exist between the two.

Where data was missing and could not be traced on review of clinical records it was indicated as "unknown" on the case report form. Oxygen saturation above 90% on room air was considered acceptable for the procedure.(5) A Hb level of greater than or equal to 10 grams per decilitre (g/dl) was considered safe for bronchoscopy, whilst a Hb of less than 10g/dl was considered low. An INR result greater than and equal to 1.4 was considered elevated and not suitable to undertake biopsy during bronchoscopy. A cluster of differentiation 4 (CD4) count of less than or equal to 200 cells per cubic millimetre (cells/$mm^{3)}$ was considered to be in keeping with acquired immunodeficiency syndrome and baseline viral loads were not routinely requested or recorded. At the time of the study, eligibility to commence ART, in South Africa, included a CD4 count of less than 200 cells/mm^3 irrespective of World Health organisation HIV clinical stage, a CD4 count of less 350 cells/mm^3 in pregnant patients or patients with TB/HIV, World Health Organisation HIV clinical stage IV disease or MDR TB or XDR TB.(93)

Microbiology, histopathology and cytology results on sputa, broncho-alveolar lavage (BAL) samples and biopsy samples were obtained from clinical records, the National Health Laboratory Service (NHLS) TrakCare computerised laboratory record system or from the relevant laboratory site. When it was uncertain if a sample was sent for analysis, it was indicated as "unknown". For cases where the

sample was sent for analysis but no result could be traced it was indicated as “no result.” Tuberculosis (TB) was diagnosed on sputum with between one and nine acid fast bacilli (AFB) per 100 oil immersion fields (scanty positive), polymerase chain reaction (PCR), line probe assay or a TB culture growing *mycobacterium tuberculosis*. GeneXpert®, which is a PCR test, was only available from October 2011 at this site.

All chest computed tomography (CT) scans were obtained either from the clinical records or from the picture archiving and communication system (PACS) computerised radiology programme. The chest CT scan reports were all reported and authorised by a radiologist.

4.7. Data Management and Analysis

On completion of all the case report forms the data was entered on an Excel® workbook using Microsoft Office 2010® software. The data was thereafter further analysed using Epi-info Version 3.5.4® and Stata/IC 13.0® statistical analysis computer programmes. The demographic, bronchoscopy and chest CT scan findings were summarised with descriptive summary measures and expressed as means ± standard deviation (SD) and/or medians with the range for quantitative variables. Percentages, frequencies and proportions were used to describe categorical variables.

The profile of the patients who underwent bronchoscopy was described on a table indicating the frequencies of relevant categories pertaining to demographic data (age, gender, race, referral centre) and clinical characteristics (family history of lung malignancy, HIV status, ART therapy, smoking status and exposure to lung irritants). Age was categorised into groups of 10 years and the distribution of age was indicated using a bar graph. A table indicating median, SD, range and 95% confidence intervals (CI) was constructed for the continuous variables; age, Hb, INR, CD4 and oxygen saturation.

The frequency of individual indications for bronchoscopy was determined and more broadly classified into infective, non-infective, either infective or non-infective (where the aetiology was unclear) or unknown categories (where the indication was missing).

The description of the chest CT scan reports was tabulated to indicate the frequency. The frequency of microbiological findings on sputa and BAL samples were also tabulated. The results of microbiological cultures and TB detection methods (Ziehl Nielsen, PCR and TB culture) on sputum and BAL samples were compared on a table.

Histology samples were analysed according to benign pathology, malignant pathology, no pathological diagnosis, non-representative and no result categories.

The demographic and clinical profile of the patients diagnosed with lung cancer was tabulated.

Radiological detection of lung mass was compared to the macroscopic appearance of lung mass during bronchoscopy. Using bronchoscopy as the more accurate means of assessment, the sensitivity, specificity, positive predictive value and negative predictive value was calculated.(94)

A comparison was made between the indication for the bronchoscopy and the results of the samples collected during bronchoscopy. This was tabulated. The data was used to determine the diagnostic outcomes of bronchoscopy.

4.8. Ethical Considerations

Permission to conduct this retrospective study using clinical record review was obtained from the Biomedical Research and Ethics Committee (Reference number 144/13) (Appendix 4 and 5) at the University of KwaZulu Natal as well as the Greys Hospital management (Appendix 6). Each patient was allocated a unique study number. Only this unique number was reflected on the case report form to maintain patient confidentiality. This link between the study number and the patient record was stored separately and will be destroyed on acceptance of the dissertation. There was no direct contact between the researcher and the participants for data collection purposes or at any point during the study for study related purposes.

CHAPTER 5

RESULTS

5.1. Patient Population

A total of 129 bronchoscopies were requested between 1 January and 31 December 2011. Of this, 22 patients were excluded due to failure to meet the inclusion criteria. A total of 107 patients met the inclusion criteria of the study and were analysed. (Figure 5.1)

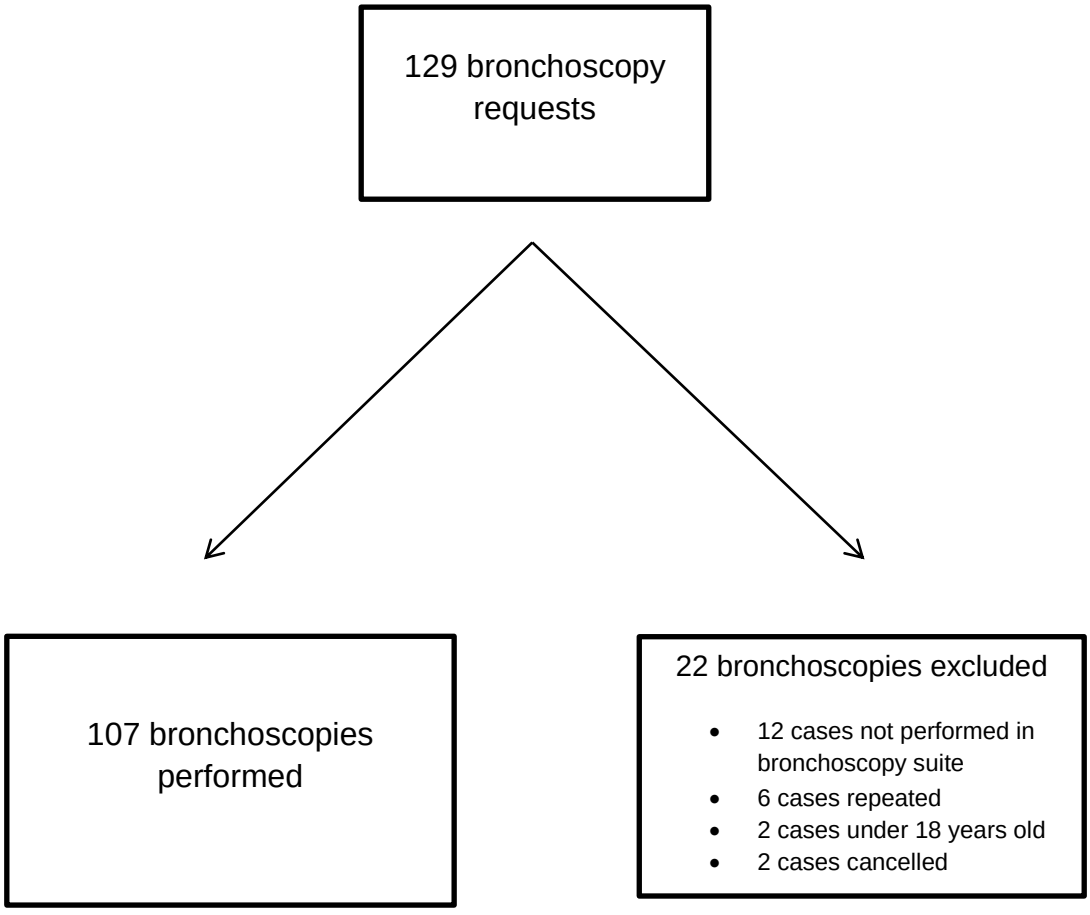


FIGURE 5.1. The sample population; all patients who had bronchoscopy at Greys Hospital in 2011

5.2. Baseline Demographic and Clinical Characteristics

The majority of the patients were indigenous African (70.1%) compared with Indian (16.8%), White (11.2%) and Coloured (1.9%) patients. (Table 5.1) There were more males (63.6%) than females (36.4%) in the study group. The median age was 55±14.4 years and 66% of patients were between 40 and 70 years. (Figure 5.2)

All of the patients, who were referred from a tertiary institution, were in-patients at Greys Hospital (84.1%). The remainder of the patients were from Edendale Hospital, a neighbouring regional hospital (13.1%) and Northdale Hospital which offers district level services (1.9%). One (0.9%) patient was referred from a correctional services facility. (Table 5.1)

Smoking was prevalent in 34.6% of the patients whilst 12.1% had never smoked. A smoking history was unknown for 53.3% of the sample group. Information about a family history of lung malignancy and exposure to lung irritants was available for 21.5% of the sample group. (Table 5.1) A positive family history of lung malignancy was noted in 5.6% of the study group and 7.5% of the patients had been exposed to lung irritants such as gold (1.9%), insecticides (1.9%), diesel fumes (0.9%), rubber (0.9%), factory smoke (0.9%) and dust (0.9%). The use of respiratory protective equipment during exposure to lung irritants was not documented in the case notes hence this information was not available for analysis.

The human immunodeficiency virus (HIV) status of the study group was unknown for 36.4% of the sample group, due to either no recorded HIV status in the case records or the patient being unaware of their HIV status at the time of bronchoscopy. (Table 5.1) A total of 28 (26.2%) patients were HIV infected with a median cluster of differentiation 4 (CD4) count of 254 cells per cubic millimetre (cells/mm^3) (95%CI 197-324 cells/mm^3). (Table 5.2)

A CD4 count of 200 cells/mm^3 and below was present in 12 (42.9%) HIV infected patients. Nine (32.1%) patients were eligible to commence antiretroviral therapy (ART) but were ART naïve at the time of bronchoscopy. Of all the HIV infected patients, 10 (35.7%) patients were on ART at the time of bronchoscopy. (Table 5.2) The median CD4 count for those on ART was 248 cells/mm^3 (95%CI 161-331 cells/mm^3). There were no HIV viral loads available for any of the HIV infected patients.

TABLE 5.1: Baseline demographic and clinical findings of the study patients (N=107), who had a bronchoscopy at Greys Hospital, in 2011

CHARACTERISTICS	FREQUENCY (number)	PERCENTAGE (%)
Demographic characteristics		
Age (years)		
21 – 40	23	21.5
41 – 70	71	66.4
>=70	13	12.2
Gender		
Male	68	63.6
Female	39	36.4
Race		
African	75	70.1
Indian	18	16.8
White	12	11.2
Coloured	2	1.9
Referral Centre		
Tertiary	90	84.1
Regional	14	13.1
District	2	1.9
Other	1	0.9
Risk factors for lung pathology		
Smoking status		
Never smoked	13	12.1
Ever smoker	37	34.6
Unknown smoking history	57	53.3
Family history of lung malignancy		
Yes	6	5.6
No	17	15.9

Unknown	84	78.5
Exposure to lung irritants		
Present	8	7.5
Absent	15	14
Unknown	84	78.5
HIV status		
Infected	28	26.2
Uninfected	40	37.4
Unknown	39	36.4
Antiretroviral drug therapy		
Regimen 1*	9	32.1
Regimen 2**	1	3.6
Not on treatment	18	64.3

**Regimen 1*: two nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI):

Lamivudine, Tenofovir, Efavirenz (4 cases)

Lamivudine, Stavudine, Efavirenz (3 cases)

Lamivudine, Stavudine, Nevirapine (1 case)

Lamivudine, Zidovudine, Efavirenz (1 case)

***Regimen 2*: two nucleoside reverse transcriptase inhibitors (NNRTI) and one protease inhibitor (PI):

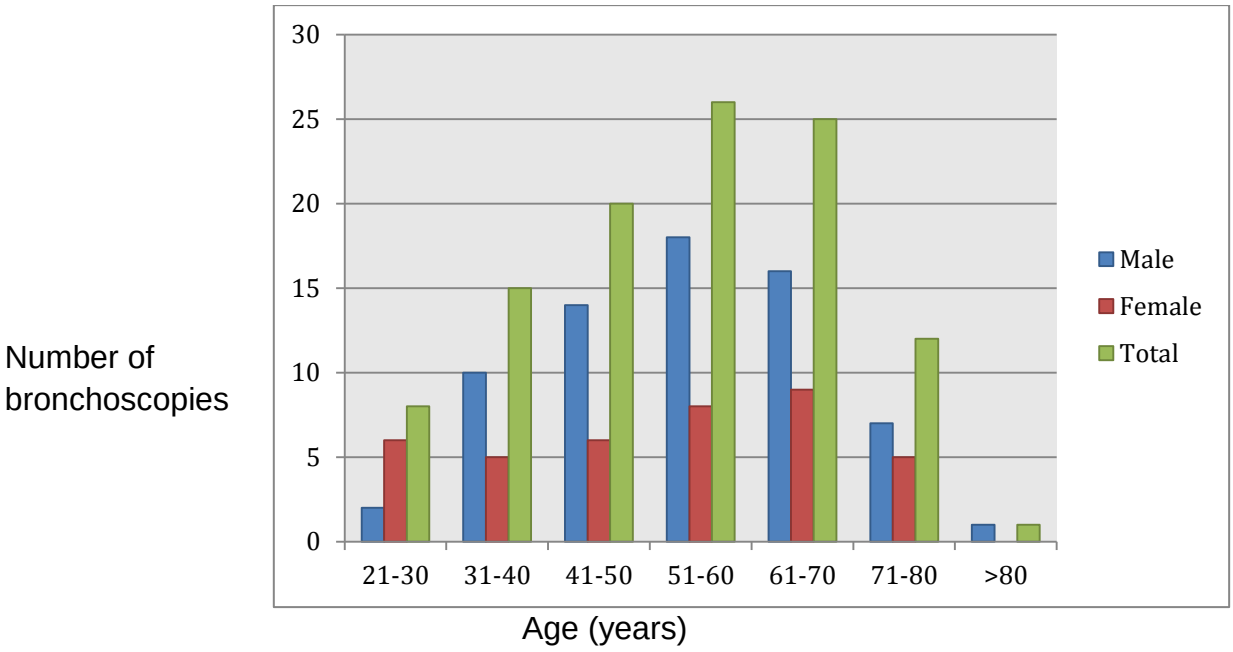
Lamivudine, Tenofovir, Lopinavir/Ritonavir (1 case)

Abbreviations- HIV: Human immunodeficiency virus, NRTI: Nucleoside reverse transcriptase inhibitors, NNRTI: Non-nucleoside reverse transcriptase inhibitor, PI: Protease inhibitor

TABLE 5.2: Laboratory safety and clinical investigations performed on the patients prior to bronchoscopy procedure

PARAMETER	MEDIAN (SD)	RANGE	95%CI	Inter-quartile Range
Haemoglobin (g/dl)	11.8 ±2.11	6.8 - 16.5	11.23 - 12.04	10.4-13.1
INR	1.09 ± 0.19	0.9 - 2.11	1.09 - 1.17	1.02-1.19
CD4 of all HIV infected (cells/mm^3)	254 ±164	52 - 665	197 - 324	126-366.5
CD4 of patients on ART (cells/mm^3)	248 ± 126	52 - 445	161 - 331	152-325.5
Oxygen Saturation (%)	99 ±1.93	91 - 99	97.58 - 98.42	97-99

Abbreviations- ART: Antiretroviral therapy, Cells/mm^3: Cells per cubic millimetre, CD4: Cluster of differentiation 4, CI: Confidence interval, g/dl: Grams per decilitre, SD: Standard deviation



Age (years)	*Male*	*Female*
21-30	*2*	*6*
95%CI	*35.0-85.0*	*40.35-109.65*
31-40	*10*	*5*
95%CI	*37.86-96.14*	*8.22-74.22*
41-50	*14*	*6*
95%CI	*46-94*	*6.67-77.7*
51-60	*18*	*8*
95%CI	*47.63-90.27*	*1.05-63.05*
61-70	*16*	*9*
95%CI	*40.48-87.52*	*4.64-67.36*
71-80	*7*	*5*
95%CI	*21.44-94.56*	*1.26-85.26*
>80	*1*	*0*
95%CI	*0*	

FIGURE 5.2: Distribution of age of the patients who had bronchoscopy at Greys Hospital in 2011

5.3. Pre-procedure Laboratory Investigations

A haemoglobin (Hb) result was available for all patients prior to bronchoscopy. An Hb of greater than or equal to 10 grams per decilitre (g/dl), which was considered safe for bronchoscopy, was present in 86 (80.4%) patients. The median Hb was 11.8g/dl (95%CI 11.23-12.04 g/dl). (Table 5.2) It is unknown if the 21 (19.6%) patients with Hb less than 10g/dl received a blood transfusion prior to bronchoscopy.

An INR result was available for 104 patients. Of this, 98 patients (91.6%) had an INR result less than or equal to 1.4 and 6 patients (5.6%) had a high INR of greater than 1.4. The median INR was 1.09 (95%CI 1.09-1.17). (Table 5.2) Bronchoscopy was still performed on the patients with an elevated INR result, but the biopsy was deferred. If a biopsy was necessary, bronchoscopy was repeated when the INR result was normal. This was done for one patient in the cohort. One patient who had an INR result of 2.1 was on enoxaparin and warfarin for the treatment of deep venous thrombus. It was unclear as to why the other INR results were elevated.

Pre-bronchoscopy oxygen saturations were documented for 84 (78.5%) patients and all readings were greater than or equal to 90%. The median saturation was 99% (95%CI 97.58- 98.42%). (Table 5.2)

5.4. Pre-bronchoscopy Chest Computed Tomography Scan

A chest computed tomography (CT) scan was done prior to bronchoscopy as it defines the lung anatomy and assists with localising the lung lesions.(95) This assists with improving the diagnostic yield of bronchoscopy.(95) A chest CT scan was performed for 66 (61.7%) patients, either at Greys Hospital or at the referral centre. (Table 5.3) A total of 36 (54.5%) reports stated that the radiographic findings were likely to be in keeping with a non-benign process with 30 (45.5%) reports describing a mass lesion, either alone or accompanied by other pathology. There were 19 reports (28.8%) of possible lung metastases.

The remaining 30 (45.5%) chest CT scans reported pathology that was likely to be related to a benign process. There were eight (12.1%) reports concluding a diagnosis of bronchiectasis, eight (12.1%) of cavitation and five (7.6%) reported lung opacification. There was one (1.5%) chest CT scan reported as normal. The indication for bronchoscopy in this patient was unclear as the clinical records were unavailable and the bronchoscopy findings were reported as normal.

TABLE 5.3: Frequency of computed tomography (CT) scan (N=66) of the chest, performed prior to bronchoscopy at Greys Hospital in 2011

CT SCAN REPORT	FREQUENCY (N=66)	PERCENTAGE (%)
Pathology suggesting malignancy	74	
Mass	30	45.5
Metastases	19	28.8
Lymphadenopathy	16	24.2
Nodules	7	10.6
Pleural effusions	2	3.0
Pathology not suggestive of malignancy	38	
Bronchiectasis	8	12.1
Cavitation	8	12.1
Opacification	5	7.6
Fibrosis	4	6.1
Interstitial pattern	3	4.5
Other	10	
Abscess	*2*	3.0
Bullous	*2*	3.0
Emphysema	*2*	3.0
Granuloma	*2*	3.0
Cyst	*1*	1.5
Haemothorax	*1*	1.5
Normal	1	1.5

TOTAL	113*	100

*The total exceeds 66 as more than 1 finding was evident on the chest CT scans.

Abbreviations- CT: Computed tomography

5.5. Indications for and Findings at Bronchoscopy

The commonest indications for bronchoscopy were non-infective (36%) rather than infective pathologies (32%). (Figure 5.3) Non-infective pathology included lung mass (35.8%) and trachea-oesophageal fistula (0.8%), whilst infective pathology included non-resolving LRTI (15%), suspected tuberculosis (TB) (15%) and lung abscess (1.7%). Diffuse pulmonary infiltrates (12.5%) and haemoptysis (9.2%) were categorised as either infective or non-infective as the aetiologies were unclear at the time of request. There was more than one indication listed for some patients resulting in the total number (120) of indications exceeding the number of bronchoscopies performed (107). Overall the commonest indications for bronchoscopy were lung mass (35.8%), non-resolving LRTI (15%) and suspected TB (15%). (Table 5.4)

The commonest diagnoses by bronchoscopy were made in the patients referred for investigation of diffuse pulmonary infiltrates. Of the 15 patients referred, bronchoscopy assisted with the diagnosis in seven (46.7%) patients; two (13.3%) patients had positive bacterial cultures, three (20%) patients had positive fungal cultures, one (6.7%) patient had TB and one (6.7%) patient had chronic interstitial fibrosis on biopsy. (Table 5.4)

The second highest diagnostic yield was in patients referred for investigation of a lung mass. Of the 43 patients referred 20 (46.5%) bronchoscopy diagnoses were made. Thirteen (30.2%) had lung cancer, one (2.3%) had a positive bacterial culture, one (2.3%) had a positive fungal culture, one (2.3%) had TB, one (2.3%) had non-specific granulation tissue and three (7%) had no pathology. (Table 5.4)

Bronchoscopy assisted with achieving a diagnosis in five (27.8%) of the 18 patients referred for non-resolving LRTI. Of these five patients; three (16.7%) patients had a positive bacterial culture, one (5.6%) patient had a positive fungal culture and one (5.6%) patient had lung cancer. (Table 5.4)

Of the 18 patients referred for investigation of suspected TB, bronchoscopy assisted with the diagnosis on four (22.2%) of the 18 patients. Two (11.1%) patients had positive fungal cultures, one (5.6%) patient had TB and one (5.6%) patient had lung cancer. (Table 5.4)

Two (18.2%) of the 11 patients referred with haemoptysis had lung cancer, one (9.1%) patient had acute bacterial pneumonia on endo-bronchial biopsy and one (9.1%) patient had a normal endo-bronchial biopsy result. (Table 5.4)

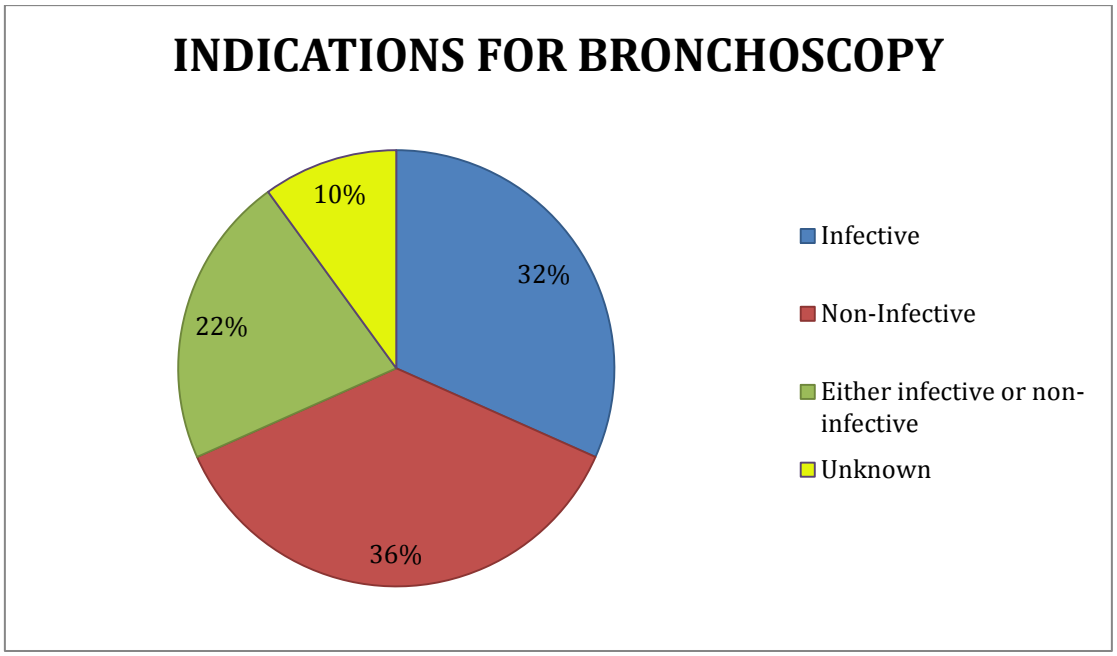


FIGURE 5.3: Infective and non-infective indications for bronchoscopy in the sample population

TABLE 5.4: Frequency of indications and findings of bronchoscopy at Greys Hospital in 2011

INDICATION		FINDINGS ON BRONCHOSCOPY					
Indication Type	**Indication Frequency N=120 (%)**	**Bacterial (%)**	**Fungal (%)**	**TB (%)**	**Lung Cancer (%)**	**Other (%)**	**Inconc-lusive (%)**
Lung mass	43 (100)	1 (2.3)	1 (2.3)	1 (2.3)	13 (30.2)	4 (9.3)	23 (53.4)
Non-resolving Lower Respiratory tract infection	18 (100)	3 (16.7)	1 (5.6)	0	1 (5.6)	0	13 (72.2)
Suspected Tuberculosis	18 (100)	0	2 (11.1)	1(5.6)	1 (5.6)	0	14 (77.8)
Diffuse pulmonary infiltrates	15 (100)	2 (13.3)	3 (20)	1(6.7)	0	1 (6.7)	8 (53.3)
Unclear indication	12 (100)	1 (8.3)	1 (8.3)	1 (8.3)	0	0	9 (75)
Haemoptysis	11 (100)	0	0	0	2 (18.2)	2 (18.2)	7 (63.6)
Other	3 (100)	0	0	0	0	0	3 (100)
Lung abscess	2 (100)	0	0	0	0	0	0
Tracheo-oesophageal fistula	1 (100)	0	0	0	0	0	0
Total	**120* (100)**	**7 (5.8)**	**8 (6.7)**	**4 (3.3)**	**17 (14.2)**	**9 (7.5)**	**75 (62.5)**

*The total of 120 exceeds the sample size of 107 as more than one indication per patient was present

5.6. Microbiological Findings on Sputa Samples Prior to Bronchoscopy

Microscopy, culture and sensitivity (MC&S) of sputa samples were requested on 48 patients prior to bronchoscopy request. (Table 5.5) There was growth of organisms on 11 (22.9%) samples. Seven (14.6%) gram negative bacterial

organisms were cultured namely; *Klebsiella pneumoniae* which was identified in two (4.2%) patients and *Haemophilus influenza, Pseudomonas aeruginosa, Citrobacter koseri, Lecercia adenocarboxylate, Morganella morganii* and *Proteus mirabilis* which was identified in six (12.5%) different patients respectively. There was one (2.1%) gram positive bacillus cultured; *Staphylococcus aureus* (sensitive to cloxacillin) and two (4.2%) fungal cultures of *Candida albicans*. On 26 (54.2%) samples there was no growth and 11 (22.9%) results could not be traced.

Of the patients with a positive bacterial or fungal culture, three were HIV infected, four were HIV uninfected and four patients had an unknown HIV status. The HIV infected patients' cultured *Klebsiella pneumoniae, Haemophilus influenza* and *Staphylococcus aureus*. Only one patient had a CD4 count available (52 cells/mm^3) and this patient was on antiretroviral therapy (ART).

TB screening was performed using either Ziehl Neelsen or auramine stains. Fifty samples (98%) stained negative and one (2%) sample leaked and was discarded. (Table 5.5) None of the samples stained positive for acid fast bacilli (AFB). Eight samples were sent for polymerase chain reaction (PCR) identification using either GeneXpert® or line probe assay (LPA). Of these, three (37.5%) samples were negative and five (62.5%) were positive for TB. One of the five positive PCR tests suggested rifampicin resistance and extreme drug resistant TB (XDR TB) on TB culture. The other four positive PCR tests were sensitive to rifampicin.

TB culture was requested in 39 patients. The results were only available post bronchoscopy and showed that 32 (82.1%) samples were negative and seven (17.9%) were positive for TB. (Table 5.5) Six (15.4%) samples were sensitive to rifampicin and isoniazid. One (2.6%) sample was resistant to isoniazid, rifampicin, streptomycin, kanamycin and ofloxacin suggesting XDR TB.

TABLE 5.5: Frequency of microbiological findings on sputum samples collected prior to bronchoscopy

TEST	**MC&S**	**ZN OR AURAMINE STAIN**	**PCR OR LPA**	**TB CULTURE**
Number of tests requested	48	51	8	39
Positive	11 (22.9%)	0 (0%)	5 (62.5%)	7 (17.9%)
Negative	26 (54.2%)	50 (98%)	3 (37.5%)	32 (82.1%)
Could Not Be Traced	11 (22.9%)	1 (2%) (leaked)	0 (0%)	0 (0%)

Abbreviations- LPA: Line probe assay, MC&S: Microscopy, culture and sensitivity, PCR: Polymerase chain reaction, TB: Tuberculosis, ZN: Ziehl Neelson

5.7. Microbiological Findings on Broncho-alveolar Lavage

There were 43 BAL samples requested for MC&S. (Table 5.6) Bacterial growth was present on six (14%) samples. *Staphylococcus aureus,* sensitive to cloxacillin, was identified on one (2.3%) sample which was not on the same individual who

cultured the organism on sputum. *Haemophilus influenzae* was identified on two (4.7%) samples. Other gram negative organisms isolated included *Klebsiella pneumonia, Pseudomonas aeruginosa* and *Serratia marcesans*. There was no growth on 24 (55.8%) samples. Results could not be traced for 13 (30.2%) patients.

Of the patients with positive bacterial cultures on BAL samples, three patients were HIV infected whilst one patient was uninfected. The HIV status of two patients was unknown. The three HIV infected patients' cultured *Haemophilus influenzae* (two patients) and *Klebsiella pneumoniae* (one patient). The two HIV infected patients with *Haemophilus influenzae* had CD4 counts of 419 cells/mm^3 and 108 cells/mm^3 respectively and were on ART. The CD4 count of the HIV infected patient with *Klebsiella pneumoniae* was unknown and the patient was ART naïve.

Fungal culture was requested on 30 samples. Growth was identified on six (20%) samples with one (3.3%) sample showing growth of two fungal organisms; *Aspergillus flavus* and *Candida albicans*. The other organisms identified were *Aspergillus niger* (one patient), *Candida albicans* (three patients) and *Cryptococcus neoformans* (one patient). Of the remaining samples, seven (23.3%) had no growth, two (6.7%) were not done due to a laboratory error and 15 (50%) results could not be traced. (Table 5.6)

Of the positive fungal cultures, one patient was HIV infected, two were HIV uninfected and three had an unknown HIV status. The one patient who was HIV infected and cultured *Aspergillus niger*, had an unknown CD4 count and was ART naïve.

Samples were requested for *Pneumocystis jirovecii* in 17 patients. The results were negative in 10 (58.8%) patients and the remainder of the results could not be traced. (Table 5.6)

BAL samples were sent for AFB analysis for 38 patients. (Table 5.6) AFB was observed in two (5.3%) samples. Both these patients were HIV infected. The CD4 count of one patient was 270 cells/mm^3 and this patient was not on ART. The other patients CD4 count was 141 cells/mm^3 and it was unknown if this patient was on ART. Results were negative in 22 (57.9%) samples and the remaining 14 (36.8%) samples could not be traced.

Seven samples were sent for PCR either using GeneXpert® or LPA. Of these, three (42.9%) were positive, two (28.6%) were negative, one (14.3%) sample leaked and no result could be traced for the remaining sample (14.3%). Of the three patients with a positive PCR test, the HIV status of two patients were unknown. The third patient also had a positive AFB result, was HIV infected, had a CD4 count of 270 cells/mm^3 and was ART naive. All three (42.9%) of the samples that were positive on PCR were sensitive to rifampicin and isoniazid.

BAL samples were also sent for TB culture for 35 patients. (Table 5.6) Results were positive for three (8.6%) samples (two of these samples were already positive on the PCR test; one patient with an unknown HIV status and the other patient was HIV infected, CD4 270 cells/mm^3 and was ART naive). Both these samples were sensitive to rifampicin and isoniazid. The third patient cultured *mycobacterium other than tuberculosis*. This third patient was HIV infected, had a CD4 count of 141 cells/mm^3 and had an unknown ART history. TB culture was negative for 18 (51.4%) samples. The results of 14 (40%) samples could not be traced. A total of four (11.4%) patients were diagnosed with TB using AFB, PCR and/or TB culture on BAL samples.

TABLE 5.6: Frequency of microbiological findings on broncho-alveolar lavage collected at the time of bronchoscopy

TEST	MC&S	FUNGAL	PCP	AFB	PCR/ LPA	TB CULTURE
Number of tests requested	43	30	17	38	7	35
Positive	6 (14%)	6 (20%)	0 (0%)	2 (5.3%)	3 (42.9%)	3 (8.6%)
Negative	24 (55.8%)	7 (23.3%)	10 (58.8%)	22 (57.9%)	2 (28.6%)	18 (51.4%)
Could not be traced	13 (30.2%)	15 (50%) 2 (6.7%) (error)	7 (41.2%)	14 (36.8%)	1(14.3%) 1(14.3%) (leaked)	14 (40%)

Abbreviations- AFB: Acid fast bacilli, LPA: Line probe assay, MC&S: Microscopy, culture and sensitivity, PCR: Polymerase chain reaction, PCP: Pneumocystis jirovecii pneumonia, TB: Tuberculosis

5.8. Comparison between Sputum and Broncho-alveolar Lavage Microbiological Culture

When the sputum and BAL microbiological cultures were compared, the same organism was cultured on only one patient. This was for *Pseudomonas aeruginosa*. There were 10 (20.8%) samples where the organism was only cultured on sputum and 12 (11.6% bacterial and 20% fungal) samples where it was only cultured on BAL.

Amongst sputum smear negative patients, two (5.7%) cases were positive for AFB on BAL samples. PCR was positive in both sputum and BAL samples for one (2.85%) patient. One (2.85%) patient had a positive TB culture on sputum whilst the BAL culture was negative. This patient had XDR TB and was on appropriate treatment for an unknown duration when BAL was performed. Another patient had a positive culture on BAL and a negative culture on sputum. (Table 5.7)

TABLE 5.7: Diagnosis of TB amongst sputum and broncho-alveolar sampling performed on the sample population

	SPUTUM			BAL		
	ZN	**PCR**	**TB Culture**	**ZN**	**PCR**	**TB Culture**
1. XDR TB	Negative	Positive	Positive	No result	Not requested	Negative
2.	Negative	Positive	Positive	Positive	Positive	Positive
3.	Negative	Not requested	Positive	Negative	Negative	No result
4.	Negative	Positive	Positive	Not requested	Not requested	Not requested
5.	Not requested	Positive	Positive	Not requested	Not requested	Not requested
6.	Negative	Not requested	Positive	Not requested	Not requested	Not requested
7.	Negative	Negative	Positive	Not requested	Not requested	Not requested
8.	Not requested	Positive	Not requested	No result	Negative	No result
9.	Not requested	Not requested	Not requested	No result	Positive	Positive
10.	Negative	Not requested	Negative	Positive	Not requested	Positive
11.	Leaked	Not requested	Not requested	No result	Positive	Not requested

Abbreviations- BAL: Broncho-alveolar lavage, PCR: Polymerase chain reaction, XDR TB: Extreme drug resistant tuberculosis, ZN: Ziehl Neelson

5.9. Cellular Findings on Sputa and Broncho-alveolar Lavage

Cytology was requested on sputa and BAL samples. There were 40 sputa samples sent for cytology. Atypical cells were present on three (7.5%) samples and one (2.5%) of these was noted as squamous cell carcinoma (SCC). All three sputa samples with atypical cells had normal BAL cytology and biopsy results. No malignant cells were present in 17 (42.5%) samples; six (15%) samples were unrepresentative, whilst 14 (35%) results could not be traced.

BAL samples for cytology were requested for 61 samples. There were atypical cells present on eight (13.1%) samples: one (1.6%) with SCC, one (1.6%) with adenocarcinoma, one (1.6%) with reactive bronchial cells and five (8.2%) with atypical cells. Both BAL cytology samples suggestive of lung cancer were proven on biopsy. Of the five (8.2%) patients with atypical cells on BAL cytology: two (3.3%) patients had SCC on biopsy, one (1.6%) patient had atypical cells on biopsy also, and two (3.3%) patients had no biopsy taken. The one (1.6%) patient with reactive bronchial cells on BAL cytology had no biopsy taken. There were no malignant cells on 37 (60.7%) samples and no result could be traced for 16 (26.2%) samples.

5.10. Macroscopic Appearance on Bronchoscopy

During the bronchoscopy procedure, there were 23 (53.5%) cases were a mass was visible macroscopically. In addition, there were nine (8.4%) cases were external compression of the airway was visible suggesting that a lung

parenchymal mass was present. When the macroscopic appearance of bronchoscopy was compared to chest CT findings; bronchoscopy detected masses in an additional 76.6% of patients. With bronchoscopy used as the gold standard test, the sensitivity and specificity of chest CT scan for detection of lung masses was 73.7% and 70% respectively. The positive predictive value and negative predictive value were 46.7% and 86.1% respectively.

5.11. Histological Findings on Biopsy

The decision to undertake biopsy was dependent on the macroscopic findings and accessibility of the lesion during bronchoscopy. There were 27 (25.2%) biopsy samples sent for histology. (Table 5.8) From these 16 (59.2%) malignant pathologies and three (11.1%) benign pathologies were identified. The malignant pathologies included 10 SCC (37%), two adenocarcinomas (7.4%), three carcinomas of unknown origin (11.1%) and one sample of atypical cells (3.7%). These atypical cells could not be further differentiated. Of the three unknown carcinoma samples: one specimen had features resembling adenocarcinoma and a neuroendocrine tumour, the second specimen was likely to be SCC and the third sample the carcinoma could not be differentiated. The ratio of SCC: adenocarcinoma diagnosed was 5:1.

The three benign pathologies identified included acute bacterial pneumonia, chronic interstitial fibrosis and non-specific granulation tissue. No organism was identified on the biopsy sample with pneumonia, but gram negative cocci were reported. Sputum and BAL MC&S for this patient were also inconclusive. There

were three (11.1%) samples for which no pathological diagnosis could be made, two (7.4%) samples that were non representative and three (11.1%) samples where no result could be traced. (Table 5.8)

TABLE 5.8: Frequency of histological findings of lung biopsy (N=27) obtained during bronchoscopy at Greys Hospital in 2011

HISTOLOGY	FREQUENCY(N=27)	PERCENTAGE (%)
Malignant Pathology		
Squamous cell carcinoma	10	37.0
Adenocarcinoma	2	7.4
Carcinoma (uncertain type)	3	11.1
Atypical cells	1	3.7
Benign Pathology		
Acute bacterial pneumonia	1	3.7
Chronic interstitial fibrosis	1	3.7
Non-specific granulation tissue	1	3.7
No pathological diagnosis	3	11.1
Non-representative	2	7.4
No result	3	11.1
TOTAL	27	100

5.12. Demographic and Clinical Profile of Patients with Lung Cancer

The mean age of males with lung cancer was 57.8 years whilst for females it was 63.7 years. Overall lung cancer was more common in males than females (4:1) and was associated mainly with the indigenous African population compared to other races. (Table 5.9) A family history of lung malignancy was noted in one (6.25%) patient with SCC and one (6.25%) patient with unknown carcinoma. Two (12.5%) patients had exposure to lung irritants; one (6.25%) with SCC had exposure to gold and the other (6.25%) with unknown carcinoma had exposure to insecticides. Unfortunately, use of respiratory protective equipment during exposure to lung irritants for these two patients was unknown. None of the patients with lung cancer were HIV infected.

More patients who smoked were diagnosed with cancer. The ratio between smokers and non-smokers who were diagnosed with lung cancer was 2:1.

TABLE 5.9: Demographic and clinical profile of 16 patients with confirmed lung cancer

	SCC (N=10)	**ADENOCARCINOMA (N=2)**	**CARCINOMA (N=3)**	**ATYPICAL CELLS (N=1)**	**TOTAL (N=16)**
Gender					
Male	9 (56.25%)	2 (12.5%)	2 (12.5%)	0 (0%)	13 (81.25%)
Female	1 (6.25%)	0 (0%)	1 (6.25%)	1 (6.25%)	3 (18.75%)
Race					
African	9 (56.25%)	1 (6.2%)	3 (18.75%)	0 (0%)	13 (81.25%)
White	0 (0%)	0 (0%)	0 (0%)	1 (6.25%)	1 (6.25%)
Indian	1 (6.25%)	1 (6.25%)	0 (0%)	0 (0%)	2 (12.5%)
Coloured	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lung Irritants					
Yes	1(gold) (6.25%)	0 (0%)	1 (insecticide) (6.25%)	0 (0%)	2 (12.5%)
No	2 (12.5%)	0 (0%)	0 (0%)	0 (0%)	2 (12.5%)
Unknown	7 (43.75%)	2 (12.5%)	2 (12.5%)	1 (6.25%)	12 (75%)
Family History					
Yes	1 (6.25%)	0 (0%)	1 (6.25%)	0 (0%)	2 (12.5%)
No	2 (12.5%)	0 (0%)	0 (0%)	0 (0%)	2 (12.5%)
Unknown	7 (43.75%)	2 (12.5%)	2 (12.5%)	1 (6.25%)	12 (75%)
Smoking					

Yes	5 (31.25%)	1 (6.25%)	1 (6.25%)	0 (0%)	7 (43.75%)
No	3 (18.75%)	0 (0%)	1 (6.25%)	0 (0%)	4 (25%)
Unknown	2 (12.5%)	1 (6.25%)	1 (6.25%)	1 (6.25%)	5 (31.25%)
HIV Status					
Infected	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Uninfected	8 (50%)	2 (12.5%)	3 (18.75%)	0	13 (81.25%)
Unknown	2 (12.5%)	0 (0%)	0 (0%)	1 (6.25%)	3 (18.75%)

Abbreviations- HIV: Human immunodeficiency virus. SCC: Squamous cell carcinoma

5.13. Summary of Outcomes of Bronchoscopy

The three most common indications for bronchoscopy were suspected lung mass, non-resolving LRTI and suspected TB. However, bronchoscopy confirmed the most diagnoses in 7 of 15 patients (46.7%) referred with diffuse pulmonary infiltrates. Bronchoscopy also confirmed the histological diagnosis in 20 of 43 patients (46.5%) referred for suspected lung mass, 5 of 18 (27.8%) patients referred for investigation of non-resolving LRTI, and 4 of 18 patients (22.2%) referred for suspected TB. (Table 5.4)

Bronchoscopy assisted with the diagnosis of broncho-respiratory pathology in 38 (35.5%) patients. No pathological diagnosis could be made for 46 (43%) patients.

There were 23 (21.5%) patients where no diagnosis could be made due to missing BAL results.

5.14. Complications related to Bronchoscopy

There were four (3.7%) patients who experienced procedure related complications. Three (75%) patients experienced oxygen desaturation during bronchoscopy and responded well to supplemental oxygen. One (25%) patient had haemoptysis post biopsy but the volume of blood loss was not documented. All patients recovered without further complications.

5.15. Summary of Results

The most common indications for bronchoscopy were investigation of a lung mass, non-resolving LRTI and suspected TB. Bronchoscopy assisted with microbiological, cytological or histological diagnosis of broncho-respiratory pathology in 35.5% of all patients in the study. Bronchoscopy confirmed infection in the majority of patients referred for investigation of diffuse pulmonary infiltrates. Microbiological findings on sputum and BAL revealed gram positive and negative cocci and fungi. Cytological findings on sputum were inconsistent with that of BAL samples. Cytology on BAL samples detected lung malignancy in 3.3% of cases which was confirmed on biopsy. Only 11.1% (two of 18 patients) of smear negative TB suspects had a diagnosis of TB confirmed by bronchoscopy. Squamous cell carcinoma was the commonest lung cancer detected by histology. Overall the bronchoscopy complication rate was 3.7%.

When the macroscopic appearance of bronchoscopy was compared to chest CT findings; bronchoscopy detected masses in an additional 76.6% of patients. With bronchoscopy used as the gold standard test, the sensitivity and specificity of chest CT scan for detection of lung masses was 73.7% and 70% respectively. The positive predictive value and negative predictive value were 46.7% and 86.1% respectively.

CHAPTER 6

DISCUSSION

Our study explored the outcomes of bronchoscopy over a one year period, at Greys Hospital. The objectives of our study were to describe bronchoscopy indications and microbiological, cellular and histological findings on bronchoscopy samples. The prevalence of TB diagnosed on broncho-alveolar lavage (BAL) amongst smear negative patients, in this high TB/HIV prevalence area, and the common types of lung cancer diagnosed on biopsy during bronchoscopy, were also described. This was the first study in Pietermaritzburg describing outcomes of bronchoscopy in the public sector.

The key findings of the study were that bacterial cultures, fungal cultures and cytology performed on broncho-alveolar lavage (BAL) samples assisted with achieving a microbiological or cytological diagnosis where sputum microscopy, culture or cytology could not. Microscopy, polymerase chain reaction (PCR) and TB culture on BAL specimens were useful means for TB detection amongst sputum smear negative patients. Squamous cell carcinoma (SCC) and adenocarcinoma were the two most common lung cancers diagnosed on histology.

6.1. Indications for Bronchoscopy

There is a scarcity of studies describing indications for bronchoscopy in South Africa. A study from Cape Town listed suspected lung malignancy and TB as the two most common indications for bronchoscopy.(31) The findings of our study are similar as suspected lung mass was the most common indication for bronchoscopy followed by an equal proportion of requests for non-resolving lower respiratory tract infection (LRTI) and suspected TB. Non-resolving LRTI could not be defined in our study and there may be significant overlap between this indication and that of suspected TB. Despite this, the results were also comparable to studies in the United States of America (15) and Middle East where infection and suspected lung malignancy were also the most common indications for bronchoscopy.(16, 17, 30)

6.2. Pre-Bronchoscopy Chest Computed Tomography Scan on Suspected Lung Cancer Patients

The value of chest computed tomography (CT) scan in lung cancer detection remains controversial. The sensitivity and specificity of lung mass detection on chest CT scan compared to bronchoscopy in this study was 73.7% and 70% respectively. The National Lung Screening Trial reported a higher sensitivity and specificity of 93.8% and 73.4% respectively for low dose CT scan at detecting lung cancer.(78) In the National Lung Cancer Screening Trial, however, not all patients enrolled had a bronchoscopy and biopsy and histological confirmation of lung cancer was by analysis of specimens obtained during mediastinoscopy, thoracoscopy, thoracotomy or percutaneous trans-thoracic or extra-thoracic

access.(78) This may have accounted for the higher sensitivity and specificity in the National Lung Cancer Screening Trial.

The National Lung Cancer Screening Trial demonstrated a positive and negative predictive value of 3.8% and 99.9% respectively.(78) The trial was associated with a large number of false positives contributing to the low positive predictive value.(78) A study by Lecourtois B et al, also demonstrated that low dose chest CT screening for lung cancer may be associated with false positive rates of 43%.(85) In our study, the positive and negative predictive values were 46.7% and 86.1% respectively. However, in our study, low dose chest CT scan was not used. Chest CT scans were performed and reported on by different radiology departments, depending on the referral hospital, and not all patients referred for bronchoscopy with a suspected lung mass had a chest CT scan. Nevertheless, the results of our study suggest that chest CT scan is a valuable rule out test, and should not be used in isolation for the diagnosis of lung cancer.

6.3. Findings at Bronchoscopy

6.3.1. Microbiological Findings on Bronchoscopy

The microbiological cultures on BAL demonstrated a variety of organisms including gram positive and negative bacilli and fungi. In South Africa, community acquired pneumonia in immune-competent patients is commonly caused by *Streptococcus pneumoniae, Klebsiella pneumoniae* and *Haemophilus influenzae*.(3) Community acquired pneumonia in immune-compromised patients

is caused by opportunistic pathogens namely; *Mycobacterium tuberculosis, Pneumocystis jiroveccii* and invasive fungi such as *Aspergillus fumigatus.*(3) Microbiology of nosocomial pneumonia in South Africa implicates; *Streptococcus pneumoniae, Haemophilus influenza, Staphylococcus aureus, Moraxella catarrhalis, Enterobacter species (spp), Klebsiella spp, Proteus spp, Serratia marcescens, Pseudomonas spp* and *Acinetobacter spp* in its aetiology.(96) Therefore, none of the microbiological findings on bronchoscopy, in our study, were unexpected. All bacteria cultured on BAL, have been recognised as common respiratory pathogens in South Africa, according to local community acquired pneumonia and nosocomial pneumonia guidelines.(3, 96)

A study examining the use of BAL in immune-compromised hosts, identified *Pneumocystis jirovecii (50%), Aspergillus spp (25%)* and *Candida albicans* in the cohort.(97) *Pneumocystis* and *Aspergillus* were pathogenic in 50% and 25% of cases respectively whilst *Candida* was found to be a coloniser and not a pathogen.(97) In our study, *Candida albicans* was cultured on four samples. Due to the retrospective nature of our study, it was unclear from available records whether *Candida albicans* was a coloniser or a pathogen as all patients with positive cultures were HIV uninfected or HIV status unknown and other reasons for immunosuppression were not explored.

Another study, conducted in Uganda amongst HIV infected, smear negative patients cultured *Pneumocystis jirovecci* and *Cryptococcus neoformans* on BAL.(8) At this site, *Cryptococcus neoformans* and *Candida albicans* were

detected in two HIV uninfected patients. Other aetiologies of immunosuppression and risk factors for cryptococcosis could not be definitively excluded on review of the clinical records. Although fungal culture on BAL is associated with immunosuppression, infrequently it may occur in immune-competent individuals.(98) There was insufficient information available to conclude if the fungi cultured in our study were among immune-compromised or competent individuals.

The use of bronchoscopy in the detection of TB has been described in literature for over 30 years.(7) Detection rates using acid fast bacilli (AFB) and TB culture on BAL and trans-bronchial needle biopsy samples vary from 30% to 83%.(9, 16). These detection rates were calculated as the percentage of TB detected on bronchoscopy amongst TB suspects and were not performed exclusively on smear negative patients. In our study, BAL samples were analysed for AFB, PCR and TB culture and no trans-bronchial needle biopsies were performed. The detection rate of TB using these three tests was 22.2%, which is lower than that reported in other studies. A study from Cape Town, South Africa, described TB diagnosis in 67.4% of smear negative patients only from bronchial brushings and trans-bronchial biopsy.(7) BAL samples were not used to make a diagnosis of TB as was used in our study. Although TB bronchoscopy did not assist significantly with TB diagnosis in our study, it is still a valuable tool in the diagnosis of smear negative TB.(7, 8)

Possible reasons for a low TB detection in our retrospective study may include; a low suspicion of smear negative TB at peripheral referring hospitals or non-referral of patients as empiric TB treatment may be commenced without microbiological

confirmation. This may have resulted in a smaller sample group being referred for further TB investigation in this area. Other considerations include a relatively healthy sample population even amongst HIV infected patients. The median cluster of differentiation 4 (CD4) count of 254 ±164 cells per cubic millimetre (cells/mm^3) was high and hence TB was less likely to be the cause of the lung pathology.

6.3.2. Cytological Findings on Bronchoscopy

The results of cytological analysis were non-specific. Cytological examination of BAL samples detected pathology in 13.1% of all samples analysed. A definitive diagnosis of SCC was only made on 2.5% of cases and the remaining samples were inconclusive. Although the National Institute for Health and Clinical Excellence (NICE) and American College of Chest Physicians (ACCP) guidelines mention that sputum cytology is not routinely advocated due to a low yield, there is no recommendation about cytological analysis on BAL samples.(52, 75) Three studies suggest that the test may be of value with 33%, 42.6% and 46% of cases detecting lung cancer.(99-101) It was unclear how these detection rates were calculated. Our study had a very low detection rate (3.3%), for lung cancer compared to other studies. The reasons for this are unclear.

6.3.3. Histological Findings on Bronchoscopy

Squamous cell carcinoma (SCC) was the most frequent malignant finding on histology. Large, multicentre Egyptian and Scottish studies reviewing 3 980 and 2

238 bronchoscopies respectively, demonstrate a 70% and 78% diagnostic yield of lung cancer.(17, 102) In the Scottish study, multiple biopsy specimens were taken, which may have contributed to this higher yield of malignancy on biopsy.(102) Where a 'frank tumour' was seen on bronchoscopy, histological confirmation ranged between 80% and 95%, whereas lesions viewed as 'possible malignant or normal' confirmed only 4% of malignancy.(102) In other studies from Singapore, London, England, United States and Norway suggest a wide range of diagnostic yields of lung cancer with a range of 22.4% to 88% diagnosed on biopsy.(100, 103-105) Discrepancies in detection rates may be attributed to differences in sample sizes, demographics, risk factors and number of biopsy samples taken.

Our study demonstrates a 46.5% diagnostic yield of malignancy on bronchoscopy. It is unknown how many biopsy samples were taken during each bronchoscopy and this may have negatively affected the diagnostic yield. Other reasons for the yield of this study being lower than other studies may include technical difficulties in accessing the tumours during bronchoscopy.

In our retrospective sample one patient worked on a gold mine and was therefore at risk for silicosis. It is unclear if there is an exposure-response relationship between lung cancer and silicosis.(62, 106) Hypothesized theories include that silicosis may lead to inflammation of lung parenchyma with metaplastic changes.(106) There was no evidence of silicosis in the patient who worked on a

gold mine and it remains unclear if occupational exposure to silica dust was a risk factor for lung cancer.

6.4. Tuberculosis Diagnosis and Broncho-alveolar Lavage in Sputum Smear Negative Patients

Tuberculosis is the leading cause of mortality in HIV infected and uninfected South Africans.(2) The diagnosis of TB is particularly challenging when sputum for AFB is negative but a high index of suspicion for TB infection remains.(37, 107) Analysis of BAL for AFB or TB culture has been very useful in TB diagnosis.(46, 108-110) Detection of AFB on BAL samples ranges between 12% and 26% (46, 108-111). TB detection using culture performed on BAL samples ranges between 25% and 30%.(16, 46)

Despite our study being conducted in a high TB/HIV prevalence area, the detection of TB on bronchoscopy was low. BAL for AFB had a yield of 11.1% in smear negative patients. The mean CD4 count of HIV individuals in this study was 254 ±164 cells/mm^3. These HIV infected individuals were therefore relatively "healthy" and did not have TB. This suggests healthy user bias. Referral bias whereby all TB suspects with a negative smear were not appropriately referred and survival bias whereby smear negative TB patients died prior to referral, may also have contributed to the findings.

GeneXpert® performed on BAL samples offers the advantages of a reduced time to diagnosis, early identification of rifampicin resistance and a higher sensitivity than sputum smears and culture.(47) In a study conducted in Cape Town, the sensitivity of GeneXpert® performed on BAL samples, amongst smear negative cases, was 60%.(47) A negative predictive value of 15% was found in those with HIV infection.(47) As our study was conducted in 2011 and GeneXpert® was not available for the entire study period, the value of GeneXpert® in bronchoscopy specimens, at this site, could not be assessed.

6.5. Common Types of Lung Cancer on Biopsy

Squamous cell carcinoma was the most frequently diagnosed lung cancer, followed by adenocarcinoma. The findings in our cohort are similar to study findings in Bloemfontein and Johannesburg where SCC was more common than adenocarcinoma.(18, 19) In Cape Town, however, adenocarcinoma was the most commonly diagnosed lung malignancy.(20) Internationally, the histological subtypes of lung cancer vary geographically, with adenocarcinoma more common in North America(75) and SCC more common in Algeria, Tunisia, Morocco and Kuala Lumpur.(103, 112)

Recent studies suggest that adenocarcinoma is more common than previously reported.(75) A North American series describes a recent change of the most common lung cancer from SCC to adenocarcinoma.(75) However, these studies included more females with lung cancer than males.(75) Other areas namely; Egypt, Jordan, Palestine, Saudi Arabia and United Arab Emirates also describe

higher adenocarcinoma to SCC ratios in females.(112) This may be due to hormonal factors or change in smoking trends. In the Cape Town study, a gender difference was not described,(20) but in the Bloemfontein and Johannesburg study, adenocarcinoma was more common in males compared to females.(18, 19) Similarly, in our study, adenocarcinoma was only diagnosed in males, perhaps reflecting similar smoking trends or social habits to Bloemfontein and Johannesburg. The unequal distribution of males and females in this study may account for SCC being the predominant lung cancer type.

The majority of the SCC patients in our study were African males. When compared to the Bloemfontein study, SCC was almost equally distributed between African and White racial groups with the coloured population having the least SCC diagnoses.(18) Conversely, SCC was most frequent in the White population in the Johannesburg cohort.(19) Variation regarding types of lung cancer in South Africa appears to differ geographically rather than racially.

Cigarette smoking is a well described risk factor for SCC.(55, 56) A study conducted in Spain links an 80 pack year smoking history exclusively with SCC(56). Unfortunately, specific smoking pack year history was poorly documented in the chart notes in this retrospective study. Nevertheless, smoking is likely to be the main risk factor for SCC in patients in our setting too.

6.6. Outcomes of Bronchoscopy

Bronchoscopy assisted with the diagnosis of broncho-respiratory pathology for 35.5% of the sample group. Studies in Saudi Arabia and Egypt demonstrated higher overall diagnostic yields of 58% and 67% respectively.(17, 30) In these studies, the majority of bronchoscopy diagnoses were in patients referred for suspected lung cancer or TB.(17, 30) In our study, the highest diagnostic rates was in patients referred for investigation of diffuse pulmonary infiltrates and suspected lung mass with 46.7% and 46.5% achieving a microbiological and histological diagnosis respectively. Although suspected TB was the second most requested indication for bronchoscopy, it was not a common finding on samples obtained by bronchoscopy.

As South Africa has the leading HIV associated incident TB cases globally and smear negative TB is more common in HIV infected patients, a high index of suspicion for the diagnosis of TB should be maintained.(33) Patients with true smear negative TB, maybe hypoxic and unable to tolerate a bronchoscopy, which may have contributed to an underestimation of TB, using BAL in the diagnosis of TB in our study.

In our study, biopsy confirmed lung cancer in 46.5% of all bronchoscopies performed for suspected malignancy. This result demonstrates a low yield compared to other studies. Detection rates of lung cancer on biopsy during bronchoscopy vary from 57% to 94% in different studies.(16, 30, 88-90) This was calculated as the percentage of lung cancer detected on biopsy during bronchoscopy amongst those patients referred with suspected malignancy.

6.7. Complications of Bronchoscopy

The documented complication rates of bronchoscopy vary from 0.5% to 11%.(28, 29, 113-115) In a large multicentre study of 20 986 bronchoscopies in Italy, the complication rate was 1.08% and the largest number of complications were associated with trans-bronchial needle biopsy.(29) The common complications were haemoptysis (0.45%), hypoxaemia (0.12%) and pneumothorax (0.1%).(29) In this study, 3.7% of patients experienced a complication during or after bronchoscopy. These complications were hypoxaemia and haemoptysis and were recognised complications in other studies.(28, 29, 114, 115)

The frequency of complications of our study was within the described and expected range. In the study by Dreiser et al, which noted a higher complication rate of 11%, bronchoscopy procedures were performed mainly by pulmonary fellows in training reflecting less developed technical skills and was associated with trans-bronchial needle biopsies mainly.(113) Due to the prospective study design it was more likely to detect even minor complications that may have been overlooked in retrospective studies.(113) In our study, however, all bronchoscopies were performed by a trained expert and the design was retrospective in nature. Furthermore, no trans-thoracic needle biopsies were undertaken. It is unclear whether the smaller sample size attributed to the relatively higher frequency of complications.

6.8. Study Limitations

There are several limitations that were identified in our study. The study was conducted at a tertiary referral hospital and hence referral bias limits the generalisation of the findings. In addition, patients who undergo bronchoscopy need to be medically “fit” to tolerate the bronchoscopy as hypoxia is a pre-procedure exclusion criterion. Patients who are very ill with respiratory illness and are unstable for transfer to the tertiary level may also not be eligible for bronchoscopy. These factors may have contributed to selection bias.

Due to the retrospective study design, information was limited to what was already noted in clinical records. Missing and incomplete records resulted in missing data. This further contributed to bias.

The sample size was limited by the number of bronchoscopies done in the year. Although data was collected for a year, not every patient with suspected smear negative TB, suspected malignancy and/ or other respiratory pathologies where no definitive diagnosis was made, was referred for a bronchoscopy. This resulted in referral bias.

CHAPTER 7

CONCLUSIONS

The value of the bronchoscopy in the diagnosis of lower respiratory tract infections (LRTI) and lung cancer is well established. Studies describing the indications and outcomes of bronchoscopy are mainly from the Middle East, United Kingdom and United States of America and there is a lack of information from South Africa and other resource limited settings.

Bronchoscopy assisted with microbiological, cytological or histological diagnosis of broncho-respiratory pathology in over one third of patients in this study. Although the commonest indication for bronchoscopy was the investigation of a lung mass, a diagnosis was more commonly established in those referred for investigation of diffuse pulmonary infiltrates. Bronchoscopy confirmed a microbiological diagnosis of LRTI in just under half of all patients referred for investigation of diffuse pulmonary infiltrates. This is an important finding in South Africa because pneumonia and tuberculosis (TB) are the leading causes of mortality.(2) Samples collected during bronchoscopy aid in microbiological diagnoses which leads to prompt and directed antibiotic treatment. This may assist in reducing mortality from pneumonia, however, further studies assessing the value of bronchoscopy in the treatment and mortality of non-resolving LRTI in KwaZulu Natal are needed.

KwaZulu Natal has the highest prevalence of drug resistant TB in South Africa.(33) TB is the main cause of mortality in this region.(2) In this study, the prevalence of TB on smear negative patients was 11.1% of those referred with suspected TB. This finding was lower than that described in other studies. There are, however, few studies of this nature in South Africa. The low TB detection rate in this study was attributed to referral and survival bias as the study was conducted at a tertiary hospital. Bronchoscopy as a means to detect TB appears to be largely under-utilised in this setting.

According to the national cancer registry of 2006, lung cancer was the fourth most common type of cancer in males.(12) Due to a lack of an updated cancer registry since 2006 the true burden of cancer in South Africa is unknown. Information regarding lung cancer in South Africa has therefore only been available through limited studies. In this study bronchoscopy confirmed a histological diagnosis of lung cancer in over one third of patients referred with a suspected lung mass.

Different subtypes of lung cancer appear to be endemic to different areas in South Africa. In Cape Town, adenocarcinoma is the most frequent lung cancer,(20) whilst in Bloemfontein and Johannesburg squamous cell carcinoma (SCC) is more common.(18, 19) In this study, SCC was the most frequently diagnosed lung cancer. The reasons for the geographical difference in histological subtypes of lung cancer are unknown and may be attributed to smoking trends and different types of environmental risk factors.

There are several limitations that were identified in this study. Referral and survival bias were therefore major limitations in the study as Greys Hospital is a tertiary hospital that requires patients to be referred by surrounding district hospitals before they receive special investigation and/ or treatment.

Due to the retrospective study design and lack of standardised clinical case record information, availability of data was limited to clinical and laboratory records only. Missing and incomplete records further contributed to the bias of the findings. This limits the generalisation of the findings in this study.

CHAPTER 8

OPERATIONAL RECOMMENDATIONS AND FUTURE DIRECTIONS

8.1. Respiratory tract infection is the leading cause of natural deaths in South Africa.(2) This study demonstrates that bronchoscopy was useful in assisting with microbiological diagnosis of pneumonia in patients with diffuse pulmonary infiltrates and non-resolving lower respiratory tract infections (LRTI). Prompt referral of patients with diffuse pulmonary infiltrates and non-resolving LRTI is recommended to prevent the morbidity and mortality associated with pneumonia.

8.2. Bronchoscopy is a limited resource in KwaZulu Natal. It is performed by specialist pulmonologists and therefore the services are currently only available at two public sector hospitals in KwaZulu Natal. Bronchoscopy, nevertheless, remains a valuable resource in the diagnosis of smear negative tuberculosis (TB). The prevalence of TB on broncho-alveolar lavage (BAL) in smear negative cases was 11.1% which is lower than other centres. The use of BAL for the diagnosis of TB is largely an underutilised resource in Greys Hospital. Timeous referral of smear negative TB suspects is recommended to establish a diagnosis and ameliorate the mortality associated with TB.

8.3. This study was conducted during transition from using sputum acid fast bacilli (AFB) to GeneXpert® for the diagnosis of TB in South Africa. GeneXpert® was

available toward the end of 2011. GeneXpert® in the diagnosis of TB is now commonly utilised in South Africa. Although it can be used on gastric lavage samples, GeneXpert® on BAL samples is not yet advised by the Department of Health. (Appendix 1) More studies are required to establish the operational efficacy of GeneXpert® on BAL samples in the diagnosis of TB.

8.4. In this study, squamous cell carcinoma (SCC) was the most common lung cancer diagnosed. There appears to be a geographic variation of histological subtypes of lung cancer across South Africa, however, the reasons for this are still to be established. A multicentre, prospective study of risk factors and incidence of lung cancer is recommended to determine the risk factors and burden of disease for the different subtypes of lung cancer.

8.5. Human immunodeficiency virus (HIV) and TB have an unclear causal relationship with lung cancer. Due to the high co-infection rates of TB/HIV in South Africa this is an ideal site to explore this association.

8.6. In this study chest computed tomography (CT) scan was compared to bronchoscopy in the detection of lung cancer. Chest CT scan had a high negative predictive value suggesting that it was a useful rule out test. Chest CT scan is therefore recommended prior to bronchoscopy in all patients in whom lung cancer is suspected.

8.7. Lack of standardised reports of patients undergoing bronchoscopy resulted in missing information and results of investigations. It is recommended that all patients be reviewed by the pulmonology team prior to and post bronchoscopy to ensure that all sputum, bronchoscopy and radiology results are indexed in a standardised format and followed up.

8.8. This bronchoscopy unit lacks more updated bronchoscopy equipment. The unit is unable to perform endobronchial ultrasounds, auto-fluorescent bronchoscopy, electromagnetic bronchoscopy and navigational bronchoscopy. This limits the outcomes of bronchoscopy in this setting.

CHAPTER 9

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APPENDICES

APPENDIX 1

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

Private Bag X828, PRETORIA, 0001, 87th Floor, Room 2710, Civitas, Cnr Thabo Sehume & Struben Street, PRETORIA, 0001
Tel: +27 (0) 12 395 8000, Fax: +27 (0) 12 395 5422

Dr S Zungu
Head of Health
Department of Health: KwaZulu-Natal Province
Private Bag X9051
PIETERMARITZBURG
3201

Dear Dr Zungu

RE: REVISED POLICY ON THE USE OF THE XPERT MTB/RIF IN DIAGNOSING TB AND RIFAMPICIN RESISTANT TB IN CHILDREN AND ADULTS

As you are aware, we have completed the roll out of the Xpert MTB/ RIF assay throughout the country. This assay has been used as the first test in diagnosing TB in all people who present with symptoms of pulmonary TB in our health facilities. The diagnosis of extra pulmonary TB still remained a challenge especially in people living with HIV and children less than 5 years, who are the most affected by this form of TB disease.

The World Health Organisation has since revised the policy on the use of Xpert MTB/RIF based on recent evidence. The implications of these recommendations are that:

1) Xpert MTB/RIF will now be used to diagnose TB;
    - In gastric washing, gastric lavage specimens obtained from children and adults.
    - In cerebrospinal fluid obtained from children and adults presenting with symptoms of TB meningitis
    - In pleural biopsy specimen obtained from children and adults presenting with pleural effusions

- Lymph node fine needle aspirates obtained from children and adults presenting with lymphadenitis

2) Only one specimen should be collected for initial diagnosis using Xpert MTB/RIF
3) Microscopy and culture remains essential for monitoring treatment and for performing drug susceptibility testing to other drugs.

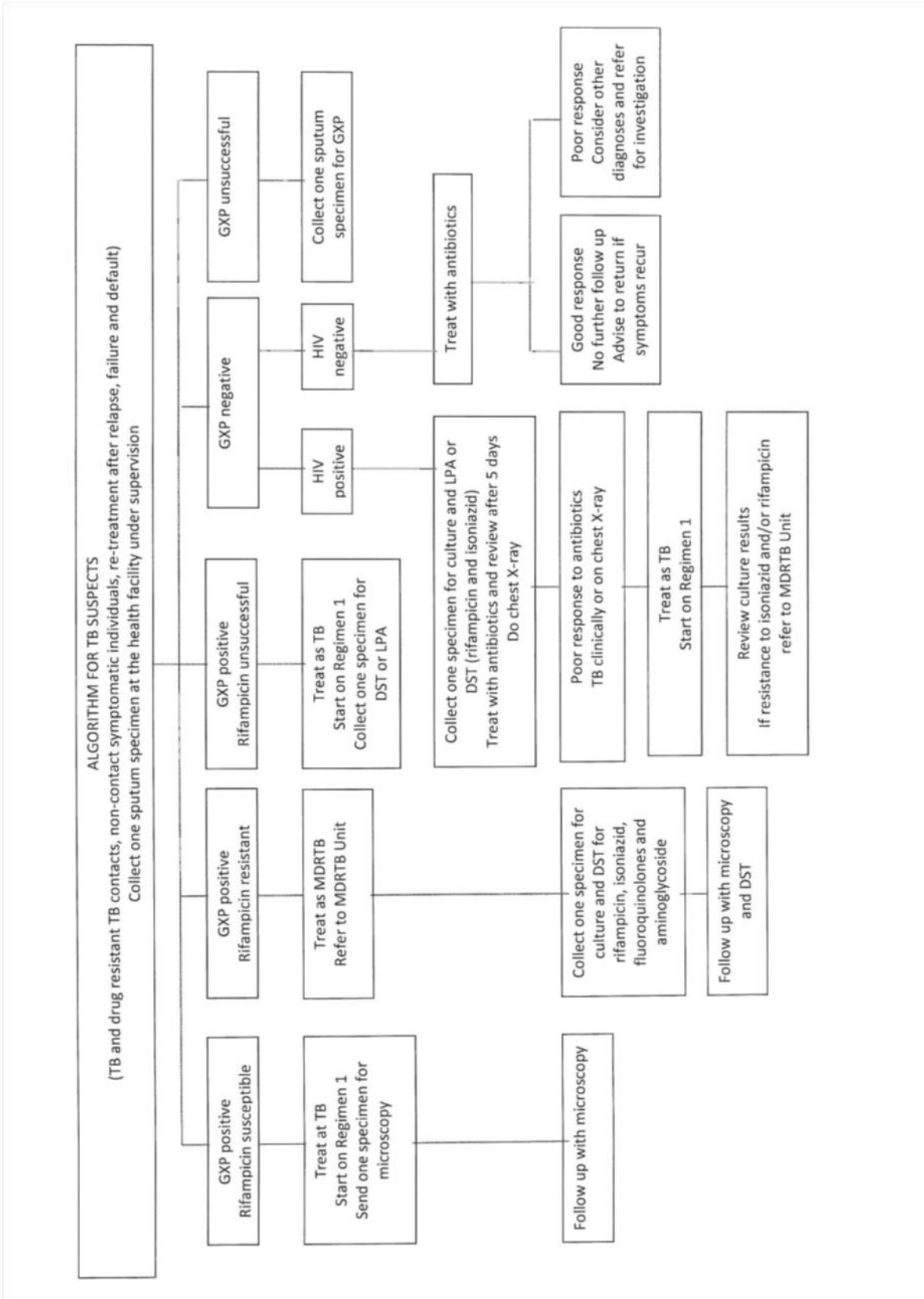
The National Health Laboratory Services (NHLS) has developed the laboratory standard operating procedures on the specimen preparation required for the testing and all laboratories informed. The clinical standard operating procedures will finalised and circulated during March by the Cluster: TB Control and Management. In the mean time for any additional information, Dr Lindiwe Mvusi may be contacted at MvusiL@health.gov.za or 012 395 8815.

These recommendations can be implemented with immediate effect. I write to request your assistance in ensuring that this information is communicated to all health facilities to ensure early diagnosis and appropriate treatment for TB in children and people living with HIV and AIDS.

Kind regards

DR YOGAN PILLAY
DEPUTY DIRECTOR GENERAL: HIV/AIDS, TB & MCWH
DATE: 12/2/2014

APPENDIX 2


ALGORITHM FOR TB SUSPECTS
(TB and drug resistant TB contacts, non-contact symptomatic individuals, re-treatment after relapse, failure and default)
Collect one sputum specimen at the health facility under supervision
GXP positive
Rifampicin susceptible
Treat at TB
Start on Regimen 1
Send one specimen for microscopy
Follow up with microscopy
GXP positive
Rifampicin resistant
Treat as MDRTB
Refer to MDRTB Unit
Collect one specimen for culture and DST for rifampicin, isoniazid, fluoroquinolones and aminoglycoside
Follow up with microscopy and DST
GXP positive
Rifampicin unsuccessful
Treat as TB
Start on Regimen 1
Collect one specimen for DST or LPA
Review culture results
If resistance to isoniazid and/or rifampicin refer to MDRTB Unit
GXP negative
HIV positive
Collect one specimen for culture and LPA or DST (rifampicin and isoniazid)
Treat with antibiotics and review after 5 days
Do chest X-ray
Poor response to antibiotics
TB clinically or on chest X-ray
Treat as TB
Start on Regimen 1
HIV negative
Treat with antibiotics
Good response
No further follow up
Advise to return if symptoms recur
Poor response
Consider other diagnoses and refer for investigation
GXP unsuccessful
Collect one sputum specimen for GXP

APPENDIX 3

CASE REPORT FORM

IDENTIFICATION

Identification study number: _ _ _ _ _ _

DEMOGRAPHICS

Date of birth _ _ _ _/ _ _/ _ _

Age:

Gender: Female ☐ Male ☐

Race: African ☐ White ☐ Coloured ☐ Asian ☐ Other ☐

REFERRAL

Was the patient referred to Greys Hospital? Yes ☐ No ☐

- If yes, name of the referral hospital/clinic/GP? _ _ _ _ _ _ _ _ _ _

What is the final outcome of the patient?

Discharged ☐ Died ☐ RHT ☐ Transferred ☐ other_ _ _ _ _ _

PRE-BRONCHOSCOPY ASSESSMENT

What is the patient's haemoglobin? _ _ _ _ _g/dl

What is the patient's INR? _ _ _ _ _

What is the oxygen saturation of the patient? _ _ _ _ _%

INDICATIONS

What was the indication for the bronchoscopy?

Haemoptysis ☐ Lung Mass ☐ Non resolving LRTI ☐ Suspected TB ☐ Diffuse pulmonary infiltrates ☐ Other ☐ Specify _ _ _ _ _ _

What is the patients HIV status? Positive ☐ Negative ☐ Unknown ☐

- If positive what is the corresponding CD4 count? _ _ _ _
- Is the patient on antiretrovirals? Yes ☐ No ☐
- If yes what medications? 3TC ☐ TDF ☐ EFV ☐

  D4T ☐ AZT ☐ NVP ☐

  ABC ☐ Ritonavir/loponivir ☐ other: _ _ _ _ _ _ _

Does the patient smoke? Yes ☐ No ☐

- If yes, how many pack years? _ _ _ _ _ _ _
- If no, has the patient ever smoked previously? Yes ☐ No ☐

Family history of malignancy? Yes ☐ No ☐

- Primary lung malignancy ☐ other ☐ _ _ _ _ _ _ _

Occupational history?

- Exposure to lung irritants? Yes ☐ No ☐
- What irritant? _ _ _ _ _ _ _ _ _ _ _ _ _
- Protective equipment used? Yes ☐ No ☐

Was sputum collected? Yes ☐ No ☐

- MC+S result? _ _ _ _ _ _ _
- Ziel Neelson AFB result? Positive ☐ Negative ☐

  culture result? _ _ _ _ _

  sensitivity result? _ _ _ _ _
- cytology result? Malignant cells ☐ No malignant cells ☐ other _ _ _

RADIOLOGY

Was a chest radiograph done? Yes ☐ No ☐

- If yes does it report a: mass ☐ infiltration ☐ infection ☐ other specify _ _ _

Was a CT Chest done? Yes ☐ No ☐

- If yes does it report a: mass ☐ infiltration ☐ infection ☐ other specify _ _ _

DESCRIPTION AND SAMPLING AT BRONCHOSCOPY

Endobronchial biopsy Yes ☐ No ☐

Transbronchial biopsy Yes ☐ No ☐

BAL: MCS ☐ AFB ☐ TB Culture ☐ Fungal ☐ PCP ☐

Cytology ☐

Was a mass visualised? Yes ☐ No ☐

Description of mass _ _ _ _ _ _ _

RESULTS

Was the result of the sample available? Yes ☐ No ☐

- If yes specify_ _ _ _ _ _ _ _ _ _ _ _ _ _
- Histology result_ _ _ _ _ _ _ _ _ _
- If no could it be traced? Yes ☐ No ☐

Was the specimen sufficient? Yes ☐ No ☐

Did the sample leak? Yes ☐ No ☐

Was there a review by pulmonology? Yes ☐ No ☐

COMPLICATIONS

Where there any procedure related complications? Yes ☐ No ☐

If yes specify: Haemoptysis ☐ Pneumothorax ☐ Bronchospasm ☐

Laryngospasm ☐ Other _ _ _ _ _ _ _

APPENDIX 4

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

19 June 2013

Dr. Y Ramkilawan
P O Box 612
Luxmi
3207
yramkillawan@yahoo.com

PROTOCOL: A description of the profile of the patients and outcomes of fiberoptic bronchoscopies, performed at a tertiary care hospital in KwaZulu- Natal, South Africa, between January to December 2011. REF: BE144/13.

EXPEDITED APPLICATION - RATIFICATION

This letter serves to notify you that at a full sitting of the Biomedical Research Ethics Committee meeting held on **11 June 2013**, the Committee **RATIFIED** the sub-committee's decision to approve the above study.

Yours sincerely

Mrs A Marimuthu
Senior Administrator: Biomedical Research Ethics

APPENDIX 5

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI

RESEARCH OFFICE
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Westville Campus
Govan Mbeki Building
Private Bag X 54001
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4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 260-4609
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/ResearchEthics/BiomedicalResearchEthics.aspx

19 June 2014

Dr. Y Ramkilawan
P O Box 612
Luxmi
3207
yramkillawan@yahoo.com

PROTOCOL: A description of the profile of the patients and outcomes of fiberoptic bronchoscopies, performed at a tertiary care hospital in KwaZulu- Natal, South Africa, between January to December 2011. REF: BE144/13.

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	31 May 2014
Expiration of Ethical Approval:	30 May 2015

I wish to advise you that your application for Recertification dated 30 May 2014 for the above protocol has been noted and approved by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The approval will be ratified by a full Committee at a meeting to be held on **08 July 2014**.

Yours sincerely

Professor D Wassenaar
Chair: Biomedical Research Ethics Committee

APPENDIX 6

PERMISSION TO CONDUCT A RESEARCH STUDY/TRIAL

This must be completed and submitted to the Medical Superintendent/s / Hospital Manager for signature.

For King Edward VIII Hospital (KEH) and Inkosi Albert Luthuli Central Hospital (IALCH) studies please submit together with the following:

i) Two copies of the final, approved protocol
ii) Letter giving provisional ethical approval
iii) Details of other research presently being performed by yourself (individually or as a collaborator)
iv) Details of any financial or human resource implications to King Edward VIII Hospital
v) If a clinical trial, please produce proof of payment or intention thereof to KEH

Once the document has been signed it should be returned to this office so that full ethical approval can be granted.

To: Hospital Manager

PROTOCOL

Permission is requested to conduct the above research study at the hospital/s indicated below:

Site 1 address: Investigator/s:

Greys Hospital
Private bag X9001
Pietermaritzburg

Principal: Dr Y Ramkillawan
Supervisor: Dr H Dawood

Co-Investigator: Dr KT Naidoo

HOSPITAL MANAGER
GREY'S HOSPITAL
PRIVATE BAG X9001
PIETERMARITZBURG

Signature of Hospital Manager : _____ Date: 17/5/2013

Site 2 address: Investigator/s

_____ Principal: _____
_____ Co-investigator: _____
_____ Co-Investigator: _____

Signature of Hospital Manager :
_____ Date: _____

NB: Hospital Manager/s to send a copy of this document to Natalia.

LIST OF ABBREVIATIONS

ACCP - American College of Chest Physicians

AFB - Acid fast bacilli

ART - Antiretroviral therapy

BAL - Broncho-alveolar lavage

Cells/mm^3 - Cells per cubic millimetre

CD4 - Cluster of differentiation 4

cfu - Colony forming units

CI - Confidence interval

cm - Centimetres

COPD - Chronic obstructive pulmonary disease

CT - Computed tomography

DNA - Deoxyribonucleic acid

DST - Drug susceptibility testing

e.g. - as an example

EGFR - Epidermal growth factor receptor

ERBB2 - v-erb-b2 avian erythroblastic leukaemia viral oncogene homolog 2

g/dl - Grams per decilitre

Hb - Haemoglobin

HIV - Human immunodeficiency virus

INR - International normalised ratio

IQR – Interquartile range

KRAS - Kirsten rat sarcoma viral oncogene homolog

KZN - KwaZulu Natal

LPA - Line probe assay

LRTI - Lower respiratory tract infection

MC&S - Microscopy, culture and sensitivity

MDR TB - Multidrug resistant tuberculosis

mg/kg - Milligrams per kilogram

ml - Millilitres

MTB/RIF - Mycobacterium tuberculosis/ rifampicin

NHLS - National health laboratory service

NICE - National institute for health and clinical excellence

NNRTI - Non-nucleoside reverse transcriptase inhibitor

NRTI - Nucleoside reverse transcriptase inhibitor

PACS - Picture archiving and communication centre

PCP - Pneumocystis jirovecii pneumonia

PCR - Polymerase chain reaction

PI - Protease inhibitor

SCC - Squamous cell carcinoma

SD - Standard deviation

Spp - Species

TB - Tuberculosis

XDR TB - Extreme drug resistant tuberculosis

ZN - Ziehl Neelsen