

https://dspace.org/

Determining the aetiology of exudative pleural effusions in patients undergoing pleural biopsy for diagnosis at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)

Sirriram, Sanvir

2017

https://hdl.handle.net/10539/25618

Downloaded from a DSpace repository. https://dspace.org/

DETERMINING THE AETIOLOGY OF EXUDATIVE PLEURAL EFFUSIONS IN PATIENTS UNDERGOING PLEURAL BIOPSY FOR DIAGNOSIS AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL (CMJAH)

Sanvir Sirriram

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine

2017, Johannesburg

DECLARATION:

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

_____

_____ day of_____ , 2017

ABSTRACT

Background and objectives: The vast range of potential aetiologies of exudative pleural effusions frequently poses a diagnostic dilemma for the treating physician. It is important for the physician to have a basic knowledge of the common conditions which cause exudative pleural effusions in their setting in order to direct their investigations appropriately and cost effectively. The aims of this study were to describe the demographic profile of patients admitted for investigation of exudative pleural effusions to Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in Johannesburg, South Africa, and to describe their aetiologies, as determined by the investigations performed.

Methods: This was a retrospective record review of 162 patients with exudative pleural effusions, confirmed on pleural fluid analysis, admitted to CMJAH for investigation over a period of one year from the 1st January 2015 to the 31st December 2015. An analysis of patient demographics, HIV status, smoking status, results of investigations and the patient diagnoses were undertaken.

Results: Of the 162 patients, 100 (61.7%) were male and 62 (38.3%) were female. The mean age, overall, was 44 years. With regard to HIV status, 78 (48.1%) patients were HIV-seronegative, 76 (46.9%) were HIV-seropositive and 8 (4.9%) were of unknown HIV status. HIV-seropositive patients were younger (mean age 39.2 years) than the HIV-seronegative patients (mean age 49.5 years; $p < 0.01$). There was no difference in gender in those patients who were HIV-seropositive ($p = 0.502$) and racial influence could not be evaluated due to the fact that most patients were of African origin. Overall, on pleural biopsy, 45 of the patients (27.8%) were diagnosed as having tuberculosis (TB), 40 (24.7%) had bacterial empyema, 37 (22.8%) had

malignancy, 29 (17.9%) had chronic pleuritis, 8 (4.8%) had an inconclusive diagnosis and 3 (1.9%) had a combined diagnosis of TB and malignancy.

Amongst the HIV-seropositive patient group, the diagnoses were as follows; 26 patients had TB, 24 patients had bacterial empyema, 12 patients had chronic pleuritis, 9 patients had malignancy, 3 cases were inconclusive and a combination of both TB and malignancy was present in 2 patients. There was a higher incidence of infective aetiologies in HIV-seropositive patients. Amongst the HIV-seronegative patient group, the diagnoses were as follows; 28 patients had malignancy, 16 patients had bacterial empyema, 16 patients had chronic pleuritis, 14 patients had TB, 3 cases were inconclusive and a combination of both TB and malignancy was present in 1 patient. There was a higher incidence of malignancy in the HIV-seronegative patients. TB was predominantly found in the younger, HIV-seropositive patients ($p = 0.01$ and $p = 0.006$, respectively). Patients that were female, older patients (mean 57.5 years) and those of negative HIV status were more likely to have malignancy ($p = 0.04$, $p = 0.01$ and $p = 0.006$, respectively). Although there was a trend to an association between smoking status and the occurrence of malignancy ($p = 0.081$), this did not reach statistical significance. This could simply be a consequence of small patient numbers and it was not recorded if patients were previous smokers. The causative organisms for the bacterial empyemas found in this study included: *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Discussion: Overall the main aetiologies of exudative pleural effusions of patients at CMJAH were TB, bacterial empyema, chronic pleuritis and malignancy. These finding were similar to studies done in other third world countries.

ACKNOWLEDGEMENTS

I am extremely grateful to Professor Charles Feldman and Dr Kate Dudgeon for their supervision and guidance from beginning to end of my MMed. I would also like to thank Dr Naseema Vorajee, Anatomical Pathologist at the National Institute of Occupational Health (NIOH), who assisted me with accessing the data for my MMed. I would also like to acknowledge Dr Preesha Premsagar for her support and assistance.

TABLE OF CONTENTS	Page
• **DECLARATION** • **ABSTRACT** • **ACKNOWLEDGEMENTS** • **TABLE OF CONTENTS** • **LIST OF TABLES** • **LIST OF ABBREVIATIONS**	**ii** **iii** **v** **vi** **ix** **x**
1. LITERATURE REVIEW	1
1.1 Introduction	1
1.2 Anatomy and pathophysiology	2
1.3 Clinical presentation of pleural effusions 1.3.1 History 1.3.2 Symptoms of pleural effusion 1.3.3 Signs of pleural effusion	3 3 4 5
1.4 Radiology and confirmation of an effusion	6
1.5 Investigations for a pleural effusion 1.5.1 Thoracentesis sampling 1.5.1.1 Appearance and odour of pleural fluid 1.5.1.2 Differentiating a transudate from an exudate on pleural fluid analysis 1.5.1.3 Aetiology for the exudative effusion 1.5.1.4 Tests done routinely on pleural fluid 1.5.1.5 Ancillary tests done on pleural fluid for rarer diagnoses that cause an exudative effusion 1.5.2 Sputum investigation 1.5.3 Pleural biopsy sampling	6 6 7 8 9 10 12 13 13
1.6 Management	14

TABLE OF CONTENTS	Page
2.1 Aim Of The Study	15
2.2 Objectives	15
2.3 Methods	15
2.3.1 Study population and data collection methods	15
2.3.2 Patient variables collected	17
2.3.3 Sample size	17
2.3.4 Data analysis	17
3. RESULTS	17
3.1 Patients included in the study group	18
3.2 Description of the study group	18
3.2.1 Demographics and clinical features	18
3.2.2 Thoracentesis results	19
3.2.3 Aetiology	20
3.2.4 Risk factors	21
3.3 Association between demographics and clinical factors	22
3.3.1 HIV status and demographics	23
3.3.2 Influence of biopsy method, department, demographics and risk factors on aetiological findings	24
3.3.3 The factors associated with a diagnosis of TB	27
3.3.4 The factors associated with a diagnosis of malignancy	28
3.3.5 Comparison of the sampling methods used, the investigations performed on the samples, and the results obtained	29
3.3.6 Investigations yielding bacterial pathogens (other than TB) on different samples	31
3.3.7 Pathogens isolated causing empyema	32
3.4 Overall conclusions of the study	33

TABLE OF CONTENTS	Page
4. DISCUSSION	34
5. POTENTIAL LIMITATIONS OF THE CURRENT STUDY	41
6. CONCLUSION	42
7. FUTURE RESEARCH	42
8. APPENDIX A – DATA SHEET	44
9. APPENDIX B – ETHICS APPROVAL	46
10. REFERENCES	47

LIST OF TABLES	Page
1.1. Relationship of exudative pleural fluid appearance and odour to the aetiology	7
1.2. Light's criteria, for differentiating transudative from exudative pleural effusions	8
1.3. Aetiology of exudative pleural effusions	9
1.4. Characteristics of pleural fluid which indicates an empyema	10
1.5. Examples of ancillary tests done on pleural fluid, when indicated	12
2.1. Inclusion and exclusion criteria for the study	16
3.1. Demographics and clinical features	19
3.2. Thoracentesis results	19
3.3. Aetiology of exudative pleural effusions in 162 patients	20
3.4. Aetiology amongst HIV-seropositive patients	21
3.5. Aetiology amongst HIV-seronegative patients	21
3.6. Summary of the statistical analyses undertaken	23
3.7. Relationship between HIV status and demographics	24
3.8. Influence of biopsy method, department, demographics and risk factors on aetiological findings	26
3.9. Factors associated with TB	27
3.10. Factors associated with a diagnosis of malignancy	28
3.11. Comparison of sampling methods used, investigations performed on the samples, and the results obtained	30
3.12. Bacterial organisms identified from investigations	31
3.13. Bacterial pathogens causing empyema (other than TB)	32

LIST OF ABBREVIATIONS

TB	Tuberculosis
MCS	Microscopy, culture and sensitivity
PA	Postero-anterior
CXR	Chest radiograph
CT scan	Computerised tomographic scan
LDH	Lactate dehydrogenase
ADA	Adenosine deaminase
HIV	Human immunodeficiency virus
ICD	Intercostal drain
PCR	Polymerase chain reaction
LE cells	Lupus erythematosus cells
ANA	Anti-nuclear antibody
CMJAH	Charlotte Maxeke Johannesburg academic hospital
NHLS	National health laboratory systems
NIOH	National institute for occupational health
AFB	Acid fast bacillus
SD	Standard deviation
SE	Standard error
N	Total number of patients in the study
WHO	World health organisation

1. LITERATURE REVIEW

1.1 Introduction

The lungs are lined by two membranes that are in continuation with one another. These membranes are known as the pleura and between them is a potential space. When this space is filled by the abnormal accumulation of fluid, the condition is known as a pleural effusion, and this indicates an underlying disease process (McGrath & Anderson, 2011). There are many disease processes that may result in a pleural effusion. Therefore, when a patient presents to the respiratory physician with a pleural effusion, further evaluation is necessary to determine the underlying aetiology. Understandably, the respiratory physician requires an approach to a patient with a pleural effusion in order to establish a diagnosis and to direct further treatment.

The chest radiograph is used to confirm the clinical suspicion of a pleural effusion, and to determine the size and location of the effusion. The clinician then performs a thoracentesis to obtain a sample of fluid which establishes whether the effusion is an exudative or a transudate using Light's criteria (see later), which is the gold standard.

Exudative effusions may indicate a sinister local pathology as opposed to transudative effusions. Some causes of transudative effusions include cardiac failure, nephrotic syndrome and renal failure. Exudative pleural effusions can be caused by tuberculosis (TB) and lung malignancies, both of which are common in our population. However, despite this high burden of lung disease in South Africa, very little research has been performed determining the common aetiologies of exudative pleural effusions amongst South African patients. In addition to

establishing whether the effusion is a transudate or exudate, a number of other investigations, including microscopy, culture and sensitivity (MCS), cytology, and TB testing (GeneXpert MTB/Rif assay, acid fast bacilli, TB culture) are undertaken on the pleural fluid, in the case of exudative effusions, to try and determine the underlying aetiology. Similar microbiological and cytological testing may also be undertaken on sputum if the patient is productive of sputum. For those patients with an exudative effusion, in whom thoracentesis and sputum investigation are unable to assist in establishing an aetiology, further investigation with a pleural biopsy is indicated. The pleural biopsy specimen will be investigated histologically and additional microbiological testing, as indicated above, may also be performed on the specimen.

This research reports aims to describe the common aetiologies of exudative pleural effusions in patients admitted to a tertiary Academic Hospital in Johannesburg, South Africa.

1.2. Anatomy and pathophysiology

The two membranes surrounding the lungs are known as the visceral and parietal pleura. The visceral pleura encases the lungs directly and the parietal pleura lines the thoracic cavity (Diaz-Guzman & Budev, 2008). These layers are in fact continuous with one another with a potential space between them known as the pleural space. Within this pleural space lies a small physiologic quantity of pleural fluid, which is approximately 0.1mg/kg body weight in volume (Saguil *et al*. 2014). Physiological pleural fluid lubricates the two pleural membranes, which reduces friction and creates a negative intra-thoracic pressure (Bruwer *et al*. 2013). In this

way, the fluid protects the lungs by facilitating lung movement, supporting their shape, and preventing over-distension of the alveoli at the pleural surface (Diaz-Guzman & Budev, 2008).

The movement of fluid into, and out of, the normal pleural space occurs at a continuous and equal rate (McGrath & Anderson, 2011), which is responsible for maintaining a constant physiological volume. A pleural effusion occurs when this equilibrium is disrupted. The Starling equation (Starling & Tubby, 1989) explains the pathophysiology of this disruption that causes pleural effusions. According to Starling, the rate of production of pleural fluid is controlled by three factors, which are: osmotic pressure, hydrostatic pressure and membrane permeability. Conversely, the re-absorption of the fluid that has formed is undertaken by the widespread lymphatic system of the parietal pleura (McGrath & Anderson, 2011). An imbalance in the local factors governing the volume of pleural fluid may result in the increased accumulation of pleural fluid as an effusion (Saguil *et al*. 2014). This imbalance may involve either:

- An increased inflow into the pleural space. This is a consequence of increased permeability of local capillaries and/or pleural membranes, and/or a decrease in oncotic pressure. These disproportions promote the increased flow of fluid into the pleural space (Saguil *et al*. 2014).
- A decreased outflow from the pleural space caused by lymphatic obstruction (Saguil *et al*. 2014).

1.3. Clinical presentation of pleural effusions

1.3.1. History

A comprehensive history may provide essential clues to the cause of the pleural effusion. A patient with an existing malignancy may present with a malignant effusion. Pulmonary emboli may be precipitated by the presence of deep vein thrombosis (McGrath & Anderson, 2011). A recent TB contact and the presence of constitutional symptoms may suggest a tuberculous effusion (Bruwer *et al*. 2013). The presence of pneumonia suggests a para-pneumonic effusion and this may be further complicated by an empyema, which is pus in the pleural space (McGrath & Anderson, 2011). A good history should also include an enquiry into previous occupations to elicit asbestos exposure, recent trauma or surgical procedures, and medication use (Bruwer *et al*. 2013). Interrogation about the patient's drug history may disclose risk factors for an exudative pleural effusion. Implicated drugs are amiodarone, nitrofurantoin, phenytoin, methotrexate, cyclophosphamide and carbamazepine (Bruwer *et al*. 2013; Medford & Maskell, 2005).

1.3.2. Symptoms of pleural effusions

The triad of cough, shortness of breath and pleuritic chest pain remain the most common symptoms related to a pleural effusion (Porcel & Light, 2006). However, these symptoms are non-specific for pleural effusions (Rahman *et al*. 2004).

Cough is usually non-productive, dry, and results from pleural inflammation or bronchial wall compression (Diaz-Guzman & Budev, 2008). However, if the cough is productive of purulent sputum, a pneumonia is suggested. If the cough produces

blood (haemoptysis) then TB, malignancy or a pulmonary embolus should be considered (Diaz-Guzman & Budev, 2008).

The pathophysiology of dyspnoea related to pleural effusions is multifactorial. Chief mechanisms include decreased compliance of the chest wall, depression of the diaphragm, ventilation-perfusion mismatch and stimulation of reflexes because of a reduced lung volume (Rahman *et al*. 2004). In the case of massive effusions, lung volume may be significantly decreased, which commonly causes dyspnoea (Diaz-Guzman & Budev, 2008).

The presence of pleuritic chest pain suggests that there may be involvement of the parietal pleura, chest wall, nerves, or ribs (Rahman *et al*. 2004). This could be secondary to an inflammatory process, such as infection. The nature of pleuritic chest pain is usually localised and positional (aggravated by lying down, cough and deep inspiration). However, pain may be referred to the ipsilateral shoulder if the diaphragmatic parietal pleural is involved (Diaz-Guzman & Budev, 2008).

1.3.3. Signs of pleural effusions

Clinical signs of the presence of an effusion become apparent when there is more than 300ml of pleural fluid. Furthermore, these findings become more prominent when there is more than 1500ml of pleural fluid present (Diaz-Guzman & Budev, 2008). Basic inspection of the chest wall will typically reveal tachypnoea and decreased chest expansion on the side of the effusion. The affected area will also have unequal chest expansion. Tactile fremitus may be reduced in the presence of an underlying effusion. Tactile fremitus is the vibration felt by the doctor's hand when

placed on the chest wall while the patient speaks (Diaz-Guzman & Budev, 2008). Depending on the size of the pleural effusion, the trachea maybe deviated away from the side of the pleural effusion. Over the affected area, percussion will be stony dull (Porcel & Light, 2006). Auscultation may reveal reduced or absent breath sounds on the side of the effusion and occasionally bronchial breathing is heard above the level of the effusion.

1.4. Radiology and confirmation of an effusion

Imaging of the chest will assist with confirming the presence of a pleural effusion and denote the size and location of it. A simple chest radiograph will usually suffice. The erect postero-anterior (PA) chest radiograph (CXR) will usually demonstrate a pleural effusion once the pleural fluid volume is more than 200ml (Maskell & Butland, 2003). The characteristic feature is costophrenic angle blunting for which a volume as small as 50ml of fluid can be detected by a lateral film (Rahman *et al*. 2004). There also is dense opacification at the lung base, and obliteration of the costophrenic angle and the formation of a meniscus (Porcel & Light, 2006). Pleural fluid can be differentiated from pleural thickening by using the lateral decubitus films, which will allow fluid to shift with gravity (Medford & Maskell, 2005). Furthermore, the CXR may also help detect the underlying aetiology of the pleural effusion, e.g. pneumonia or malignancy (Rahman *et al*. 2004). When confirmatory doubt still exists, a chest ultrasound or computerised tomographic (CT) scan of the chest may be indicated (Bruwer *et al*. 2013).

1.5. Investigations for a pleural effusion

Sampling of pleural fluid through thoracentesis is one of the first steps in the assessment of a patient with pleural effusion, although sputum analysis, additional testing and even a pleural biopsy may be required.

1.5.1. Thoracentesis sampling

Thoracentesis refers to the aspiration of pleural fluid using a syringe and needle under sterile conditions. This diagnostic procedure is minimally invasive, relatively safe and cost-effective (Bruwer *et al*. 2013).

1.5.1.1. Appearance and odour of pleural fluid

The appearance and odour of the pleural fluid can help identify the likely aetiology. (Table 1.1)

Table 1.1: Relationship of exudative pleural fluid appearance and odour to the aetiology

Pleural fluid odour/appearance	**Aetiology**
Straw coloured	Tuberculosis Benign asbestos related effusion
Putrid odour/turbid	Anaerobic empyema
Turbid	Empyema Tuberculosis
Bloody	Trauma/post cardiac injury Malignancy Pulmonary embolus Benign asbestos-related effusion

	Dressler's Syndrome
"Anchovy" brown paste	Ruptured amoebic liver abscess
Food particles	Oesophageal rupture
White/milky	Chylothorax/pseudochylothorax
Bile stained	Cholethorax (biliary fistula)
Urine odour/ clear or yellow	Urinothorax

Data from Bruwer *et al*. 2013; Maskell & Butland, 2003; McGrath & Anderson, 2011

1.5.1.2. Differentiating a transudate from an exudate on pleural fluid analysis

The pleural fluid is first sent for biochemical analysis to determine if the effusion is an exudate or a transudate, using Light's criteria. Pleural fluid protein and LDH levels form the basis for this biochemical evaluation (McGrath & Anderson, 2011) (Table 1.2).

Table 1.2: Light's criteria, for differentiating transudative from exudative pleural effusions

If one or more of the following criteria are met, then the pleural fluid is an exudate

Protein	Ratio of pleural fluid to serum protein greater than 0.5
LDH	Pleural fluid LDH to serum LDH ratio greater than 0.6
Pleural fluid/LDH	Pleural fluid LDH greater than two-thirds the normal upper limit for serum LDH

(Light, 2002), LDH=lactate dehydrogenase

1.5.1.3. Aetiology for the exudative effusion

Once the pleural effusion has been documented to be an exudate, various possible diagnoses need to be considered and excluded. There are several causes for an exudative pleural effusion, some more common than others (Table 1.3).

Table 1.3: Aetiology of exudative pleural effusions

Frequency	**Exudate**
Common	Tuberculosis (TB) Malignancy Parapneumonic effusion and empyema
Less common	Pulmonary embolism (with infarction) Rheumatoid arthritis Systemic lupus erythematosus Dressler's syndrome (post-myocardial infarction syndrome) Benign asbestos effusion Post-coronary artery bypass
Rare	Yellow nail syndrome Drugs Fungal infection Uraemia Chylothorax Radiotherapy Oesophageal rupture Subhepatic, hepatic or splenic abscess

Data from Bruwer *et al*. 2013; Medford & Maskell, 2005; McGrath & Anderson, 2011; Rahman *et al*. 2004.

1.5.1.4. Tests done routinely on pleural fluid

- Biochemistry consisting of pH, LDH, protein and glucose
- Microscopy, culture and sensitivity
- Cytology
- Tests for TB include GeneXpert MTB/Rif assay, microscopy for acid fast bacilli and TB culture, and measurement of adenosine deaminase (ADA), which is no longer part of current practice. Although ADA is sensitive for TB effusions, it has a poor specificity since ADA can be increased in other aetiologies of exudative pleural effusions such as in empyema, rheumatoid arthritis and malignant effusions (Bruwer *et al*. 2013). ADA levels can also be falsely low in HIV-seropositive patients with TB effusions (Bruwer *et al*. 2013).

Gram stain and cultures are important for the diagnosis of an empyema in order to implement culture-directed antibiotic treatment. Light has also had proposed specific characteristics of pleural fluid, which can assist the Respiratory Physician in confirming the presence/absence of an empyema (Light, 2002). (Table 1.4).

Table 1.4: Characteristics of pleural fluid which indicate an empyema

If one or more of the following are present, then the exudative pleural fluid is an empyema

pH	<7.0
Glucose	<3.3 mmol/l
ICD insertion	Frankly purulent or Gram stain positive

(Data from Light, 2002), ICD=Intercostal drain

Normal physiologic pleural fluid pH is 7.6 because of the accumulation of bicarbonate in the pleural space (Rahman *et al*. 2004). Neoplasms, inflammatory cells and bacteria in the pleural cavity can increase production of lactic acid because of their high metabolic rate. This in turn decreases both pH and glucose in pleural fluid (Rahman *et al*. 2004). Common causes of a low pH in pleural fluid include complicated para-pneumonic effusions, malignancy, empyema, tuberculosis, rheumatoid arthritis and lupus pleuritis (Rahman *et al*. 2004). Pleural fluid with a pH <7.0 is an empyema and needs urgent intercostal drain insertion in order to control the source of sepsis.

In an empyema or pleural fluid infection, pH is a better indicator of infection than glucose (Maskell & Butland, 2003). Other causes of a reduced pleural fluid glucose level of <3.3mmol/l in an exudative effusion include TB, empyema, neoplasm, lupus pleuritis and oesophageal rupture (Bruwer *et al*. 2013). Rheumatoid arthritis causes the lowest glucose level of <1.6mmol/l in the exudative effusion (Maskell & Butland, 2003).

Cytology of pleural fluid involves microscopic evaluation of free cells suspended in these body fluid samples. It is particularly important in a suspected malignant effusion because malignant cells can be identified in 60% of patients with neoplastic disease (McGrath & Anderson, 2011). Repetitive sampling is advised because this 60% yield can be amplified when more samples are sent for cytology (Maskell & Butland, 2003). Cytology can assist in identifying the presence of a malignancy; however, it may be unhelpful in confirming the type of malignancy. In this instance a pleural biopsy is beneficial.

1.5.1.5. Ancillary tests done on pleural fluid for rarer diagnoses that cause an exudative effusion

These additional tests are not routinely performed. They should be done when clinically indicated, (Table 1.5)

Table 1.5: Examples of ancillary tests done on pleural fluid, when indicated

Pleural fluid test finding	**Aetiology implied**
Elevated amylase	Pancreatitis Oesophageal rupture
Cholesterol levels >5.18mmol/l (No chylomicrons)	Pseudochylothorax
Triglycerides >1.24mmol/l	Chylothorax
Rheumatoid factor	Rheumatoid arthritis
LE cells positive Anti-nuclear antibody positive	Systemic lupus erythematosus
PCR	Pneumonia *e.g. Streptococcus pneumoniae*
Creatinine > than in serum	Urinothorax
Tumour markers positive	Relevant malignancy

LE Lupus erythematosus, ANA Anti-nuclear antibody, PCR Polymerase chain reaction

Data from Light, 2002; Bruwer *et al*. 2013; Maskell & Butland, 2003; McGrath & Anderson, 2011.

1.5.2. Sputum investigation

Sputum may be produced by the patient or induced with hypertonic saline nebulisation. Sputum examination may help determine the likely aetiology, particularly of an exudative pleural effusion. Microscopy, culture and sensitivity are done on the sputum with consideration of a possible infective cause (parapneumonic effusion). Sputum cytology investigates for malignant/atypical cells, and if positive warrants further investigations for a malignancy. If a tuberculous effusion is suspected then sputum should be sent for GeneXpert MTB/Rif assay, microscopy for acid fast bacilli and TB culture to try to confirm the diagnosis by isolating *Mycobacterium tuberculosis* bacilli.

1.5.3. Pleural biopsy sampling

A pleural biopsy is a tissue sample of the parietal pleural membrane, taken for histological evaluation and additional investigations. A blind/closed pleural biopsy is a sterile bed-side procedure performed using an Abram's needle. Ideally, a thoracic ultrasound first identifies the point of maximal pleural thickening and the largest collection of fluid. This is the best site for pleural biopsy to be performed (Bruwer *et al*. 2013). For optimal diagnostic yield, the clinician preferably collects a minimum of four specimens from the site. The pleural tissue must be sent to the laboratory in 10% formaldehyde for histology and sterile saline for TB culture (Medford & Maskell, 2005). Complications caused by pleural biopsies are pneumothorax, pain, bleeding, haematoma and vasovagal reactions. Fortunately, however, a complication is seldom encountered (Rahman *et al*. 2004). If the aetiology of the effusion is still unknown after closed biopsy sampling, open biopsy sampling, undertaken in theatre usually by a cardiothoracic surgeon, may subsequently be performed. Histological evidence for TB is classically granulomatous inflammation with or without necrosis.

While TB bacilli may be visualized on the pleural biopsy, additional tests available for the detection of TB organisms includes the GeneXpert MTB/Rif assay, microscopy for acid fast bacilli and TB culture.

1.6. Management

Management of pleural effusions is directed towards treating the aetiology of the effusion. Complicated para-pneumonic effusions and empyemas are treated with antibiotics and insertion of intercostal drains to achieve source control. Tuberculous effusions are treated with anti-TB drugs and sometimes an intercostal drain. Malignant effusions can be persistent and may require pleurodesis using sclerosing agents such as talc, tetracycline or chemotherapeutic agents e.g. bleomycin, instilled into the pleural cavity via a chest drain (Medford & Maskell, 2005).

2.1. AIM OF THE STUDY

The aims of this study were to determine the aetiologies and describe the clinical characteristics of patients with exudative pleural effusions admitted for investigation to a tertiary academic hospital in Johannesburg.

2.2. OBJECTIVES

- To determine the aetiology of exudative pleural effusions in patients admitted to CMJAH for investigation of pleural effusion
- To describe the demographic profile and laboratory results obtained in these patients
- To compare the aetiologies in different patient groups (if possible), including HIV-seropositive versus HIV-seronegative patients, males versus females, young versus elderly (≥ 65 years)

2.3. METHODS

2.3.1 Study population and data collection methods

This was a retrospective record review. The study was based at CMJAH, undertaken over a period of one year from 1 January 2015 to 31 December 2015. (Table 2.1)

Table 2.1: Inclusion and exclusion criteria for the study	
Inclusion Criteria	**Exclusion Criteria**
18 years of age or older	Transudative effusions
Admitted for investigation at CMJAH	Lost to follow up prior to completion of investigations
Exudative pleural effusion	
Underwent thoracentesis with or without sputum analysis	
Underwent a pleural biopsy	

The study population comprised adult patients (≥18 years) admitted to CMJAH for investigation of an exudative pleural effusion. All the patients in this study underwent either open or closed pleural biopsy as part of their diagnostic work up. The National Health Laboratory Systems (NHLS) and National Institute for Occupational Health (NIOH) were the laboratory institutions responsible for processing of the laboratory-based investigations for the patients attending CMJAH. This included the processing of pleural biopsy samples. The pleural biopsy reports of all the patients who underwent pleural biopsies at CMJAH in the relevant departments (Respiratory, Infectious Diseases and Cardiothoracic Units) were found on the NHLS and NIOH databases and recorded. Data were extracted on these patients from the pleural biopsy reports according to the stipulated variables. The Human Research Ethics Committee of the University of the Witwatersrand granted ethics approval for the study on the 1st August 2016 (Appendix A, Ethics Clearance number M160755).

2.3.2 Patient variables collected

The NHLS and NIOH laboratory databases provided the pleural biopsy reports from which demographic data and results of the pleural biopsies (histology, MCS, GeneXpert MTB/Rif assay, AFB microscopy, TB culture and ancillary test results), sputum analysis (MCS, GeneXpert MTB/Rif assay, auramine and cytology results), results of thoracentesis (biochemistry, MCS, cytology, GeneXpert MTB/Rif assay, AFB microscopy, TB culture and ancillary test results) and TB Bactec results were extracted (Appendix A). If the required data on the pleural biopsy result reports were inadequate, patient ward discharge summaries were used to retrieve the required data.

2.3.3 Sample size

Overall 162 patients fulfilled the inclusion criteria for this study.

2.3.4 Data analysis

Data on the study group was captured on a Microsoft Excel spreadsheet and thereafter transferred to Graphpad Instat for analysis. Descriptive statistics, as appropriate, were performed for demographic details, clinical information and the laboratory results using numbers (percentages), means and standard deviations and median and ranges, as appropriate. For the comparison of the data in the different patient groups (HIV-seronegative versus HIV-seropositive, male versus female, young versus elderly) continuous variables were compared using the Student's unpaired t-test or the Mann Whitney U test, as appropriate, and categorical variables were compared using the Chi-squared or Fisher's exact (2-tail) test, as appropriate. A p value of < 0.05 was considered to be significant.

3. RESULTS

3.1 Patients included in the study group

During the study period (1 January 2015 to 31 December 2015), there were 162 pleural biopsies reported on, as per the records from the Department of Anatomical Pathology. Further evaluation indicated that these biopsies were performed within three clinical disciplines; namely the Respiratory Unit (52 biopsies), the Cardiothoracic Unit (91 biopsies) and the Infectious Diseases Unit (19 biopsies). The methods used to obtain the pleural biopsies were closed biopsy (82 patients), open biopsy (74 patients), and both methods done after the closed biopsy failed to yield an optimal sample and an open biopsy was performed thereafter (6 patients).

3.2 Description of the study group

3.2.1 Demographics and clinical features

Of this cohort of 162 subjects, 100 were males and 62 were females. Race could not be evaluated as a contributing factor towards diagnosis because most of the patients were of African origin (Table 3.1). The mean age (standard deviation (SD)) of the study group was 44 years (±16.3), with a range from 19 years to 88 years. With regard to HIV status, 78 (48.1%) patients were HIV-seronegative, 76 (46.9%) were HIV-seropositive and 8 (4.9%) were of unknown HIV status.

Table 3.1: Demographics and clinical features			
		Number	**Percentage**
Gender	Female	62	38.3%
	Male	100	61.7%
Race	Black	141	87.0%
	White	14	9.9%
	Indian	4	2.5%
	Coloured	1	0.6%
Smoking status	Non-smoker	119	73.5%
	Smoker	25	15.4%
	Unknown	18	11.1%
HIV status	Negative	78	48.1%
	Positive	76	46.9%
	Unknown	8	4.9%

3.2.2 Thoracentesis results

The mean LDH level was 536.1 U/L, with a range from 10 U/L to 3742 U/L. The mean glucose level was 4.9 mmol/L, with a range from 0.2 mmol/L to 19.8 mmol/L. The mean total protein was 44.2 g/L with a range from 30 g/L to 74 g/L. The mean

Table 3.2: Thoracentesis results				
	Min	**Max**	**Mean**	**SD**
LDH (U/L)	10	3742	536,1	589,1
Glucose (mmol/L)	0,2	19,8	4,9	2,5
Protein (g/L)	30	74	42	10,6
ADA (U/L)	1,0	160,2	11,2	26,2

ADA level was 11.2 U/L with a range from 1.0 U/L to 160.2 U/L, (Table 3.2).

LDH= lactate dehydrogenase, ADA=Adenosine deaminase, Min=minimum, Max=maximum, SD=Standard deviation

3.2.3 Aetiology

Aetiologies were determined from the collective results of all the investigations that were performed. The aetiologies included: TB in 45 patients, bacterial empyema in 40, malignancy in 37, chronic pleuritis in 29, a combination of both TB and malignancy in 3, and the results were inconclusive in 8 patients (Table 3.3). Amongst the HIV-seropositive patient group, the diagnosis were as follows; TB in 26 patients, bacterial empyema in 24, chronic pleuritis in 12, malignancy in 9, inconclusive in 3 and a combination of both TB and malignancy in 2 (Table 3.4). Amongst the HIV-seronegative patient group, the diagnosis were as follows; malignancy in 28 patients, bacterial empyema in 16, chronic pleuritis in 16, TB in 14, inconclusive in 3 and a combination of both TB and malignancy in 1 (Table 3.5).

Table 3.3: Aetiology of exudative pleural effusions in 162 patients

Aetiology	Number	Percentage
TB	45	27.8%
Bacterial empyema	40	24.7%
Malignancy	37	22.8%
Chronic pleuritis	29	17.9%
Inconclusive	8	4.8%
TB and malignancy	3	1.9%

Table 3.4: Aetiologies amongst the HIV-seropositive patients			
Outcome	**Number**	**Percentage**	**Mean CD4 count**
TB	26	34.2%	222
Bacterial empyema	24	31.6%	251
Chronic pleuritis	12	15.8%	166
Malignancy	9	11.8%	318
Inconclusive	3	3.9%	241
TB and malignancy	2	2.6%	269

Table 3.5: Aetiologies amongst the HIV-seronegative patients		
Outcome	**Number**	**Percentage**
Malignancy	28	35.9%
Bacterial empyema	16	20.5%
Chronic pleuritis	16	20.5%
TB	14	17.9%
Inconclusive	3	3.8%
TB and malignancy	1	1.2%

3.2.4 Risk factors

There were 78 HIV-seronegative patients, 76 HIV-seropositive patients, and 8 patients whose HIV status was unknown. Of the 76 patients who were HIV-seropositive, the mean CD4 count was 249 cells/uL (±193 cells/uL) with a range from 10–937 cells/uL. There were 25 patients who smoked, 119 patients who declared that they did not smoke, and in 18 patients the smoking history was unknown.

3.3 Associations between demographics and clinical factors

Table 3.6 is a summary of all the statistical analyses undertaken, which included the following; i) an evaluation of the relationship between age and gender and the HIV status of the patients, ii) an evaluation of the relationship between method of biopsy, department in which the biopsy was performed, gender, smoking status and HIV status and the overall diagnosis made in the patients, iii) an evaluation of the relationship between gender, age and HIV status and a final diagnosis of TB in the patients and iv) an evaluation of the relationship between gender, age, HIV status and smoking status and a final diagnosis of malignancy in the patients.

3.3.1 HIV status and demographics

HIV-seropositive patients were younger than HIV-seronegative patients (mean age 39.2 years versus 49.5 years; p<0.001) (Table 3.7).There were no significant gender differences in relationship to the HIV status of the patients (p = 0.502). Since most patients were of African origin it was not possible to show any relationship between race and HIV status (Table 3.6, 3.7). The relationships of the analyses undertaken as demonstrated in Table 3.6 are discussed in more detail in the results and discussion sections below.

Table 3.6: Summary of the statistical analyses undertaken

Independent variable	Dependant variable	P value
Age	HIV status	<0.001
Gender	HIV status	0.502
Method of biopsy	Diagnosis	0.04
Department	Diagnosis	0.05
Gender	Diagnosis	0.109
Smoking	Diagnosis	0.009
HIV status	Diagnosis	0.118
HIV positive status	Diagnosis	0.001
Gender	TB diagnosis	0.233
Age	TB diagnosis	0.01
HIV status	TB diagnosis	0.006
Gender	Malignancy diagnosis	0.04
Age	Malignancy diagnosis	<0.01
HIV status	Malignancy diagnosis	0.001
Smoking status	Malignancy diagnosis	0.081

Table 3.7: Relationship between HIV status and demographics					
		HIV status			
		Negative	Positive	Unknown	Total
Gender	**Female**	33	27	2	**62**
	Male	45	49	6	**100**
	Total	**78**	**76**	**8**	**162**
Race	**Black**	63	72	6	**141**
	White	12	3	1	**16**
	Indian	3	0	1	**4**
	Coloured	0	1	0	**1**
	Total	**76**	**78**	**8**	**162**
Age and HIV status – Student's unpaired t-test					
Age		**Mean**	**SD**	**p value**	
	Negative	49.5	18.8	<0.001	
	Positive	39.2	11.4		
	Unknown	40	16.2		

SD=Standard deviation

3.3.2 Influence of biopsy method, department, demographics and risk factors on aetiological findings

The aetiologies documented on the pleural biopsy samples were similar across all departments and for both pleural biopsy methods used (Tables 3.8). A total of 162 (100%) patients underwent pleural biopsy, of which 74 (45.6%) patients had a diagnostic open pleural biopsy, 82 (50.6%) had a diagnostic closed pleural biopsy, and 6 (3.7%) required both open and closed pleural biopsy. The Cardiothoracic Unit conducted 91 pleural biopsies, the Respiratory Unit 52 biopsies and the Infectious Diseases Unit 19 biopsies. TB was the final diagnosis in 13 (8%) of female patients

and 32 (19.7%) male patients, TB being more common in males than females in this study. Malignancy was the final diagnosis in 21 (12.9%) female patients and 16 (9.8%) male patients, malignancy being more common in females than males in this study. Race could not be analysed because most of the patients in this study were of African origin 141 (87%). With regard to smoking status, 25 (15.4%) patients were smokers, 119 (23.5%) did not smoke, and 18 (11.1%) smoking status was not reported on in the patient records. Although there was a trend to an association between smoking status and the occurrence of malignancy ($p = 0.081$), this did not reach statistical significance. This could simply be a consequence of small patient numbers.

Table 3.8: Influence of biopsy method, department, demographics and risk factors on aetiological findings								
		In-conclusive	**TB**	**Malignancy**	**Bacterial empyema**	**Chronic Pleuritis**	**TB & Malignancy**	**Total**
Method and aetiologies – cross-tabulation								
Method of pleural biopsy	**Open**	5	28	19	12	9	1	**74**
	Closed	3	15	15	28	19	2	**82**
	Both	0	2	3	0	1	0	**6**
	Total	**8**	**45**	**37**	**40**	**29**	**3**	**162**
Department and aetiologies – cross-tabulation								
Department	**Respiratory**	3	12	19	10	7	1	**52**
	Infectious disease	1	15	1	1	1	0	**19**
	Cardio-thoracics	4	18	17	29	21	2	**91**
	Total	**8**	**45**	**37**	**40**	**29**	**3**	**162**
Gender and aetiologies – cross-tabulation								
Gender	**Female**	2	13	21	14	10	2	**62**
	Male	6	32	16	26	19	1	**100**
	Total	**8**	**45**	**37**	**40**	**29**	**3**	**162**
Race and aetiologies – cross-tabulation								
Race	**Black**	7	44	27	32	26	3	**141**
	White	1	0	7	6	2	0	**16**
	Indian	0	1	1	1	1	0	**4**
	Coloured	0	0	0	1	0	0	**1**
	Total	**8**	**45**	**37**	**40**	**29**	**3**	**162**
Smoking and aetiologies – cross-tabulation								
Smoking	**Smoker**	2	2	10	1	7	3	**25**
	Non-smoker	6	40	25	28	20	0	**119**
	Unknown	0	3	2	11	2	0	**18**
	Total	**8**	**45**	**37**	**40**	**29**	**3**	**162**
HIV status and aetiologies – cross-tabulation								
HIV Status	**Negative**	3	14	28	16	16	1	**78**
	Positive	3	26	9	24	12	2	**76**
	Unknown	2	5	0	0	1	0	**8**
	Total	**8**	**45**	**37**	**40**	**29**	**3**	**162**

3.3.3 The factors associated with a diagnosis of TB

With regard to the diagnosis of TB, there were 45 patients who had TB only, and 3 who had TB together with malignancy. Thus overall there were 48 patients with TB, and 114 patients who did not have TB. There were no gender differences with regard to a diagnosis of TB (p = 0.233), but younger patients compared to older patients (mean 37.9 years versus 46.9 years; p<0.01) and those who were HIV-seropositive compared with those that HIV-seronegative were more likely to have TB (p = 0.006) (Table 3.9).

Table 3.9: Factors associated with TB

		TB		
		Negative	**Positive**	**Total**
Gender and TB				
Gender	**Females**	47	15	**62**
	Males	67	33	**100**
	Total	**114**	***48**	**162**
Age and TB				
Age	**Mean**	46,9	37,9	
	SD	16,3	14,5	
	SE	1,5	2,1	
	N	**114**	***48**	**162**
HIV status and TB				
HIV Status	**Negative**	63	15	**78**
	Positive	48	28	**76**
	Unknown	3	5	**8**
	Total	**114**	***48**	**162**

*-there were 48 with a TB diagnosis, 45 with TB only and 3 with TB and Malignancy, SD=Standard deviation, SE=Standard error, N= total number of patients in the study

3.3.4 The factors associated with a diagnosis of malignancy

With regard to the aetiology of malignancy, there were 37 patients who had malignancy only, and 3 who had TB with malignancy. Thus overall 40 patients had malignancy, while the remaining 122 patients did not have malignancy. Older patients compared with younger patients (mean 57.5 years versus 39.8 years; p=0.01), HIV-seronegative versus HIV-seropositive patients (p=0.001) and, female patients compared with male patients (p=0.04) were more likely to have malignancy. Although there was a trend, smoking could not be demonstrated, in the current study, to be a significant contributing factor to the diagnosis of malignancy (p = 0.081) (Table 3.6 and 3.10).

Table 3.10: Factors associated with a diagnosis of malignancy

		Malignancy		
		Absent	**Present**	**Total**
Gender	**Females**	39	23	**62**
	Males	83	17	**100**
	Total	**122**	***40**	**162**
Age	**Mean**	39.8	57.5	
	SD	13.8	16.4	
	SE	1.25	2.6	
	N	**122**	***40**	**162**
HIV Status	**Negative**	49	29	**78**
	Positive	65	11	**76**
	Unknown	8	0	**8**
	Total	**122**	***40**	**162**
Smoking	**Negative**	91	28	**119**
	Positive	15	10	**25**
	Unknown	16	2	**18**
	Total	**122**	***40**	**162**

*-40 patients had malignancy as a diagnosis, 37 with Malignancy alone and 3 with TB and a malignancy, SD=Standard deviation, SE=Standard error, N= total number of patients in the study.

3.3.5 Comparison of the sampling methods used, the investigations performed on the samples, and the results obtained

There were four sampling methods performed, namely; thoracentesis, sputum analysis, pleural biopsy and bronchoscopy. On the thoracentesis samples, tests performed were GeneXpert for MTB/Rif assay, TB Bactec, cytology for malignant cells and microscopy, culture and sensitivity (MCS). On the sputum samples, tests performed were GeneXpert for MTB/Rif assay, Auramine O staining, cytology for malignant cells and MCS. On the biopsy samples, Ziehl Neelsen staining was done; necrotising granulomata and histological findings of malignant cells, pus cells for organising empyema, and chronic pleuritis were reported upon, and microscopy and culture for pathogens was performed. Bronchoscopy, which is not routinely performed for exudative pleural effusions, was only conducted on 12 patients and cytology for malignant cells was tested for. A full series of all tests were performed on all pleural biopsy samples, but for the sputum and thoracentesis samples, there were many tests not done (Table 3.11).

Table 3.11: Comparison of sampling methods used, investigations performed on the samples, and the results obtained											
Procedure	**Test**	**Negative**		**Positive**		**Not done**		**Bloody**		**Total**	
			%		**%**		**%**		**%**	**n**	**%**
Thoracentesis	**GeneXpert**	69	*37,0*	8	*4,9*	81	*50,1*	13	*8*	162	*100*
	TB Bactec	114	*70,4*	3	*1,9*	45	*28,7*	0	*0*	162	*100*
	Malignant cells	76	*46,9*	16	*9,9*	70	*43,2*	0	*0*	162	*100*
	MCS	113	*69,8*	16*	*9,9*	33	*20,4*	0	*0*	162	*100*
Sputum	**GeneXpert**	63	*38,9*	17	*10,5*	82	*50,6*	0	*0*	162	*100*
	Auramine	33	*20.4*	3	*1.9*	126	*77.8*	0	*0*	162	*100*
	Malignant cells	10	*6.2*	3	*1.9*	149	*92.0*	0	*0*	162	*100*
	MCS	4	*2,5*	45**	*27,8*	113	*69,8*	0	*0*	162	*100*
Biopsy	**Necrotising Granuloma's**	136	*84*	26	*16*	0	*0*	0	*0*	162	*100*
	Culture	158	*97.5*	4	*2.5*	0	*0*	0	*0*	162	*100*
	Ziehl Neelsen	153	*94.4*	9	*5.6*	0	*0*	0	*0*	162	*100*
	Malignant infiltrate	130	*80.2*	32	*19.8*	0	*0*	0	*0*	162	*100*
	MCS	158	*97.5*	4***	*2.5*	0	*0*	0	*0*	162	*100*
	Chronic pleuritis	138	*85,2*	24	*14,8*	0	*0*	0	*0*	162	*100*
	Empyema	135	*83,3*	27	*16,7*	0	*0*	0	*0*	162	*100*
Bronchoscopy	**Malignancy**	7	*4.3*	5	*3.1*	150	*92.6*	0	*0*	162	*100*

16* positive microscopy, cultures and sensitive's(MCS) on thoracentesis: *Staphylococcus aureus*=1, *Streptococcus pneumoniae*=8, *Pseudomonas aeruginosa*=4, *Klebsiella pneumoniae*=3

45** positive MCS on sputum: *Staphylococcus aureus* = 38, *Streptococcus pneumoniae* = 1, *Pseudomonas aeruginosa* = 1, *Klebsiella pneumoniae* = 5

4*** positive MCS on pleural biopsy: *Staphylococcus aureus* = 1, *Streptococcus pneumoniae* = 1, *Pseudomonas aeruginosa*= 2

GeneXpert = GeneXpert MTB/Rif Assay

3.3.6 Investigations yielding bacterial pathogens (other than TB) on different samples

There were four pathogens that were found on MCS testing amongst the different samples. These pathogens included: *Staphylococcus aureus* (40 isolates), *Streptococcus pneumoniae* (10 isolates), *Klebsiella pneumoniae* (7 isolates) and *Pseudomonas aeruginosa* (8 isolates). (Table 3.12).

Table 3.12: Bacterial organisms identified from investigations

Pathogen identified	Sputum MCS	%	Thoracentesis MCS	%	Pleural biopsy MCS	%	Total pathogens
Normal	4 (normal flora)	*2.5*	0 (no bacterial growth)	*0*	0 (no bacterial growth)	*0*	
Staphylococcus aureus	38	*23.5*	1	*0.6*	1	*0.6*	40
Streptococcus pneumonia	1	*0.6*	8	*4.9*	1	*0.6*	10
Pseudomonas aeruginosa	1	*0.6*	4	*2.5*	2	*1.2*	7
Klebsiella pneumonia	5	*3.1*	3	*1.9*	0	*0*	8
Not done	113	*69.8*	33	*20.4*	0	*0*	unknown
Total	162	*100*	162	*100*	162	*100*	65

MCS=microscopy, culture and sensitivity

3.3.7 Pathogens isolated causing empyema

Amongst the aetiologies, 40 patients were found to have bacterial empyemas. Empyema was confirmed if there was identification of a bacterial pathogen in the thoracentesis specimen. Some pathogens were found in sputa with no diagnosis of empyema rendered, suggesting a para-pneumonic effusion. Some pathogens which were isolated in the thoracentesis specimen did not correlate with the overall clinical picture or results from other investigations done. Hence these were concluded to be contaminants. The causative organisms for the bacterial empyemas found in this study included: *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeuriginosa* respectively (Table 3.13).

Table 3.13: Bacterial pathogens causing empyema (other than TB)

	Microscopy and culture								
	Thoracentesis			**Sputum**			**Pleural biopsy**		
	No empyema	**Empyema**	**Total**	**No empyema**	**Empyema**	**Total**	**No empyema**	**Empyema**	**Total**
No bacterial growth	93	20	113	4	0	4	121	37	158
Staphylococcus aureus	1	0	1	19	19	38	1	0	1
Streptococcus pneumoniae	3	5	8	1	0	1	0	1	1
Pseudomonas aeruginosa	1	3	4	0	1	1	0	2	2
Klebsiella pneumoniae	0	3	3	3	2	5	0	0	0
Not done	24	9	33	95	18	113	0	0	0
Total	**122**	**40**	**162**	**122**	**40**	**162**	**122**	**40**	**162**

3.4 Overall conclusions of the study

The main aetiologies of the exudative pleural effusions obtained from the pleural biopsy sampling, in conjunction with other sampling methods (thoracentesis, sputum analysis and bronchoscopy when indicated) were TB, bacterial empyema and malignancy. Out of 162 patients, 45 had TB, 40 had bacterial empyema and 37 had malignancy (included 3 patients with malignancy and super-imposed TB). HIV-seropositivity was associated with younger age. HIV infection and younger age were risk factors for TB. Older HIV-seronegative patients were more likely to have a malignancy. There was an unexpected finding that female patients had a higher rate than male patients of malignancy as a cause of their exudative pleural effusion. Smoking was not documented to be a significant risk factor for the occurrence of TB or malignancy in this study.

Another significant finding was the high incidence of bacterial empyema amongst those patients in whom adequate testing was done. However, data revealed that there was a paucity of relevant investigations done on the thoracentesis, sputum and bronchoscopy samples to affirmatively exclude bacterial empyema in all subjects.

4. DISCUSSION

The main findings of the study were as follows. The aetiologies of patients being investigated for exudative pleural effusions at CMJAH were documented, in descending order, to be TB, bacterial empyema, malignancy, chronic pleuritis and an inconclusive diagnosis. Overall, three patients had concomitant malignancy and TB. HIV-seropositive patients tended to be younger. Interestingly, gender was not related to the HIV status of the patients in this study. Race could not be analysed because most of the patients were of African descent, which represents the demographics of the South African population at large. There was a higher incidence of infective aetiologies namely, TB and bacterial empyema, as the cause of the exudative pleural effusions in HIV-seropositive patients, which was expected. TB was predominantly found in the younger HIV-seropositive patients while malignancy was more common in older HIV-seronegative female patients. With regard to the causative organisms for the bacterial empyemas in the study, these included: *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Out of a total of 162 patients in this study, 45 (27.8%) patients had TB, 40 (24.7%) had bacterial empyema, 37 (22.8%) had malignancy, 29 (17.9%) had chronic pleuritis, 3 (1.9%) patients had a combination of TB and malignancy and in 8 (4.8%) patients the diagnosis was inconclusive. This answers the main objective of this study. The largest proportion of patients had an infective aetiology causing the exudative pleural effusion which was followed by malignancy. The higher occurrence of infective aetiologies, namely TB and bacterial empyema, as compared to

malignancy, in this study reflects similar findings in other studies carried out in third world countries such as Nigeria and Qatar (Mbata *et al*. 2015; El-Sameed *et al*. 2014; Khan *et al*. 2011). A 5 year retrospective study done in Eastern Nigeria which looked at the clinical presentation, aetiology and mortality in patients with pleural effusions revealed that infectious aetiologies were the predominant finding (Mbata *et al*. 2015). Of a total of 199 patients in this eastern Nigerian study, 42.2% of patients had TB, 14% had parapneumonic effusions and 12% of the patients had malignancy (Mbata *et al*. 2015).

Many factors in South Africa favour the fact that infective aetiologies, and particularly TB, were the most common aetiologies found in the current study, ranging from the high burden of HIV/AIDS, poor socioeconomic status of the majority of the patients attending government hospitals, overcrowding and impoverished living conditions, poor nutritional status of patients, poor health seeking behaviour and access to health-care, amongst other factors, which increases the risk of communicable diseases (van Staden & Badenhorst, 2009).

Furthermore, South Africa has the third highest burden of TB globally, and in 2013 it was projected that there were 450000 cases of active TB overall in South Africa (WHO, 2014). Over the last 15 years, there has been a 400% increase in the incidence of TB (WHO, 2014). HIV infection and TB were both present concomitantly in an estimated 60-73% of these 450000 cases (WHO, 2014).

A review article by a group of physicians in Pretoria, which provides a clinical approach to effusions in HIV-seropositive patients, focused on the common aetiologies in this subset of patients (Mostert & Pannell, 2009). That review indicated that tuberculous parapneumonic effusions, other infective parapneumonic effusions

and empyemas were the most common causes of exudative effusions; and that Kaposi's sarcoma, lymphoma, pulmonary embolus and pancreatitis were less common causes (Mostert & Pannell, 2009).

In the current study HIV-seropositive patients were of a younger, possibly more sexually active age group. This finding reflects similar results of other studies done on the patterns and prevalence of HIV infection in South Africa (Shisana *et al*. 2012).

Interestingly, there was no relationship of gender to the HIV status of the patients in the current study. In contrast, traditionally in South Africa, females have had higher rates of HIV/AIDS as compared to men (van Staden & Badenhorst, 2009). Factors which may contribute to this include different cultural beliefs and myths, gender inequality, gender-based violence, health seeking-behaviour, where woman are more likely to seek medical attention earlier compared to men, voluntary counselling and testing (VCT) during antenatal care (ANC) with detection of more females with HIV infection, to name a few (van Staden & Badenhorst, 2009).

This study showed a higher prevalence of malignancy in the older HIV-seronegative patient group, which correlates with the current pattern in the literature (Pinzone *et al*. 2012; Pakkala & Ramalingam, 2010; Koegelenberg *et al*. 2016). The rate of lung cancer in SA stands at 5.8 per 100000, which seems to be an under-representation (Sheridan & Collins, 2013). The upsurge in lung malignancy in South Africans is multi-factorial. Increased accessibility to cigarettes, the sprawl of population into city centres, lack of anti-smoking interventions and lifestyle changes all contribute to the high incidence of lung cancer in SA (Sheridan & Collins, 2013). Conventional risk

factors for lung cancer still prevail which include smoking, asbestos and silica exposure in the mining industry (an integral part of SA history), biomass fuel exposure and outdoor air pollution (Sheridan & Collins, 2013). In the current study there was a trend, but no definitive relationship between smoking and malignancy. The reason for this is uncertain, although it may be due to statistical analyses with relatively small patient numbers.

Interestingly, the current study demonstrated that only 9 patients (11.8%) of the HIV-seropositive cohort were found to have malignancy as the primary aetiology. Although this representation of malignancy in HIV-seropositive patients is small, it is not reflective of the current South African and international trends (Pinzone *et al*. 2012), (Pakkala & Ramalingam, 2010), (Koegelenberg *et al*. 2016). Advances in HIV research and ARV treatment options have resulted in a paradigm shift. HIV-seropositive patients are living longer and are at lower risk of AIDS associated opportunistic infections if initiated early on ARV therapy and adherent to their treatment. This advent has revealed a new entity of non-AIDS defining cancers which are becoming more rampant in HIV-seropositive patients (Pinzone *et al*. 2012). The most common non-AIDS defining cancer occurring in HIV-infected individuals appears to be lung cancer (Pinzone *et al*. 2012). The high incidence of lower respiratory tract infections in younger HIV-seropositive patients tends to mislead the attending physician and delay the diagnosis of lung cancer in this younger group of patients (Pakkala & Ramalingam, 2010). Despite this, lung cancers in HIV-seropositive patients are usually diagnosed a decade earlier than their HIV-seronegative counterparts (Pakkala & Ramalingam, 2010). There are many theories postulated as to the higher incidence of lung cancer in HIV-seropositive patients. HIV

has a potential oncogenic role, it decreases immune system surveillance and induces immunosuppression. This immune-deficient state predisposes to recurrent lower respiratory tract infections leading to ongoing chronic inflammation which may potentiate the effect of tobacco carcinogens and ultimately increasing the risk of lung cancer (Pakkala & Ramalingam, 2010).

A recent South African (SA)-based study, conducted in Cape Town, compared the incidence of lung cancer between HIV-seropositive and seronegative patient groups. Their findings paralleled other international studies, which showed that HIV-seropositive patients with lung cancer were younger, that squamous cell lung cancer was more common in HIV-seropositive patients and that the patients presented with more advanced disease. Adenocarcinoma was the most common type of lung cancer in HIV-seronegative patients (Koegelenberg *et al*. 2016). The types of malignancies diagnosed on pleural biopsy in the current study included adenocarcinoma, squamous cell carcinoma, lymphoma and mesothelioma (data not shown).

The current study revealed an association between female gender and an increased rate of malignancy as the cause of the exudative pleural effusion. Other studies have also noted this finding. A study conducted in Germany (Lienert *et al*, 2000) using the cancer registry, not done primarily on pleural effusions, concluded that there was a twofold rise in the number of young woman being diagnosed with lung cancer. In the German study, females at an age of less than 46 years old were defined as being young. Adenocarcinoma was the most frequent histology of the lung cancer in that population group, with smoking being the main contributory factor (Lienert *et al*,

2000). Notably, this finding in that study of woman being at a higher risk for lung cancer and presenting at a younger age is not a new discovery. A study done in Japan between 1984-1998 which looked at the characteristics and life expectancy in female patients treated surgically for lung cancer shared this finding of a higher incidence of lung cancer in younger women (Minami *et al*, 2000).

Oestrogen has been associated with an increased risk for lung malignancy. Overall, 45-70% of non-small cell lung cancers express oestrogen receptors in both males and females; and hence oestrogen may have a stimulatory effect on lung malignancy cell growth. This does raise concerns for an increased risk for lung cancer in post-menopausal woman using hormone replacement therapy (Fitzgerald *et al*, 2016).

Although there was a trend, the current study did not document smoking to be a risk factor for malignancy. This could be related to the small patient numbers in the current study.

Another incidental finding in this study was that the pleural biopsy sampling method yield was equivalent amongst all the departments. This study also highlights the importance of pleural biopsy as a modality used to diagnose the aetiologies of exudative pleural effusions. A study done in India which looked at the role of pleural biopsy in the diagnosis of pleural effusions found that in 62 patients out of a cohort of 72 patients who underwent pleural biopsy, the latter were successful in attaining pleural tissue for analysis (Pandit *et al*, 2010). Although closed pleural biopsy with an Abrams needle is a blind procedure, which is user dependent, a significant number of the patients in this current study did have a positive yield of pleural tissue on closed biopsy.

Finally, with regard to the causative organisms for the bacterial empyemas in the cyrrent study, implicated pathogens included: *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* These organisms are typical of organisms which cause lower respiratory tract infections and which may complicate leading to the development of an empyema. Other studies conducted in third world countries also found a similar pattern of organisms causing empyemas (Khan *et al*, 2000).

5. POTENTIAL LIMITATIONS OF THE CURRENT STUDY

This study has potential limitations. Firstly, it is a single centre study and the results may not be generalized to other institutions or to the entire country. Being a retrospective record review, the study relied on recorded data and not all the data required for the study had been recorded, nor were all the procedures which were needed for the analysis of the research performed on every patient. Furthermore, there were inconsistencies in the details provided on pleural biopsy request forms and discrepancies in the fullness of the diagnostic investigations performed especially when comparing the surgical and medical departments used in the study. For example, patients were more extensively investigated by the Infectious Disease and Respiratory Departments than they were in the Cardiothoracic Surgery Department, but this may simply have been due to the fact that the medical units were the units that did the initial patient workup whereas the surgical units were the units to which the patients were referred purely for further surgical procedures. Furthermore, there was a lack of uniformity of baseline investigations performed in individual cases, particularly with regard to thoracentesis, sputum analysis, TB Bactec done on blood and pleural fluid, as well as the performance of closed pleural biopsy, the reasons for which were potentially multifactorial. This could include patient factors, for example refusal of consent for closed pleural biopsy, and even contraindications to the performance of closed pleural biopsies, as well patients with non-productive cough who were therefore not able to produce sputum samples. Nevertheless this study is the first that comprehensively reported on the aetiology and associated features for exudative pleural effusions in patients hospitalised for investigation at a Tertiary care institution in South Africa.

6. CONCLUSION

Overall the main aetiologies of exudative pleural effusions of patients at CMJAH included TB, bacterial empyema, malignancy, chronic pleuritis and inconclusive findings, respectively. This result was similar to research done in other third world countries. HIV-seropositive patients were of a younger age. Gender did not influence HIV status in this study. Race could not be analysed because most of the patients were of African descent which represented the demographics of the South African population at large. There was a higher incidence of infective aetiologies in HIV-seropositive patients. TB was predominantly found in the younger HIV-seropositive patients while malignancy was more common in older HIV-seronegative female patients. The causative organisms for the bacterial empyemas found in this study included: TB, *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* respectively.

7. FUTURE RESEARCH

This study does highlight certain areas where further research needs to be done. This includes the need to investigate the higher prevalence of lung malignancies which present as exudative pleural effusions in female patients. This study also sheds light on the need to improve our strategies against communicable diseases, namely HIV/AIDS and TB in South Africa. Even though this study did not show an increased rate of lung malignancy as a cause of exudative pleural effusions in HIV-seropositive patients, current international studies do show a rise in the incidence of non-AIDS defining cancers in HIV positive patients especially lung cancers and

hence clinicians should be aware of this irrespective of the patients age. This was the first study that attempted to document a comprehensive overview of exudative pleural effusions in South Africa and it would be important to conduct this study in other centres in South Africa and compare the findings.

8. APPENDIX A- DATA SHEET

8.1. Demographics:

Study number		HIV status	
Date of admission		CD4 count	
Age		Viral load	
Gender		Anti-retrovirals	
Race		Smoker	

8.2. Thoracentesis results:

Biochemistry		Tests for TB		Cytology	MCS	Other
LDH		GeneXpert MTB/Rif assay				
Glucose		ADA				
Protein						
pH						
Albumin		AFB microscopy +/- TB culture				

8.3. Sputum analysis:

MCS	GeneXpert MTB/Rif assay	AFB +/- TB culture	cytology

8.4. Pleural biopsy results:

Histology Report conclusion				
Tests for TB	GeneXpert MTB/Rif assay		AFB +/- TB culture	
MCS				
Other ancillary tests				
Open pleural biopsy		Closed pleural biopsy		
Date performed				

8.5. TB Bactec result - specimen:

Positive		Negative		Not done	

8.6. Other investigations results:

9. APPENDIX B (ETHICS APPROVAL)

10. REFERENCES

Bruwer JW, Batubara E, Koegelenberg CFN. A practical approach to diagnosing pleural effusion in South Africa. *CME* 2013; 31(9):320-325

El-Sameed Y, Waness A, Mehta AC. Pleural diseases in the Middle East: A 2013 review. *International Journal of Medical Science and Public Health* 2014; 3(2):116-120

Fitzgerald T, Glyrin A, Waheed A. Lung cancer: A Women's Health Imperative. A Report of the Mary Horrigan Connors Center for Women's Health and Gender Biology at Brigham and Women's Hospital 2016; [online] Available at: http://www.brighamandwomens.org/connorscenter [accessed 1 Mar. 2017]

Hamm H, Light RW. Parapneumonic effusion and empyema. *European Respiratory Journal* 1997; 10(5):1150-1156

Khan FY, Alsamawi M, Yasin M, Ibrahim AS, Hamza M, Lingawi M, Abbas MT, Musa RM. Etiology of pleural effusion among adults in the State of Qatar: a 1-year hospital-based study. *Eastern Mediterranean Health Journal* 2011; 17(7):611-618

Koegelenberg CFN, Van Der Made T, Taljaard JJ, Irusen EM. The impact of HIV infection on the presentation of lung cancer in South Africa. *South African Medical Journal* 2016; 107(7):666-668

Laniado-Laborin R. Adenosine Deaminase in the Diagnosis of Tuberculous Pleural Effusion. *Chest Journal* 2005; 127(2):417-418

Light RW. Pleural effusion. *New England Journal of Medicine* 2002; 346(25):1971-1977

Lienert T, Serke M, Schönfeld N, Loddenkemper R. Lung cancer in young females. *European Respiratory Journal* 2000; 16(5): 986-990

Maskell NA, Butland RJA. BTS guidelines for the investigation of a unilateral pleural effusion in adults. *Thorax* 2003; 58(2): ii8-ii17

Mbata GC, Ajuonuma BC, Ofondu EO, Okeke EC, Chukwuonye I, Aguwa EN. Pleural Effusion: Aetiology, Clinical presentation and Mortality Outcome in a Tertiary health Instituition in Eastern Nigeria – A Five Year retrospective Study. *Journal of AIDS & Clinical Research* 2015; 6(2)

McGrath EE, Anderson PB. Diagnosis of pleural effusion: a systematic approach. *American Journal of Critical Care* 2011; 20(2):119-127

Medford A, Maskell N. Pleural effusion. *Postgraduate Medical Journal* 2005; 81(961):702-710

Minami H, Yoshimura M, Miyamoto Y, Matsuoka H, Tsubota N. Lung cancer in woman: Sex-Associated Differences in Survival of Patients Undergoing Resection for Lung Cancer. *CHEST* 2000; 6(118):1603-1609

Pakkala S, Ramalingam SS. Lung Cancer in HIV-Positive Patients. *Journal of Thoracic Oncology* 2010; 5(11):1864-1871

Pandit S, Chaudhuri AD, Bhuniya S, Datta S, Dey A, Bhanja P. Role of pleural biopsy in etiological diagnosis of pleural effusion. *Lung India* 2010; 27(4):202-204

Pinzone MR, Fiorica F, Rosa MD, Malaguarnera G, Malaguarnera L, Cacopardo B, Zanghi G, Nunnari G. Non-AIDS-defining cancers among HIV-infected people. *European Review for Medical and Pharmacological Sciences* 2012; 16(10):1377-1388

Porcel JM, Light RW. Diagnostic Approach to Pleural Effusion in Adults. *American Academy of Family Physicians* 2006; 73(7):1211-1220

Rahman NM, Chapman SJ, Davies RJO. Pleural effusion: a structured approach to care. *British Medical Bulletin* 2004; 72(1):31-47

Scott LE, Beylis N, Nicol M, Nkuna G, Molapo S, Berrie L, Duse A, Stevens WS. Diagnostic Accuracy of Xpert MTB/RIF for Extrapulmonary Tuberculosis Specimens:

Establishing a Laboratory Testing Algorithm for South Africa. *Journal of Clinical Microbiology* 2014; 52(6):1818-1823

Sheridan J, Collins AM. Adult lung cancer in Southern Africa: epidemiology and aetiology. *African Journal of Respiratory Medicine* 2013; 8(2):10-12

Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Zungu N, Labadarios D, Onoya D. South African National HIV Prevalence, Incidence and Behaviour Survey, 2012. Cape Town, *Human Sciences Research Council Press* 2014.

Van Staden A, Badenhorst G. Reviewing gender and cultural factors associated with HIV/AIDS among university students in the South African context. *Curationis* 2009; 32(4):19-28

Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CFN. Tuberculous pleural effusions: advances and controversies. *Journal of Thoracic Disease* 2015; 7(6):981-991

World Health Organization. *Global Tuberculosis Report 2014*.Geneva: WHO