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Histopathological diagnoses on pleural biopsy specimens at Chris Hani Baragwanath Academic Hospital over a 15-year period: a retrospective review

Edgar, Jason

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Histopathological diagnoses on pleural biopsy specimens at Chris Hani Baragwanath Academic Hospital over a 15-year period: A retrospective review

Jason Edgar

A research report submitted to the University of the Witwatersrand, Johannesburg in fulfilment for the requirements of the degree of Master of Medicine.

Declaration

I, Jason Edgar, declare that this research report is my own work which is being submitted for the degree Master of Medicine (in the submissible format with my protocol and an extended literature review) in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other university.

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…… day of ……2018

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Professor Menezes, for agreeing to be my second supervisor. Your experience in the research process and guidance were invaluable in allowing me to navigate the complexities of this project.

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To Dr Olorunfemi for his endless patience, assistance and teaching around statistical analysis and interpretation.

Abstract

Background

Pleural effusions are a common reason for presentation to health care facilities. The clinicians' approach to the investigation of exudative pleural effusions often requires pleural biopsies. Blind closed pleural biopsy can be a useful tool, especially in resource-limited settings to diagnose the cause of exudative pleural effusions.

Objectives

To determine the variety, frequency and change in profile of histopathological diagnoses of closed pleural biopsies at Chris Hani Baragwanath Academic Hospital over the period from 1st January 2001 to 31st December 2015.

Methods

A retrospective review of pleural biopsies performed on patients from 1st January 2001 to 31st December 2015 at Chris Hani Baragwanath Academic Hospital were examined by the Department of Anatomical Pathology at the National Health Laboratory service. Patients' age, gender, HIV status and histopathological diagnosis were obtained from two databases (DISA and TrakCare).

Results

A total of 1 013 samples were included in the study. The most common diagnosis was granulomatous inflammation in 48% (n=375), with the most common type being necrotizing granulomatous inflammation in 73.8% (n=276). Ten percent (n=78) of biopsies showed malignancy, most commonly adenocarcinoma, with 46% (n=36) metastatic and 23% (n=18) primary lung adenocarcinoma. The odds of being

diagnosed with malignancy showed increasing statistical significance above the age of 40 years: 40-49 years (OR 8.7, 95% CI 1.1-66.9, p=0.038), 50-59 years (OR 12.4, 95% CI 1.6-95.0, p=0.015), 60 years and greater (OR 23.0, 95% CI 3.1-171.3, p=0.002). The odds of being diagnosed with malignancy in this study was greater in HIV negative patients (OR 0.5 95 CI 0.2-1.0, p=0.040), with greater odds of a “non-cancer” diagnosis in HIV positive patients (including granulomatous inflammation and pleuritis (OR 2.16, 95% CI 1.03-4.51, p=0.040)).

Conclusion

Blind closed pleural biopsy has a role to play in the diagnosis of exudative pleural effusions in resource-limited settings, particularly for patients suspected to have tuberculosis or malignancy. Tuberculosis remains a common cause of exudative pleural effusions. Patients with an exudative pleural effusion (in whom the diagnosis is not obvious by other means) should have a pleural biopsy performed. Sampling technique is important to obtain specimens of adequate quality for assessment. There was a high frequency of inadequate specimens noted in this study suggesting that further training in pleural biopsy technique may be of benefit.

CHAPTER 1 Table of Contents

Declaration II

Acknowledgements III

Abstract IV

List of Figures VIII

Abbreviations IX

CHAPTER 1 : PROTOCOL WITH EXTENDED LITERATURE REVIEW 1

1.1 Introduction 1

2. Study aims and objectives 8

2.2 Study Objectives 8

Primary objective 8

Secondary objectives 8

2.3 Methods 9

2.3.4 Data analysis 12

2.3.5 Ethics 12

2.3.6 Timing 13

2.3.7 Funding 13

2.3.8 Study Limitations 13

3. References 15

CHAPTER 2 : SUBMISSIBLE ARTICLE 19

CHAPTER 3 : APPENDICES 40

Appendix 1 40

List of Tables

Table 1-1 Comparison of diagnostic yield and diagnoses obtained on closed pleural biopsy according to study 5

Table 2-1 Number of diagnoses obtained on pleural biopsy classified according to HIV status 33

List of Figures

Figure 1-1 Comparison of malignancy diagnoses by study 5

Figure 2-1 Study enrollment 34

Figure 2-2 Sample number according to year 35

Figure 2-3 Distribution of percentage of inadequate samples submitted by year 36

Abbreviations

ADA	Adenosine deaminase
CHBAH	Chris Hani Baragwanath Academic Hospital
GI	Granulomatous inflammation
HIV	Human immunodeficiency virus
NGI	Necrotizing granulomatous inflammation
NNGI	Non-necrotizing granulomatous inflammation
TB	Tuberculosis
ZN	Ziehl-Neelsen

CHAPTER 2 : PROTOCOL WITH EXTENDED LITERATURE REVIEW

1.1 Introduction

Pleural effusions are a common reason for presentation to health care facilities. It is estimated that pleural disease affects approximately 300 persons per 100 000 population per year worldwide.[1] Local data suggests pleural effusions are being encountered more frequently in the South African population then in the past perhaps due the increasing rates of tuberculosis. Data from the Department of Chemical Pathology and Internal Medicine at the University of Stellenbosch and Tygerberg Hospital showed 10.7 new cases of pleural effusion per week in 1993.[2] Establishing the underlying cause for a pleural effusion is important in guiding the clinician's therapeutic strategy and ultimately impacts on outcomes for that patient.

Current recommendations are that all patients with more than a minimal pleural effusion should undergo diagnostic thoracocentesis.[3] Classification of the fluid obtained into transudates or exudates based on various published guidelines and criteria assists in narrowing the differential diagnosis. The most commonly used is Light's criteria, which compares serum and pleural fluid protein and lactate dehydrogenase levels, as well as the degree of the increase in lactate dehydrogenase level in the pleural fluid.[4] However, this still does not provide a specific diagnosis, necessitating further diagnostic tests.

1.2 Role of closed pleural biopsy

Pleural tissue sampling provides a more direct method of making a definitive diagnosis. Percutaneous pleural biopsy is currently recommended for undiagnosed

exudative pleural effusions when tuberculosis or malignancy is suspected.[4] In published work done by Light *et al.* in 2001, it was suggested that the combination of histology and culture of pleural biopsy tissue revealed the diagnosis of tuberculosis in up to 90% of cases.[5]

Abrams and Cope needles have been shown to have equal efficacy, diagnostic yield and complication rates in closed pleural biopsy.[20] Use of imaging techniques such as ultrasound and CT (computed tomography) scan increase the diagnostic rates of pleural biopsy compared to blind pleural biopsy. For example when assessing for malignancy, ultrasound guided pleural biopsy had a diagnostic yield of 89.7%[6], CT guided pleural biopsy had a diagnostic yield of 87%[7] and blind closed pleural biopsy had a diagnostic rate of 50%.[8] Medical thoracoscopy can increase this yield further to 91-95% for malignancy.[9] However these modalities require more equipment, expertise and subsequently have a higher cost associated with them. This becomes important in resource limited settings.

There are conflicting opinions in the literature regarding the benefit of closed pleural biopsy compared to pleural fluid analysis in making a diagnosis of malignancy. A study done at the Mayo clinic in 1985 analysed 414 cases where closed needle biopsy was compared with cytological analysis of pleural fluid obtained by thoracocentesis, and found cytological analysis had superior sensitivity to pleural biopsy tissue examination for detecting malignancy.[10] However, since this study, a number of articles have been published contradicting this and suggesting that pleural biopsy tissue examination provides a higher yield when looking for malignancy, albeit a small to moderately increased difference of between 7 and 27%.[11,12]

1.3 Pleural biopsy diagnoses

When reviewing the incidence of various diagnoses on pleural biopsy specimens, the geographical and socioeconomic background of the population studied should be kept in mind. For example, communicable diseases such as tuberculosis are more common in developing countries than in First World countries. Therefore, caution should be exercised when comparing South African data to studies done in First World settings.

However, studies from other developing countries looking at diagnostic yield of a variety of diagnoses on pleural biopsy tissue specimens. A Malaysian study of 100 patients conducted in 1991 showed a 46% positive yield for patients with tuberculous pleural effusions and 67% for malignant disease. The study also included diagnoses of transudative pleural effusions and bacterial infection.[13] A similar study from North Lebanon using a sample population of 165, found a diagnosis of tuberculosis (43.7%), malignancy (32.1%), empyema (13.3%) and parapneumonic effusion (10.9%).[14] Both studies looked at closed pleural biopsy using an Abrams needle; however, the sample sizes were relatively small. An analysis of pleural biopsy diagnostic yields done in Iran in 2013 showed a positive diagnostic yield for tuberculosis in 32.7%, and 55.6% for malignancy in a sample of 171 patients. No other diagnoses were found in this sample population. This Iranian study also documented the number of inadequate specimens submitted (9%) and the number of inconclusive results (28%).[15]

These last two categories are important aspects to consider when deciding whether closed pleural biopsy is a clinically useful test to perform in patients with

undiagnosed exudative pleural effusions. Of note, all pleural biopsies done in this Iranian study were performed by pulmonary specialists.

This Iranian study also used closed pleural biopsy with an Abrams needle. The Abrams needle was first described in 1958[16], and has become the standard way to obtain pleural biopsy specimens due to its ease of use, safety and relatively low cost.

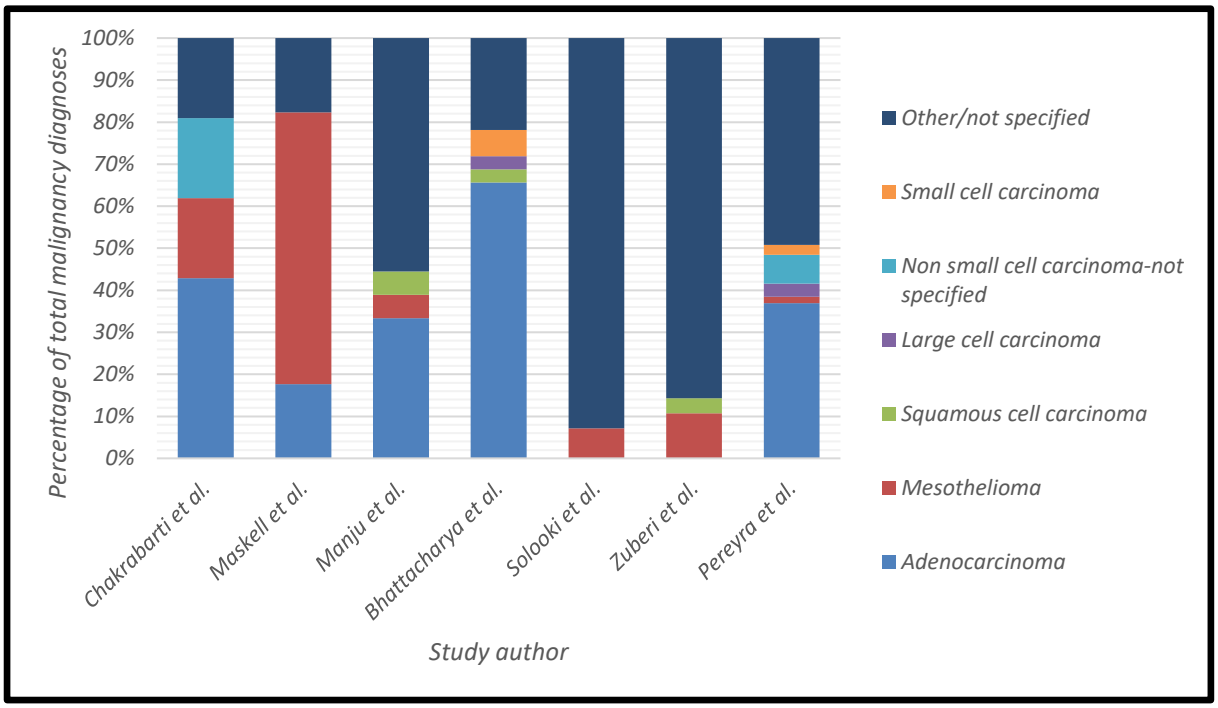
One of the most recent studies published in 2016 in Pakistan looked at 94 pleural biopsy specimens performed using a closed pleural biopsy technique. Of these specimens, 6 (6.4%) showed non-specific inflammation, 14 (14.9%) were inconclusive, 46 (48.9%) showed tuberculosis and 28 (29.8%) showed malignancy.[17] Once again, the predominant diagnoses were tuberculosis and malignancy.

1.4 Tuberculosis and malignancy

A variety of other studies have also researched the diagnoses made on closed pleural biopsy samples with the most frequently identified diagnoses being tuberculosis and malignancy. The frequency of tuberculosis diagnosed within the samples obtained varies across studies from 0-64%.[7,8,13,15,18-20] The studies reflecting a 0% frequency of `tuberculosis were predominantly developed countries with the exception of a study from India. Diagnoses of malignancy on pleural biopsy specimens range across studies from 18.5-100%.[7,8,13,15,18-20] Adenocarcinoma is the most common malignancy diagnosed on review of the literature. However, studies

show a variation in predominant malignancy subtype dependent on sample number

Figure 2-1 Comparison of malignancy diagnoses by study



and population (Figure 1-1). The frequency of diagnoses of tuberculosis and malignancy across a variety of studies, as well as the diagnostic yield of closed pleural biopsy is summarized in Table 2-1. The diagnostic yield of any investigation is an important determinant in evaluating the value of performing an investigation.

Table 2-1 Comparison of diagnostic yield and diagnoses obtained on closed pleural biopsy according to study

Study	Region of study	Sample number (n)	Number of diagnostic pleural biopsies (% yield)	Tuberculosis positive*	Malignancy positive*
Ngoh *et al.*	Malaysia	64	33 (51.6%)	13 (39.4%)	20 (60.6%)
Solooki *et al.*	Iran	171	108 (63.2%)	52 (48.1%)	56 (51.9%)
Al-Shimemeri *et al.*	Saudi Arabia	110	54 (49.1%)	35 (64.8%)	10 (18.5%)
Chakrabarti *et al.*	England	75	59 (78.7%)	0 (0.0%)	20 (33.9%)
Maskell *et al.*	England	24	23 (95.8%)	0 (0.0%)	17 (73.9%)
McLean *et al.*	Scotland	24	10 (41.7%)	0 (0.0%)	10 (100%)
Manju *et al.*	India	30	17 (56.7%)	0 (0.0%)	17 (100%)

1.5 Adenosine deaminase

Although this study focuses on pleural biopsy specimens only and not pleural fluid analysis, this study will also examine any documented adenosine deaminase (ADA) values recorded in the pleural biopsy report. Multiple studies have demonstrated that elevated levels of ADA are associated with tuberculous pleural effusions.[23-27] The suggested cut-off level for which an ADA value may be considered sensitive for the diagnosis of tuberculosis varies amongst studies, from > 30 IU/L[23] to > 70 IU/L.[27] ADA may provide useful supporting data to narrow the differential diagnosis. A local study researched a variety of diagnostic tests for tuberculosis, ADA had a sensitivity (using a clinical cut-point of >30 IU/L) of 79% and a specificity of 92.7%. This study also showed the value of interferon gamma levels in pleural fluid as a diagnostic tool for tuberculosis with a sensitivity of 92.5% and a specificity of 95.9%.[28]

1.6 Ziehl-Neelsen stain

Ziehl-Neelsen (ZN) staining of specimens is used for the detection of acid fast bacilli, therefore assisting in the diagnosis of tuberculosis. This stain has been reported to have a sensitivity of 70% and a specificity of 97.1%.[29] The ZN stain is used routinely by the histopathologists where tuberculosis is suspected. Results of this stain will be recorded in this study to assist in guiding the determination of a final diagnosis.

1.7 Closed blind pleural biopsy vs other techniques for pleural biopsy

Alternative methods of obtaining pleural tissue, such as ultrasound-guided closed pleural biopsy and thoracoscopic pleural biopsy are utilized in some centres. This is in comparison to “blind” pleural biopsy which refers to sampling of the parietal pleura without direct visualization of the underlying pleura. These alternative methods are

more expensive, require specially trained personnel and specialized and often expensive equipment. Therefore, the question that is raised is whether simple closed needle pleural biopsy, which is more easily accessible to clinicians as a diagnostic tool, is still a valuable and effective means for making a diagnosis in exudative pleural effusions, especially in resource-limited settings.

1.8 The South African setting

There is a paucity of evidence with regards to pleural biopsy yield and diagnoses in the South African setting. CHBAH is a large academic hospital in Soweto, Johannesburg in the province of Gauteng, South Africa. An analysis of pleural biopsy histopathological diagnoses at this large academic hospital has never been done. The aim of this study is to provide a South African perspective with respect to the diagnostic yield of pleural biopsies and to compare this with international data. Closed pleural biopsies at CHBAH are done exclusively by the Department of Internal Medicine using the Abrams needle, since there is no Cardiothoracic Surgery department at CHBAH. The pleural biopsies in this setting are done predominantly done by registrars under supervision.

There is a high burden of human immunodeficiency virus (HIV) and associated opportunistic infections, particularly tuberculosis, in Sub-Saharan Africa. The joint United Nations programme on HIV and AIDS (UNAIDS) estimated that there were 6.8 million South Africans living with HIV in 2014.[30] It was estimated in 2013 that 450 000 new cases of tuberculosis were diagnosed in South Africa, with 270 000 of those cases being HIV and TB co-infected.[31] Comparison of the incidence of tuberculosis diagnoses on pleural biopsy specimens in this South African setting with the incidence in other developing countries as described above will also provide more

information to South African clinicians, policy makers and researchers to guide management decisions.

Although it may be suspected that in South Africa the majority of exudative pleural effusions are secondary to tuberculosis, there is currently no evidence published to support this suspicion. This study will therefore analyse all diagnoses obtained at this large academic institution, not only in an effort to assess the number of tuberculosis diagnoses made on closed pleural biopsy, but also to provide insight into other diagnoses made over the period of study.

2. Study aims and objectives

2.1 Aims

The aim of this study was to determine the variety of diagnoses on blind pleural biopsies done at CHBAH for comparison with other population groups. It also aimed to determine any associations between the diagnoses made and the age and gender of the patients assessed.

2.2 Study Objectives

Primary objective

1. To determine the variety, frequency and change in profile of histopathological diagnoses made on closed pleural biopsy specimens at CHBAH from 1st January 2001 to 31st December 2015.

Secondary objectives

1. To determine the most common diagnoses made on closed pleural biopsy.

2. To determine possible relationships between age, gender and histopathological diagnosis.
3. To document the number of inadequate pleural biopsy specimens submitted for assessment.
4. To determine correlation between various diagnoses and HIV status if latter recorded on histopathology report.
5. To determine correlation between various diagnoses and ADA (adenosine deaminase) level if recorded on the final histopathology report.

2.3 Methods

2.3.1 Site of study

Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg is the third largest hospital in the world, with approximately 3200 beds and 6760 staff members. The hospital is associated with the University of the Witwatersrand.

2.3.2 Size

A database search of all pleural biopsies done from 1st January 2001 to 31st December 2015 -1266 were identified. These were then examined to determine if they met the inclusion criteria for this study. Biopsy specimens were excluded if the age of the patient was not specified, originated from a patient less than 18 years old or were performed at another hospital. This resulted in 253 specimens being excluded.

2.3.3 Data collection

This study made use of 2 databases (one online database and one offline) kept and managed by the National Health Laboratory Service (NHLS).

The first database is the “DISA” database (offline database) and the second, the “Trakcare” database (online database). The DISA database, used prior to July 2013, was replaced by the Trakcare database. Searches were done on both databases for pleural biopsy specimens submitted and evaluated at CHBAH over a period of 15 years (from 1st January 2001 to 31st December 2015). From the biopsy reports extracted from the database, the following information was captured:

- Age of patient.
- Gender of patient.
- Adequacy of specimen for histopathological assessment.
- Diagnosis based on the assessment of the histopathologist reviewing the specimen, including a diagnosis of “inconclusive” where a definitive diagnosis could not be made.
- ADA level, if reported on the final histopathology result.
- ZN stain result if reported.
- HIV status of patient if stated on the final histopathology report.

Please see below for data collection sheet that will be used during data capturing:

Specimen #	Month	Year	Age	Gender		Adequate		HIV status	ADA result	ZN result	Diagnosis		Inconclusive
				M	F	Y	N					If malignant, type of malignancy	

Specimen #: This is a number allocated to each specimen consecutively according to date of submission.

Month: This reflects the month that the pleural biopsy specimen was collected from the patient.

Year: This reflects the year that the pleural biopsy specimen was collected from the patient.

Age: This reflects the age of the patient that the specimen was collected from (at the time of collection).

Gender: Documentation of whether the patient was male (M) or female (F). A tick was made in the relevant block.

Adequate: This allowed for documentation of whether the pleural biopsy specimen submitted was adequate for assessment of pleural histology: Yes (Y) or No (N). A tick was made in the relevant block.

HIV Status: If HIV status was supplied to the histopathologist by the requesting doctor, this was noted in this column.

ADA result: The ADA result if noted on the final histopathology report.

ZN result: The ZN result if noted on the final histopathology report.

Diagnosis: Documentation of diagnosis suggested by the attending histopathologist on assessment of the pleural biopsy specimen. If malignancy is diagnosed, the type of malignancy was specified.

Inconclusive: A tick was made in this column if after assessment of the specimen no conclusive diagnosis could be suggested.

Of note, no patient names or hospital numbers were captured as part of the study and no patient medical notes were accessed beyond the result of their pleural biopsy

specimen. If malignancy was found on the specimen, the histological type of malignancy (if given) was documented to further define the frequency of distinct types of malignancy on the pleural biopsy specimens reviewed.

2.3.4 Data analysis

Data will be analysed using Stata® software version 13.1 (StataCorp USA). The total number of the various diagnoses will be added up as a simple descriptive assessment of the total number of cases of each diagnosis per year. Data was manually examined for repeated biopsy specimens on the same patient.

The relationship between age and rate of various diagnoses will be compared to each other using correlation statistics. Testing for normality will be done using skewness/kurtosis testing. Where possible, logistic regression analysis will be conducted to determine odds ratios. Where possible, sensitivity, specificity, positive and negative predictive values will be determined for ADA testing and ZN staining.

2.3.5 Ethics

This study only made use of data already in the NHLS database. It did not use patient names and complete anonymity and privacy of data was ensured.

Permission to access data from the National Health Laboratory Service was granted by the Department of Anatomical Pathology.

Permission was also granted by CHBAH management to conduct the research at the hospital.

Ethics clearance was obtained from the University of the Witwatersrand Human Research Ethics Committee – see appendix 1.

2.3.6 Timing

Please see below for graphic representation of the expected timing for completion of the various aspects of this research project.

	Mar '16	April '16	May '16	June '16	July '16	Aug '16	Sept '16	Oct '16	Nov '16	Dec '16	Jan '17	Feb '17	Mar '17	Apr '17	May '17
Literature review															
Preparing protocol															
Protocol assessment by supervisors and committee															
Ethics application															
Data collection															
Data analysis															
Write up															
Submission for examination															

2.3.7 Funding

No funding will be required for this study.

2.3.8 Study Limitations

- Currently at CHBAH it is not routine to perform a pleural biopsy on all exudative pleural effusions. Therefore, the decision regarding whether a pleural biopsy should be done remains the decision of the attending physician and therefore this may influence the ability for this study to give a true reflection of the variety of diagnoses.

- Due to resource constraints there have been periods of sporadic availability of Abrams needles, with some periods where no needles were available for physicians to perform biopsies. This may give the false impression of less pleural effusions presenting to the hospital when in fact the limitation was in performing the test.
- As this study is a purely retrospective analysis, the samples included lack standardization with respect to technique used in obtaining samples with the Abrams needle, patient demographics submitted with samples, and histopathologist assessing the sample. The nature of this study also does not allow for secondary assessment/ confirmation of diagnoses by a second histopathologist.
- Pleural biopsies done in this setting are done predominantly by registrars under supervision and therefore lack of experience may influence the adequacy of samples.

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CHAPTER 3 : SUBMISSIBLE ARTICLE

Title: Histopathological diagnoses on pleural biopsy specimens at Chris Hani Baragwanath Academic Hospital over a 15-year period: A retrospective review

Authors:

1. J. Edgar [a]
2. M. L. Wong [b]
3. M. Hale [c]
4. C. N. Menezes [d]

Affiliations:

[a]Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

[b]Division of Pulmonology, Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand and Chris Hani Baragwanath Academic Hospital, Soweto, South Africa.

[c]Department of Anatomical Pathology, National Health Laboratory Services.

[d]Division of Infectious Diseases, Department of Internal Medicine, Faculty of Health Sciences, University of the Witwatersrand and Chris Hani Baragwanath Academic Hospital, Soweto, South Africa.

Conflict of interest: Nil

Keywords: Pleural biopsy, malignancy, tuberculosis, South Africa

Corresponding Author:

Jason Edgar

Email: jasonedgar2210@hotmail.com

Mobile: +27768123585

Postal Address: P.O. Box 1585, Mondeor, Johannesburg South Africa, 2110

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Abstract

Background. Pleural effusions are a common reason for presentation to health care facilities. The clinicians' approach to the investigation of exudative pleural effusions often requires pleural biopsy. Blind closed pleural biopsy can be a useful tool, especially in resource limited settings to diagnose the cause of exudative pleural effusions.

Objective. To determine the aetiology, frequency and change in profile of histopathological diagnoses made at Chris Hani Baragwanath Academic Hospital over the period from 1st January 2001 to 31st December 2015.

Methods. Retrospective review of pleural biopsies performed at Chris Hani Baragwanath Academic Hospital and analysed by histopathologists from the National Health Laboratory service at Chris Hani Baragwanath Academic Hospital from 1st January 2001 to 31st December 2015 were recorded from two databases (DISA and TrakCare). The subjects' ages, genders, HIV status, histopathological diagnoses as well as ADA and ZN results were recorded.

Results. A total of 1 013 samples were included in the study. The most common diagnosis was granulomatous inflammation 48% (n=375), with the most common type being necrotizing granulomatous inflammation 73.8% (n=276). Ten percent (n=78) of biopsies showed malignancy, most commonly adenocarcinoma, with 46% (n=36) metastatic and 23% (n=18) primary lung adenocarcinoma. The odds of being diagnosed with malignancy showed increasing statistical significance above the age of 40 years: 40-49 years (OR 8.7, 95% CI 1.1-66.9, p=0.038), 50-59 years (OR 12.4, 95% CI 1.6-95.0, p=0.015), 60 years and greater (OR 23.0, 95% CI 3.1-171.3, p=0.002). The odds of being diagnosed with malignancy in this study was greater in

HIV negative patients (OR 0.5 95 CI 0.2-1.0, p=0.040), with greater odds of a “non-cancer” diagnosis in HIV positive patients (including granulomatous inflammation and pleuritis (OR 2.16, 95% CI 1.03-4.51, p=0.040)).

Conclusion. Blind closed pleural biopsy has a role to play in the diagnosis of exudative pleural effusions in resource-limited settings, particularly for patients suspected to have tuberculosis or malignancy. Tuberculosis remains a common cause of exudative pleural effusions. Patients above the age of 40 years presenting with an exudative pleural effusion should have a pleural biopsy performed routinely. This study however shows that there remains a high frequency of inadequate specimens submitted to the laboratory for assessment. Training in the performance of this procedure in an effort to increase diagnostic rates is recommended.

Introduction

Pleural effusions are a common reason for patients to present to health care facilities around the world. The diagnostic work-up of exudative pleural effusions may require a pleural biopsy to obtain a histopathological assessment in order to reach a diagnosis.[14] Closed blind pleural biopsy is a relatively cost-effective investigation that can be used to diagnose the cause of an exudative pleural effusion. The term “blind” in this setting refers to the reliance on obtaining tissue from the parietal pleura without directly visualizing the area being sampled.

The predominant diagnoses found on closed pleural biopsy in other studies included tuberculosis and malignancy. The prevalence of tuberculosis across studies varies up to 64.8%.[1-5] These tuberculosis rates vary depending on the population studied, with higher rates in studies carried out in developing nations. The rate of malignancy diagnoses varies from 18.5%[5] to 100% in two studies performed in Scotland and India.[3,4] The most common malignancies diagnosed on pleural biopsy across these studies were adenocarcinoma (both primary lung and metastatic disease) and mesothelioma. Other malignancies included: lymphomas, anaplastic carcinoma, chondrosarcoma and atypical carcinoid tumours.

There is a paucity of evidence for the use of closed blind pleural biopsy in the South African setting. Chris Hani Baragwanath Academic Hospital (CHBAH) is the largest hospital in South Africa, and indeed, the southern hemisphere. Despite this there have been no studies investigating pleural biopsies in the work-up of exudative pleural effusions at this hospital.

Methods

Study population and data collection

A retrospective review was conducted of closed blind pleural biopsy specimens using an Abrams needle at CHBAH in Soweto, Johannesburg over the period from 1st January 2001 to 31st December 2015. Pleural biopsies performed during this period were submitted to the National Health Laboratory Service where they were analysed by the Department of Anatomical Pathology. Two databases (DISA and TrakCare) were accessed and the reports issued were reviewed. The following information was recorded: age of patient (excluded from study if under the age of 18 years), gender of patient, HIV status if noted in the report, adequacy of specimen submitted, histopathological diagnosis made and pleural fluid adenosine deaminase (ADA) result as well as Ziehl-Neelsen (ZN) stain result if noted on the final report.

Statistical analysis

Data was analysed using Stata® version 13.1 (StataCorp USA). Continuous variables were summarized using means and standard deviations (SDs). Tests for normality were conducted using skewness/kurtosis testing. Significant associations in contingency tables were assessed using Pearson's Chi squared test and in some cases, logistic regression analysis was conducted. Results were taken as statistically significant for p-values of less than 0.05.

Results

A total of 1266 pleural biopsies were reviewed from reports extracted from the NHLS records. Of these, 253 samples were excluded from the study: 222 samples had no patient age specified, 21 samples were specified with an age less than 18 years and

10 samples were submitted from other hospitals. 23 patients had repeat specimens submitted: 14 of these samples were inadequate on first sampling with 5 reflecting an inadequate sample once again on repeat sampling, 4 had a malignancy diagnosis on the first sample confirmed on the second sample, 3 samples reflected pleuritis on the first sample confirmed on the second sample, 1 sample could not be processed on first sampling due to a laboratory error and therefore a second sample was taken which confirmed pleuritis, 1 specimen was inconclusive on the first sample, the second sample reflected primary adenocarcinoma. Samples that had a diagnosis made and confirmed on the second sample were included in the study as a single entry to avoid duplication of diagnoses, however all inadequate samples and the inconclusive sample were included as distinct entries to allow a more accurate assessment of inadequate sampling rates.

A total of 1013 samples were included in the study. Of these, 573 (56.6%) were males and 440 (43.4%) were females. Of samples included in the study, 780 (77%) were considered adequate; 233 (23%) were inadequate (Figure 2-1). The reasons for samples being considered inadequate for assessment included: lack of pleural tissue in the sample, lack of adequate pleural tissue in the sample, and samples damaged to an extent that adequate histological assessment could not be performed. Of the samples considered adequate, 43 were inconclusive following assessment (no specific histopathological diagnosis was made).

Figure 2-2 shows the distribution of total samples submitted according to year. The greatest number of samples submitted was in 2001 (n=157) and the least performed in 2006 (n=33). The greatest percentage of inadequate samples was submitted in 2015 (36.7%) with a trend upwards in the last 5 years (Figure 2-3). The majority of specimens submitted had no HIV status specified for the patient: 364 (35.9% of

specimens) had HIV status specified; of these 162 (44.5%) were HIV negative and 202 (55.5%) were HIV positive.

The range of histopathological diagnoses made on pleural biopsy in this study have been divided into 4 categories: granulomatous inflammation (necrotizing, non-necrotizing and undefined granulomatous inflammation), malignancy, non-specific pleuritis and suppurative pleuritis.

Of the adequate specimens submitted, 67 (8.6%) were normal pleural tissue on histopathological examination. Granulomatous inflammation was found in 375 of the 780 specimens (48%) (Table 3-1). Of these, 276 (73.6%) demonstrated necrotizing granulomatous inflammation (NGI), 36 (9.6%) showed non-necrotizing granulomatous inflammation (NNGI) and 63 (3.75%) showed granulomatous inflammation (GI) that was not defined as either necrotizing or non-necrotizing. The mean age for the diagnosis of granulomatous inflammation was 44 years (SD=15). There was no significant association between HIV status and any type of granulomatous inflammation (p=0.382). Only 18 of the granulomatous specimens had an ADA result noted. There was no statistically significant association between any level of ADA and granulomatous inflammation; however this may merely be a reflection of the small number of ADA results included.

This study also recorded the Ziehl-Neelsen stain result, if done, on the histopathological specimen. Of note, this stain was only done if the pathologist found features of granulomatous inflammation or, in some cases, pleuritis. However, not all specimens with features of granulomatous inflammation had the stain performed: 298 specimens were stained, and 258 of these had granulomatous inflammation (126 of these were ZN positive and 132 were ZN negative). There was also no

statistically significant association between a positive ZN result and necrotizing or non-necrotizing granulomatous inflammation (p=0.793).

Of the 780 specimens, 78 (10%) showed malignancy Table 3-1. Of these, the majority were metastatic adenocarcinoma (46%), with primary lung adenocarcinoma (determined on immunohistochemical staining) second most common (23%), followed by mesothelioma (18%). The mean ages for patients diagnosed with metastatic adenocarcinoma was 58 ±14 years, primary lung adenocarcinoma 57 ±12 years and mesothelioma 57 ±14 years. Other malignancy diagnoses made included: 4 cases of adenocarcinoma of unknown primary, 3 cases of squamous cell carcinoma of unknown primary and one case of plasmablastic lymphoma. There was no statistically significant variance in age group affected when different malignancy diagnoses were compared with each other. Forty-eight percent of patients diagnosed with malignancy were at least 60 years of age. Logistic regression analysis showed statistically significant increased odds of a cancer diagnosis in the age groups 40-49 years (OR 8.7, 95% CI 1.1-66.9, p=0.038), 50-59 years (OR 12.4, 95% CI 1.6-95.0, p=0.015), 60 years and greater (OR 23.0, 95% CI 3.1-171.3, p=0.002). There was no significant difference between malignancy diagnoses based on gender (p=0.450). There was also no significant association between HIV status and malignancy diagnosis. When logistic regression analysis was performed, it demonstrated lower odds for being diagnosed with cancer in samples from patients that were HIV positive (OR 0.5 95 CI 0.2-1.0, p=0.040).

In HIV positive patients, the odds of being diagnosed with a “non-cancer” diagnosis (including granulomatous inflammation and pleuritis) was higher than in HIV negative patients (OR 2.16, 95% CI 1.03-4.51, p=0.040).

The diagnosis of pleuritis was made in 217 (27.8%) of the specimens. Acute pleuritis accounted for 114 (52.5%) of these specimens. Chronic pleuritis was found in 82 (37.8%). Suppurative pleuritis was found in 21 (9.7%) of specimens. There was no statistical significance between the distinct types of pleuritis and HIV status.

Discussion

Pleural effusions are a common presentation to medical units around the world and the work-up of a patient presenting with an exudative pleural effusion is therefore extremely important for timely diagnosis and treatment. Blind closed pleural biopsy is a tool that has been used as a relatively inexpensive means to reach a diagnosis. The majority of pleural biopsies done at CHBAH is done by junior staff (registrars and medical officers) and not by specialist pulmonologists as is the standard in First World settings. Despite this, 72.8% of all samples submitted led to a histopathological diagnosis, suggesting that pleural biopsy remains a valuable tool in this setting. However, the rate of inadequate samples submitted at this hospital per year averaged 22.6% with a trend upwards in the years since 2010. This likely reflects inexperience and inadequate training of staff in correct sampling technique as this compares with rates ranging from 4.2% to 21% in other studies.[1,2]

There have been resource-related difficulties experienced at CHBAH and this may have impacted on sample numbers in some years. These difficulties included a shortage of, or damaged, Abrams needles. There are no clear retrospective records of which years this may have affected.

Almost half (48%) of all adequate samples reviewed, demonstrated some form of granulomatous inflammation, with the predominant form being necrotizing granulomatous inflammation (73.6%). The presence of granulomata in a histological

specimen cannot be considered diagnostic for *Mycobacterium tuberculosis* and other causes of granulomatous inflammation should be considered. However, in the developing world with a high prevalence of tuberculosis, the presence of granulomata should raise concerns for TB as a top differential diagnosis. One hundred and thirty-four out of the total 780 adequate specimens (17.2%) showed positive ZN staining for acid fast bacilli. However, not all specimens were stained and a substantial proportion of specimens that showed granulomatous inflammation also were not stained for acid-fast bacilli. This study also showed that there is no statistically significant association between ZN positivity and the type of granulomatous inflammation (necrotizing vs non-necrotizing). The incidence of TB in other studies conducted in developing countries have shown a TB incidence on pleural biopsy of 32.7-48.9%.[6-8] Despite the higher prevalence of TB in HIV positive individuals, this study failed to show a significant association between HIV positive status and granulomatous inflammation (p=0.382). Unfortunately, due to the small number of samples with HIV status and ZN result included, no meaningful statistics could be derived on the association between HIV status and ZN positivity.

Numerous studies have found an association between high ADA results and tuberculosis. [9-13] The level above which an ADA result should be considered suggestive has varied across studies, from a level of 30 IU/L to a level of 70 IU/L.[9,13] In some cases in this study, ADA results were provided on the report (obtained from pleurocentesis specimens submitted). This study had a very low number of ADA results included; therefore the lack of statistical significance in the relationship between ADA and granulomatous inflammation is most likely simply a result of low sample numbers.

Malignancies were diagnosed in 10% of specimens in this study. This compares to rates of 18.5%-100% in other studies.[1-9,14-21] This study showed that the majority of malignancy diagnoses were adenocarcinomas, with 46% of all malignancies metastatic adenocarcinoma and 23 % primary lung adenocarcinoma (determined using immunohistochemical staining). This was also reflected by Pereyra *et al*. and Bhattacharya *et al*. that showed rates for adenocarcinoma of 40.3%[21] and 65%[18] of all malignancy diagnoses respectively. Mesothelioma (a primary pleural malignancy) accounted for 14% of malignancies diagnosed in this study. This was comparable to other studies that showed rates of 9.4-19%.[1,6,7]

This study supported the finding by Kalaajieh that malignancy diagnosis rates on pleural biopsy increase with increasing age above 50 years.[6] Our study showed that there are statistically significant increased odds of malignancy with older age, with an increasing odds ratio for every decade.

Interestingly in this study there was no statistically significant association between HIV status and granulomatous inflammation or malignancy. Despite only 35.9% of specimens having HIV status noted, when logistic regression analysis was conducted, our study demonstrated that the odds of being diagnosed with malignancy was higher in the HIV negative subgroup of patients, and the odds of being diagnosed with a “non-cancer” diagnosis (including pleuritis and granulomatous inflammation) were significantly greater in the HIV positive subgroup of patients. The HIV positive subgroup of patients was younger than the HIV negative group. Perhaps the lower prevalence in this study of malignancy in HIV positive patients simply reflects a phenomenon where the number of HIV positive patients surviving to the age groups that reflect a higher malignancy prevalence are lower than that of HIV negative individuals.

Pleuritis is a non-specific histopathological finding. In this study, cases of pleuritis were subdivided into acute, chronic and suppurative. Twenty-seven-point eight percent of all cases showed some form of pleuritis in this study. Of these cases, only 6 had a positive ZN result associated with them (3- chronic pleuritis, 2- suppurative pleuritis, 1- acute pleuritis).

Study limitations

The major strength of this study is the large sample size compared to other studies done. However, despite this, these findings must be considered in the light of potential complications. This study was focused on a retrospective review of laboratory records. Patient records (kept as paper records at this hospital) were not utilized to add more clinical information such as original indication for performing the biopsy, other co-morbidities, clinical status of the patient, as well as other findings that may provide supportive information for the diagnosis. Secondly, this study relied on reports generated by the Department of Anatomical Pathology at the National Health Laboratory service. As this was a retrospective review, it did not include second party validation of the diagnosis proposed on the original report. Samples obtained may have been influenced by the experience level of the clinician performing the procedure and histopathological diagnosis may be influenced by the experience level of the histopathologist assessing the specimen. Finally, there was no standardized information form submitted when the original biopsy was submitted to the laboratory. Retrospective review of the reports indicated that a substantial proportion of the biopsy specimens submitted had information missing, such as HIV status and ADA result.

Conclusion

Patients that present with exudative pleural effusions require work-up to diagnose the cause of the pleural effusion. On occasion, the cause may be elucidated in other ways (e.g. sputum microscopy, bronchoscopy, cytology etc.). However, this study has demonstrated that blind closed pleural biopsy remains a valuable tool in the work-up of these patients, especially in resource limited settings. The high numbers of inadequate biopsy specimens submitted suggest that further training in sampling technique may assist in further improving diagnostic rates. The role of pleural biopsy has been shown particularly with respect to the diagnosis of granulomatous disease and malignancy. With the prevalence of HIV being high, especially in developing countries, this study has demonstrated the role of this investigation in the diagnosis of tuberculosis. In the setting of older patients presenting with exudative pleural effusions, pleural biopsy is important when considering a possible diagnosis of malignancy.

Acknowledgements

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Funding

None

Conflicts of interest

None

Table 3-1 Number of diagnoses obtained on pleural biopsy classified according to HIV status

	Normal	Granulomatous inflammation			Non-small cell carcinoma				Small cell carcinoma	Metastatic disease			Mesothelioma	Pleuritis			Inconclusive
		NGI	NNGI	GI	Primary lung adenocarcinoma	Primary lung SCC	Large cell	Poorly differentiated	Small cell neuroendocrine carcinoma	Adenocarcinoma	SCC	Poorly differentiated		Acute	Chronic	Suppurative	
HIV POS	10	65	5	10	6	0	0	0	1	5	0	2	1	23	15	6	5
HIV NEG	5	36	2	6	3	0	0	1	1	12	1	0	1	17	10	3	13
HIV NS	52	175	29	47	9	0	0	0	0	19	1	3	12	74	57	12	25
TOTAL	67	276	36	63	18	0	0	1	2	36	2	5	14	114	82	21	43

HIV – Human immunodeficiency virus, HIV NS – HIV not specified, NGI – Necrotizing granulomatous inflammation, NNGI – Non-necrotizing granulomatous inflammation, GI – Granulomatous inflammation, SCC – Squamous cell carcinoma

*All figures in table represent total number of specimens.

Figure 3-1 Study enrolment

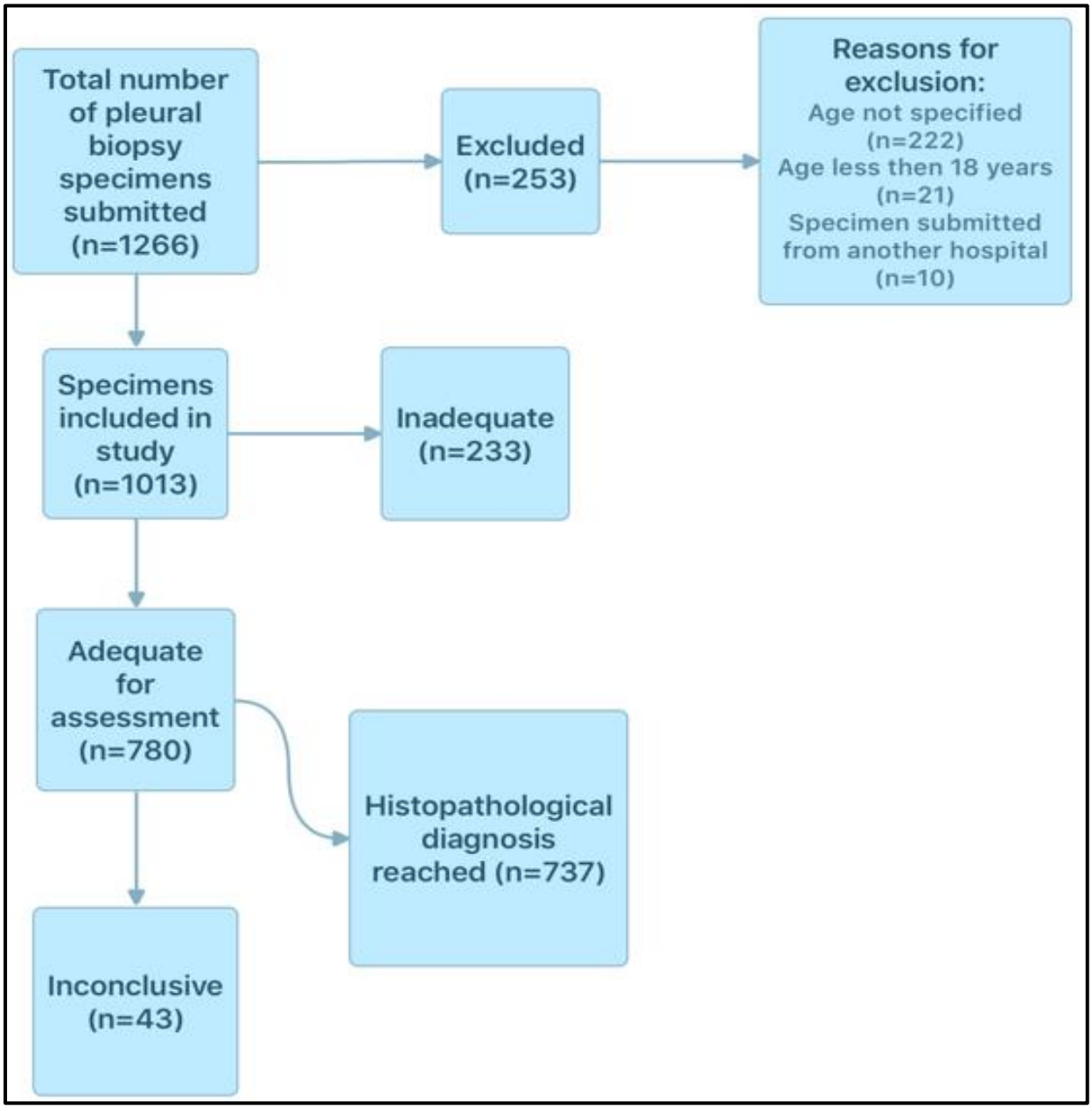


Figure 3-2 Sample number according to year

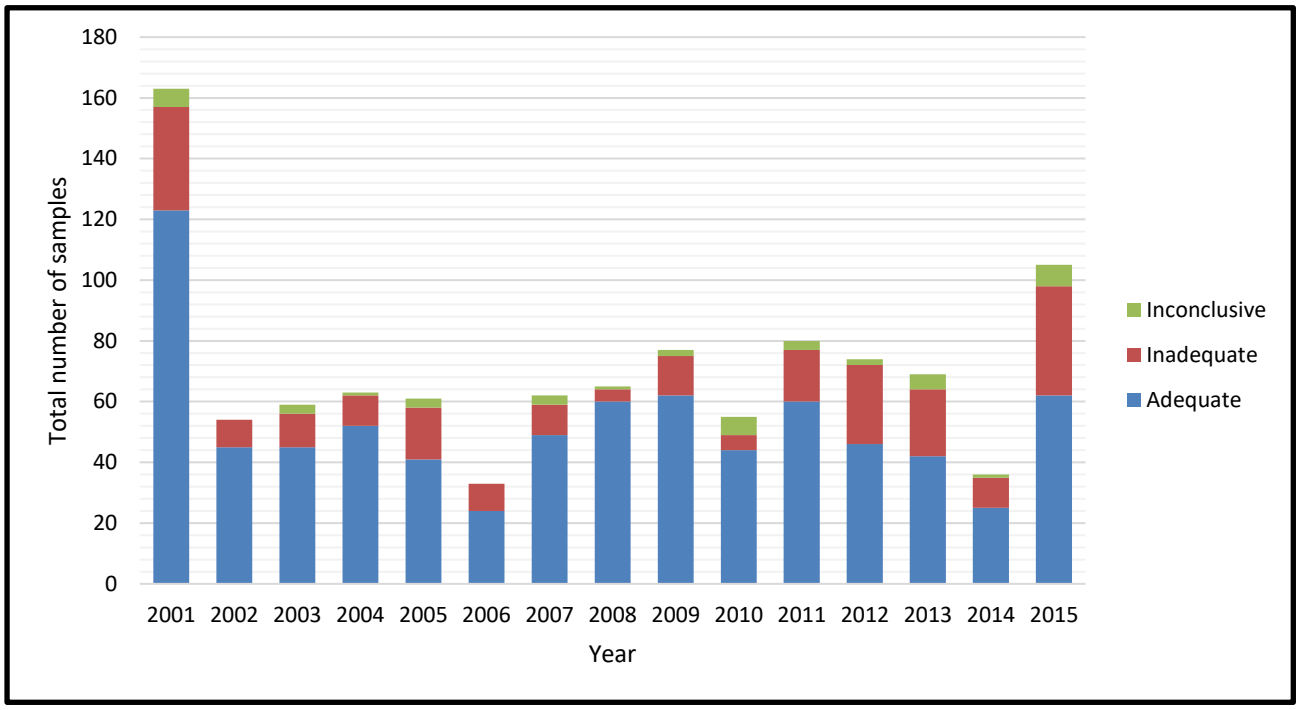
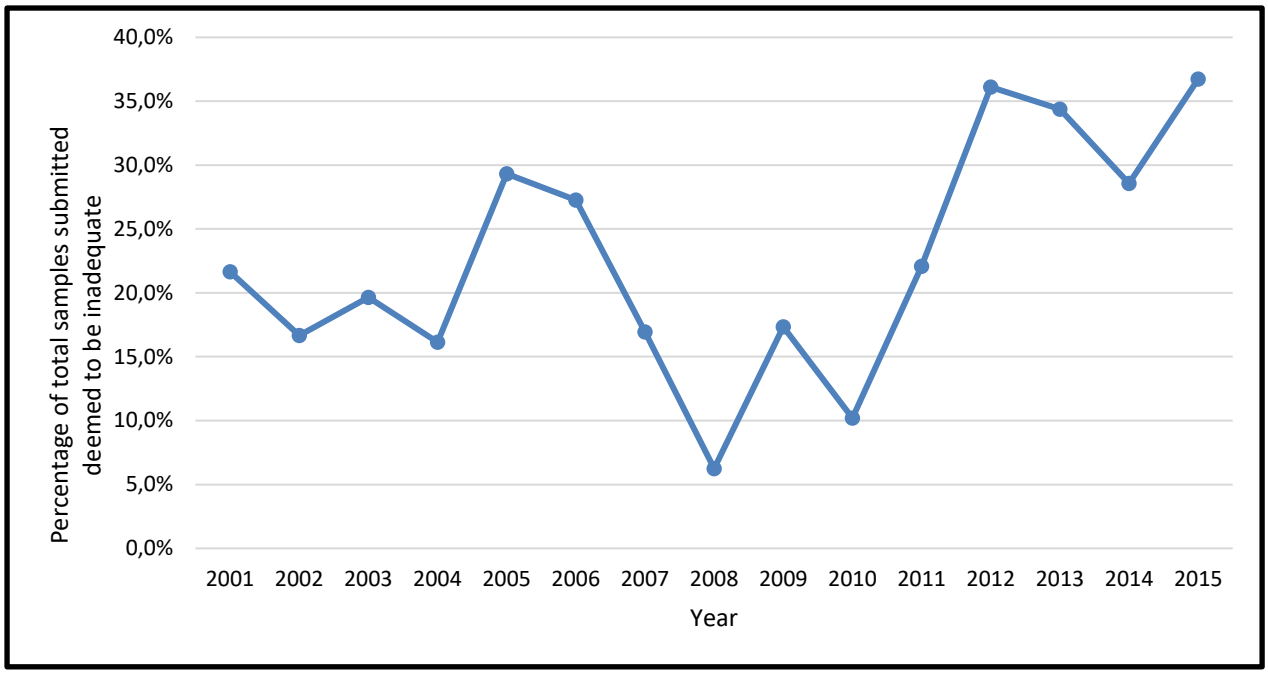


Figure 3-3 Distribution of percentage of inadequate samples submitted by year



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CHAPTER 4 : APPENDICES

Appendix 1

R14/49 Dr Jason Edgar

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160772

NAME: (Principal Investigator)	Dr Jason Edgar
DEPARTMENT:	Internal Medicine Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Histopathological Diagnoses on Pleural Biopsy Specimens at Chris Hani Baragwanath Academic Hospital over a 15 Year Period: A Retrospective Review
DATE CONSIDERED:	29/07/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Michelle Wong and Prof Colin Menezes
APPROVED BY:	Professor P Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	26/08/2016

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

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004, 10th floor, Senate House/3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in July and will therefore be due in the month of July each year.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES