Automated Sputum Screening using the BD FocalPoint™ Slide Profiler: Correlation with Transbronchial and Transthoracic Needle Aspirates in a High Risk Population.

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"Thesis presented in fulfilment of the requirements for the degree of Master of Pathology in the Faculty of Anatomical Pathology at Stellenbosch University"

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Greta Neethling

Date: 26 February 2014

ABSTRACT

Background:

Sputum is a non-invasive, economic investigation whereby bronchogenic carcinoma can be identified. Manual cytological screening is labour intensive, time-consuming and requires a continuous high level of alertness. Automation has recently been successfully introduced in gynaecological cytology. Since sputum samples are similar to cervical smears, the question arises as to whether they are also suitable for automated screening.

Objective:

This study presented with various objectives: 1) To test automated sputum screening using the BD FocalPoint™ Slide Profiler (FP) and compare with manual sputum screening. 2) To determine the sensitivity and specificity of sputum in identification of bronchogenic carcinoma. 3) To ascertain if any clinical, radiological or bronchoscopy findings would be predictors for bronchogenic carcinoma. 4) To determine the significance of adequacy.

Method:

Sputum samples were collected prospectively from patients attending the Division of Pulmonology at Tygerberg hospital for a transbronchial fine needle aspiration biopsy (TBNA) or a transthoracic fine needle aspiration biopsy (TTNA) for the period from 2010 to 2012. A pre-bronchoscopy sputum was collected and submitted for processing. Stained slides were put through the FP for automated screening. After slides were qualified, sputum slides were put back in the routine screening pool. Correlation was done using the TBNA/TTNA result as the standard to evaluate the sputum results.

Results:

108 sputum samples were included in this study. Of the 84.3% malignant (n=91) and 15.7% benign (n=17) cases confirmed with a diagnostic procedure, sputum cytology had a sensitivity of 38.5% (35/91 malignant cases), and a specificity of 100% (17/17 benign cases). Automated screening had a better sensitivity of 94.3% (33/35 positive sputum cases), while manual screening showed a sensitivity of 74.3% (26/35 positive sputum cases) when compared to the final sputum result.

Individual parameters with a significant association with positive sputum included the presence of an endobronchial tumour, partial airway obstruction / stenosis, round mass, spiculated mass (negative association), loss of weight (negative association) and squamous cell carcinoma as the histological subtype. Adequacy was not as significant as hypothesised since 85.3% of true positive sputum, but also 65.5% of false negative sputum, had large numbers of alveolar macrophages present.

Conclusion:

Sputum cytology remains an important part of the screening programme for bronchogenic carcinoma in the public health sector of South Africa. Results confirm that sputum cytology is very specific, and automated screening improves sensitivity. Automated screening proved to be more time efficient, resulting in 83.1% reduction ($p<0.0001$) in the screening time spent per case by a cytotechnologist.

Results confirm that the quantity of alveolar macrophages is not directly proprtional to pathology representation. Positive sputum results did however improve with sputum adequacy, but had no significant association.

Recommendations from this study include adopting automated sputum screening.

ABSTRAK

Agtergrond:

Die verkryging van ‘n sputummonster is ‘n nie-indringende, ekonomiese ondersoek waardeur bronguskarsinoom identifiseer kan word. Nie-geoutomatiseerde sitologiese ondersoek is arbeidsintensief, tydrowend en vereis ‘n deurlopende hoë vlak van konsentrasie en fokus. Outomatisering is onlangs suksesvol geïmplementeer in ginekologiese sitologie-ondersoeke. Aangesien sputummonsters soortgelyk aan servikale monsters is, het die vraag ontstaan of sputummonsters ook geskik sou wees vir geoutomatiseerde sifting.

Doelwit:

Hierdie studie het verskeie doelwitte gehad: 1) Om geoutomatiseerde sifting van sputummonsters te toets deur gebruik te maak van BD Focal Point ™ Slide Profiler (FP), en te vergelyk met nie-geoutomatiseerde sputum sifting. 2) Om die sensitiwiteit en spesifisiteit van sputum in die identifikasie van bronguskarsinoom te bepaal. 3) Om vas te stel of enige kliniese, radiologiese of brongoskopiese bevindings bronguskarsinoom sou kon voorspel. 4) Om die belang van ‘n verteenwoordigende monster te bepaal.

Metode:

‘n Prospektiewe studie van die pasiënte wat die Divisie van Pulmonologie by Tygerberg Hospitaal vir transbrongiale nodale aspirasie (TBNA) of ‘n transtorakale aspirasie (TTNA) vanaf Julie 2010 tot Mei 2012 bygewoon het, is gedoen. ‘n Pre-brongoskopiese sputum is geneem en gestuur vir prosessering. Die gekleurde skuifies is deur die FP gestuur vir geoutomatiseerde ondersoek. Indien die sputumskuifies gekwalifiseer het vir geoutomatiseerde sifting, is hulle in die groep vir ondersoek ingesluit. ‘n Korrelasiestudie, om die sputumresultate te evalueer, is uitgevoer deur die TBNA/TTNA bevindings as standaard te gebruik.

Resultate:

Vir hierdie studie is 108 sputummonsters ingesluit. Vanuit die 84.3% maligne (n=91) en 15.7% benigne (n=17) gevalle, bevestig deur ‘n diagnostiese prosedure, het sputumsitologie ‘n sensitiwiteit van 38.5% (35/91 maligne gevalle) en ‘n spesifisiteit van 100.0% (17/17 benigne gevalle), getoon. Geoutomatiseerde sifting het ‘n beter sensitiwiteit met 94.3% (33/35 maligne gevalle), terwyl nie-geoutomatiseerde (ondersoek) ‘n sensitiwiteit van 74.3% (26/35 maligne gevalle) wanneer met die finale resultaat vergelyk, gevind.

Individuele parameters met ‘n betekenisvolle assosiasie het die teenwoordigheid van ‘n endobrongiale tumor, gedeeltelike lugwegobstruksie / stenose, ronde massa, ‘n spekuleerde massa (negatiewe assosiasie), gewigsverlies (negatiewe assosiasie) en plaveiselkarsinoom as die histologiese subtipe, ingesluit. Geskiktheid van die monster was nie so betekenisvol as wat in die hipotese gestel is nie: aangesien 85.3% van ware positief gediagnoseerde sputummonsters, maar ook 65.5% van die vals negatiewe sputummonsters, groot hoeveelhede alveolêre makrofae ingesluit het.

Gevolgtrekking:

Sputumsitologie bly steeds ‘n belangrike deel van die siftingsprogram vir bronguskarsinoom in die openbare gesondheidssektor in Suid-Afrika. Resultate van hierdie studie bevestig dat sputumsitologie baie spesifiek is en dat geoutomatiseerde sifting die sensitiwiteit verbeter. Ge-outomatiseerde sifting het bewys dat dit meer tydsbesparend is, met ‘n 83.1% vermindering ($p<0.0001$) in die siftingstyd wat deur een sitotegnoloog per geval bestee word.

Resultate het bevestig dat die hoeveelheid alveolêre makrofae nie direk proporsioneel verwant is tot die patologie nie. Hoe meer verteenwoordigend die sputummonster was, hoe groter was die kanse om ‘n akkurate positiewe diagnose te maak. Die assosiasie van die geskiktheid van die sputummonster en die positiewe resultate het egter nie ‘n statisties betekenisvolle resultaat getoon nie.

Aanbevelings vir hierdie studie sluit in die aanwending van geoutomatiseerde sputumondersoeke.

DEDICATION

To **Henry, Marli, Erika** and the rest of my family for their support, patience and love.

To all the dedicated **cytotechnologists and other colleagues** at NHLS/TBH for their contribution and support.

Also to my mentors, **Prof. CA Wright** and **Prof. PAB Wranz** for their encouragement, motivation and faith in me as a professional.

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LIST OF ABBREVIATIONS

ATS	American Thoracic Society
BAC	Bronchioloalveolar carcinoma
CT	Computed tomography
DX	Diagnosis
ERS	European Respiratory Society
FDA	United States Food and Drug Administration
FNA	Fine needle aspiration biopsy
FOV's	Fields-of-view
FP	BD FocalPoint^{tm} Slide Profiler
GS	Guided Screening
LOW	Loss of weight
Mtb	Mycobacterium tuberculosis
N	Lymph node station
N/C	Nuclear to cytoplasmic
NHLS	National Health Laboratory Service
NOS	Not otherwise specified
PR	Process review
ROC	Receiver operating characteristic
T	Primary tumour
TAT	Turn-around-time
TBH	Tygerberg Academic Hospital
TBNA	Transbronchial fine needle aspiration biopsy
TNM	Tumour-Node-Mestastasis
TTNA	Transthoracic fine needle aspiration biopsy
US	Ultrasound

The terminology used throughout this thesis is that which is used in standard British text.

LIST OF APPENDICES

1. INTRODUCTION

This prospective study was designed to test automated sputum screening on pre-bronchoscopy sputum in a high risk population, and compare with manual sputum screening. The study population comprised of patients referred to the Division of Pulmonology at Tygerberg Academic Hospital (TBH) for a transthoracic fine needle aspiration biopsy (TTNA) or a transbronchial fine needle aspiration biopsy (TBNA) between July 2010 and June 2012. These patients were clinically and radiologically suspected to have intra-thoracic neoplasms.

The automated screening system under investigation, the BD FocalPoint^{tm} Slide Profiler (FP), is an United States Food and Drug Administration (1) approved automated computerised primary screening system for cervical cytology smear preparations (Pap smears).(1, 2) The FP is intended to identify slides with evidence of squamous cell carcinoma or adenocarcinoma and their precursor conditions.(3) Sputum smears and cervical cytology smears (Pap smears) share similar characteristics: abnormal cells have similar morphological criteria; squamous and glandular malignancies are the most common malignant tumours; mucus and contaminants are present in the background; both are hypercellular; cytology screening is time-consuming, both requiring a continuous high level of alertness; and both are a common screening tool in South Africa. When carcinoma cells are present in sputum, they are often few in number requiring greater diligence from staff to screen these cases. For these reasons, and to reduce the false negative rate of sputum screening, it was decided to investigate automated sputum screening.

After automated FP screening, the same smears were manually screened by cytotechnologists who were blinded to the FP result. These results were compared to the final outcome/diagnosis of the patient determined on FNA or surgical biopsy. Furthermore, this study also explored the correlation between improved quality of sputum with improved sensitivity. Sputum samples were weighed and alveolar macrophages were counted to see if an increasing number of macrophages corresponded with increased sensitivity.

A comprehensive list of clinical, radiological and bronchoscopy findings were documented and reviewed to determine the strongest predictors for malignancy.

2. LITERATURE REVIEW

Lung cancer has been reported as the most common cancer worldwide for the past several decades, accounting for 13% of the total cancer burden in 2008 (all carcinomas, lymphoma, leukaemia, myeloma, melanoma and tumours of the brain and nervous system).(4) South Africa is not exempt from this burden, with 1 in 82 males and 1 in 259 females being affected by lung cancer during their lifetime.(5)

2.1 Aetiology of lung cancer

Cigarette smoking (tobacco) is by far the dominant risk factor for lung cancer. Exposure to other carcinogens such as asbestos, radon, arsenic, beryllium, bis-choromethyl ether, cadmium, chromium, nickel, polycyclic aromatic hydrocarbons, silica, crystalline, vinyl chloride and other heavy metals has been shown to increase risk for lung cancer.(6) Subsequently, greater incidence of lung cancer has been observed in industries such as mining, coal-gas, metal refining and smelting processes, as well as in painting and welding.(6) With the relatively high incidence of employment in mining in South Africa, these minor environmental risks may also contribute to our increased burden of lung cancer.

2.2 Clinical features

The majority of patients with bronchogenic carcinoma are symptomatic at presentation.(7) A chronic cough with or without sputum production is the most common symptom. Excessive sputum production is an occasional feature of bronchioloalveolar carcinoma / adenocarcinoma-in-situ. Haemoptysis, a common feature in patients with bronchogenic carcinoma, frequently prompts patients to seek medical attention. Localised symptoms are caused by invasion of the chest wall or nerves. This study included various clinical parameters that were recorded to determine potential predictors of bronchogenic carcinoma.

Recurrent pneumonia raises the possibility of an obstructive lesion in the airways and should prompt further investigation.

2.3 Anatomy of respiratory tract

Respiratory cytology is primarily concerned with disease from the lower respiratory tract. This includes the trachea, bronchi, bronchioles and alveoli. Pseudostratified, ciliated columnar epithelium, a few goblet, reserve and neuroendocrine cells line the trachea, bronchi and bronchioles, while pneumocytes and alveolar macrophages are found in the alveoli. Sputum consists predominantly of cells from the upper respiratory tract which consists of squamous cells (stratified, non-keratinised squamous epithelium).(8) The lower respiratory tract is usually further subdivided into central (trachea and major bronchi) and peripheral (minor bronchi and alveoli) portions.

The lower airways begin at the cricoid cartilage in the trachea, dividing at the carina into the left and right main stem bronchi.(9) The distal trachea and main carina are important sites for examination because bronchogenic carcinoma often metastasises to mediastinal lymph nodes.(9) During a TBNA, the mediastinal lymph node station (N) conferring the highest tumour staging when found to contain malignant cells is aspirated first, followed by lower staged stations and finally the primary tumour (T) (i.e. $N_3 \rightarrow N_2 \rightarrow N_1 \rightarrow T$). This approach provides vital staging information. As soon as malignant cells are found, the procedure can be concluded as both diagnosis and staging are established.

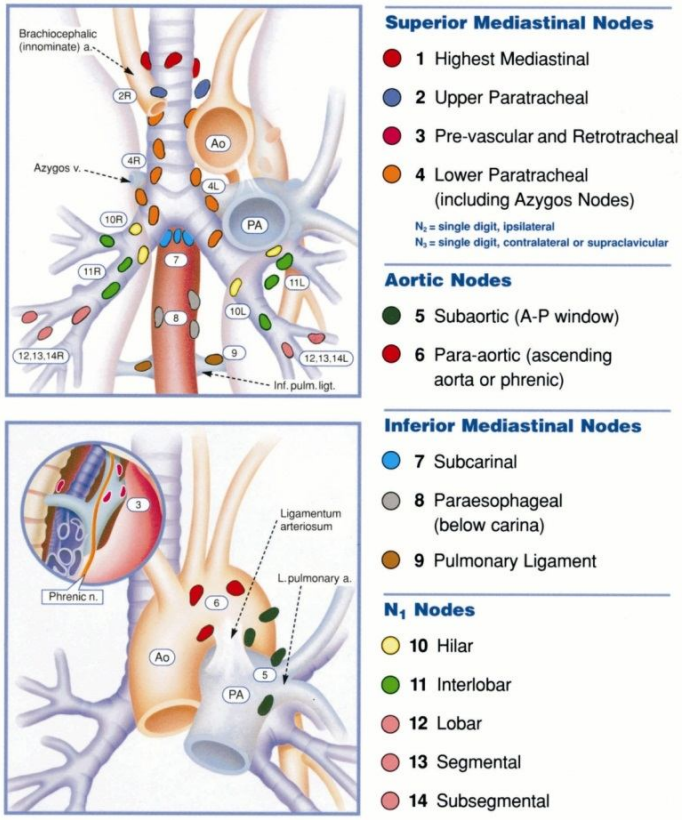

Figure 2.1: *American Thoracic Society and the European Respiratory Society (ATS/ERS) lymph node map (10)*

2.4 Blood and inflammation

Haemoptysis is a worrying sign of a more serious pulmonary lesion, including *Mycobacterium tuberculosis* (Mtb) or carcinoma. Macroscopically bloody sputum is strongly associated with finding malignant cells in the specimen.(11) Extremely bloody background may lead to false negative results as blood may obscure diagnostic cells or inhibit staining of cells resulting in poorly stained smears.

Neutrophils normally occur in low numbers (without infection or inflammation) and may be increased in cigarette smokers. Numerous neutrophils usually suggest pneumonia or an abscess.(8) It is important to note that the presence of an inflammatory exudate does not exclude malignancy. The concurrence of pneumonia and malignancy is a potential and significant diagnostic dilemma for cytologists (cytopathologist and cytotechnologist). Marked inflammatory exudates in the background of malignancy may lead to false negative results. Diagnostic cells may be obscured by inflammatory cells or cytologists may decide that the sputum merely represents an inflammatory condition.

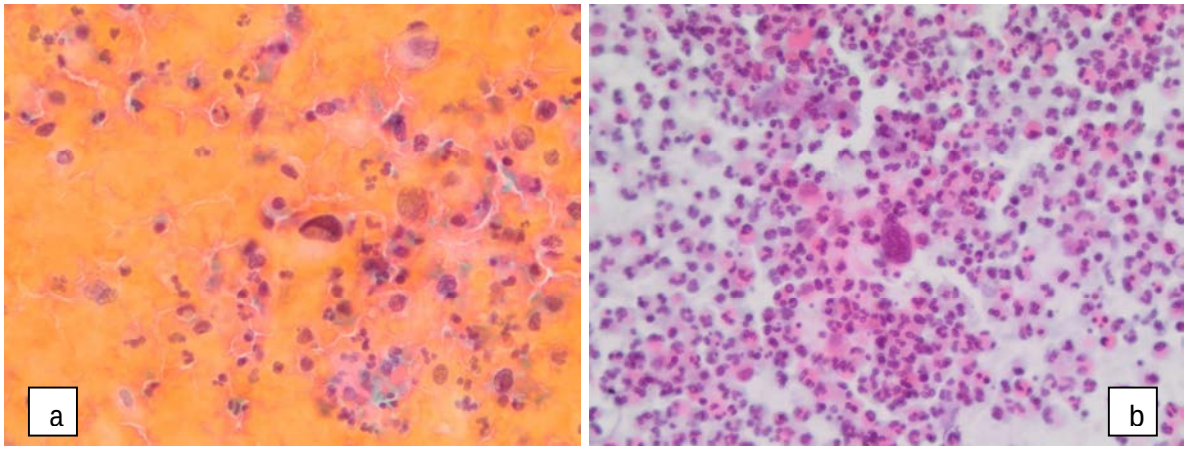

Figure 2.2: *Bloody sputum (a) obscuring malignant cells and result in poor staining. Marked inflammatory exudate present (b) partially obscuring diagnostic cells.*

2.5 Patient management

Optimum management, diagnosis and care of patients with bronchogenic carcinoma requires a ‘’team approach” of dedicated groups of multidisciplinary specialists. These include pathologists, radiologists, pulmonologists, surgeons and oncologists. This study included pathology, radiology and bronchoscopy and hypothesised that valuable data would be generated following this approach.

The Division of Pulmonology at TBH is well established with a good research record. There is an excellent working relationship with the Cytology Unit. Cytopathologists and cytotechnologists/technicians regularly assist with rapid on-site evaluation and diagnosis (12) (12) in the outpatient based, bronchoscopy theatre.(13) Approximately 450 TBNA/TTNA's are performed annually at TBH. As shown by Diacon et al., ROSE (12) of TBNA is a highly useful, accurate and cost-effective addition to routine diagnostic bronchoscopy.(13) On-site discussions with the cytopathologist reduces the incidence of inadequate specimens and allow the pulmonologists to focus on specific areas of sampling.(14) This communication can increase cellular yield, and reduce bronchoscopy time when diagnostic cells are found quickly.(13)

Figure 2.3: *Cytotechnologist (a) and cytopathologist assisting with ROSE in the bronchoscopy theatre (b).*

2.6 Prognosis

The main prognostic factors for bronchogenic carcinoma include the cell type of the tumour, general performance status of the patient, any co-morbidities that could affect treatment options, the disease staging or extent of the tumour and the treatment modality.(6) The most important prognostic factor is determining mediastinal lymph node involvement.(15) Patients with stage I disease who are amenable to surgery have a five-year survival rate of over 60%, whereas patients with stage III or IV and are only amenable to palliative treatment have a five-year survival rate of less than 10%.(16) Almost 90% of patients with non-small cell bronchogenic carcinoma attending TBH have advanced disease at presentation (stage IIIB or stage IV).(17) These patients will still require a cytological or histological diagnosis before starting oncology therapy, and the diagnosis is usually obtained with a TBNA/TTNA.

2.7 Radiology

Low-dose computed tomography (18) may be used in the evaluation and as a radiological screening test for asymptomatic people at risk of lung cancer. Unfortunately this has its own side-effects. The use of CT as a routine method to scan asymptomatic patients is a controversial topic.(19) At this institution, a chest X-ray and staging CT scan of the thorax and abdomen is performed on patients suspected of bronchogenic carcinoma to obtain information about the extent of disease and plan the diagnostic procedure (i.e. TBNA or TTNA). This provides information on the primary lesion and will reveal possible involvement of adjacent structures or metastases required for staging of the carcinoma.(6) Chest X-rays are usually performed at referral clinics or satellite hospitals.

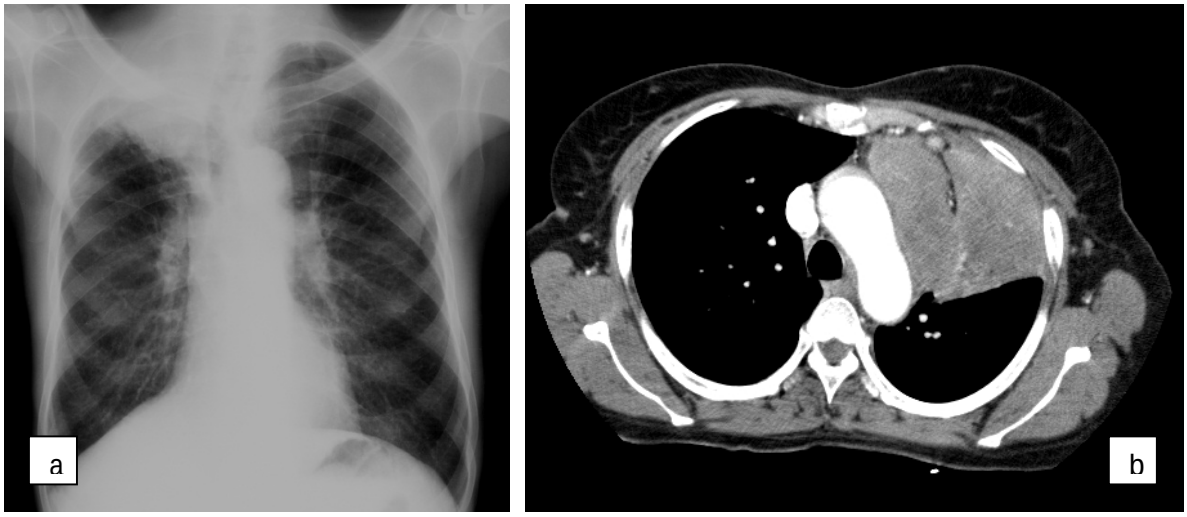

Figure 2.4: *Chest X-ray shows a right apical lung tumour (a). Chest CT scan of large, peripheral lesion (b) with extension to the pleura, amenable to a TTNA.*

Transthoracic ultrasound (US) is used when performing a TTNA on pleural based or peripheral lung tumours.(20) US can visualise parenchymal pathology provided there is contact with the pleura.(21)

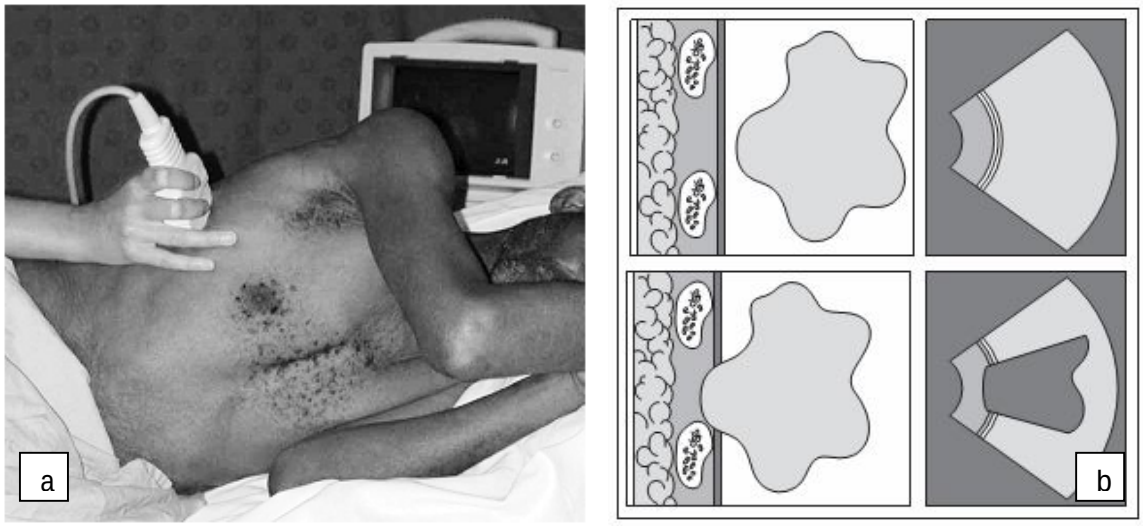

Figure 2.5: *One of the scanning positions for chest US (a). Schematic presentations of peripheral lung lesions without (top) and with (bottom) pleural contact (b). Only the lesion with pleural contact is visible on ultrasound (15)*

2.8 Sampling techniques

There is a range of procedures to choose from when patients are investigated for bronchogenic carcinoma. Sputum cytology is an easy, repeatable, non-invasive test and is particularly helpful if the patient is not a surgical candidate. Further invasive staging might be avoided in such patients.(15) At this institution, if metastases are confirmed radiologically with a positive sputum, the patient is not a candidate for surgery or curative treatment. If a patient has no metastases with positive sputum, the patient will still have to undergo a TBNA for staging purposes.

If there is palpable lymph node involvement or easy access to a metastatic site such as a pleural effusion, these should be aspirated initially. All other patients will undergo either bronchoscopy (fine needle aspiration biopsy, brush, wash or lavage) or a TTNA. The main objective of a TBNA is to diagnose and stage the patient's tumour at the same time.

Due to the high yield of TBNA/TTNA combined with ROSE, only 81 tissue biopsies were performed during 2012 at this institution. This includes 11 Tru-cut pleural biopsies and 38 Abrams' needle pleural biopsies (compared to 453 cytology cases during the same year). Schubert et al. suggested that US-guided TTNA should be the first-line investigation in patients suspected of a lung malignancy, provided the lesion is accessible.(22) Overall sensitivity was superior in FNA of epithelial lung neoplasms compared to cutting-needle biopsy.(22) Tumours of mediastinal origin, haematopoietic or sarcomatous tumours should however undergo FNA and cutting-needle biopsy to harvest tissue, making ancillary investigations possible.

Proietti et al. aimed to compare results from cytology and biopsy samples.(23) Newer targeted therapy demands accurate histological subtyping of tumours. The diagnostic concordance of cytology samples with surgical samples was high ($P<0.0001$). Definitive histological subtype of carcinoma on cytology specimens were accurately reported in 92.8% (206/222) of cases. They concluded that the diagnostic approach to lung cancer does not require more invasive procedures.

In a study by Sackett et al. they confirmed that it is safe practice to use cytology specimens obtained during bronchoscopy as a diagnostic test.(24) Of the 231 cases in their cohort, 97.4% had concordant diagnosis between the different pathologists that interpret these cases routinely.

Due to the emerging differences in medical treatment between adenocarcinoma and squamous cell tumours, accurate histological subtyping of carcinomas has become increasingly relevant in developed countries. Nizzoli et al. investigated the accuracy of FNA cytology in non-small cell carcinoma compared to histology.(25) They reported that when an experienced pathologists is reporting on the cases, the results can be used to plan medical treatment on, especially when more invasive procedures are not feasible. Immunocytochemistry may also be used as an ancillary test to improve diagnostic yield.

In recent years there has been development of prognostic and predictive biomarkers and targeted therapeutic agents. Personalised therapy based on tumour molecular profile can improve treatment efficacy.(26) Most molecular techniques including in situ hybridisation, polymerase chain reaction, and transcriptional profiling can be done on FNA/cytology specimens. Hasanovic et al. concluded that FNA has proven to be an invaluable tool for diagnostic accuracy of pulmonary carcinomas, while also reliable, adequate and a suitable source for molecular testing.

2.8.1. Percutaneous transthoracic fine needle aspiration biopsy (TTNA)

Transthoracic ultrasound (US) is frequently used for the assessment of pleural thickening, pleural or peripheral lung tumours as well as chest wall abnormalities.(20) US can visualise parenchymal pathology provided there is contact with the pleura.(21) US increases the diagnostic yield and minimises risk when compared to blind procedures.(21) It is therefore ideal for image-guided chest wall, pleural, peripheral pulmonary and mediastinal interventions, including diagnostic thoracentesis and biopsy.(20)

US-assisted TTNA has the added advantage that it may be performed outside of theatre, an important practical consideration in patients with advanced disease.(20) TTNA is generally performed under local anaesthesia with a 22-gauge needle.(20)

Diacon et al. demonstrated that US-assisted TTNA with ROSE by a cytopathologist of tumours abutting the chest wall has a diagnostic yield of 82%.(27) US-guided FNA had a low complication rate, with pneumothoraces observed in only 1.3% of cases.(27) Studies also showed that US-guided TTNA is significantly superior to tissue biopsy in confirming a diagnosis of bronchogenic carcinoma (95% vs. 81%, p = <0.05), whereas tissue biopsy is superior in cases of non-carcinomatous tumours and benign lesions.(22, 27) Investigators concluded that tissue biopsy may be reserved for cases where cytology is non-contributory and a diagnosis other than bronchogenic carcinoma is suspected.(22, 27) Because TTNA results have been validated extensively against histology at this institution, it could be used in this study as one of the gold standard tests.

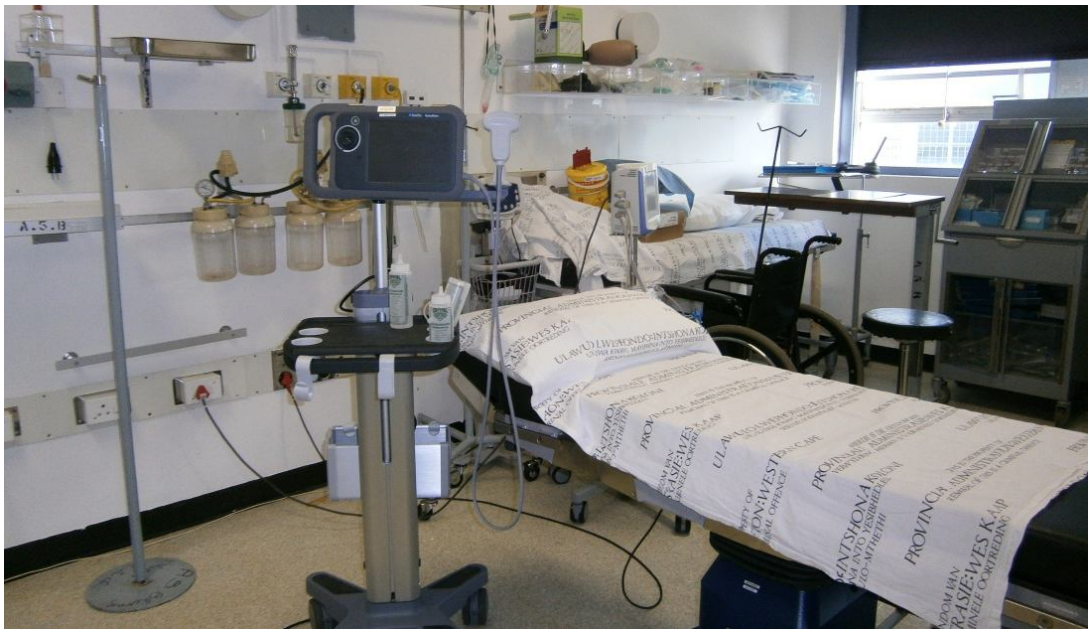

***Figure 2.6**: Theatre where TTNA is performed, equipped with US.*

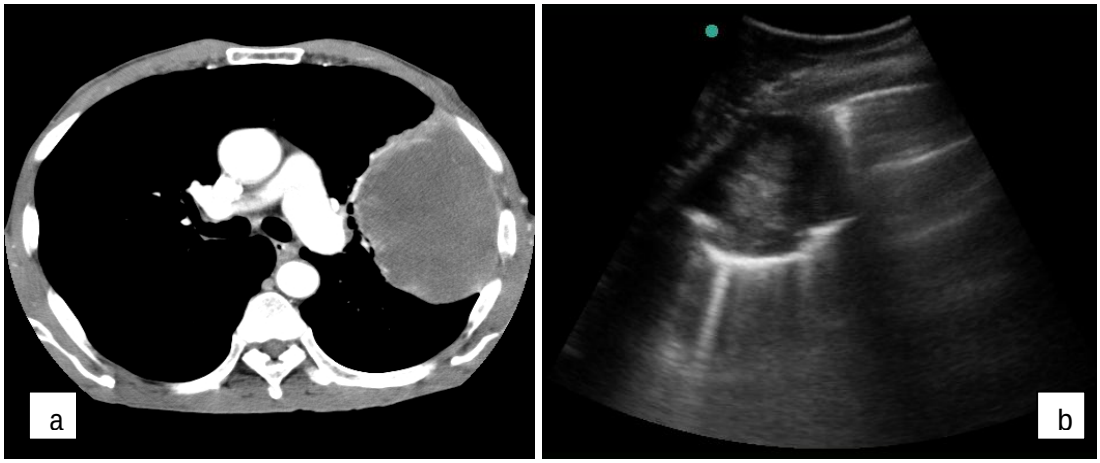

Figure 2.7: *Chest CT (a) and US (b) of peripheral lesions abutting the chest wall amenable to TTNA.*

2.8.2 Transbronchial (Wang) fine needle aspiration biopsy (TBNA)

Flexible bronchoscopy is an investigation which permits direct visual examination of the major airways down to the subsegmental level and thus can verify suspected lung cancer in those anatomical areas. A short, protected and disposable needle attached to a flexible plastic catheter is introduced via flexible bronchoscope and can be inserted into the lesion of interest under direct vision.(28) Jabbing action allows cytological material to be aspirated into the needle while suction is applied to the other end with a syringe.(15) The aspirated material is expelled and spread onto slides so that ROSE can be performed. Modern video enabled bronchoscopes have a dedicated instrument channel for introduction of TBNA needles under sonographic vision.

In bronchogenic carcinoma, TBNA usually establishes the diagnosis and provides staging information in a single procedure.(29) It also facilitates various ancillary sampling methods including bronchial brushing and washing for cytology or tissue biopsies for histology. Direct vision improves the adequacy and quality of the specimens. TBNA enables sampling of mediastinal and hilar lymph nodes providing diagnostic and staging information. It is important to plan this procedure according to the CT scans thus highlighting the “team approach”.

TBNA is an invasive procedure performed only at selected institutions by a pulmonologist. At this institution, yield is improved markedly by the availability of a cytopathologist in the bronchoscopy theatre. This enables immediate evaluation of specimens obtained to assess adequacy, assists with guidance of further required specimens and often providing an immediate diagnosis.(13) This is a time-consuming procedure for a cytopathologist and therefore costly.

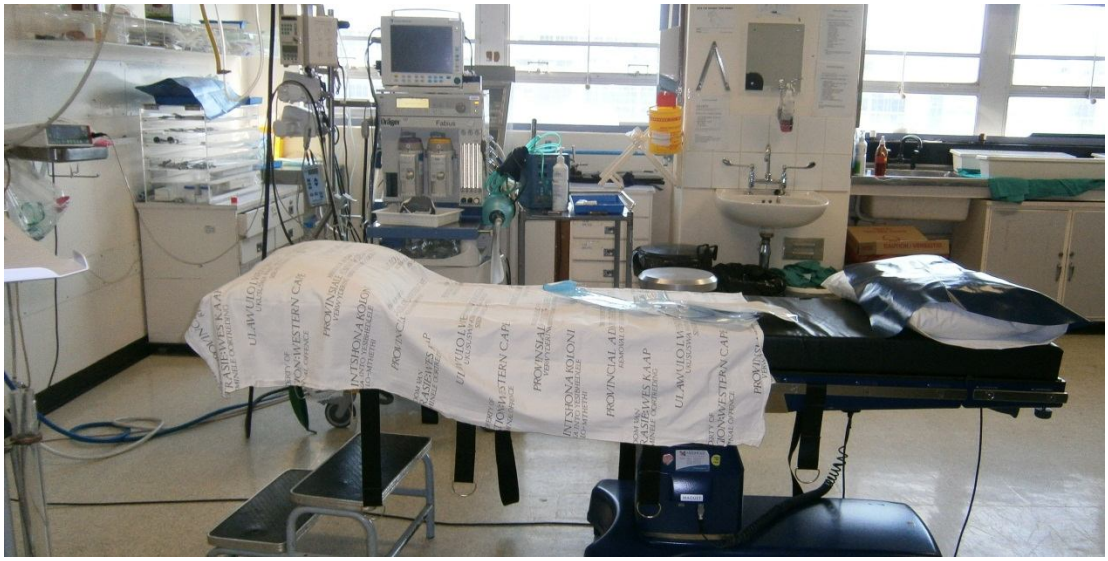

Figure 2.8: *Bronchoscopy theatre where TBNA is performed, equipped with monitors to allow direct vision during bronchoscopy.*

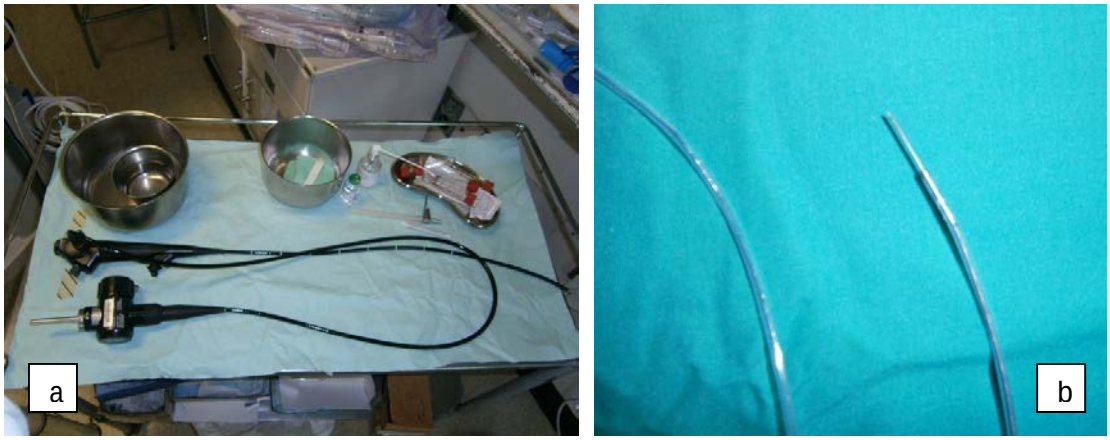

Figure 2.9: *Flexible bronchoscope (a) and disposable needle (b) attached to a plastic catheter which is introduced via the bronchoscope when performing a TBNA.*

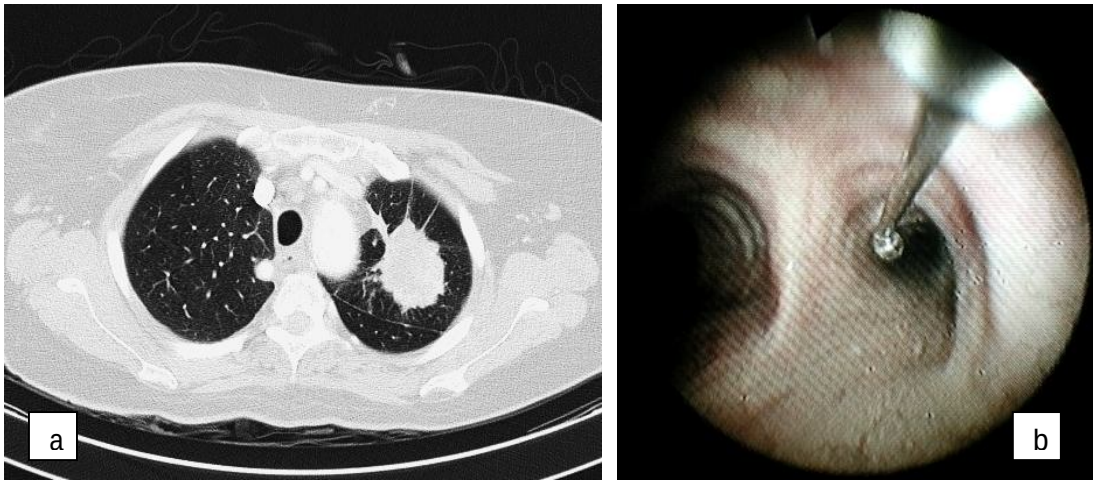

Figure 2.10: *Chest CT scan reveals a central and right sided lung mass which is examined before the TBNA to plan the procedure (a). Distal bronchus with fine needle extended from the bronchoscope (b).*

2.8.3 Sputum

Significant sputum production indicates the presence of pulmonary disease, therefore most patients with bronchogenic carcinoma can spontaneously produce sputum.(8) This study only involved patients producing spontaneous sputum. Although Neumann et al. reported improved sensitivity using induced sputum, induction was not intended for this study.(30) This was not employed because we focused on a practical way to obtain sputum that could be performed anywhere at any time without special equipment or training.

Sputum cytology is an easily obtainable, low-risk, non-invasive, economical investigational tool for the assessment of respiratory diseases, including pre-invasive and invasive bronchogenic carcinoma.(31, 32) Sputum samples an extensive area and is a relatively sensitive specimen for central tumours or for lesions communicating with the airways.(33, 34) Sputum sampling has no contra-indications or complications and can be repeated many times. Raab et al. concluded that sputum cytology, particularly in central lesions, shows significant promise in lowering the costs of testing and initial treatment, lowering the lifetime costs of medical care, lowering the risk of death from testing and initial treatment, and thus improving life expectancy.(33)

Sputum cytology sensitivity is fairly low, with a wide range and little agreement between researchers. Schreiber and McCrory (2003) summarised sputum sensitivity in published evidence of 16 studies which included more than 28,000 patients.(35) These studies were published between 1948 and 1992. Sputum sensitivity ranged from 42% to 97% with an average of 66%.(35) The variables in these studies included method of sputum collection, number of samples obtained, preparation technique, tumour size and the experience of the cytopathologist.(35) The sensitivity from pre-bronchoscopy sputum reported ranged from 10% to 74% with an average of 22%. (35)

Recent studies have shown that sensitivity of sputum specimens have decreased over the last decades to less than 50%. (36, 37) It has been suggested that this decrease may be due to an increased incidence of more peripheral tumours or that clinicians are less motivated to collect good sputum samples. Sputum is only a screening test, and not a diagnostic test, while TBNA/TTNA provide fast and accurate results.(8) Bhattacharjee reported in 2010 that malignant cells were present in 33.8% of positive cases in their study.(38) Yield was better in central (72.7%) than peripheral lesions (18.2%).(38)

Miura et al. found that patients with positive sputum have a poor 5-year survival rate, implying that these patients already have advanced tumours.(39) Other disadvantages of sputum include difficulty in localising lesions, and it is even less sensitive for peripheral tumours including adenocarcinoma which has now overtaken squamous cell carcinoma as the most common primary lung carcinoma.(17, 33, 34)

In developed countries, the number of sputum samples has fallen dramatically mainly due to a combination of the relatively low diagnostic yield of sputum and increased use of bronchoscopy.(40) At this laboratory, 1300 sputum samples for cytology are processed annually and 450 TBNA/TTNA's are attended.

South Africa's medical services are decentralised in rural areas, patients have transport difficulties and limited access to hospitals with bronchoscopy units. Additionally TBNA/TTNA is expensive. This makes sputum cytology an important potential component of a screening programme for lung carcinoma, especially in health care settings with limited resources. We should rather attempt to improve the quality and adequacy of sputum obtained from patients, improve cytology screening and interpretation. For these reasons automation of sputum screening was attempted to ascertain if sputum cytology sensitivity could be improved.

Other situations in which sputum cytology is justifiable include confirming unresectable advanced carcinoma, detecting pre-malignant lesions or early bronchogenic carcinoma and screening of high-risk patients with occupational carcinogen exposure.(41, 42)

2.9 Benign changes

Squamous metaplasia is extremely common. These cells are small and angulated. Squamous metaplasia is not considered a pre-malignant condition in itself, but the milieu in which carcinoma may arise.(8) Atypical squamous metaplasia can be associated with lung injury, including reaction to inflammation or tumours, and often results in false positive diagnosis.(43) Cells have slightly enlarged nuclei with a high nuclear to cytoplasmic (N/C) ratio, the nuclear membrane appears thickened and chromatin hyperchromatic.(8)

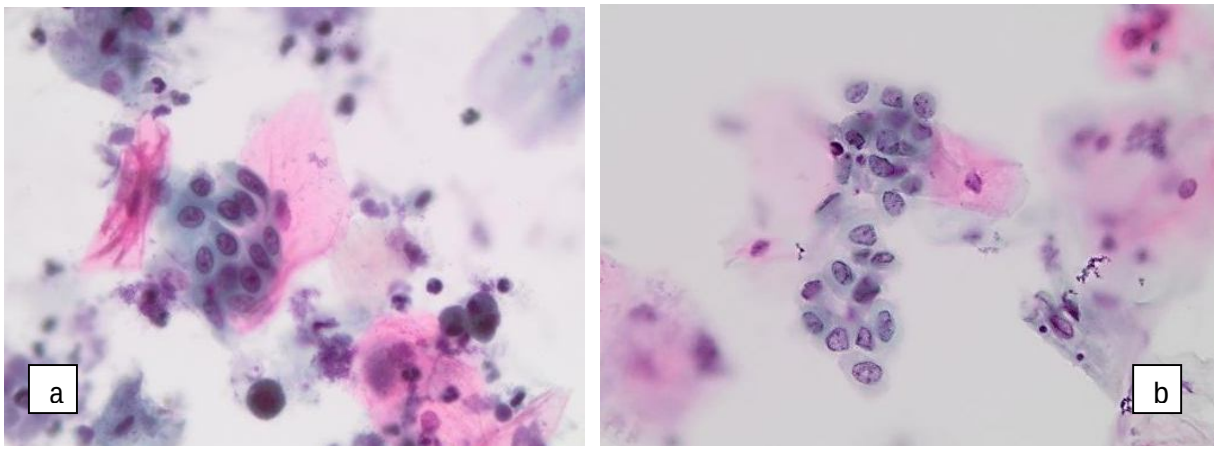

Figure 2.11: *Reactive (benign) squamous metaplasia (a) with regular nuclear membranes and small, regular nucleoli present. Slight nuclear atypia noted in squamous metaplastic cells (b) with irregular nuclear membranes and slight pleomorphism.*

2.10 Pre-invasive lesions

2.10.1 Squamous lesions:

Squamous dysplasia and carcinoma-in-situ are defined as precursor abnormalities for squamous cell carcinoma that may occur as single or multifocal lesions. The pathogenesis of squamous cell carcinoma following the steps of hyperplasia, metaplasia, dysplasia, carcinoma-in-situ and invasive carcinoma is accepted.(44) The diagnosis of dysplasia and carcinoma-in-situ can be difficult, especially when differentiating from marked reactive change associated with pneumonia.

Cells from low-grade lesions resemble squamous metaplasia, however dysplasia is more pleomorphic in size and shape, with an increased N/C ratio, coarse, hyperchromatic chromatin and thick nuclear membranes.(8) Cells from high-grade lesions have small, round to pleomorphic cells with a high N/C ratio, coarse hyperchromatic chromatin and irregular nuclear membranes. All these pre-invasive lesions lack tumour diathesis.(8) Tumour diathesis is composed of necrotic debris, fibrin, inflammatory cells and altered blood.

2.10.2 Glandular lesions:

Atypical adenomatoid hyperplasia is a small (usually less than 5mm in diameter), peripheral lesion characterised by proliferation of atypical cells lining the alveoli and terminal bronchioles. This has been seen in resection specimens associated with adenocarcinoma.(45) It is important to note that these precursor lesions cannot be detected by conventional CT scans, but may be identified at bronchoscopy. Sputum may contain atypical cells that suggest a pre-invasive lesion, leading to improved patient management and triage.(46)

Cells from atypical adenomatoid hyperplasia are fairly monotonous with slight atypia, dense chromatin, inconspicuous nucleoli and scant cytoplasm, almost resembling non-mucinous bronchioloalveolar carcinoma (BAC).(46)

2.11 Bronchogenic carcinoma

Malignant cells have higher N/C ratio's than benign cells, are more pleomorphic with irregular nuclear membranes, abnormal coarse, hyperchromatic, irregularly distributed chromatin. Malignant cells in sputum are often small and degenerate, since cells spontaneously exfoliate prior to collection time, become trapped in mucus and are coughed out eventually. Many bronchogenic carcinomas are mixtures of cell types.(8)

2.11.1 Squamous cell carcinoma

Squamous cell carcinoma comprises approximately 20% of all bronchogenic carcinomas and is strongly associated with a prolonged history of cigarette smoking.(47, 48) At this institution, Nanguzgambo et al. reported that squamous cell carcinoma comprised 25.9% of total bronchogenic carcinoma in their series of a total of 204 cases.(49) This was once the most common histological subtype of bronchogenic carcinoma, but has been surpassed by adenocarcinoma. It has been suggested that the shift has occurred due to increased awareness of smoking as a health risk. Promotion of filter cigarettes reduced the average diameter and weight of the particles inhaled with cigarette smoke. This favours deposition of carcinogens in the peripheral rather than central airways. (50)

The majority of squamous cell carcinomas are located centrally, often causing obstructive symptoms such as cough, dyspnoea, haemoptysis, wheezing, stridor or pneumonia.(47) Tumours range considerably in size from small endobronchial tumours, to large cavitating lesions that replace an entire lobe. Metastasis to lymph nodes and other organs is very frequent with an unfavourable prognosis and high mortality rate.(47)

In sputum, malignant squamous cells typically show differentiated, keratinised cells with dense cytoplasm and pyknotic nuclei.(8) Features indicative of squamous differentiation include keratinised cytoplasm, spindled tumour cells, hyperkeratosis and pearl formation.(51) Clues suggesting invasion include high cellularity, diathesis, and the presence of frankly malignant-appearing cells, i.e. bizarre shapes, heavy keratinisation, irregular chromatin with prominent nucleoli.(8)

2.11.2 Adenocarcinoma

The incidence of adenocarcinoma has recently increased, and it is now the most common primary bronchogenic carcinoma with an average of 38% reported.(17, 48, 49) The incidence in some publications has been recorded to be as high as 60%.(47) At this institution, Nanguzgambo et al. reported that adenocarcinoma comprised 55.4% of total bronchogenic carcinoma.(49) This tumour typically, but not exclusively, occurs in the peripheral areas of the lung.(8) Traditionally, adenocarcinoma was mainly divided into bronchogenic adenocarcinoma and BAC. However, it has recently been suggested that tumours should be reclassified as adenocarcinoma-in-situ (AIS), minimally invasive adenocarcinoma (MIA) and adenocarcinoma with a prominent lepidic pattern/component.(12, 52) (Appendix F)

When these tumours are small and peripheral, malignant cells are less readily seen in sputum.(51) Features typical of adenocarcinoma include nucleocytoplasmic polarity, multiple or macronucleoli and vacuolated or foamy cytoplasm.(51)

2.11.2.1 Adenocarcinoma, conventional type:

Besides the typical features of adenocarcinoma described in 2.11.2, crowded three dimensional groups, papillae, cell balls and acini can be seen with irregular or lobulated nuclear membranes and relatively vesicular nuclei.(8)

2.11.2.2 Bronchioloalveolar adenocarcinoma:

This tumour refers to various cells of origin including terminal bronchiolar cells (mucinous type), Clara cells and type II alveolar pneumocytes (non-mucinous type).(8) Cells are typically well-differentiated and uniform with numerous three dimensional aggregates, irregular sheets, minimal nuclear atypia, nuclear grooves, intranuclear cytoplasmic invaginations, fine chromatin and nucleoli. Cells vary from columnar (mucinous) to cuboidal (non-mucinous); additionally, an abundance of mucus in the background may be suggestive of this tumour.

2.11.3 Small cell carcinoma

Small cell carcinoma comprises approximately 13% of all bronchogenic carcinomas.(48) At this institution, Nanguzgambo et al. reported that small cell carcinoma comprised 14.2% of total bronchogenic carcinoma in their series.(49) This extremely aggressive neuroendocrine tumour commonly presents with distant metastases at the time of diagnosis and is associated with a high mortality rate.(47) It usually arises in a major bronchus with early hilar lymph node involvement, presenting as a peri-hilar mass.(8) Diagnostic cells are often present in sputum and it has been recognised that its central location contributes to this.(8, 11, 30, 53)

Malignant cells are often degenerate in sputum with obscured nuclear detail. Cells are typically small (1-2x size of lymphocyte) and hyperchromatic forming loosely cohesive strings in mucus strands with nuclear moulding. Other features include scant cytoplasm, inconspicuous nucleoli, round to angulated nuclei with crush artefact. The characteristic “salt-and-pepper” chromatin often appears more coarsely clumped in sputum due to degeneration of cells.(8)

2.11.4 Large cell carcinoma

This tumour is strongly associated with a history of smoking, and accounts for approximately 5% of bronchogenic carcinoma.(47, 48) At this institution, Nanguzgambo reported that large cell carcinoma comprised of only 2.4% of total bronchogenic carcinoma.(49) It tends to occur peripherally and is often attached to the pleura or invades adjacent organs.(47) According to the latest proposed classification, large cell carcinoma will be known as “Non-small cell carcinoma, not otherwise specified”.(12) These cells are easily recognised as malignant, but typically lack features of specific differentiation. Cells are large and undifferentiated, often pleomorphic with markedly irregular nuclear membranes and prominent, irregular, multiple nucleoli. Syncytial groups and single cells are a common presentation with relatively abundant cytoplasm. Keratinisation and mucin secretion are absent.

2.11.5 Adenosquamous carcinoma

These tumours are aggressive and comprise only 0.4-4% of all bronchogenic carcinomas.(47) Minor degrees of dual differentiation are common in primary bronchogenic carcinoma. These tumours have evidence of both keratin formation suggestive of squamous differentiation, and mucin secretion suggestive of glandular differentiation. Squamous features include dense cytoplasm, distinct cell borders, keratin rings and pearls. Glandular features include nucleocytoplasmic polarity, acini and mucin.(8) The newly proposed nomenclature is “Non-small cell carcinoma, with squamous cell and adenocarcinoma patterns”.(12)

2.12 Screening for bronchogenic carcinoma

Since bronchogenic carcinoma is such a common fatal malignancy (5), all attempts should be made to introduce effective screening methods to diagnose it early. However, thus far screening for bronchogenic carcinoma has been a major disappointment as it has not shown to significantly reduce mortality.(8) One of the criteria of a successful screening programme is that effective intervention must take place during the pre-symptomatic phase that will alter the outcome of the course of the disease. Currently, there are no established screening tests for lung cancer available. Of note, this study included a selected population at very high risk of lung cancer increasing the probability of a positive sputum compared to randomly selected, asymptomatic individuals included in studies of mass screening.

Jett and Midthun reported in 2004 that screening for bronchogenic carcinoma is not currently recommended. Unfortunately when patients present with symptomatic disease, the tumour is usually advanced. Their study showed that low-dose spiral CT detected smaller tumours (average 1.5 cm), compared to chest X-ray (average 3.0 cm). This resulted in 58% to 85% of non-small cell carcinomas detected while in stage IA using CT.(54)

Extensive research is being done on biomarker-based screening that could lead to diagnosis at a much earlier and more treatable stage. These include molecular markers such as heterogeneous nuclear ribonucleoprotein, matrix metalloproteinases, telomerase and transforming growth factor β with DNA analysis and fluorescent in-situ hybridisation.(55-60)

2.13 Factors affecting diagnostic sensitivity and reliability of sputum

“Diagnostic sensitivity and reliability of respiratory cytology depends on several factors, including time and method of specimen collection, number of samples submitted, tumour cell type and differentiation, size and location of the lesion.”(8) This statement from DeMay was used as the basis of one of this study’s objectives to determine if certain clinical, radiological or bronchoscopy findings would increase the probability of producing positive sputum.

2.13.1 Tumour characteristics

According to Bhattacharjee and Bocking et al., central tumours exfoliate diagnostic cells more readily in sputum samples, while the more peripheral the tumour, the fewer the number of diagnostic cells present.(34, 38) Tanaka and Lam reported that there is a rough correlation between the size of the tumour and the number of cells it sheds, making it more likely to be diagnosed on cytology.(61, 62) When tumours are too large, this can also result in diagnostic difficulties like obstruction of bronchi or lesions producing an abundance of necrosis. The ideal size for optimum exfoliation of diagnostic cells is between 3cm and 6cm.(63)

Bocking et al. demonstrated that more advanced tumours are more likely to shed diagnostic cells.(34) According to Berg et al., well differentiated squamous cell carcinoma and small cell carcinoma are the most accurately classified primary bronchogenic carcinoma, while adenocarcinoma and large cell carcinoma are less readily detected in sputum.(53) They also hypothesised that poorly differentiated tumours shed more cells due to decreased intercellular cohesion, and are more difficult to classify into a specific tumour type.(8, 63)

2.13.2 Specimen collection

Sputum sensitivity has been shown to improve when more specimens are collected for each patient. Bocking et al. have shown that sensitivity improved from 68% for a single sputum specimen, to 78% for two specimens and ultimately to 86% when three or more specimens were collected.(34) This study only collected one sample per patient since collection was performed on the same day as the scheduled diagnostic procedure.

2.13.3 Adequacy of sputum

Parameters for adequacy include sufficient volume of sputum to allow processing of 2 cellular slides, cells should be well preserved without obscuring elements and numerous alveolar macrophages should be present.(64, 65)

Alveolar macrophages indicate that that distal bronchioles and alveoli have been sampled. Their absence indicates that the specimen consists only of saliva and is not representative of the lower respiratory tract. Risse et al. found that sputum with low numbers of alveolar macrophages is often found in false negative sputum samples, indicating that the mere presence of these macrophages does not accurately represent pathology of the lung.(11) Greenberg stated that “adequacy of sputum sample is directly proportional to the number of alveolar macrophages it contains.”(66)

Very few current references are available with specific minimum number of macrophages prescribed for adequacy. Johnson and Frable used 6-10 macrophages per slide as the standard for adequacy, but without evidence based data.(67) Neumann et al. considered sputum specimens to be adequate when at least 50 alveolar macrophages were present per slide.(30) The Papanicolaou Society also merely stated that numerous alveolar macrophages should be present. (65) After their literature search, they did not find consistent data for a numerical cutpoint for macrophages.(65) The presence of ciliated columnar cells is also not sufficient for adequacy, since these cells could have originated from the upper respiratory tract.(8)

Alveolar macrophages are derived from the bone-marrow and are responsible for engulfing foreign material and debris in the alveoli.(8) These macrophages may cause diagnostic difficulty with their irregular nuclei, pleomorphism, prominent nucleoli and vacuolated cytoplasm, often mimicking carcinoma.(43) These phagocytic cells usually contain particles such as carbon, haemosiderin, lipid or mucus. Carbon particles vary in size, colour and texture, ranging from fine to large, black granules often masking the entire cell. Common sources of carbon include cigarette smoking, air pollution and anthracosis.(68)

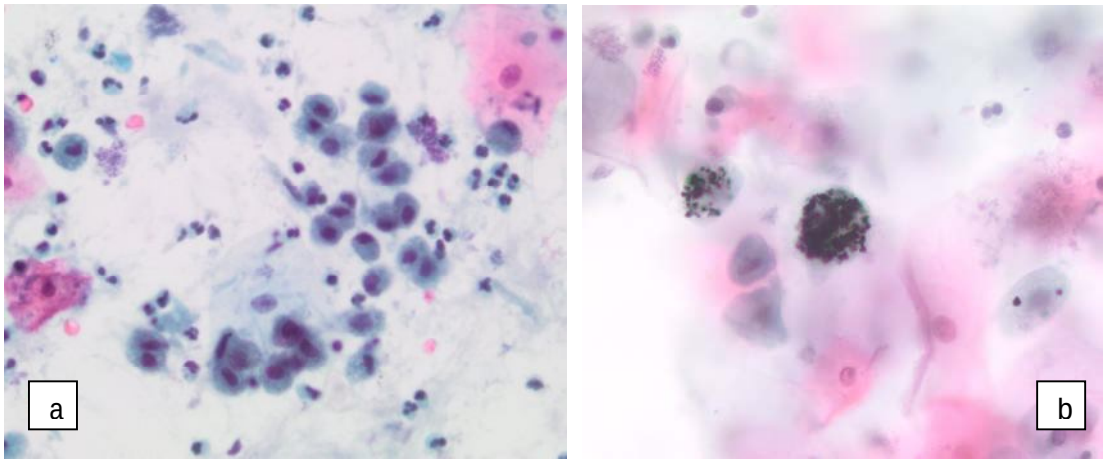

***Figure 2.12**: Images of alveolar macrophages. Numerous macrophages present with minimal carbon pigment (a), and larger, coarser green to black pigment seen in cells (b) which often obscure the nucleus.*

2.14 Automated screening

Neethling et al. demonstrated that the FP is sensitive, reproducible and identify scant, single lying atypical cells.(69) A case was identified in their laboratory with only a few abnormal cells on it after FP review triaged the case for a full rescreen. After careful manual rescreen, it was identified that there were only 3 abnormal cells present on the entire slide (suggestive of a LSIL according to the Bethesda System for reporting cervical smears). After removing the physical dots, the slide was put through the FP slide profiler in 15 different runs. 14 out of the 15 runs at least one of the abnormal cells were present in the 15 FOV's, triaging the case for a full rescreen (i.e. 93% sensitivity). When this case was slipped into a manual screeners routine work, she missed all 3 abnormal cells and called the case "within normal limits".

Wong et al. reported that the implementation of the FP significantly decreased their turn-around-time (27%, $p<0.001$) for Pap smears and substantially increased their productivity (17% improvement) while maintaining diagnostic quality.(70) Their false negative fraction decreased by 36%.

Automated screening was dealt a blow when the outcome of MAVARIC trial was presented in 2010 at the International Academy of Cytology Congress.(71) The long awaited trial was the deciding factor if automated screening would be adopted in the United Kingdom (NHS Cancer Screening Programme). Kitchener et al. evaluated the FP as well as the Thinprep® Imaging System from Hologic and it was suggested that the introduction of automated screening was not justified. Automated screening was 6.3% less sensitive compared to manual screening. The “no further review” system was very reliable in routine screening samples. Similar cost-effectiveness was determined, but with a 60-80% increase in productivity. Some of their laboratories are now using the instrument as a quality control tool.

Colgan et al. concluded in their validation study with a cohort of 10,233 patients that the diagnostic performance of the FP is no different to that of manual screening in detecting LSIL and worse lesions.(72) Manual screening identified more ASC-US cases. They stated that they did not experience similar results as the MAVARIC trial i.e. decreased sensitivity using the FP.

The RODEO Study Team also compared automated versus manual screening in their 10,165 cohort.(73) While using cervical biopsy specimens as the golden standard, they reported no statistical significant difference in the results obtained from the 2 methods. They included that the FP safely screened HSIL lesions and was valuable in high volume laboratories.

Finally Sweeney and Wilbur tested the productivity that could be gained by the FP.(74) Initially, straight after FP implementation in the laboratory, they reported a merely overall average of 2.4% increase in productivity, ranging in individual screeners up to 14.7%. After 6 months of using the FP, they reported an overall increase in the laboratories productivity of 15.4%, ranging in individual screeners up to 26.9%.

2.14.1 History, evolution and FDA approval

The need to automate Pap smear screening, was already recognised in the 1950's by Dr. Papanicolaou and his co-workers.(75) The nature of cervical cytology screening is detecting a few abnormal cells among thousands of normal cells. Failure of early attempts to automate was contributed to limited computing power and lack of software that could effectively analyse cells. Substantial pattern recognition capabilities are necessary to distinguish between cancerous and normal cells as well as other types of objects found in the specimens including debris, degenerated cells, cell clusters and contaminants.(76) With significant information technology advances over the last 15 years, computer screening systems have been more widely accepted and routinely used.(77)

One of the early instruments that was at the forefront of technological advances in automated screening of conventional smears was the AutoPap® 300 QC System developed by NeoPath, Inc. Dr Stanley Patten and his team created comprehensive cell charts that listed detailed morphologic criteria for the differentiation of different cell types.(78) (Appendix G) Engineers would then use this data to produce algorithms used in software programming.

The AutoPap® 300 was approved by the FDA in 1995 as a quality control tool only. In 1998, this instrument was again FDA approved, but this time for primary screening of conventional slides.(1) In 1999, NeoPath, Inc. and AutoCyte, Inc. merged to form TriPath Imaging, Inc.(77) In 2001, the AutoPap® 300 got FDA approval to primarily screen liquid based cytology slides (SurePath^{tm} Pap Test slides). In 2006, TriPath Imaging, Inc. agreed to be aquired by Becton Dickinson and Company, and this system is currently known as the BD FocalPoint^{tm} Slide Profiler (FP). In 2008, the BD FocalPoint^{tm} GS Imaging System was approved by the FDA to scan SurePath^{tm} Pap Test slides.(1)

2.14.2 The complete BD FocalPoint^{tm} System and operation

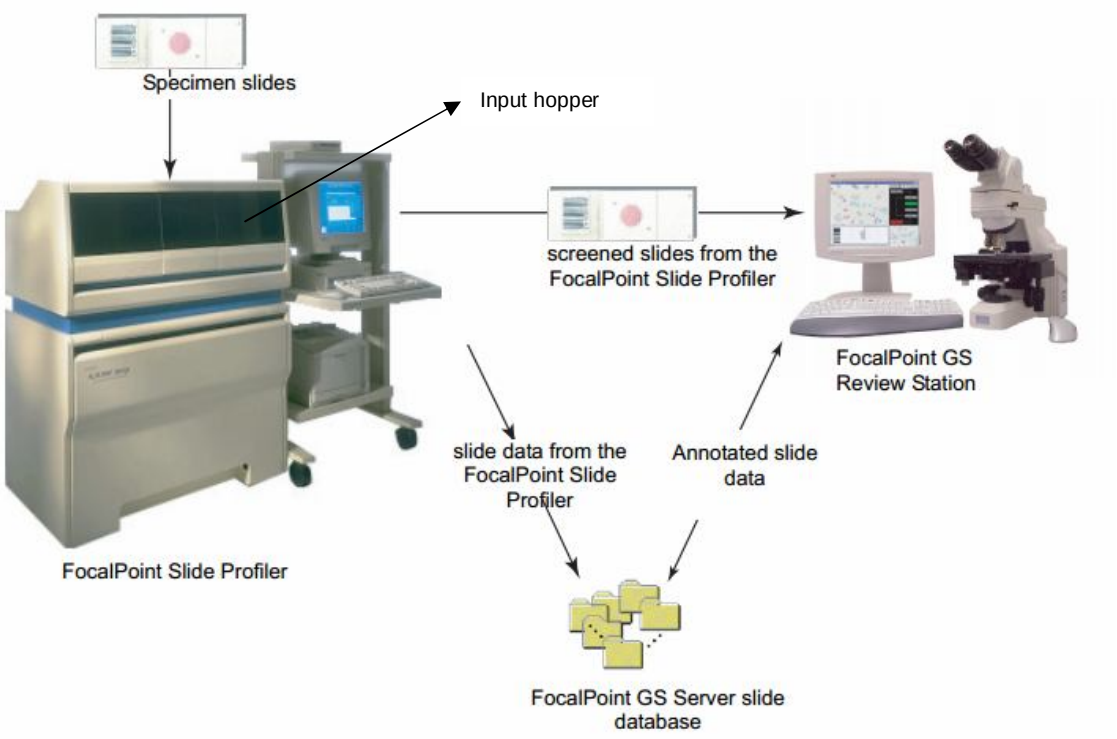

***Figure 2.13**: Stained slides are loaded into the BD FocalPoint^{tm} Slide Profiler, screened, and reviewed at the GS Review Station.*

The FP is a current, FDA approved, automated computerised primary screening system for gynaecological smear preparations.(1, 2) The instrument classifies slides using a high speed video microscope, image interpretation software, and morphology computers to image and analyse the complex images on a slide.(3) A set of algorithms is applied to analyse morphologic features. The instrument is intended to detect slides with evidence of squamous cell carcinoma or adenocarcinoma and their precursor conditions.(3)

The FP is the instrument that actually screens the barcoded slides. It literally performs the cytotechnologists' task by examining the cellular features. No physical dots are made. These are all computerised locations and stored in the database, which can be retrieved at the BD FocalPoint^{tm} Guided Screening Review Station (GS). The FP and the GS does not have to be in the same room since they share a network.

Data is transferred from the FP to the GS. Computerised locations/dots are now available at these remote stations via a network between the various components. The slide data is retrieved after scanning the slide barcode and then the automated stage on the microscope (also linked to the computer) will automatically take you to the 15 fields (FOV's).

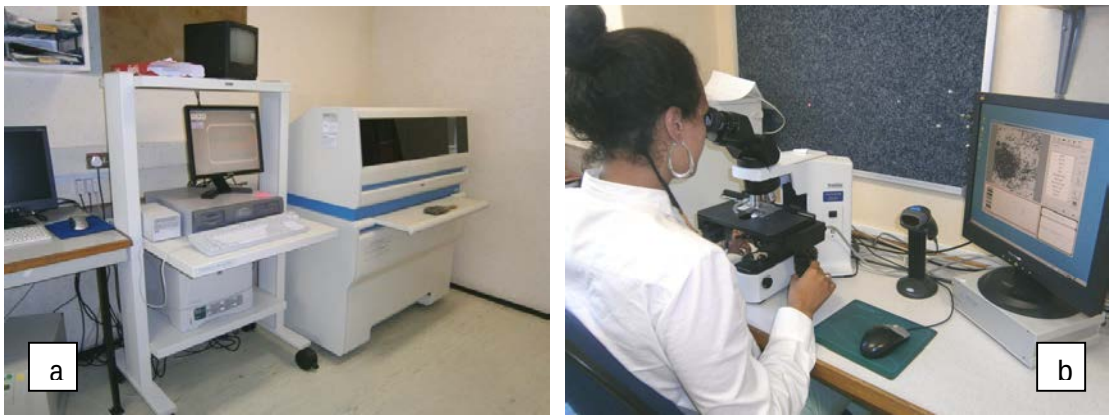

***Figure 2.14**: FP (a) is a computerised, automated screening device, networked to a review station (b) where FOV's are reviewed by a cytotechnologist.*

The FP instrument scans the entire slide area under the coverslip, and provides the cytotechnologist with 15 designated fields-of-view (FOV's) with the highest probability of containing abnormal cells. The cytotechnologist interprets these 15 FOV's at the GS Review station, documents a provisional diagnosis and then makes a triage decision. Depending on this diagnosis, slides can either undergo rapid review if benign, or be re-screened if abnormal. It is of critical importance to note that the systems' diagnostic accuracy relies on the cytotechnologist to make an accurate interpretation of the FOV's.

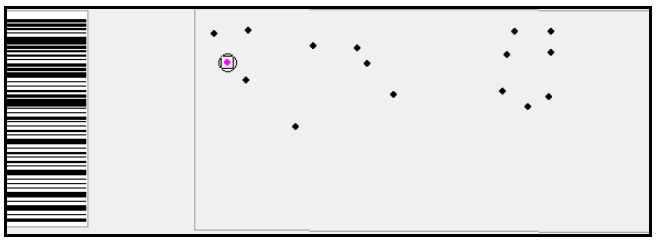

***Figure 2.15**: An example of a computerised slide created by the FP with designated FOV's to be reviewed by a cytotechnologist.*

The FP also provides the user with a Quintile ranking for each case. Quintiles are formed by dividing the print set rankings into 5 equal groups. Quintile 1 is the category of slides with the highest risk, while Quintile 5 is the category of slides with the lowest risk for abnormality. This allows the cytotechnologist to understand the risk inherent in each slide. This ranking is only accurate and of value when a random population sample is used (i.e. not all normal or all abnormal cases in a batch), and a large batch was processed (preferably 240 slides). This is important for laboratories that use the 25% "no further review" function and with low risk populations. Since South Africa does not use the 25% "no further review", as we are screening a high risk population, the quintile ranking was deemed of little importance which might create bias. It was therefore not used in this research project. In addition, sputum samples were processed either with Pap smears batches or alone in small batches. Therefore the Quintile ranking would have been of no value.

2.14.3 Literature review of automated sputum screening

The first (and only) report of automated sputum screening was published in 1996 by Hoda et al.(80) They used the PAPNET® System which was the first automated computerised interactive instrument for conventional Pap smears to be FDA approved. The study was retrospective and included 122 sputum samples that were randomly selected from their archives. 31 positive sputum samples were included in the study. After PAPNET® scanning and review, 30/31 cases were triaged for full manual review (i.e. 97% sensitivity). The case that was missed was a small cell carcinoma with only a single group of cells present. These results were encouraging for future use. The PAPNET® system has since ceased to be marketed in the USA and is no longer commercially available largely due to high costs associated with the system and logistical issues with workflow.(76, 77) This system required that glass slides be sent to central review sites with scanning stations.

Neumann et al. suggested the prospect of sputum automation to improve the screening process and diagnostic potential in 2009.(30) Their article was published just as this study commenced.

2.14.4 Instrument validation

A validation study was performed in 2010/11 at this laboratory on the FP.(see Appendix I). 9922 conventional cervical cytology slides were evaluated that was processed by the FP during a 6-month period. Only 20 cases were “missed” during this period by FP screening (i.e. 8xASC-US, 11xLSIL, 1xHSIL), which equates to 0.2% false negative/error rate. Manual screening during the same period averaged 1.7% false negative/error rate, and therefore the validation was accepted by the Quality Assurance division at NHLS.

2.14.5 Characteristics shared by Pap smears and sputum:

- Abnormal cells have similar morphological criteria
- Squamous and glandular malignancies are the most common malignant tumours
- Presence of mucus and contaminants in the background
- Time-consuming for cytological screening, requiring a continuous high level of alertness
- Hypercellularity
- High volumes – National Health Laboratory Service (NHLS), TBH processes approximately 1300 sputum samples annually
- Common screening tool

2.14.6 Benefits of automated screening:

Since Pap smears and sputum share characteristics as described in 2.14.5, this study was designed to test automated screening of sputum samples to improve sensitivity and reduce the false negative rate. Since carcinoma cells are often sparse, sputum screening demands a diligent screener with continuous high level of alertness. These factors contribute to the false negative rate of sputum. This is what makes automated screening well suited for sputum screening. The FP reduces the number of fields to be viewed and therefore decrease diligence and prolonged alertness. The FP is able to identify cells whether sparse or abundant as encountered in sputum samples.

DeMay states: “Probably the single most important source of error is loss of concentration due to screener fatigue.”(8) Screening Pap smears and sputum is very time consuming to screen and automated screening significantly reduce time spent per case. While a cytotechnologist can manually screen 50 conventional Pap smears at this laboratory per day (6.25 slides per hour), he/she can review 130 conventional Pap smears per day (16.25 slides per hour) at the GS Review Station. This laboratory has 2 GS Review Stations, and therefore 2 cytotechnologists are able to review 260 slides per day instead of screening 100 slides per day, i.e. 160% improvement in productivity of Pap smears.

Although no published data is available, this laboratory definitely experienced an improvement in the cytotechnologist's internal quality control rate (abnormal cases "missed") while reviewing FP slides compared to manual screening. Since the FP was used optimally, the laboratory's backlog was diminished and technologists were no longer requested to work overtime. These factors improved the moral of the cytotechnologists. Although the FP reduces the need for cytotechnologists, it cannot replace them since they are still vital in the interpretation of data. With the cytology laboratories under ever increasing work load pressure and shortage of qualified staff, this study, if successful, could be very beneficial.

Sputum automation benefits include:

- Increase productivity and improved turn-around-time (TAT): This will allow staff to screen the other non-gynaecological specimens while the FP screens the sputum specimens
- Increased sensitivity and reduce false negative rate: As with Pap smears, this instrument is programmed to identify single lying, sparse atypia as demonstrated by Neethling et al.(69, 77)
- Important extension to the usage of existing FP systems: Since all the national cytology laboratories in the NHLS and also some private laboratories are currently using the FP system for gynaecological work
- Improved overall job satisfaction of the cytotechnologist and decreased fatigue

Automated screening would probably also be successful in anal cytology, oesophageal brushings, oral cavity and upper respiratory specimens.

2.14.7 Limitations to automated screening

Since the cytotechnologist only have 15 fields to view (compared to an entire smear in manual screening) before deciding on a diagnosis, cells could be misinterpreted resulting in a false negative results. Kitchener et al. reported on monotony as a factor in using automated systems which could lead to decreased concentration and focus when reviewing FOV's. (71)

2.14.8 Glandular lesions and automated screening

Chute et al. reported on the FP's ability to identify atypical glandular lesions using SurePath liquid based cytology slides.(81) It could be deducted from their study that the Quintile ranking was not very reliable for glandular lesions. These cases were distributed throughout all 5 Quintile categories. At least none of these cases fell in the "no further review" category as concluded by them.

At this laboratory, the "no further review" function is not used due to the high risk population that is screened. As mentioned before, the Quintile ranking is also not relied upon, since this often creates bias.

The question arises: Since adenocarcinoma is the most common type of bronchogenic carcinoma diagnosed, could this pose a potential problem?" (49) During the past 5 years that the FP has been used at this laboratory, only 1 case of endometrial adenocarcinoma had been "missed" on FP screening (i.e. false negative), but identified by rapid review. According to the algorithms designed by Patten et al, the FP has been designed to identify adenocarcinoma and its percursor lesions. (Appendix G)

3. PILOT STUDY

A pilot study was completed in 2009 to test the prospect of automated sputum screening. This was a retrospective study of 84 cases selected from NHLS archives at TBH. These cases were received between 2005 and 2008, with an initial diagnosis of “atypia” on cytology. The final 35 cases included in the study met the additional criteria of which necessitated that both slides processed successfully on the FP.

Only 41.7% of the cases were qualified for study admission after automated screening. Possible reasons for this poor result included that slides were old (retrospective study), staining had faded and air bubbles had formed.

On follow up, all of these (35) cases were confirmed to be malignant either on subsequent tissue biopsy (16 cases), TTNA (12 cases) or TBNA (7 cases). The results were impressive with 33 of the 35 cases (94.3% sensitivity) warranting a full re-screen after FP scanning. Please note that squamous metaplasia was also placed into the “full re-screen” category.

Two cases were reported as false negative diagnoses, as they did not present diagnostic FOV’s: one adenocarcinoma and one small cell carcinoma. Specimen adequacy was not evaluated. It was concluded that larger prospective studies were required to confirm success.

4. HYPOTHESES AND AIMS

This study presented with multiple hypotheses, especially due to the collaboration with the clinicians at the Division of Pulmonology. The key purpose was to test automated sputum screening and compare with manual sputum screening. Good sensitivity would be the most important consideration. This could relieve some of the workload in understaffed laboratories nationally, and possibly worldwide.

4.1. Hypothesis 1:

The sensitivity of automated sputum screening will be comparable to manual sputum screening.

Sputum is one of the most difficult specimens for cytotechnologists to screen since abnormal cells are often sparse, specimens are very cellular and sputum often contains contamination or inflammatory exudate. Automated screening using the FP has been shown to detect sparse, single lying atypia, which is an imperative feature for sputum.(69)

4.2. Hypothesis 2:

Certain clinical, radiological and bronchoscopic findings will be able to predict the presence of diagnostic cells representative of bronchogenic carcinoma in sputum samples.

Studies have shown that tumour size, location and extent play an important role in exfoliation of diagnostic cells into respiratory secretions that appear in sputum. Various parameters were recorded in this cohort to test which factors are associated with positive sputum in patients with bronchogenic carcinoma.

4.3. Hypothesis 3:

High numbers of alveolar macrophages in a sputum sample will lead to improved sensitivity for malignancy.

Sputum with an abundance of alveolar macrophages indicates a well representative sample from the lower respiratory tract. The question arises if this optimal sputum can accurately represent pathology of the lung.

4.4 Aim 1:

To evaluate the usage of the FP in screening sputum samples.

Since automation of Pap smears has been successful in improving turn-around-time, reducing backlogs and increasing sensitivity, this could potentially be effective for sputum as well. Cellular components and malignant morphologic features of Pap smears and sputum are similar.

4.5 Aim 2:

To determine the sensitivity and specificity of pre-bronchoscopy sputum samples within a high risk population in South Africa.

Sputum sensitivity reported in the literature has a wide range. This study aimed to test sensitivity in patients suspected of bronchogenic carcinoma and attending the Division of Pulmonology for a TBNA/TTNA. This is a population at very high risk for bronchogenic carcinoma.

5. METHODOLOGY AND MATERIALS

5.1 Study settings and patient population

This prospective study was undertaken by collaborators from the National Health Laboratory Service (NHLS), Department of Pathology, Division of Anatomical Pathology (including Cytopathology) and the Division of Pulmonology, Department Medicine at TBH, in the Western Cape, South Africa. TBH is a 1300-bed teaching and referral hospital in Cape Town serving a population of 3.6 million of predominantly mixed ancestry, making it the largest hospital in the Western Cape.(82)

Specimen collection occurred over a 2 year period, July 2010 to June 2012. The sputum samples were obtained from a high risk population that included adult patients (older than 18 years of age) with a clinical suspicion of bronchogenic carcinoma. All patients able to produce spontaneous sputum were invited to participate in the study at the time of arrival to the Division of Pulmonology. Consent was obtained in either English, Afrikaans or Xhosa (Appendix D). In the anteroom (dedicated intake and recovery room) to the theatre, patients were instructed to cough deeply and expectorate sputum into a provided container.

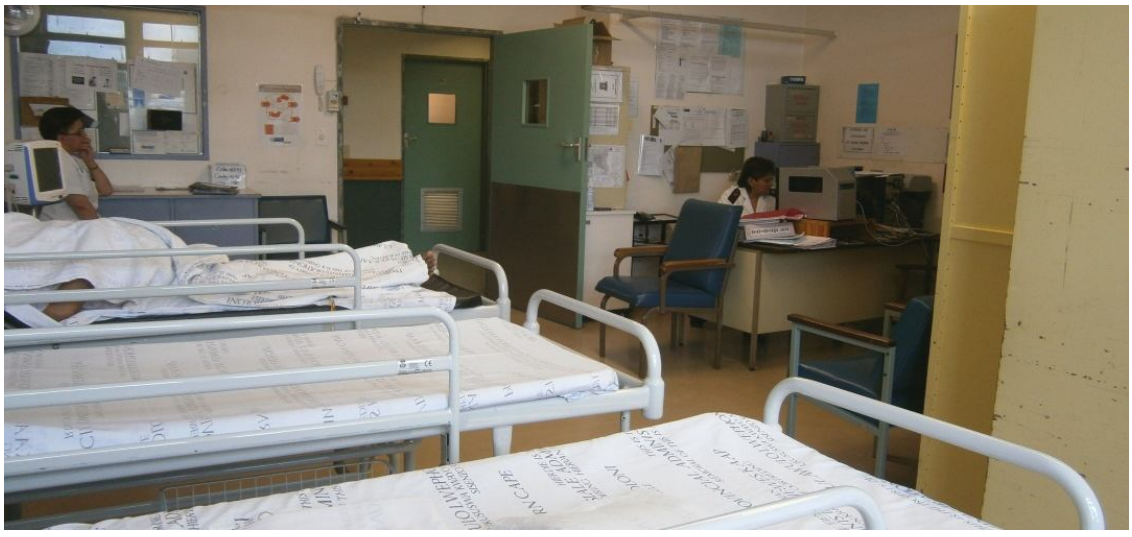

***Figure 5.1**: Dedicated intake and recovery room adjacent to the bronchoscopy theatre. Sputum was collected here before the planned, diagnostic procedure.*

These patients were all scheduled to attend the Division of Pulmonology for a diagnostic procedure, either a TBNA or TTNA. TBNA is performed on lesions situated in the central airways within reach of a flexible bronchoscope, while a TTNA is performed with US guidance on lesions extending to or originating from the pleura. A few cases were scheduled to receive FNA of a palpable lymph node or mass, pleural tap and/or tissue biopsy.

Histology or cytology from conventionally analysed TBNA/TTNA result was used as the diagnostic gold standard. Following extensive validation of cytology from TBNA/TTNA against histology in earlier studies at our institution, tissue biopsies are only performed when the TBNA/TTNA does not yield diagnostic material upon review by an on-site cytopathologist.(22, 27) Surgical interventions are rare because the diagnostic yield of non-surgical methods is high and therapeutic resections are seldom indicated as patients present with advanced disease. (49)

5.2 Data collection

5.2.1. Patient data (83)

Unique data collection sheets (Appendix C) were designed on which each patient's history and clinical, radiological and bronchoscopic findings were recorded. The collected information was obtained by clinicians (co-authors) from the direct interview, clinical notes, and any other available supportive documentation.

Table 5.1 *List of the clinical, radiological and bronchoscopic parameters recorded.*

Clinical	**Radiological**	**Bronchoscopic**
Previous tuberculosis	Location	Endobronchial lesion
Smoker	*Central*	Ulcerated lesion
Active Smoker	*Peripheral*	Exophytic lesion
Former Smoker	*Hilar mass*	Mucosal thickening
Never Smoked	*No clear mass / nodes only*	Complete Occlusion
Cough	*Right upper lobe*	Stenosis
Chronic	*Right middle lobe*	
New	*Right lower lobe*	
Chest pain	*Left upper lobe*	
Loss of weight (LOW)	*Left lower lobe*	
Increased dyspnoea	*Lingula*	
Haemoptysis	Size	
Hoarseness	*<3cm*	
Superior vena cava syndrome	*3-7cm*	
Pancoast syndrome	*>7cm*	
Neurologic symptoms	Mass characteristics	
Paraneoplastic phenomena	*Round*	
	Calcified	
	Spiculated	
	Cavitating	
	Atelectasis	
	Pleural effusion	
	Metastases	

The interviews were conducted prior to the planned diagnostic procedure. Participants were questioned as to symptoms experienced, whether this was a new or chronic problem and the estimated duration of the symptom(s). For the purposes of this study, a symptom present for longer than one year was deemed to be chronic.

Prior or on-going history of haemoptysis was documented. Any patient with a subjective experience of worsening dyspnoea (from one class to a higher class in the New York Heart Association classification) from their baseline, was included under shortness of breath, regardless of their baseline level.(84)

Patients that reported pleuritic chest pain (on coughing or deep inspiration) were noted as having chest pain. In patients with a smoking history, an attempt was made to estimate the duration as well as the amount of cigarettes smoked per day with the information supplied by the patient.

Patients were questioned about the presence of facial or upper limb oedema and examined for venous distension in the neck and distended veins in the upper chest and arms or any other features of superior vena cava syndrome. The presence of a tumour compressing the superior cava was subsequently noted on the CT of all the patients with these symptoms. Patients were questioned and examined for any focal neurological symptoms or problems with their gait. When available, family members were also questioned for confirmation. In most cases with neurological symptoms, a CT scan of the brain had been done prior to the patient's diagnostic procedure, to confirm the suspicion of metastasis/es to the brain.

Participants were asked about any weakness in the muscles of their arms and hands and were examined for small muscle wasting. They were also evaluated for the presence of Horner's syndrome. If any of these features were found, it was correlated with the CT scan to confirm the presence of a Pancoast tumour. Available laboratory findings were briefly reviewed for the presence of hypercalcaemia or hyponatraemia, suggestive of syndrome of inappropriate ADH (SIADH) and the patient was questioned about recent thrombo-embolic phenomena. If any of the findings were positive with no alternative explanation, the patient was noted as having paraneoplastic phenomena.

Tumour location

- If more than one mass was present, the lobe in which the largest mass was located was noted as the tumour location
- If the mass extended across the fissure to involve 2 lobes, both were noted as the tumour location
- Participants were sub classified as having either a central or a peripheral mass. Central: within 2cm of the carina, communicating directly with the large airways. Peripheral: more than 2cm from the carina, clearly surrounded by lung parenchyma without communication with the large airways

Tumour size

- The mass was measured using a digital measuring tool in the axial plane on a central electronic radiology review system
- The maximum diameter was taken and subcategorised as: <3cm, 3-7cm and >7cm

Specific characteristics of the mass

- Round: similar diameter in all dimensions with a rounded edge
- Spiculated: irregular edged mass

Atelectasis was deemed to be present if there was segmental or lobar collapse with volume loss. The CT scans were reviewed for the presence of pleural effusion of any size. Staging was done based on the Tumour-Node-Metastasis (TNM) 2009 guidelines.(85)

When bronchoscopy was performed, findings were looked at retrospectively. The description of the mass was based on the findings of the operator who performed the bronchoscopy. If stenosis of the airways was present, the operator subjectively noted this as a percentage.

5.2.2. Sputum collection

For optimal results of the FP screening, fairly monolayered slides needed to be prepared from well preserved, cellular samples. Sputum samples were collected in 45ml containers that were pre-filled with 10ml Shandon MUCOLEXX^{TM}. MUCOLEXX^{TM} is a mucoliquefying preservative designed for use in the preparation of mucoid cytology specimens.(86) This liquefying process is designed to produce slides that contain a high concentration of cells on the slide, with minimal clumping of cells.(86) Microscopically, the cells should not exhibit cellular distortion, destruction or affect staining. Carbowax is routinely used as the sputum fixative at this laboratory, but could easily be replaced by MUCOLEXX^{TM}.

Sputum specimens were sent to the laboratory immediately and refrigerated. Samples were prepared twice per week by 2 of the collaborators and residual material was refrigerated until the study was complete.

During the collection period, a total of 129 sputum samples were collected for processing. In the anteroom to the theatre, the clinician attending to the participating patient, allocated a matching study number to the sputum container, unique Cytology request form (Appendix B) as well as a collection sheet. The collection sheet with the patient identification was stored in the Division of Pulmonology.

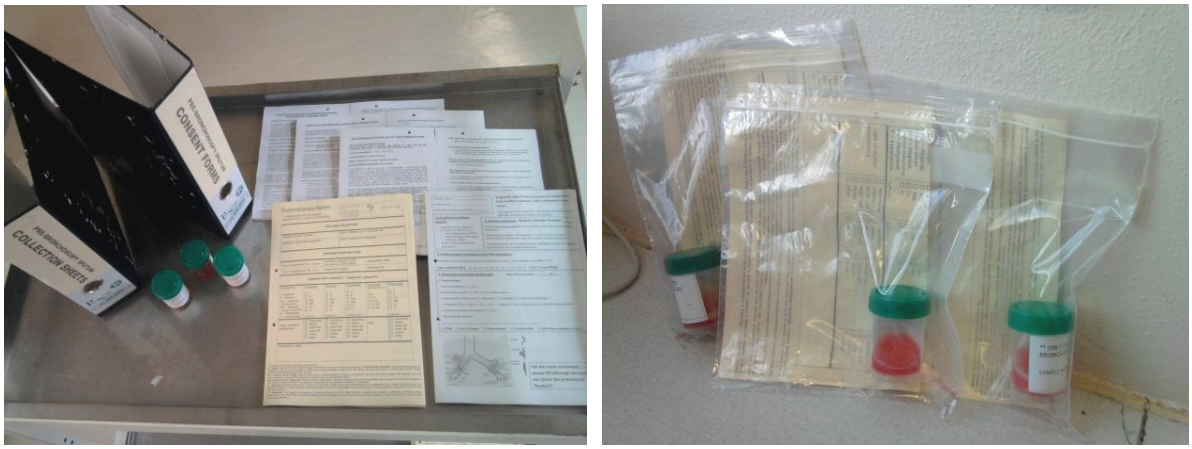

Figure 5.2: *Images of the data collection sheets, request forms, consent forms and sputum containers designed for this study*

5.3 Inclusion and exclusion criteria

Patients could participate if they were scheduled for a diagnostic procedure and were able to produce sputum spontaneously. The only initial inclusion criterion was successful automation. Patients were included when both slides were qualified after FP screening.

5.4 Safety considerations

This study's slide preparation was carried out in a Microbiological Safety Cabinet Class II (LabAire), in the Cytology Unit's designated preparation room. All preparation was performed by 2 qualified cytotechnologists. Safety precautions were strictly adhered to when samples were processed. Protective clothing was worn (laboratory coats and latex gloves) while handling the specimens. Careful attention to processing steps was followed to avoid cross contamination. The working surfaces were disinfected with 70% ethanol after processing each batch.

5.5 Specimen processing

5.5.1 Macroscopic appearance and weight

Upon arrival in the Cytology laboratory, the numbered specimens were allocated a laboratory number and registered on the DISA*LAB Laboratory System technologies, i.e. computerised database. The macroscopic appearance of the sputum was noted and classified as:

- Watery
- White
- Purulent
- Bloody

The exact weight of the specimens was also documented using an analytical balance (Sartorius AY212) to ascertain if sputum quantity would improve the sensitivity.

5.5.2 Specimen preparation

The MUCOLEXX^{TM} filled containers were vortexed for approximately 10 seconds and blended using a Saccomanno homogeniser. Sputum samples were homogenised at 9500 rpm for 5-10 seconds to further liquefy the mucus. Processing was performed in a Microbiological Safety Cabinet Class II.

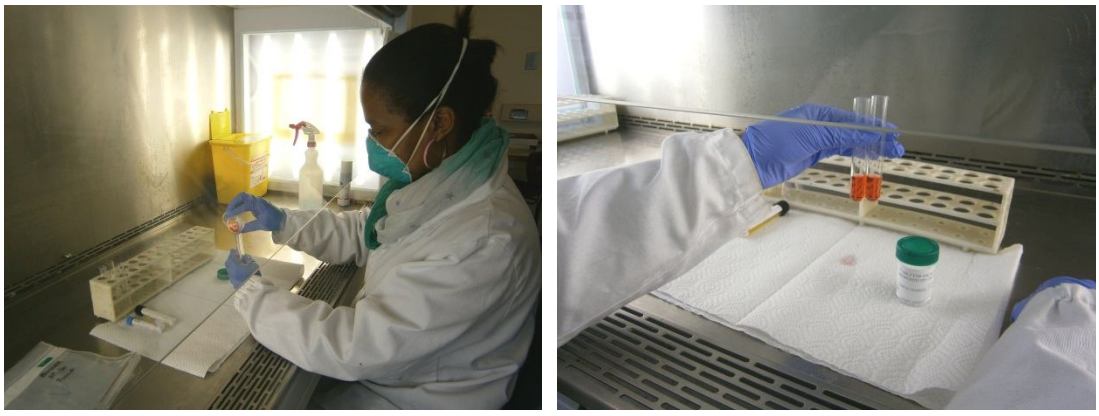

Figure 5.3: *Specimen preparation of sputum collected in Shandon MUCOLEXX^{TM}.*

Homogenised specimens were centrifuged in plastic tubes using a swing out head centrifuge (Heraeus Multifuge 3s) at 1500rpm for 10 minutes. The supernatant was decanted into the original container and the pellet dislodged to make two thin smears using labelled, frosted end glass slides. 2 drops of sediment were placed in the centre of one slide, while a second slide was gently placed upside down. Carefully but quickly the two slides were pulled apart.

Spray fixative was not used to fix smears, as the sputum was collected in a fixative, and spray fixing usually results in macroscopic clumping of the material. The residual pellet was decanted into the original container if more smears were needed. This was kept in a laboratory refrigerator at 5°C until the study was complete.

After the smears had dried completely, they were stained using a routine Papanicolaou stain (Appendix H) in an automated staining instrument (Leica ST 5020). The smears were manually mounted with glass coverslips using an optimal amount of Entellan mounting media (Merck product) to facilitate automated screening. Too little Entellan can yield air bubbles when it dries. Too much Entellan could result in a thick smear or be smeared on top of the coverslip and thus be rejected during the FP screening step.

5.5.3 Automated screening

Mounted slides were labelled with barcoded stickers generated by DISA*LAB system. This is essential for automated screening, as the instrument links data for each slide by this identifier. Slides were inserted into trays of eight slides each, and placed into the input hopper of the FP (instrument serial number 0355). These slide trays are automatically moved to the microscope stage, situated front and centre of the instrument. The instrument checked each slide for physical integrity, read the slide barcode label and scanned and analysed the slide at low power and high power fields.

Before the first tray and after each tray was processed, a comprehensive system integrity assessment of the instrument is performed automatically for quality assurance purposes. This ensures that all data collection and image analysis mechanisms are operating within specified limits.(3) The results of all these tests are compared to specific performance limits to validate the processing result for each slide in the tray. Slides can then be classified as qualified (ready to be reviewed) or process review (not able to be reviewed). The process review cases were rerun and reclassified. When slides remained process reviews, the sputum samples were re-prepared using the standard preparation procedure as described, and attempted automated screening again.

5.6 Microscopic examination

5.6.1 Manual microscopy:

Slides were allocated to the Cytology Units' screening pool. The cytotechnologists knew when they were screening study cases, but were blinded from any patient data to prevent bias. Qualified cytotechnologists (screener) were given these cases as part of their routine work. Blinded from the FP outcome, screeners applied standard criteria to screen these unmarked slides.(8, 47) Looking at all the fields systematically, screeners recorded a diagnosis. Slides were re-screened by senior cytotechnologists (checker) who recorded a final diagnosis (manual dx). This is standard protocol in the cytology laboratory. The checker was not blinded to the screener's diagnosis, but was blinded to the FP diagnosis. Diagnostic categories are as follow:

- Inadequate
- Benign
- Atypical, not otherwise specified (62)
- Suspicious for malignancy
- Malignant

For statistical purposes, "Inadequate", "Benign" and "Atypical, NOS" were considered sputum negative. "Suspicious for malignancy" and "Malignant" categories were considered sputum positive. These categories were compared to the patients finally diagnosed with malignancy. If a manual dx of "atypical, NOS" or worse was recorded, the result was changed to the Final diagnosis (see 5.6.3), since in practice these cases would be reviewed by a cytopathologist.

Table 5.2: *Diagnostic categories used for sputum samples in this study*

Inadequate	Little or no cellular material obtained or material obscured by blood, inflammation or poor preservation such that a diagnosis cannot be rendered. Also included are sputum samples not representative of the lower respiratory tract. (none, or too few alveolar macrophages present).(8, 47)
Benign	Negative, no abnormal cells present. Inflammation or reactive changes may be present. Includes benign squamous metaplasia. Sputum is adequate. Benign squamous metaplasia criteria: cells are round and seen in cobble-stone arranged fragments. Cells have a well-defined cell border and small to medium sized, smooth, central nuclei. N/C ratio fairly low. (8, 47)
Atypical, NOS	Abnormal cells are present, but of uncertain potential. Adequacy is not important. Atypical cells are usually sparse with some pleomorphism, enlarged nuclei and coarsely granular chromatin. The cytoplasm is decreased and may exhibit keratinisation.(8, 47)
Suspicious for Malignancy	Highly atypical cells present, suspicious for malignancy. Adequacy is not important. Criteria are quantitatively or qualitatively not enough for a diagnosis of malignancy. Cells are pleomorphic with enlarged nuclei, slightly irregular nuclear membranes and coarsely granular chromatin. The cytoplasm is decreased and may exhibit keratinisation.(8, 47) Cells may also appear markedly atypical, but with extreme sparse cellularity.
Malignant	Malignant cells are present. Adequacy is not important. Samples have an abundance of malignant cells with marked pleomorphism and a variable N/C ratio. Nuclear chromatin tends to be coarsely granular and hyperchromatic with markedly irregular nuclear membranes. Pearls and keratinisation may be present, or balls and other glandular arrangements. Indian filing and moulding may be seen (tumour dependant).(8, 47)

5.6.2 FocalPoint interpretation

After manual screening by cytotechnologists, the principal investigator reviewed the FP generated FOV's blinded from the manual diagnosis. This was not interpreted as bias. The FP diagnosis should reflect the instruments' capacity to identify cells of interest and should not be negatively affected by the quality of the screener to locate these cells.

After data transfer from the FP workstation to the server, slides were ready for review at the GS Imaging System. The GS provides the electronic capacity of locating diagnostically relevant locations on the slides for screening.(3) Using the GS workstation platform, a computer system networked to an automated microscope made triage screening possible. After reading the barcode, to link data to the slide, the microscope automatically positioned the slide at the first relevant location identified by the GS system. A user activated footswitch or mouse click moved the microscope to the next position until all locations were screened for suspicious cells or features.(3)

All 15 fields allocated were carefully screened using the 100x magnification (10x objective), and switched to 400x magnification (40x objective) when suspicious cells were seen. A diagnosis was recorded based only on the 15 FOV's (FP dx) using the 5 categories (Table 5.2). If a FP dx of "atypical, NOS" or worse was recorded, the result was changed to the final diagnosis (see 5.6.3), since in practise these cases would be reviewed by a cytopathologist.

5.6.3 Final review

Slides were reviewed by three independent, partially blinded (cytotechnologists marked fields to be evaluated, but the diagnosis was concealed) collaborators from the Cytology Unit. Diagnosis was individually recorded, and eventually a consensus diagnosis (final dx) was rendered. When two or more cytologists agreed on a diagnosis, this was recorded as the final dx. When all three differed in their diagnosis, (namely atypical, suspicious and malignant) the middle category (i.e. suspicious) was recorded as the final dx.

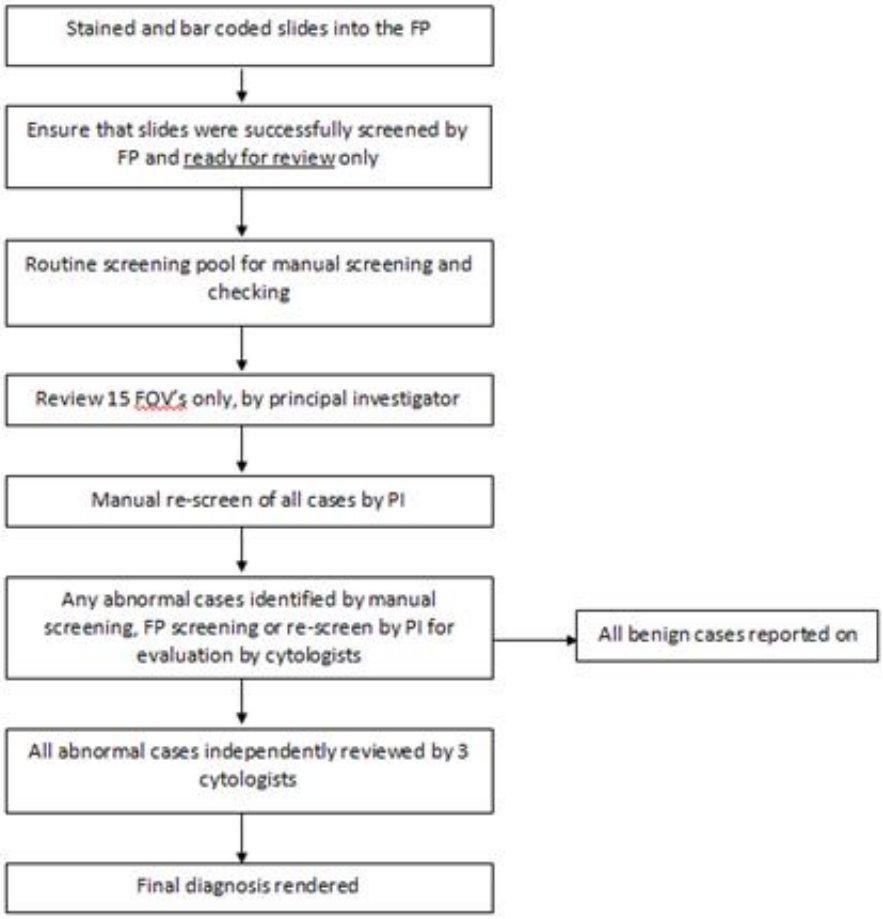

Figure 5.4: *Workflow diagram followed in this study.*

5.7 Adequacy

5.7.1 Quantity of alveolar macrophages

A random 10x10mm (100mm^2) square was marked off on one of the 2 two slides of each case. This was used to count alveolar macrophages using 400 times magnification (x40 objective). The total area of the standard coverslip used was 24x50mm (1200mm^2).

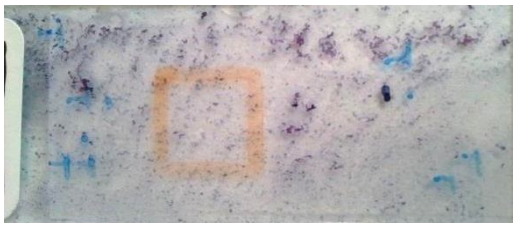

Figure 5.5: *10x10mm square on slide in which alveolar macrophages were counted.*

Table 5.3: *Bins used in counting alveolar macrophages (factor x12 used)*

Counted Alveolar Macrophages In 10x10mm area		Average Alveolar Macrophages Per slide
0		0-11
1-5		12-60
6-10		72-120
11-20		132-240
>21		252-600

5.7.2 Sputum weight

Sputum were weighed and categorised into one of four groups:

- 0-1g
- 1-3g
- 3-5g
- >5g

5.8. Ethical approval

Ethical approval was granted (Appendix A) by the Health Research Ethics Committee, Faculty of Health Sciences, Stellenbosch University, Cape Town. Ethics Reference number: **N10/04/135**.

Written informed consent for the TBNA/TTNA was obtained from all patients' prior to the bronchoscopy procedure by one of the clinicians. No patient data (name, hospital number etc.) were made public, either verbally or in any publication or presentation. The patients' remained anonymous for the purpose of this study. Patients were free to decline, while their management was not affected. All patients were reviewed at clinico-pathological-radiological meetings where individual patient management was discussed. Patients were only to produce sputum if they could, and no additional procedures were involved e.g. sputum induction. Patients that agreed to participate in the study were instructed as to what the study entailed and consent forms were signed in either English, Afrikaans or Xhosa (Appendix D).

5.9 Statistical analysis

Statistical analysis was performed by Dr. Justin Harvey of Stellenbosch University, Research Support. This study was designed as a prospective comparative study. Univariate analyses were performed and p-values calculated using Pearson's chi-square and Fischer's two-tailed test. Odds ratios were calculated for the significant factors and Cohen Kappa for the inter-observer variability.

6. RESULTS

6.1 Final diagnosis of study population

Table 6.1: *Final diagnosis of cohort obtained during diagnostic procedure.*

	Final diagnosis	n=108	% of total cohort
Malignant		91	84.3
	Adenocarcinoma, NOS	33	30.6
	Squamous cell carcinoma	30	27.8
	Small cell carcinoma	15	13.9
	Large cell carcinoma	3	2.8
	Adenosquamous carcinoma	2	1.9
	Non-small cell carcinoma, NOS	4	3.7
	Poorly differentiated carcinoma	3	2.8
	Plasmacytoma	1	0.9
Benign		17	15.7
	Mycobacterial infection	4	3.7
	Inflammation (acute or granulomatous)	4	3.7
	Benign, NOS	8	7.4
	Atypical, NOS	1	0.9

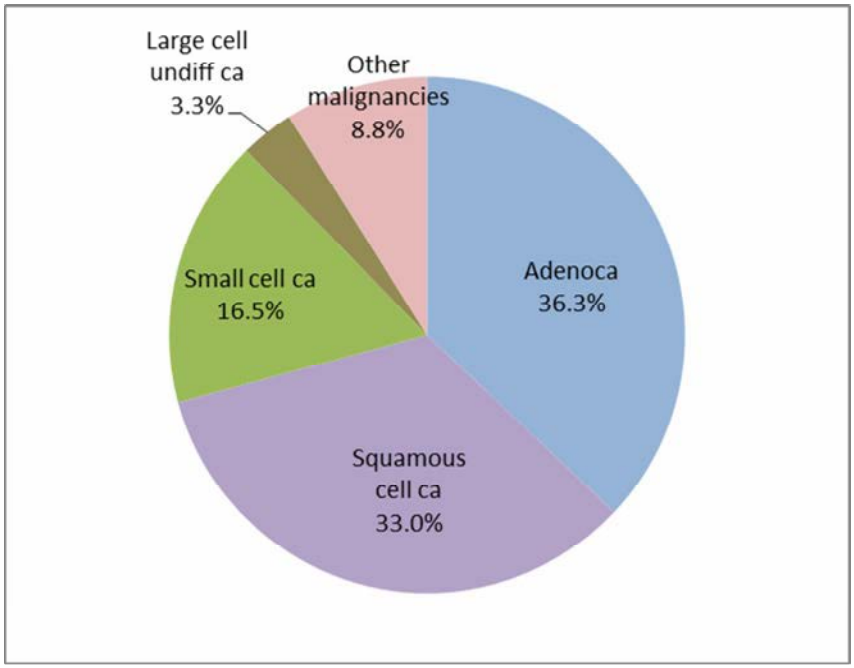

Figure 6.1: *Pie chart illustrating the proportions of the various histological subtypes*

6.2 Patient demographics of study population

Table 6.2: *Summary of age and gender of the participants in various histological subtypes of bronchogenic carcinoma.*

	Final Diagnosis	Age mean (37-81)	Gender (M : F)
Malignant		59.2	2.4 : 1
	Adenocarcinoma, NOS	60.0	2.2 : 1
	Squamous cell carcinoma	59.3	2.3 : 1
	Small cell carcinoma	60.1	2.0 : 1
	Large cell carcinoma	60.7	2.0 : 1
Benign		60.6	1.1 : 1

Results for patients ≤45 years of age:

- 3 patients with a benign diagnosis (aged 40,41,45)
- 1 patient with an adenosquamous carcinoma (aged 37)
- 2 patients with a squamous carcinoma (aged 42,45)

Results for patients ≥75 years of age:

- 3 patients with a benign diagnosis (all aged 75)
- 3 squamous cell carcinoma (2 aged 75, 77)
- 1 large cell undifferentiated carcinoma (aged 76)
- 1 small cell carcinoma (aged 81)

6.3 Patient data (83)

6.3.1 Clinical characteristics:

Table 6.3: *Clinical characteristics of patients in cohort with positive and negative sputa.*

n=90 malignant cases		% of total	Sputum +	% of parameter	Sputum -	% of parameter	p-value
Previous tuberculosis	22	24.4	10	45.5	12	54.6	0.616
Smoker							
Active smoker	42	46.7	18	42.9	24	57.1	0.520
Former smoker	41	45.6	13	31.7	28	68.3	0.278
Never smoked	7	7.8	4	57.1	3	42.9	0.424
Cough (n=82)	78	86.7	31	39.7	47	60.3	0.759
Chronic	29	35.4	10	34.5	19	65.5	0.638
New	48	58.5	21	43.8	27	56.3	0.463
Chest pain	51	56.7	22	43.1	29	56.9	0.388
LOW (n=89)	73	82.0	25	34.3	48	65.8	0.049
Increased dyspnoea (n=88)	60	68.2	25	41.7	35	58.3	0.646
Haemoptysis	29	32.2	14	48.3	15	51.7	0.250
Hoarseness (n=89)	23	25.8	9	39.1	14	60.9	1.000
SVC syndrome (n=89)	12	13.5	5	41.7	7	58.3	1.000
Pancoast syndrome (n=89)	5	5.6	3	60.0	2	40.0	0.457
Neurologic symptoms (n=89)	4	4.5	2	50.0	2	50.0	0.644
Paraneoplastic phenomena (n=89)	4	4.5	0	0	4	100	0.151

Loss of weight was significantly more often observed in patients with negative sputum cytology. Surprisingly, neither haemoptysis nor coughing was significantly more often seen in patients with positive sputum cytology.

6.3.2 Radiological characteristics:

Table 6.4: *Radiological characteristics of patients in cohort.*

n=90 malignant cases		**% of total**	**Sputum +**	**% of parameter**	**Sputum -**	**% of parameter**	**p-value**
Location							
Central (n=89)	50	56.2	22	44.0	28	56.0	0.228
Peripheral (n=89)	38	42.7	12	31.6	26	68.4	0.228
Hilar mass (n=90)	11	12.2	5	45.5	6	54.6	0.745
No clear mass / Nodes only (n=90)	6	6.7	3	50.0	3	50.0	0.674
Right upper lobe (n=79)	41	45.6	14	34.2	27	65.9	0.495
Right middle lobe (n=79)	5	5.6	0	0	5	100	0.151
Right lower lobe (n=79)	9	10.0	3	33.3	6	66.7	1.000
Left upper lobe (n=79)	22	24.4	10	45.5	12	54.6	0.444
Left lower lobe (n=79)	6	6.7	2	33.3	4	66.7	1.000
Lingula (n=79)	1	1.1	0	0	1	100	1.000
Size (n=78)							
<3cm	7	9.0	3	42.9	4	57.1	1.000
3-7cm	37	47.4	16	43.2	21	56.8	0.352
>7cm	34	43.6	10	29.4	24	70.6	0.244
Mass characteristics (n=81)							
Round	10	12.3	7	70.0	3	30.0	0.039
Calcified	3	3.7	2	66.7	1	33.3	0.555
Spiculated	68	84.0	22	32.4	46	67.7	0.026
Cavitating	7	8.6	2	28.6	5	71.4	0.702
Atelectasis (n=90)	44	48.9	17	38.6	27	61.4	1.000
Pleural effusion (n=90)	20	22.2	7	35.0	13	65.0	0.655
Metastatic (n=89)	60	67.4	24	40.0	36	60.0	1.000

A round mass was significantly more often observed in patients with positive sputum cytology, while a spiculated mass was significantly more often observed in patients with negative sputum cytology. Surprisingly, neither a central nor a right upper lobe mass was significantly more often observed in patients with positive sputum.

6.3.3 Bronchoscopy characteristics:

Table 6.5: *Findings documented during bronchoscopy of patients in cohort with positive and negative sputa.*

n=41 malignant cases		% of total	Sputum +	% of parameter	Sputum -	% of parameter	p-value
Endobronchial lesion	17	41.5	8	47.1	9	52.9	0.045
Ulcerated lesion	2	4.9	0	0	2	100	0.495
Exophytic lesion	4	9.8	2	50.0	2	50.0	0.567
Mucosal thickening (n=43)	10	23.3	2	20.0	8	80.0	0.456
Complete occlusion	8	19.5	3	37.5	5	62.5	0.672
Stenosis	11	26.8	7	63.6	4	36.4	0.007

Endobronchial lesions and bronchial stenosis were significantly more often observed in patients with positive sputum cytology.

6.4 Case selection for FP screening

Initially, the only inclusion criterion was successful automation, i.e. both slides qualified after FP screening. Starting with 129 cases, four cases could not be linked with certainty to the respective patients. A further three patients with sputum submitted did not complete TBNA/TTNA. One patient died before a final diagnosis was rendered. One patient had two sputum samples submitted since he had two diagnostic procedures performed on separate days. These sputum samples were excluded from the study.

The FP occasionally had difficulty screening these sputum slides since it was not designed for sputum samples with its diverse cellular components and contaminants. During automated screening, twelve cases were rejected due to scant cellularity, resulting in unsuccessful automation (process reviews). These sputum samples were also excluded from the study. This resulted in a final sample size of 108.

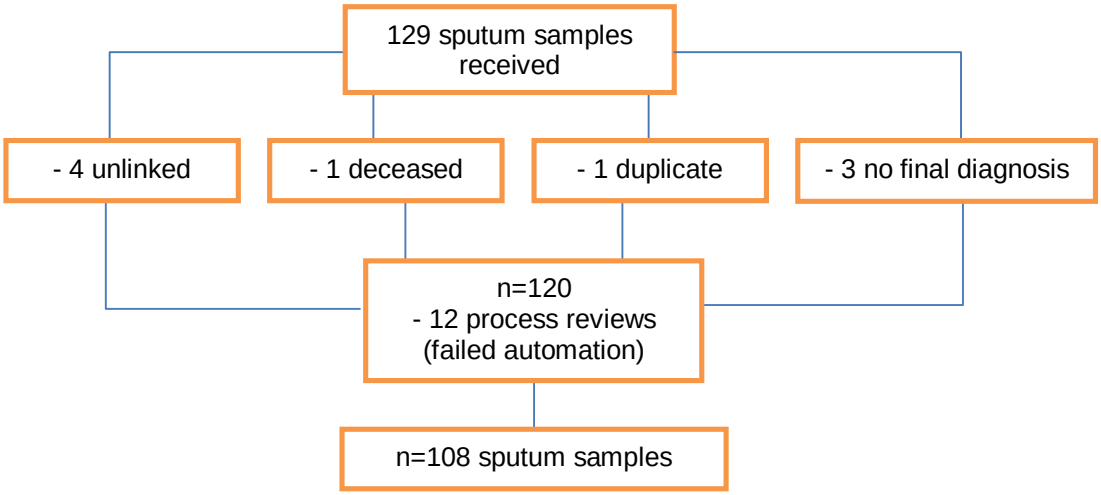

Figure 6.2: *Flow diagram depicting cases that were excluded from the study.*

6.5 Manual screening versus automated screening

6.5.1 Duration of manual screening versus automated screening

The standard coverslip size used at this laboratory is 50x24mm^2. This equates to 22 fields (x10 objective) horizontally and 11 fields (10x objective) vertically. Calculated, a manual sputum slide will have approximately 242 fields to examine using a 10x objective to cover the entire slide. Each case has 2 slides compared to the 15 FOV's generated by the FP that needs to be examined per slide.

The time spent per case (i.e. 2 slides) was recorded, and on average manual screening or checking required 19 minutes 14 seconds, while FOV interpretation required 3 minutes 15 seconds. Note that the pre-analytical events (before FOV review) were not taken into account e.g. loading, unloading and processing by the FP instrument since this does not require cytotechnologist time.

Table 6.6: *Comparison between the duration of manual screening versus FP screening.*

Efficiency parameter	Manual screening	FP screening	% reduction
Fields per slide to be reviewed	242	15	93.8
Time spent per case (mean)	19min14sec	3min15sec	83.1
			p-value <0.001

A significant time difference was established between manual screening and FP screening. There is also no overlap between the minimum time spent during manual screening and maximum of FP screening.

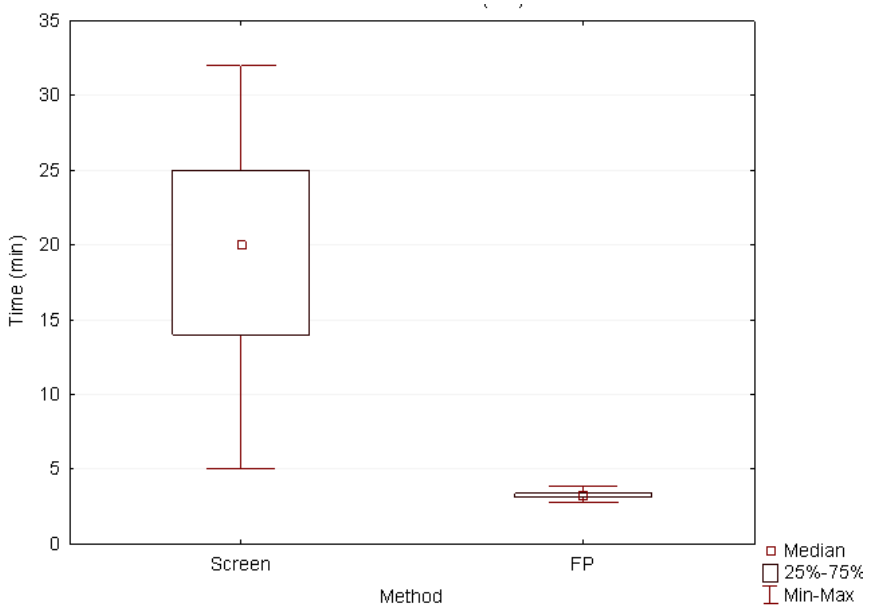

Figure 6.3: *Boxplot using Mann-Whitney test to represent the time spent per sputum performing manual screening (screen) versus FOV review (FP).*

6.5.2 Sensitivity of manual screening versus automated screening

Table 6.7: *Comparison between the sensitivity and specificity obtained in sputum samples from manual and FP screening.*

Screening method	True positive sputum samples (n=35)	% sensitivity	% specificity
Manual screening	26	74.3	100
FP screening	33	94.3	97.8
			p-value: 0.803

No statistical significant difference exists between the screening methods.

6.6 Macroscopic examination of sputum samples

6.6.1 Weight

Sputum samples were prepared twice weekly in small batches. Unused specimen containers were not exactly the same weight and slight evaporation of fixative from the sealed containers occurred. This could have resulted in some increased variability but not systematic bias. The average weight of the spontaneous sputum samples received was 1.97g with a minimum and maximum weight of 0.0g and 7.22g respectively.

Table 6.8: *Weight of sputum samples recorded in weight bins before processing.*

Weight bins	n=108	True sputum+	% in category	True sputum-	% in category	False sputum-	% in category
0-1g	39	6	17.1	7	38.9	26	47.3
1-3g	45	22	62.9	5	27.8	18	32.7
3-5g	16	5	14.3	6	33.3	5	9.1
>5g	8	2	5.7	0	0	6	10.9
							p-value: 0.005

These results seem to indicate a trend with significant differences in results obtained in the first two groups (0-1g and 1-3g) between the categories. Additional testing with more cases in the >5g group would be ideal to confirm this trend.

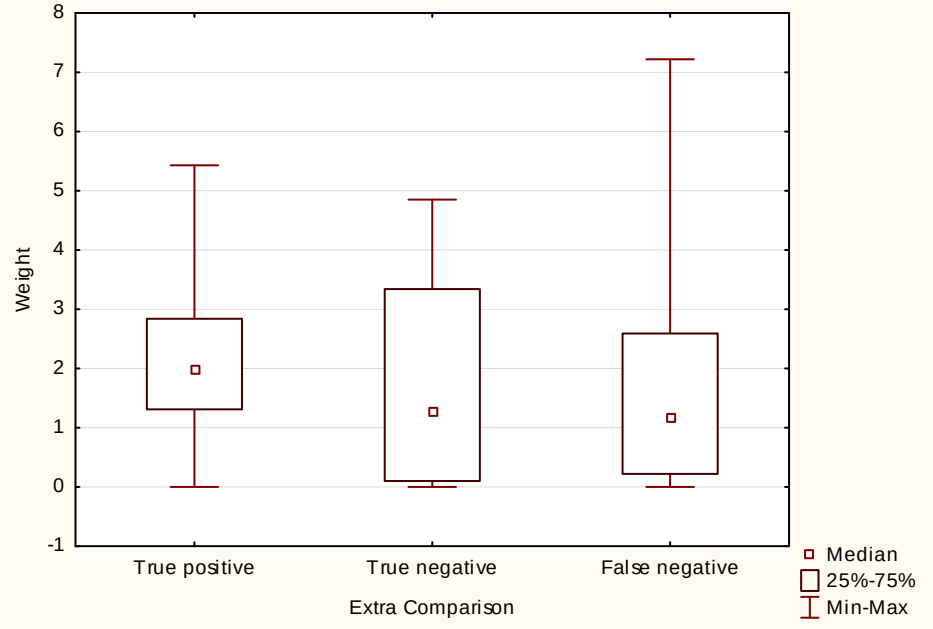

Figure 6.4: *Boxplot using Kruskal-Wallis test to represent the minimum, maximum and median of sputum weight across the results.*

6.6.2 Macroscopic appearance

Table 6.9: *Macroscopic appearance of sputum samples recorded before processing.*

Macro	n=108	True sputum+	% in category	True sputum-	% in category	False sputum-	% in category
Watery	5	0	0	1	5.6	4	7.3
White	93	29	82.9	17	94.4	47	85.5
Purulent	3	1	2.9	0	0	2	3.6
Bloody	7	5	14.3	0	0	2	3.6
							p-value 0.221

A trend cannot be established since there are too few cases in the watery, purulent and bloody categories.

All of the patients with macroscopically bloody sputum samples (100%) had bronchogenic carcinoma confirmed on TBNA/TTNA, while 5/7 (71.4%) of these bloody sputums had diagnostic cells present.

6.7 Inter observer variability

All abnormal slides were reviewed by 3 independent collaborators (2 cytopathologists and 1 cytotechnologist) and a consensus diagnosis was rendered. Cohen's kappa coefficient was calculated on the 35 positive sputum cases which measured the inter-rater agreement.

Table 6.10: *Kappa coefficient calculations between the 3 cytologists and the consensus result.*

Rater 1	Rater 2	Cohen Kappa	95% CI
Consensus dx	Cytologist 1	0.452	0.064 – 0.781
Consensus dx	Cytologist 2	0.386	0.062 – 0.688
Consensus dx	Cytologist 3	0.269	0.088 – 0.487
Cytologist 1	Cytologist 2	0.020	-0.185 – 0.325
Cytologist 1	Cytologist 3	-0.006	-0.131 – 0.104
Cytologist 2	Cytologist 3	-0.009	-0.170 – 0.174

dx=diagnosis; CI=confidence interval

No agreement was established between the three cytologists with the confidence interval including the point zero. At least some agreement was obtained between the consensus diagnosis and the individual cytologists.

6.8 Automation performance:

The FP instrument had a high rate of technical errors at the start of the study period, resulting in a high process review (23) rate. The FP image system and plate on the path extender were eventually replaced which cleared up the high process review and error rate. The PR rate should be kept as low as possible, since these slides have to go back into the manual screening pool and does not improve TAT. This also affected the automation performance and success of the sputum samples.

Table 6.11: *Process review rate for all smears (Pap smears and sputum samples) processed during the study period.*

Year	Process review rate
2010	15.1%
2011	20.0%
2012	9.0%

Table 6.12: *Automation performance including process review and failure rate.*

FP automation outcome	n=120	%
Initial automation success	85	70.8
Process review, repeated x1	13	10.8
Process review, repeated ≥x2	10	8.3
Failed automation	12	10.0

6.8.1 Reasons for process reviews / incomplete processing included:

Table 6.13: *Process review comments after FP screening.*

Process Review Status (n=35)	%
Scant cellularity	28.7
Insufficient reference cells	23.0
Bubble, 3D or scant specimen	2.1
Insufficient nuclear-cytoplasmic contrast	14.3
Specimen/coverslip too thick	14.1
Dust or dirt on the coverslip	12.0
Nuclear stain – chromatin blurring	3.1
Nuclear stain too light/dark	2.7

Pertaining to "too few squamous cells"

6.9 Inadequate sputum

Table 6.14: *Clarification of inadequate results.*

Inadequacy factor	n=108	%
Total inadequate sputum samples	16	14.8
No alveolar macrophages	9	8.3
Too few alveolar macrophages	3	2.8
>75% cells covered by inflammation	4	3.7

6.10 Inflammatory exudate

Marked inflammatory exudate was commented on when > 50% of cells was covered by inflammatory cells.

Table 6.15: *Sputum samples with marked inflammatory exudate present*

	Inflammatory exudate present	% in category
Sputum of all patients with carcinoma confirmed	36/91	40.0
All positive sputum cases, suggestive of obstructive pneumonia	17/35	48.6

6.11 Final diagnostic procedure (method of final result confirmation)

Table 6.16: *Summary of diagnostic procedures used to obtain patient diagnosis.*

Procedure	N=108	%	Sputum +	% true +	Sputum -	% false -
TBNA	45	41.7	11	28.9	34	71.1
TTNA	46	42.6	19	43.2	27	56.8
Wash/Brush	7	6.5	1	50.0	6	50.0
Biopsy	5	4.6	0	0	5	100
FNA	4	3.7	3	75.0	1	25.0
Pleural Fluid	1	0.9	1	100	0	0
						p-value < 0.001

FNA = Fine needle aspirate other than lung

A significant association between the diagnostic procedure and sputum cytology was obtained. While comparing TBNA and TTNA, there is a significant difference between the positive and negative sputum results. When patients had a TTNA as diagnostic procedure, they were more likely to have positive sputum cytology.

6.12 Sensitivity and specificity of sputum

Table 6.17: *Sensitivity and specificity of sputum results.*

Category	n=108	% of all sputum	Sensitivity	Specificity
True positive	35	32.4		
True negative	17	15.7		
False positive	0	0		
False negative	56	51.9		
Diagnostic accuracy			38.5%	100%

6.12.1 Sputum results categorised

Table 6.18: *Final sputum diagnosis in the 5 categories and comparison with final diagnosis of the patient.*

Category	n=108	% of cohort	% true +	True -	%	False -	%
Inadequate	16	14.8	-	1	6.3	15	93.8
Benign	43	39.8	-	14	32.6	29	67.4
Atypical, NOS	14	13.0	-	2	14.3	12	85.7
Suspicious for malignancy	8	7.4	100	-	-	-	-
Malignant	27	25.0	100	-	-	-	-

NOS=not otherwise specified

All patients with “suspicious for malignancy” sputum cytology were confirmed as having bronchogenic carcinoma.

6.12.2 Sputum sensitivity in histological subtypes of carcinoma

Table 6.19: *Sputum diagnosis compared to the final diagnosis obtained during the diagnostic procedure. An attempt was also made to diagnose the exact histological subtype on the sputum.*

	Final diagnosis	Sputum +	%	Sputum -	%	p-value	% correct type
Malignant		35/91	38.5	56/91	61.5		
	Adenocarcinoma, NOS	10/33	30.3	23/33	69.7	0.351	40.0
	Squamous cell carcinoma	18/30	60.0	12/30	40.0	< 0.001	83.3
	Small cell carcinoma	2/15	13.3	13/15	86.7	0.136	100
	Large cell carcinoma	1/3	33.3	2/3	66.7		
	Adenosquamous carcinoma	½	50.0	1/2	50.0		
	Non-small cell carcinoma, NOS	2/4	50.0	2/4	50.0		
	Poorly differentiated carcinoma	1/3	33.3	2/3	66.7		
	Plasmacytoma	0/1	0	1/1	100		
Benign	All benign cases	0/17	0	17/17	100		

NOS=not otherwise specified

Squamous cell carcinoma was significantly more often observed in patients with positive sputum cytology. Surprisingly, small cell carcinoma, which often has a large central tumour mass, was not significantly seen more often in positive sputum cytology but had very poor sensitivity. This might be due to the central mass representing metastatic small cell carcinoma originating from a very small peripheral primary lesion, whereas squamous cell carcinoma has its primary lesion located centrally.

6.13 Predictors of positive sputum (83)

Table 6.20 lists the unadjusted odds ratios for all factors that were found to be associated with positive sputum cytology. Data from all 6 factors were available in 39 cases of which 9 (23.1%) had positive sputum cytology.

Table 6.20: *Summarising the predictors of positive sputum cytology.*

Parameter	p-value	OR	95% CI	Association
Bronchoscopy: partial airway obstruction / stenosis	0.007	8.9	1.29-61.06	Positive
CT: round mass	0.039	5.4	1.13-26.06	Positive
Bronchoscopy: endobronchial tumour	0.045	4.7	0.74-29.83	Positive
Pathology: squamous cell carcinoma	< 0.001	1.9	0.38-9.38	Positive
CT: spiculated	0.026	0.1	0.03-0.48	Negative
Clinical: loss of weight	0.049	0.12	0.03-0.45	Negative

CT= Computed Tomography; OR=odds ratio; CI=confidence interval

6.14 Quantity of alveolar macrophages

This calculation only included 107 sputum samples. In one of the cases, cells were obscured by more than 75% blood and therefore the macrophages could not be counted.

Table 6.21: *Number of alveolar macrophages present on slides compared to the sputum result.*

M* per 1cm^2	M* per slide	n=107	True sputum + (n=34)	% in cat	True sputum- (n=18)	% in cat	False sputum- (n=55)	% in cat
0	0-11	11	1	2.9	0	0	10	18.2
1-5	12-60	10	2	5.9	2	11.1	6	10.9
6-10	72-120	1	0	0	1	5.6	0	0
11-20	132-240	8	2	5.9	3	16.7	3	5.5
> 21	252-600	77	29	85.3	12	66.7	36	65.5
								p-value: 0.128

M*= macrophages; "M* per slide" is only an estimate; cat=category

Due to a lack of power, a significant association could not be established between the quantity of alveolar macrophages and an improved sputum cytology result.

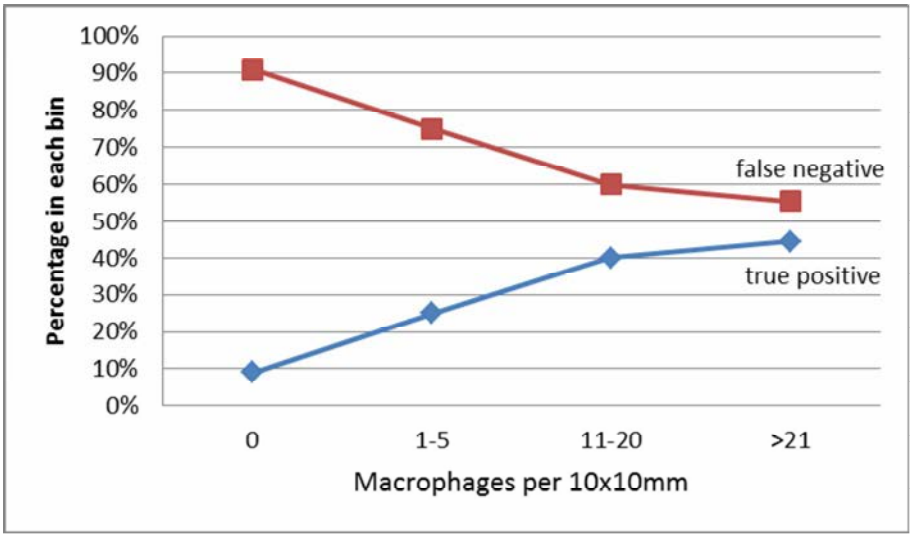

Figure 6.5: *Line graph illustrating the percentage of alveolar macrophages present in false negative and true positive sputum cases in each bin. These patients had carcinoma confirmed.*

6.15 Photomicrographs of all the positive cases

Each of the positive sputum samples (c) and its corresponding diagnostic procedure (d) has been photographed. In most cases, radiological images have been included: chest X-ray (a) and chest CT scan (b).

Case 1: 56 Male - TBNA of right upper lobe mass in an active smoker with a chronic cough.

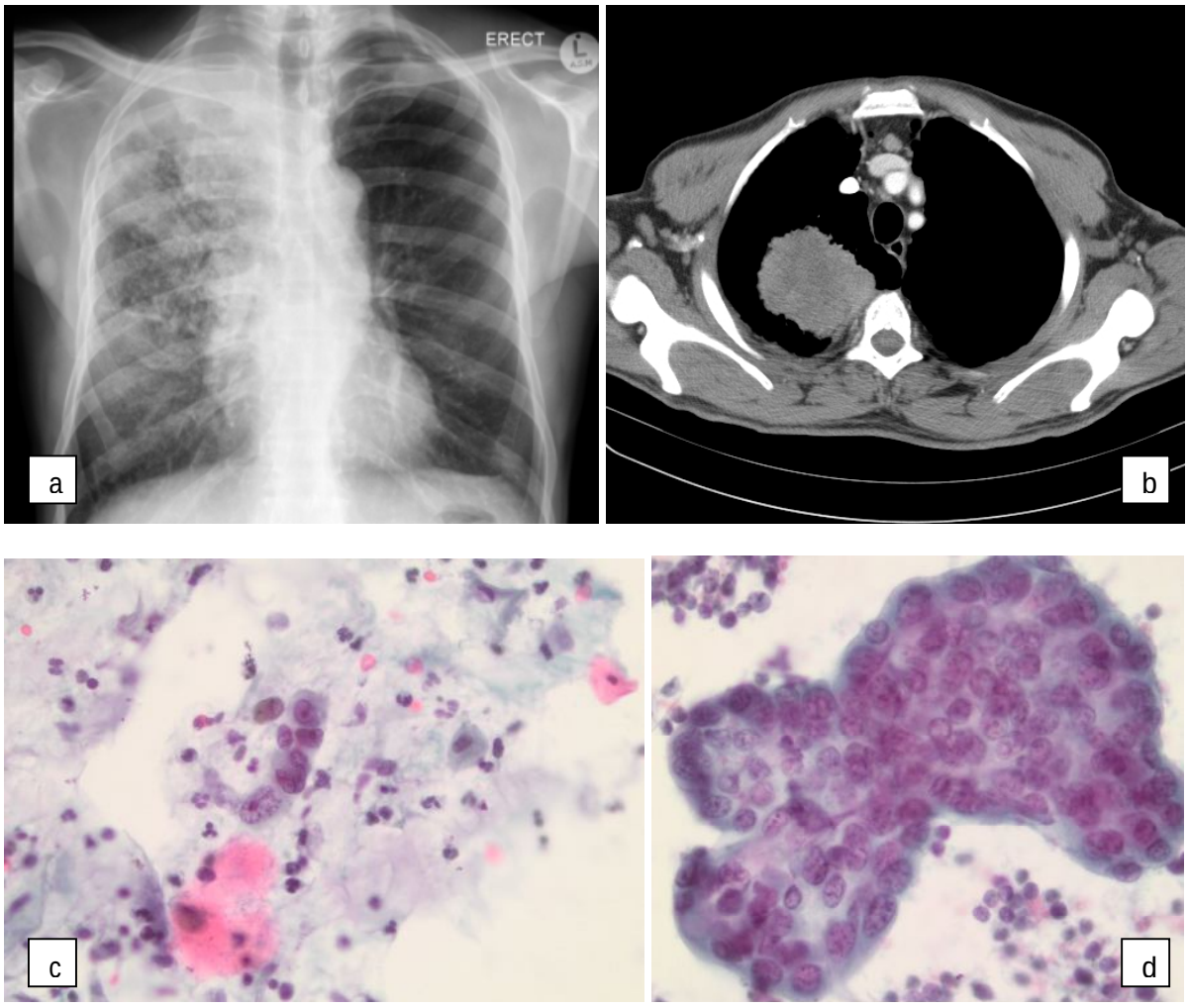

Figure 6.6: *Large peripheral right upper lung mass on chest X-ray (a) and CT (b). Sputum – malignant. Small aggregates of cells with clumped chromatin, thickened nuclear membranes and prominent nucleoli (c). TBNA - Balls of cells with characteristic features of adenocarcinoma in a background of reactive lymphocytes (d).*

Case 4: 69 Male - TTNA of lower left lobe. Chronic smoker with severe obstructive pulmonary disease.

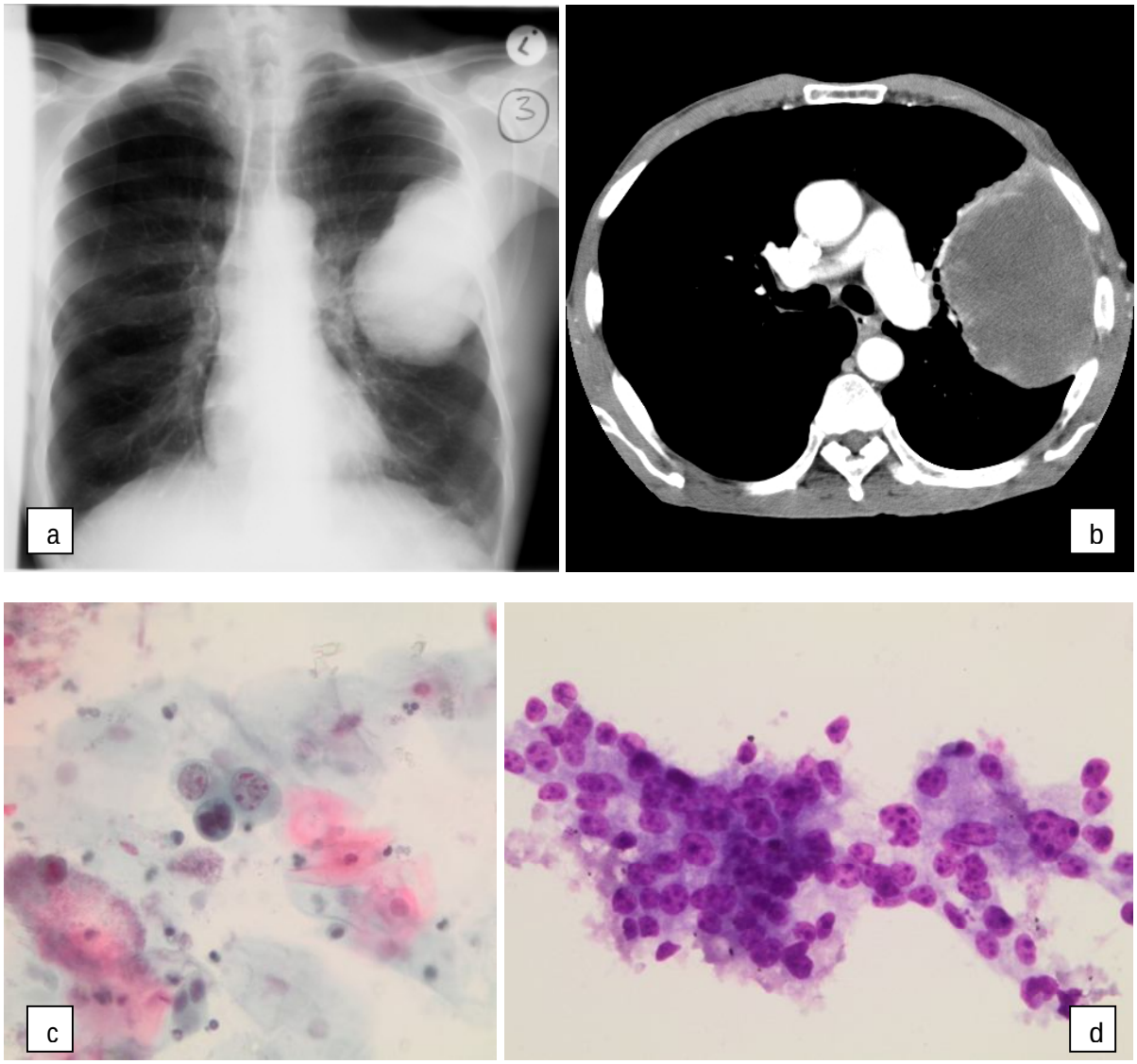

Figure 6.7: *Large mass in the left hemithorax with a broad base of contact with the pleura on chest X-ray (a) and CT (b). Sputum - suspicious for malignancy. Small cells with high N/C ratio and multiple nucleoli (c). TTNA - Adenocarcinoma with delicate cytoplasm and multiple nucleoli (d) MGG.*

Case 7: 59 Male – TBNA in a patient with weight loss, cough and dyspnoea.

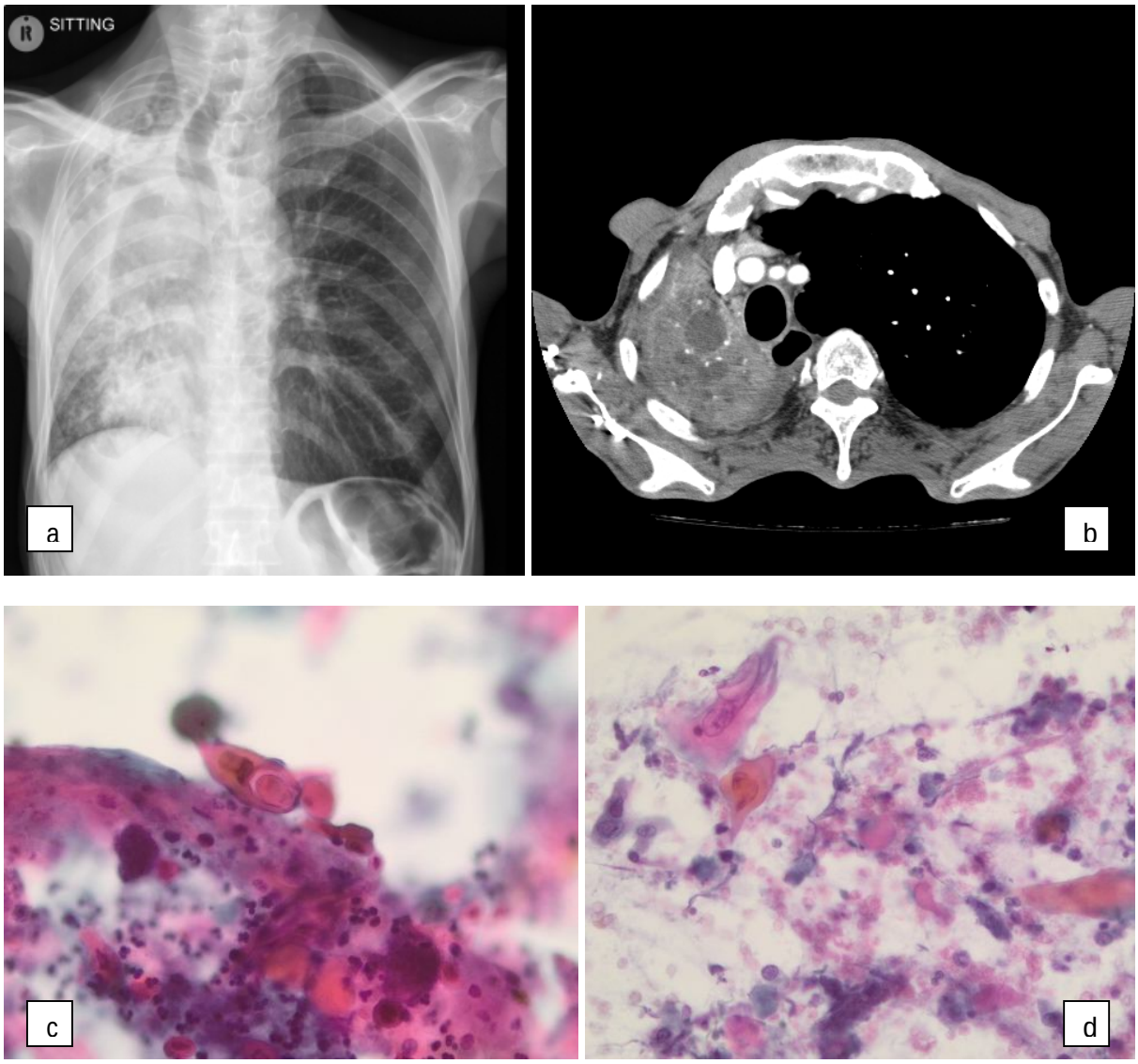

Figure 6.8: *Complete collapse of the entire right lung with a central mass and local invasion on chest X-ray (a) and CT (b). Sputum – malignant. Keratinised pearls and cells with dense cytoplasm and hyperchromatic nuclei (c). TBNA - Squamous cell carcinoma with necrotic background (d).*

Case 12: 49 Male - FNA of a right neck mass in a patient with enlarged cervical lymph node, right upper lung mass and Superior Vena Cava syndrome.

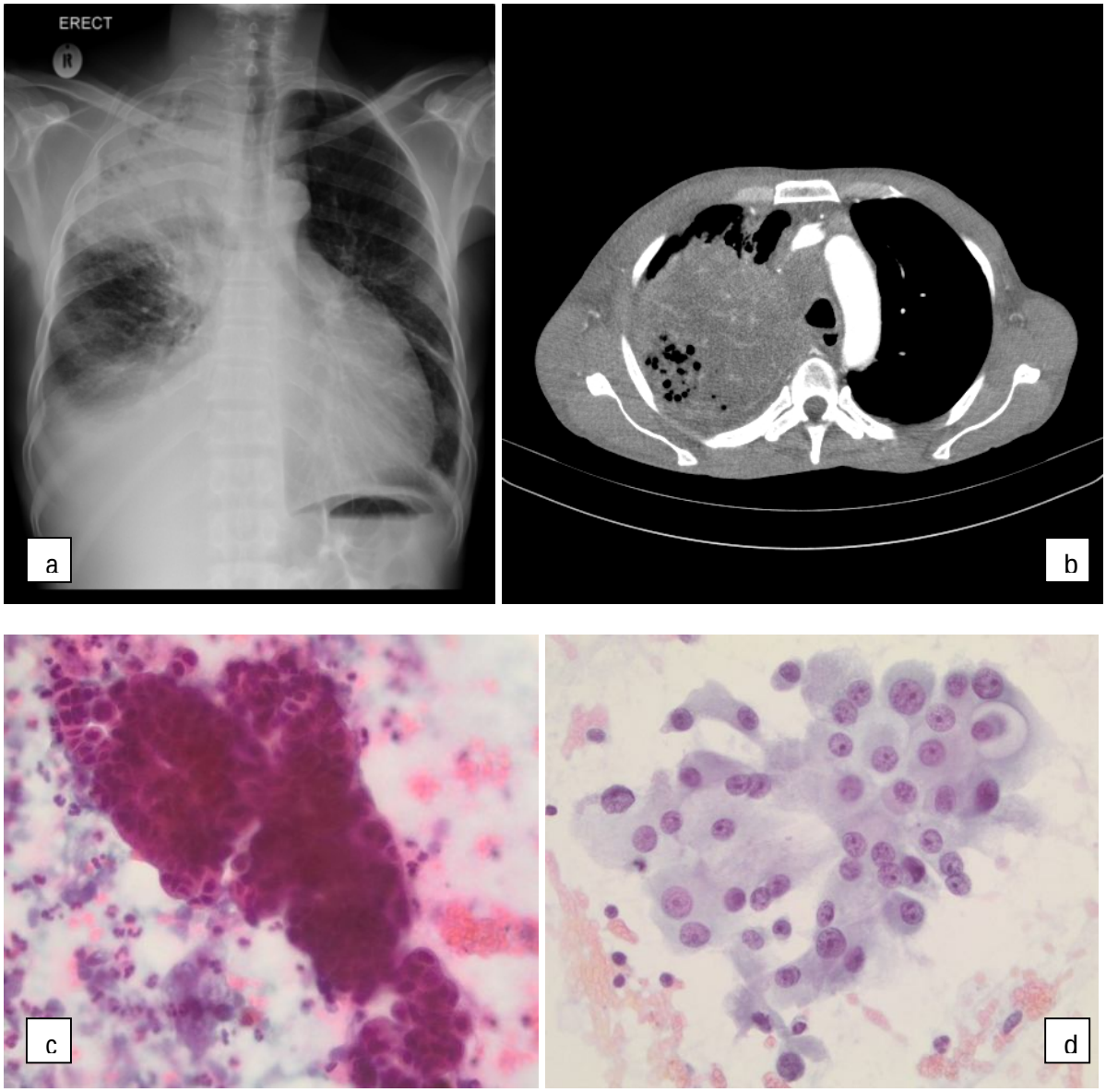

Figure 6.9: *Large, right upper lobe mass with extensive lymphadenopathy on chest X-ray (a) and CT (b). Sputum - suspicious for malignancy. Large clusters of small, moulded, degenerate cells (c). FNA – Metastatic adenocarcinoma with giant cells, abundance of cytoplasm and prominent nucleoli, subclassified as pleomorphic carcinoma (d).*

Case 13: 67 Male - FNA of a right supraclavicular mass in a patient with a strong smoking history, haemoptysis and a suspicious lung lesion on CT.

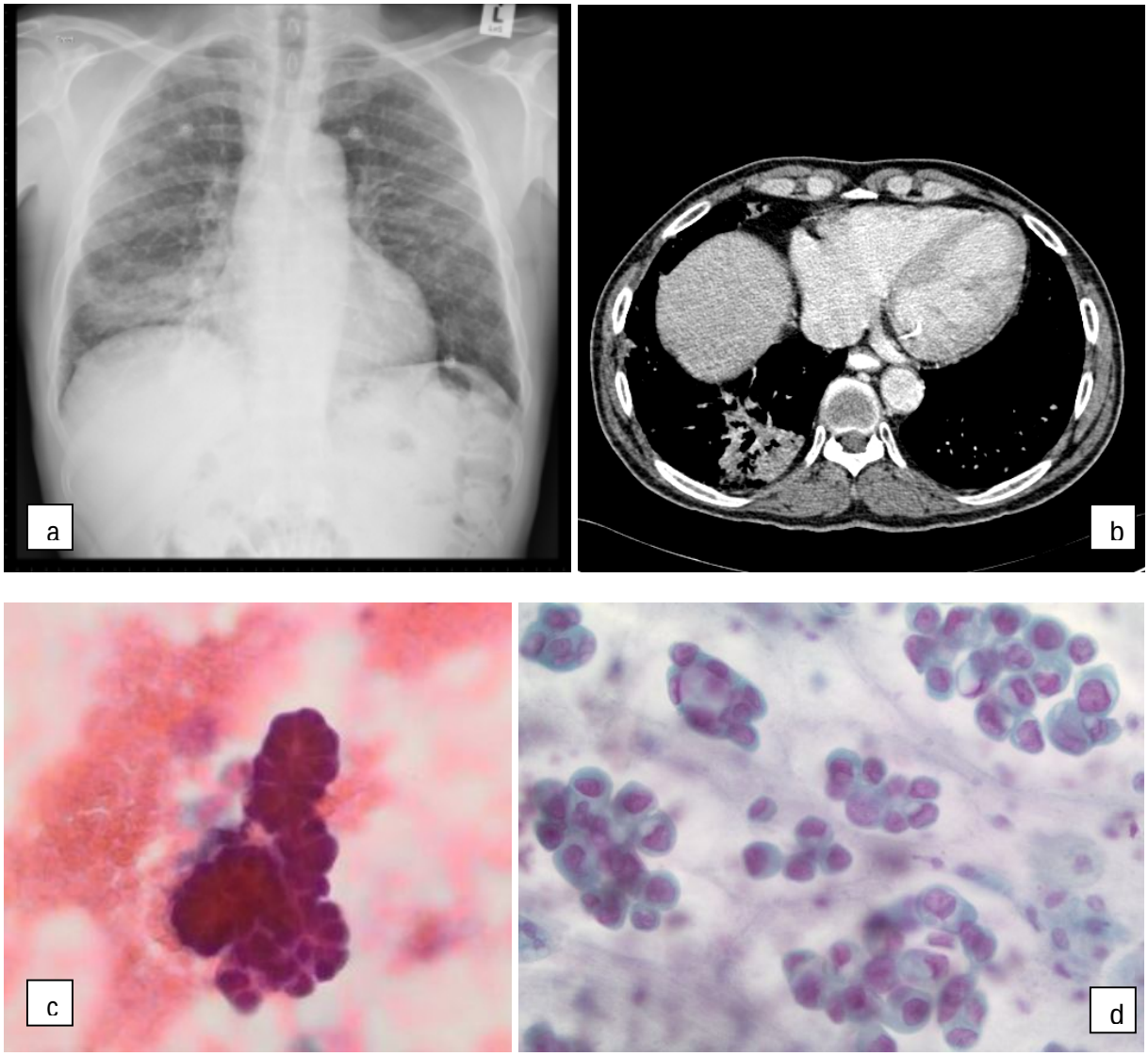

Figure 6.10: *Right middle lobe consolidation with extensive lymphadenopathy on chest X-ray (a) and CT (b). Sputum – malignant. Small, degenerate cells forming acini (c). FNA - Well differentiated adenocarcinoma with mucin in the background, papillary structures and vacuolated cytoplasm; features similar to a BAC (d).*

Case 15: 47 Male - TBNA of a patient with marked weight loss, clubbing and a lung mass suggestive of malignancy.

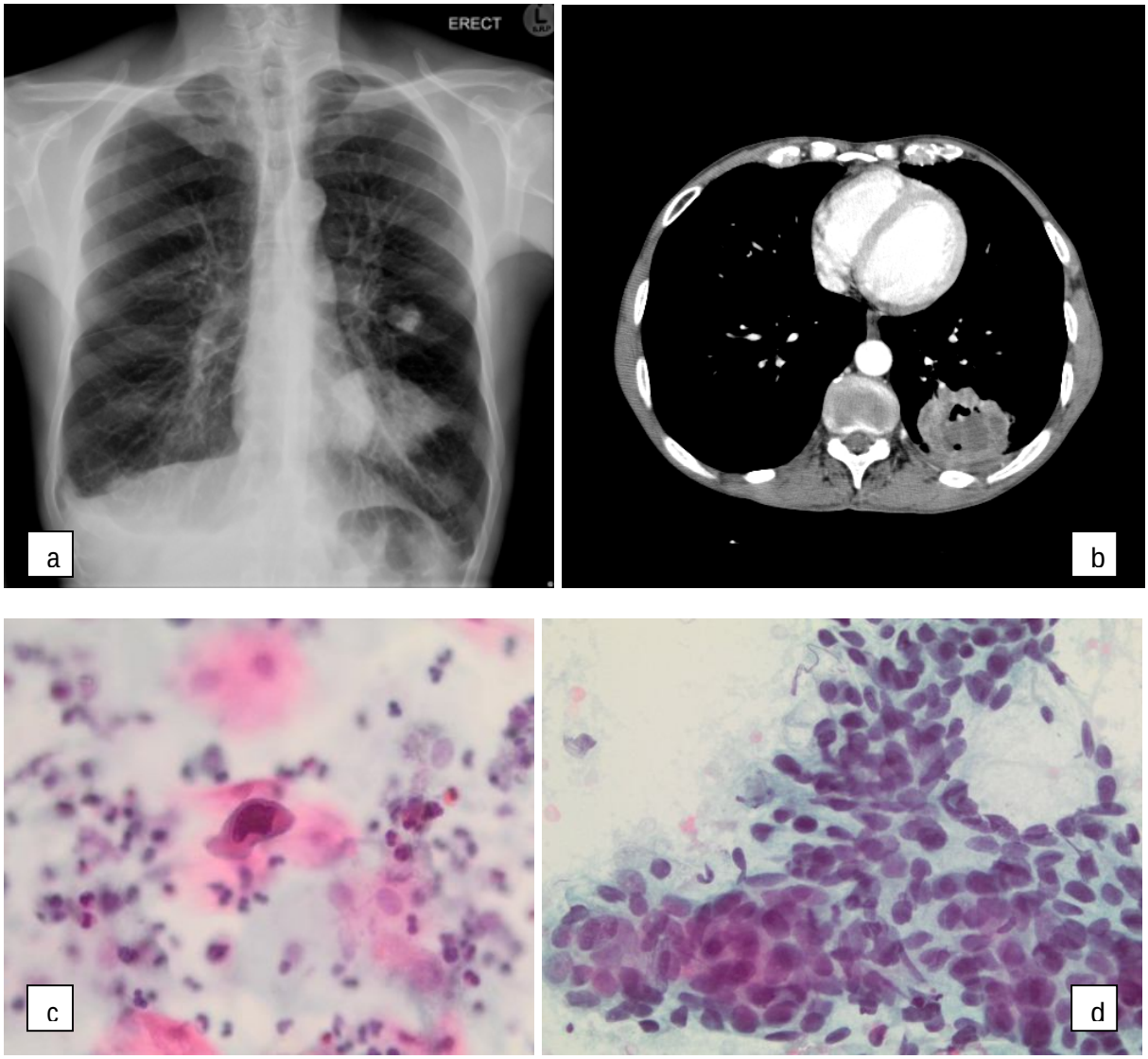

Figure 6.11: *Large heterogeneously enhancing lesion and associated pleural reaction in the posterior segment of the left lower lobe on chest X-ray (a) and CT (b). Sputum – malignant. Sparse, single lying cells with marked nuclear irregularities, hyperchromasia and dense cytoplasm (c). TBNA - Squamous cell carcinoma, predominantly non-keratinising (d).*

Case 17: 61 Male – TBNA in patient with haemoptysis and reduced sensation of right side of the body.

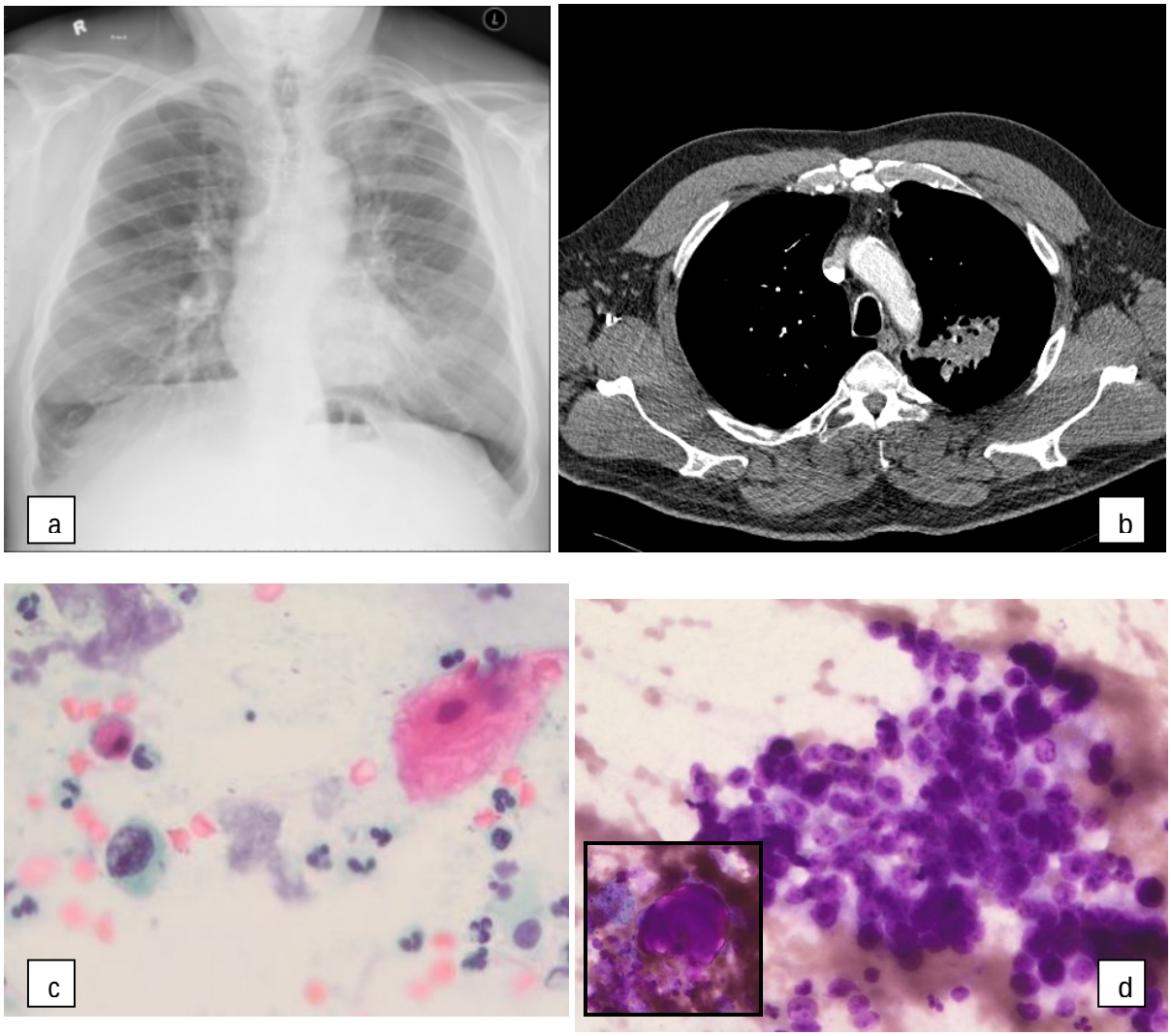

Figure 6.12: *Left hilar mass-nodal-complex with infiltration of the left main and upper lobe bronchus on chest X-ray (a) and CT (b).* Sputum *– malignant. Sparse, single lying, high N/C ratio cells with irregular chromatin distribution (c). TBNA - Adenosquamous cell carcinoma (predominant adenocarcinoma). Inlay a squamous pearl (d) MGG. CK5 positive in certain cells, BerEP4 positive in majority of cells. (images not included)*

Case 26: 46 Female - TTNA of right chest mass.

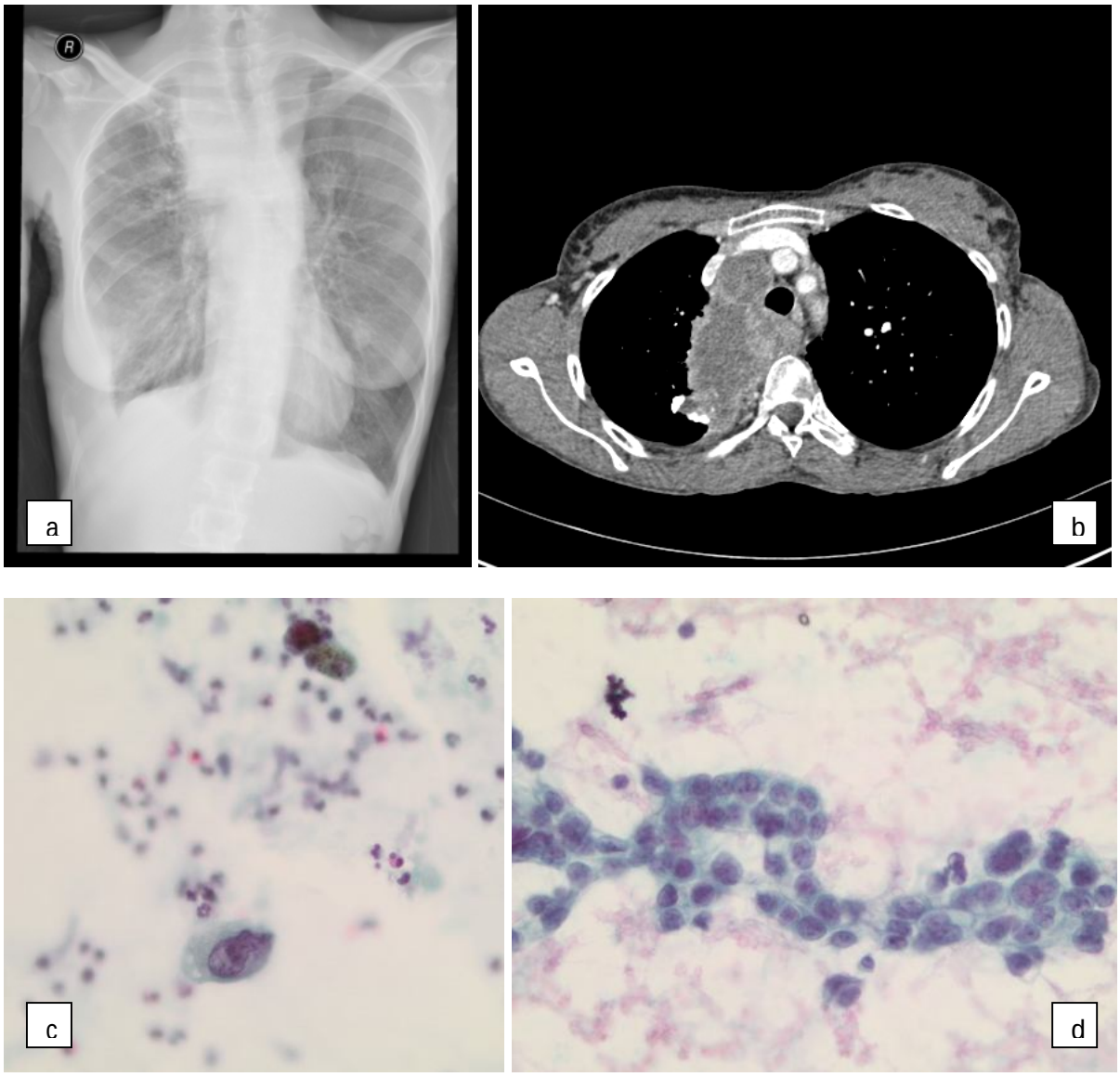

Figure 6.13: *Large right hilar mass-nodal-complex extending to the apex of the right lung on chest X-ray (a) and CT (b). Sputum – malignant. Sparse, single cells predominantly with marked nuclear irregularities and vacuolated cytoplasm (c). TTNA - Well differentiated adenocarcinoma (d).*

Case 27: 70 Male - TTNA of patient with haemoptysis and left lower lobe mass.

No chest X-ray or CT images available

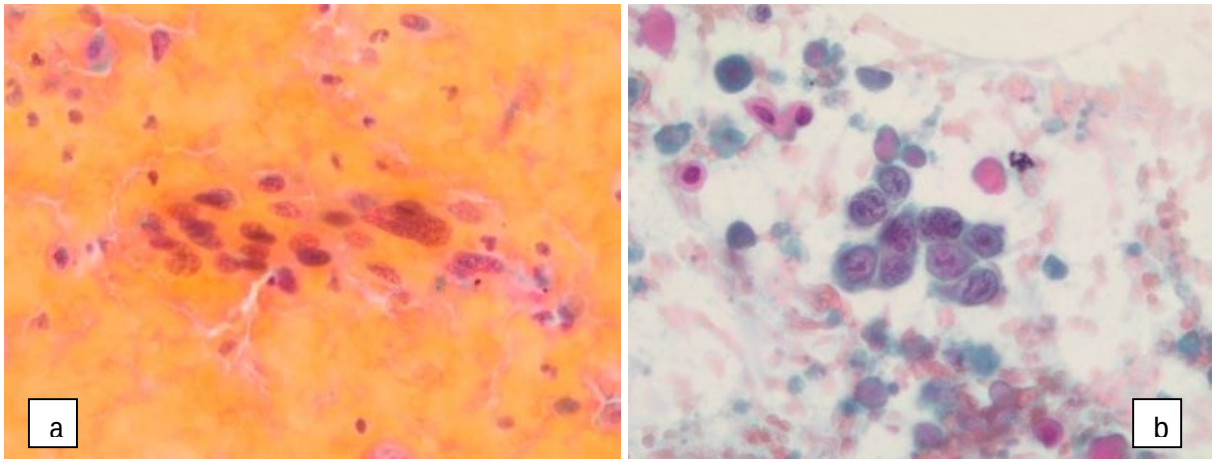

Figure 6.14: *Sputum – malignant. Extremely bloody sample. Degenerate cells have hyperchromatic nuclei (a).TTNA - Squamous cell carcinoma with necrosis (b).*

Case 29: 50 Male - TTNA of right upper lobe mass. Patient presented with haemoptysis and suspected of Pancoast tumour with thoracic cord compression.

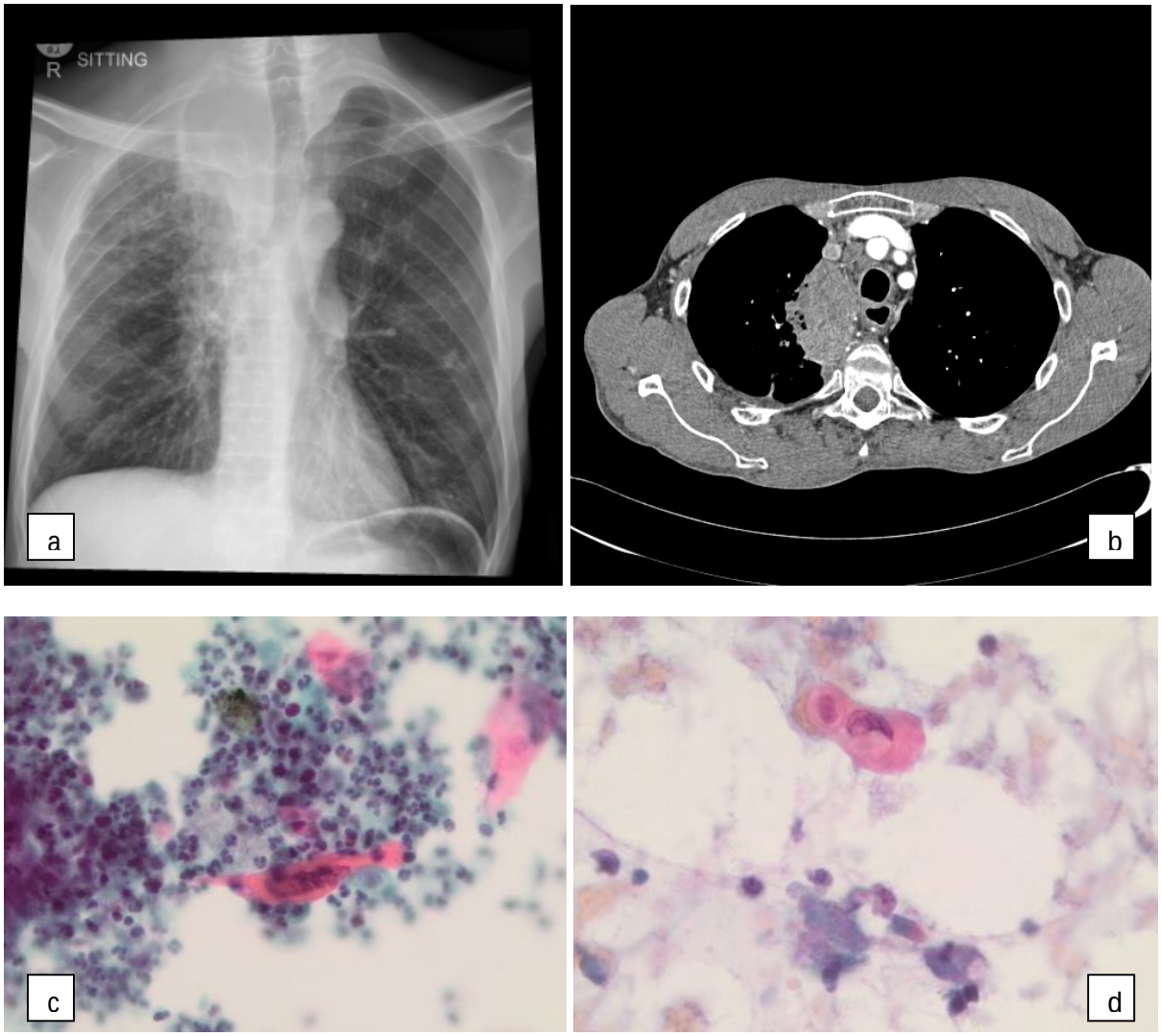

Figure 6.15: *Right apical mediastinal mass with extension into the right hilum with a right nodal mass complex on chest X-ray (a) and CT (b). Sputum – malignant. Marked inflammatory smear with keratinised, spindled cells and nuclear irregularities (c). These cells were missed with manual screening. TTNA - Squamous cell carcinoma (d).*

Case 35: 74 Female - TBNA of patient with weight loss, cough and left upper lobe mass suspected of bronchus carcinoma.

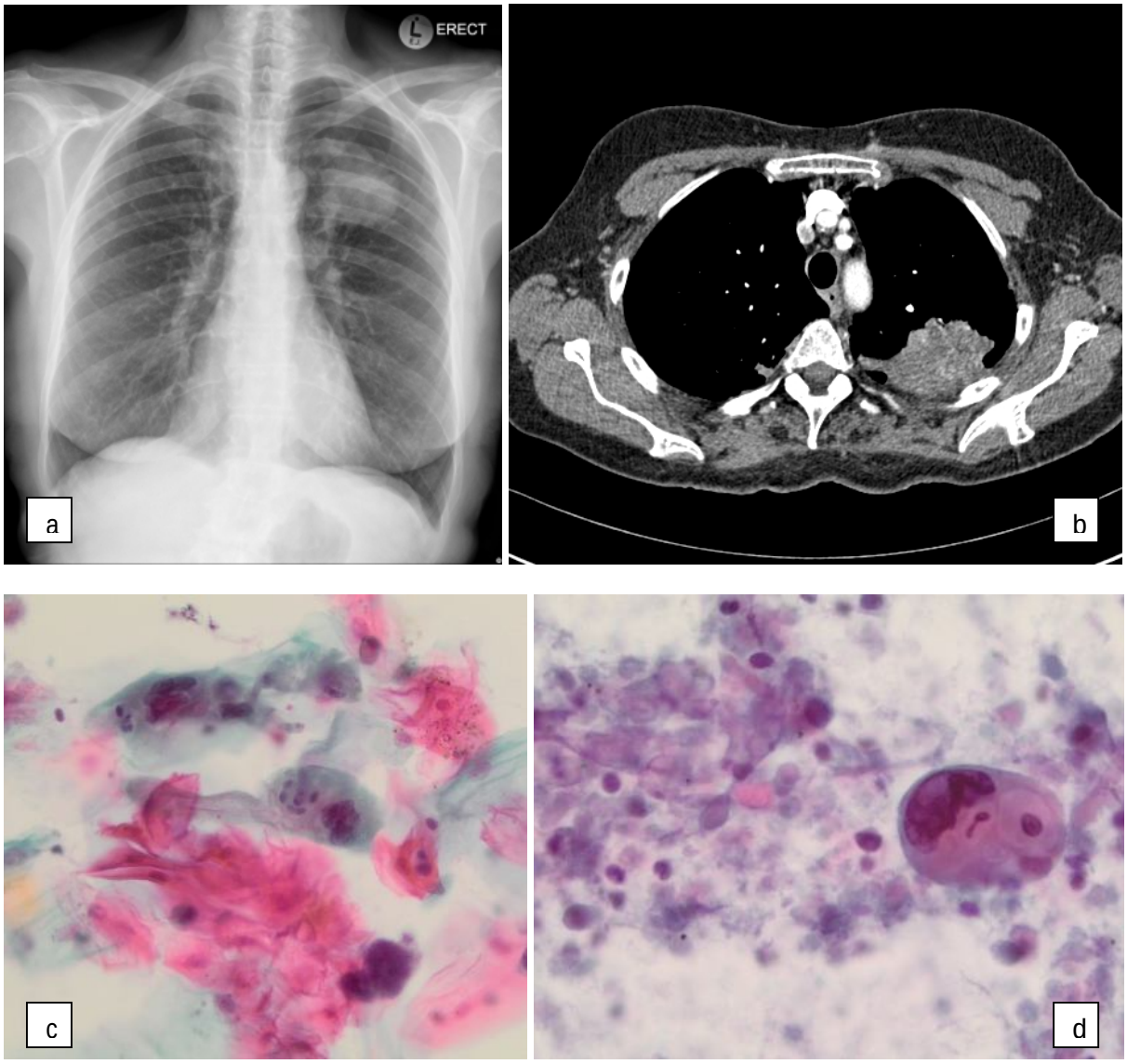

Figure 6.16: *Circumscribed, lobulated mass in left upper lobe abutting the pleura on chest X-ray (a) and CT (b). Sputum – malignant. Large cells with dense cytoplasm and nuclear irregularities (c). These cells were missed with manual screening. TBNA - Squamous cell carcinoma, predominantly keratinising type with necrosis (d).*

Case 37: 49 Male - TTNA of right lower lobe mass.

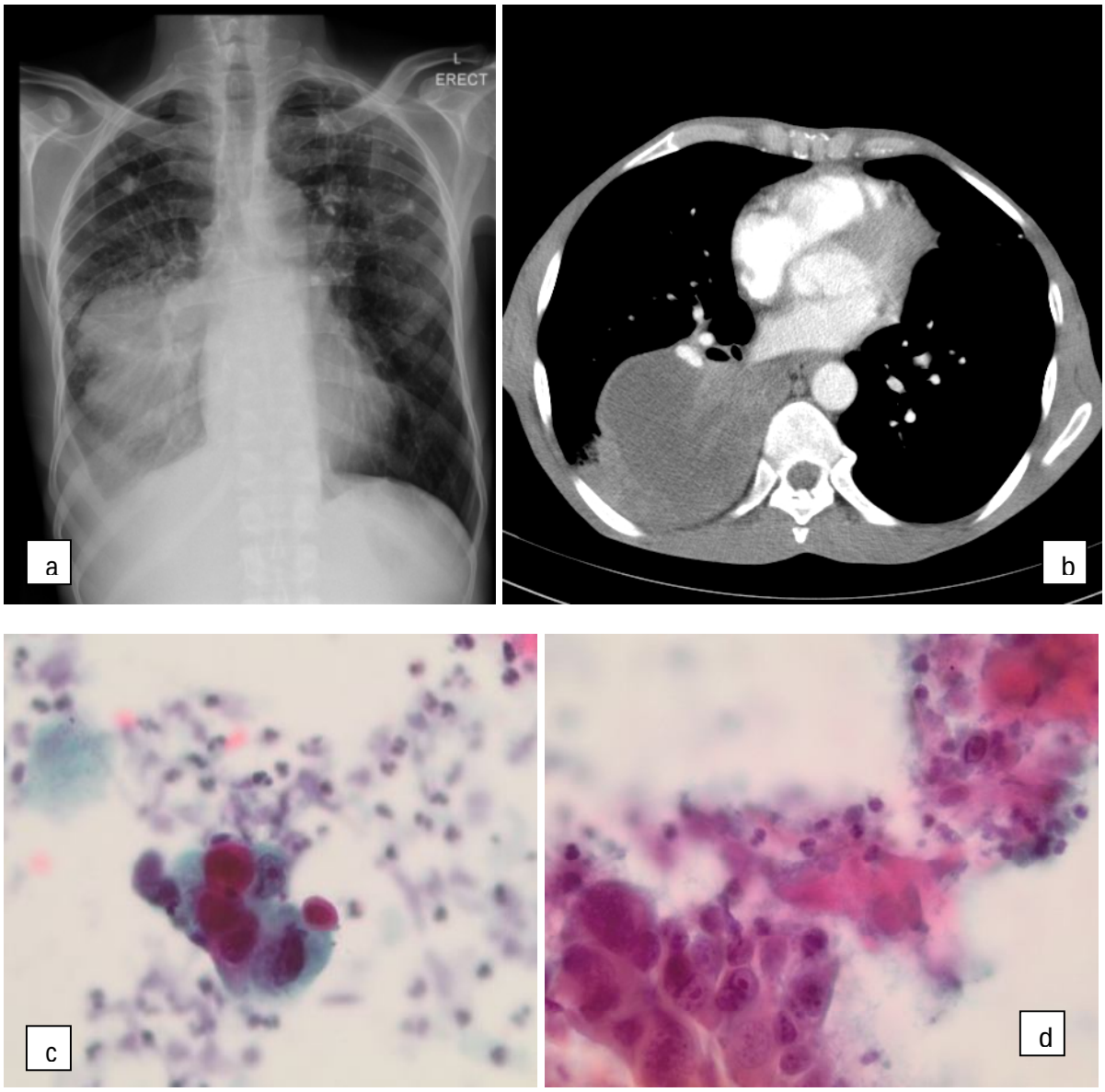

Figure 6.17: *Large central and right lower lobe mass on chest X-ray (a) and CT (b). Sputum - malignant. Small aggregates of cells with prominent nucleoli (c). TTNA - Squamous cell carcinoma (d).*

Case 47: 65 Male - TBNA of subcarinal lymph node in a heavy smoker with weight loss, dyspnoea and clubbing.

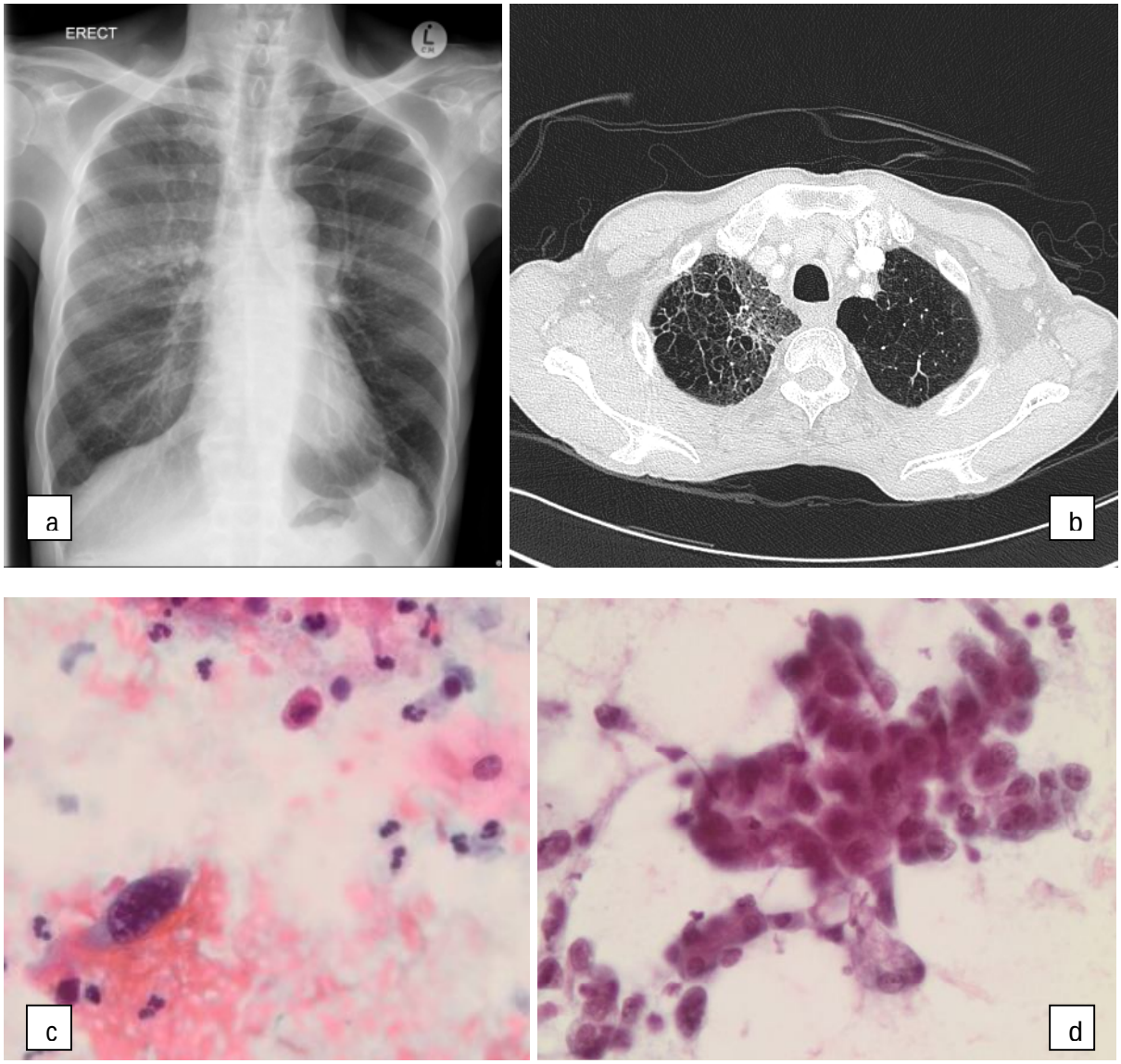

Figure 6.18: *Mediastinal lymphadenoapathy with compression and narrowing of bronchus on chest X-ray (a) and CT (b). Sputum - malignant. High N/C ratio malignant cells. These cells were missed with manual screening (c). TBNA - Adenocarcinoma with vacuolated cytoplasm (d).*

Case 48: 50 Female - FNA of soft tissue masses in a patient known with metastatic carcinoma. Masses on back, chest wall and breast.

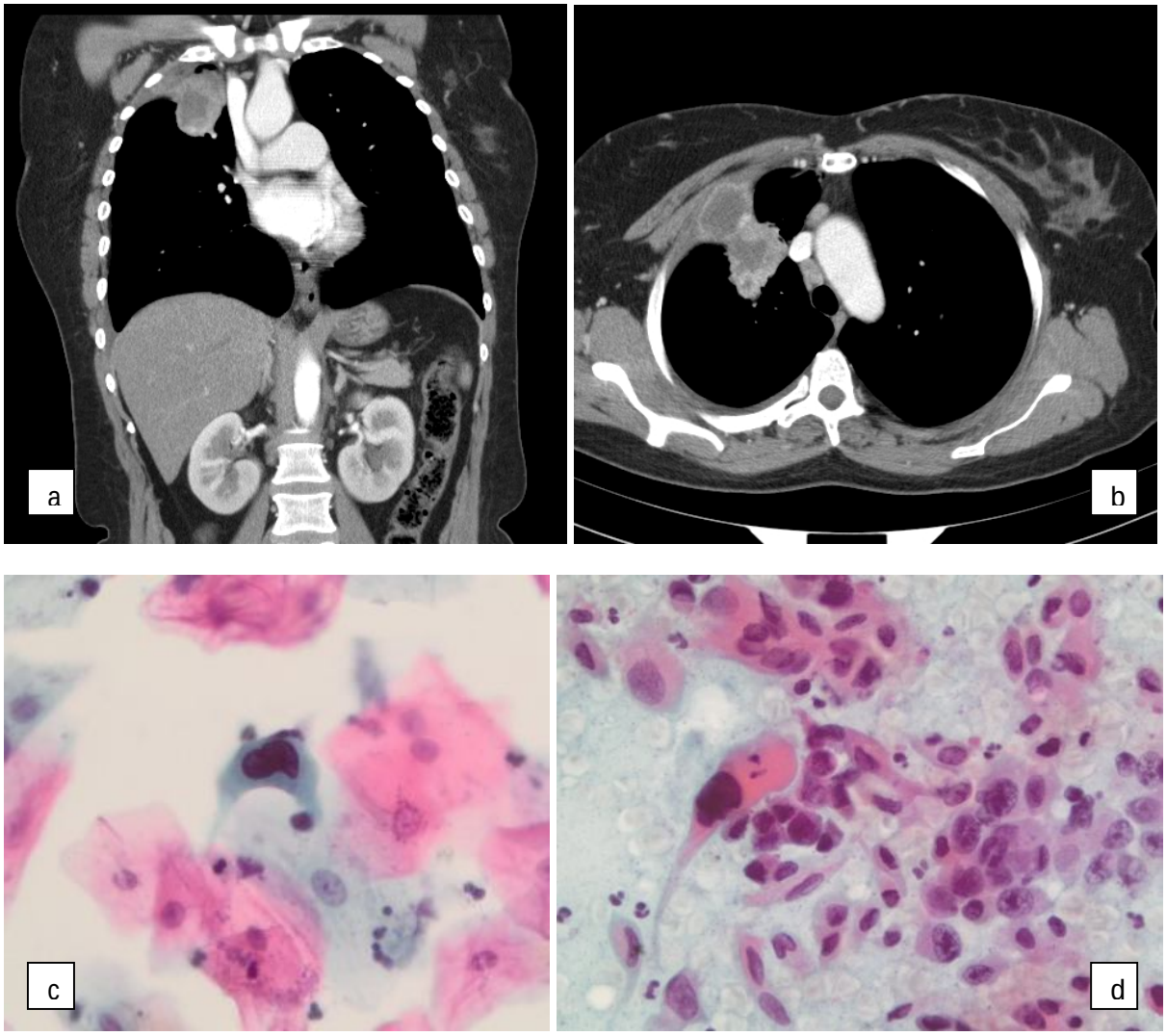

Figure 6.19: *Right upper lobe peripheral mass abutting the chest wall on chest CT (a,b) Sputum – suspicious for malignancy. Scattered atypical cells with hyperchromatic nuclei and dense cytoplasm suggestive of a squamous cell carcinoma (c). FNA – Metastatic squamous cell carcinoma (d).*

Case 49: 78 Male - TTNA of right upper lobe mass in patient with haemoptysis.

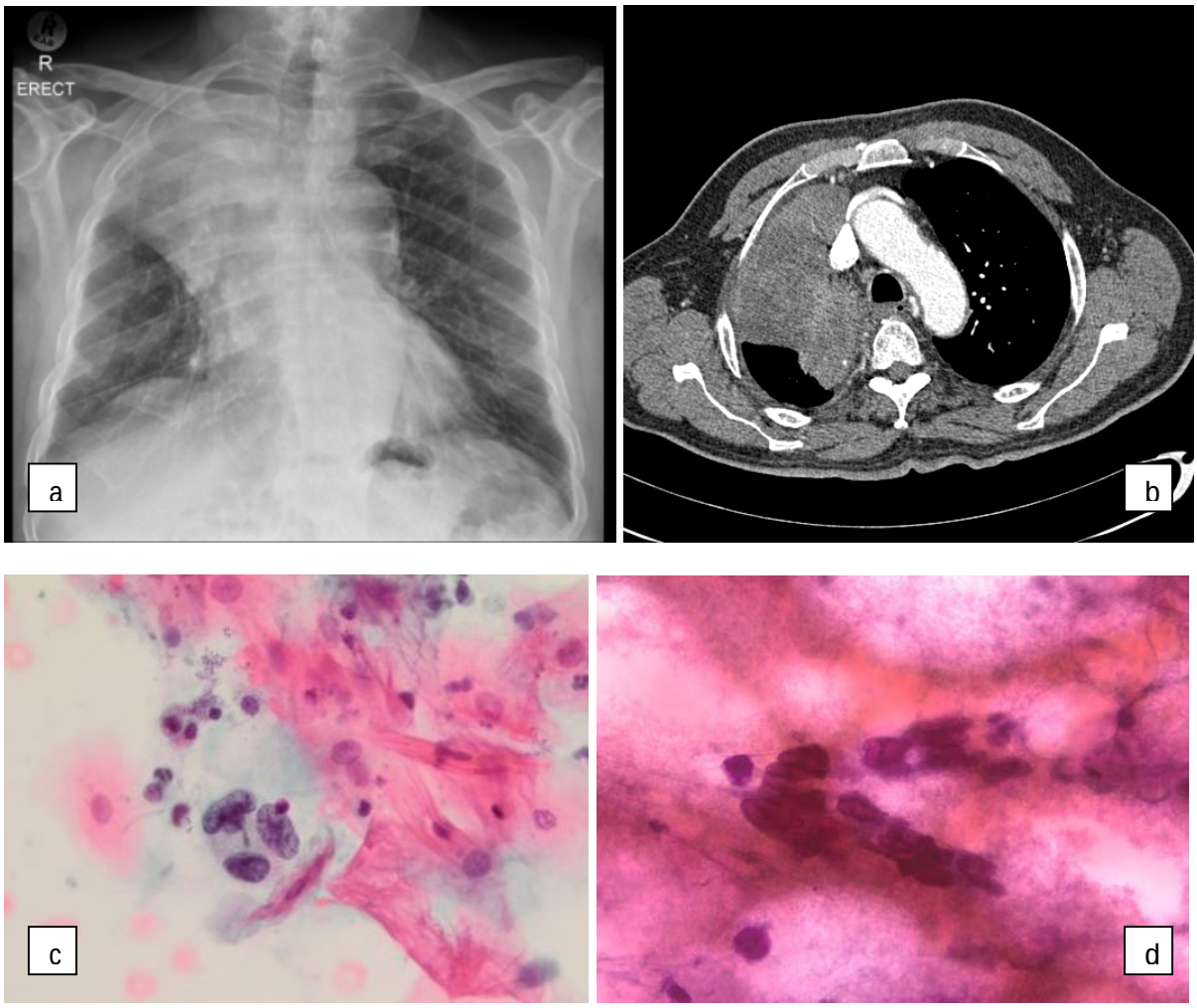

Figure 6.20: *Mass causing obstruction of the right upper lobe bronchus with complete collapse of the right upper lobe on chest X-ray (a) and CT (b). Sputum - malignant. Small balls of cells present with clear, vacuolated cytoplasm and eccentric nuclei suggestive of adenocarcinoma (c). TTNA - Poorly differentiated carcinoma, likely adenocarcinoma (d).*

Case 50: 63 Male - TTNA of right anterior mediastinal mass in ex-smoker with previous pulmonary tuberculosis.

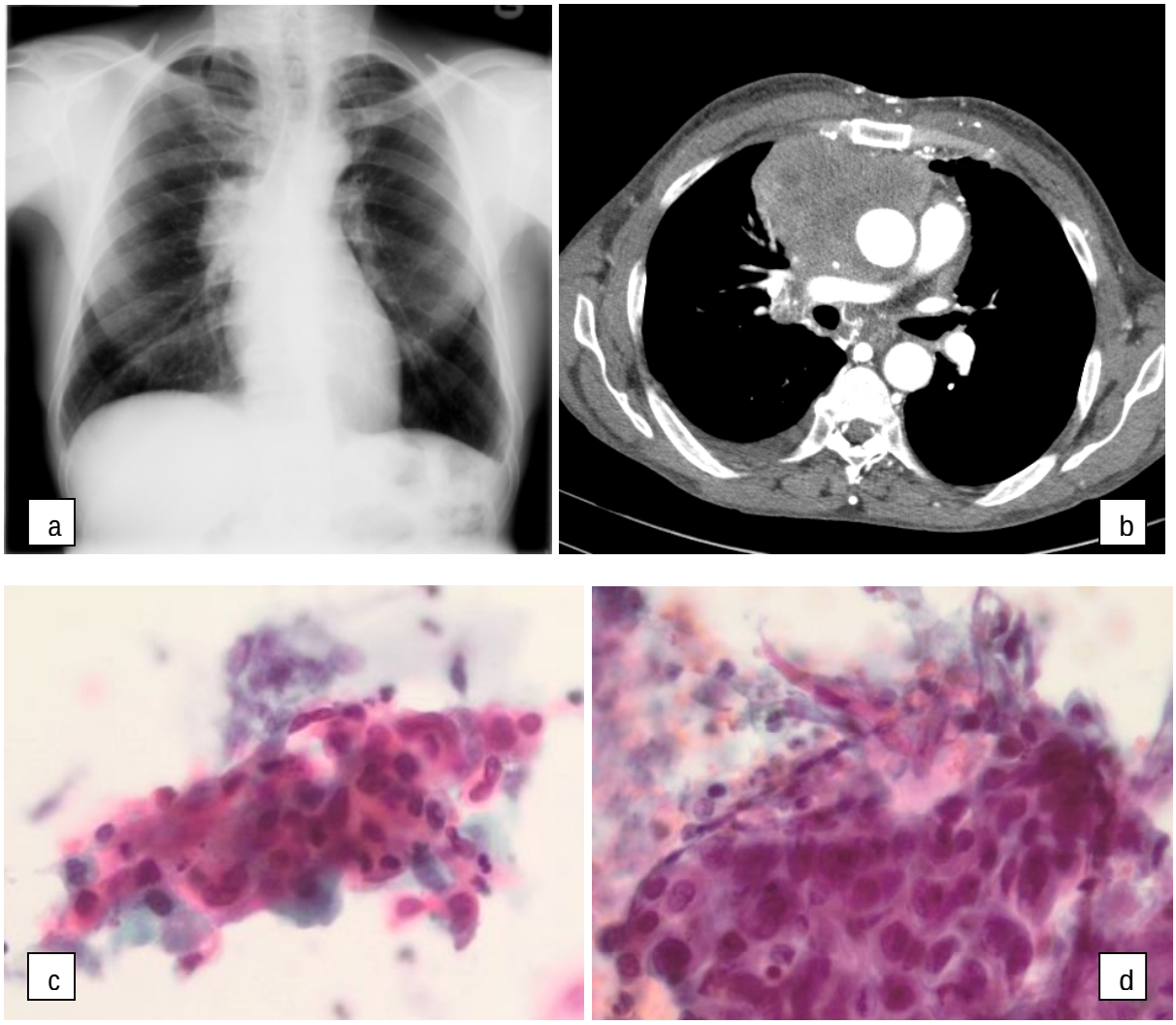

Figure 6.21: *Large mass involving the right upper lobe and invading the mediastinal structures on chest X-ray (a) and CT (b). Sputum - malignant. Clusters of cells present with hyperchromatic nuclei and keratinised cytoplasm (c). TTNA - Squamous cell carcinoma with hyperchromatic nuclei (d).*

Case 56: 59 Male - TTNA of patient with clinically bronchial carcinoma or pulmonary tuberculosis.

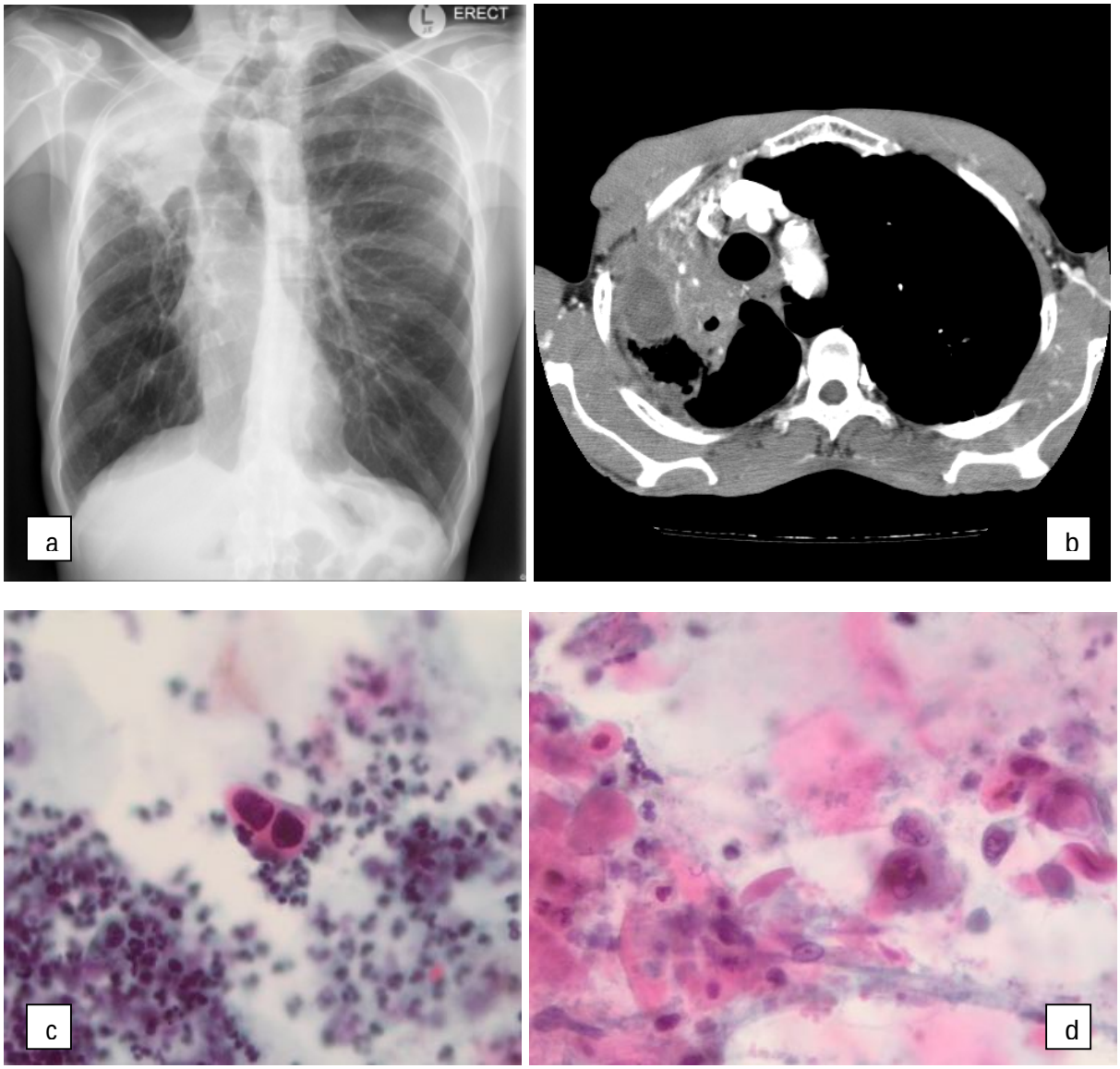

Figure 6.22: *Spiculated mass of the right upper lobe with extension to the anterior thoracic lung cage and hilar involvement on chest X-ray (a) and CT (b). Sputum - malignant. Extremely hyperchromatic nuclei with keratinised cytoplasm (c). TTNA - Squamous cell carcinoma with abundant necrosis (d).*

Case 69: 48 Female - Bronchial brushing of right upper lobe tumour in patient with haemoptysis, weight loss, chronic cough and chest pain.

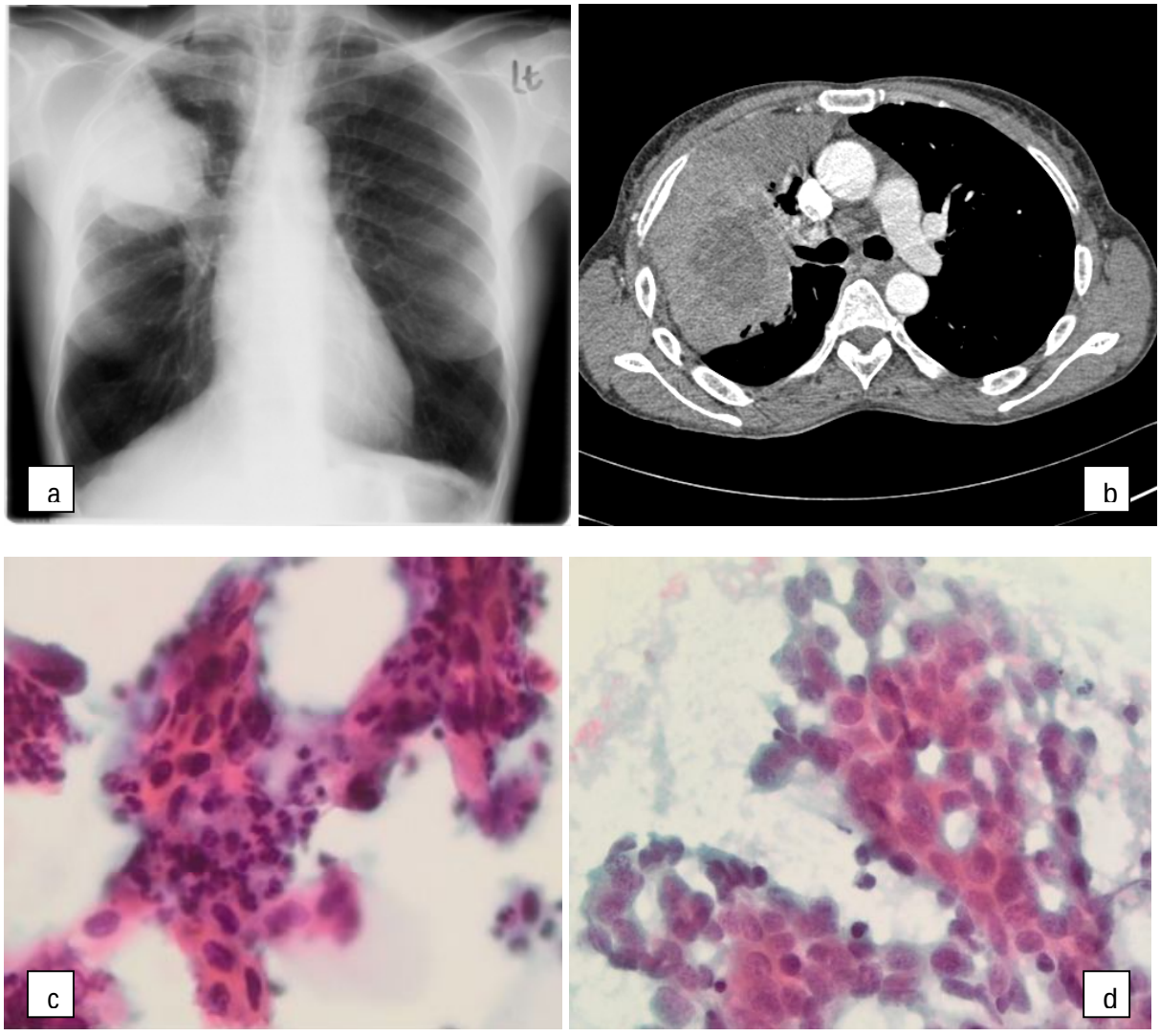

Figure 6.23: *Mass in right upper lobe with associative collapse and lymphadenopathy on chest X-ray (a) and CT (b). Sputum - malignant. Sheets of keratinised cells with elongated, hyperchromatic nuclei (c). Bronchial brushing - Squamous cell carcinoma (d).*

Case 78: 51 Male - Retrocarinal FNA of the main bronchus in patient complaining of coughing.

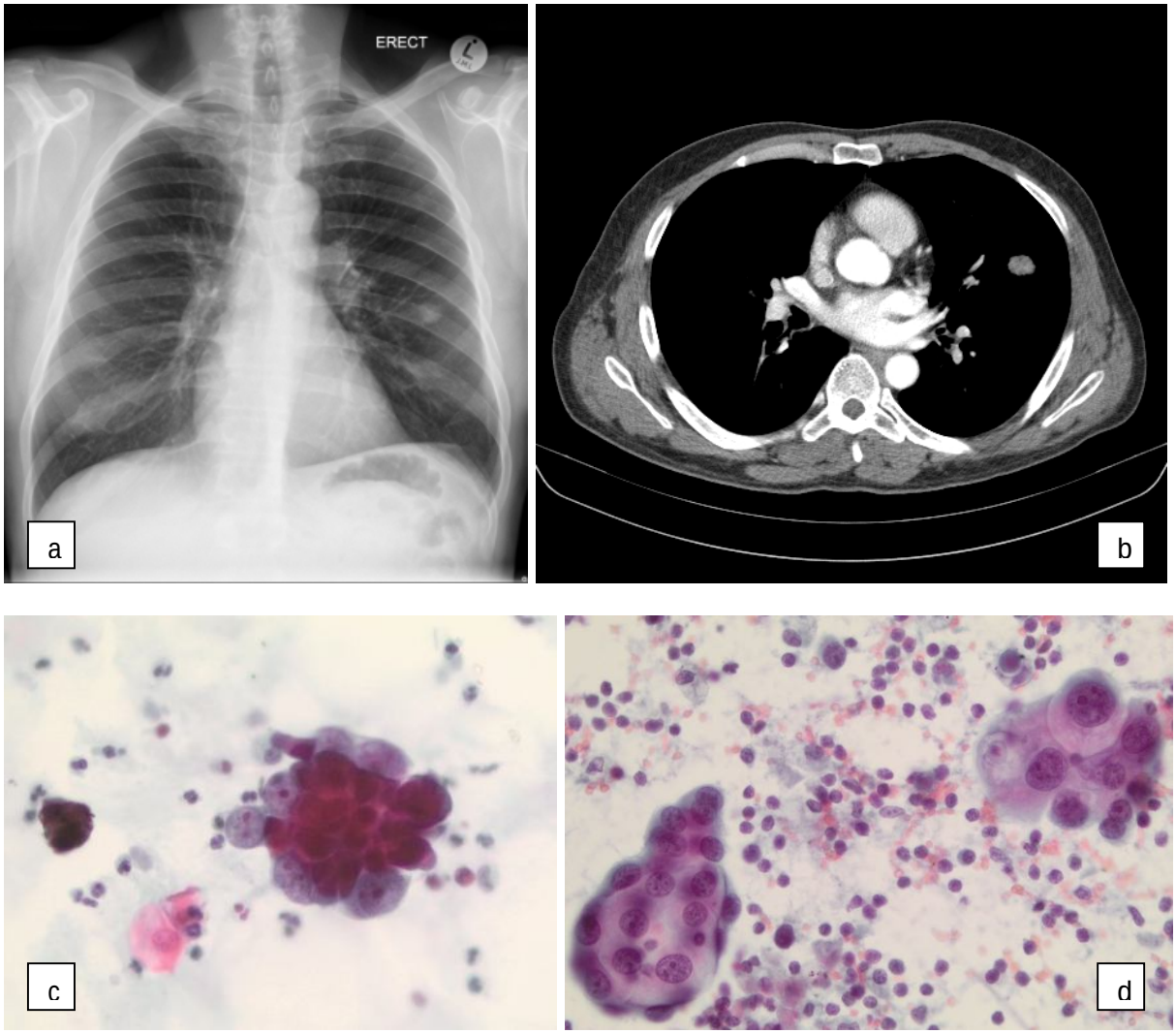

Figure 6.24: *Small, spiculated lesion in the left upper lobe with surrounding satellite nodules on chest X-ray (a), while not seen on CT (b) due to tumour position. Sputum – suspicious for malignancy. Extreme scant cellularity. Cells have high N/C ratio's with multiple nucleoli. These cells were missed with manual and automated screening (c). TBNA - Balls of vacuolated cells with characteristic features consistent with adenoarcinoma (d).*

Case 79: 66 Male - TBNA of left lingula spur in patient with weight loss and haemoptysis.

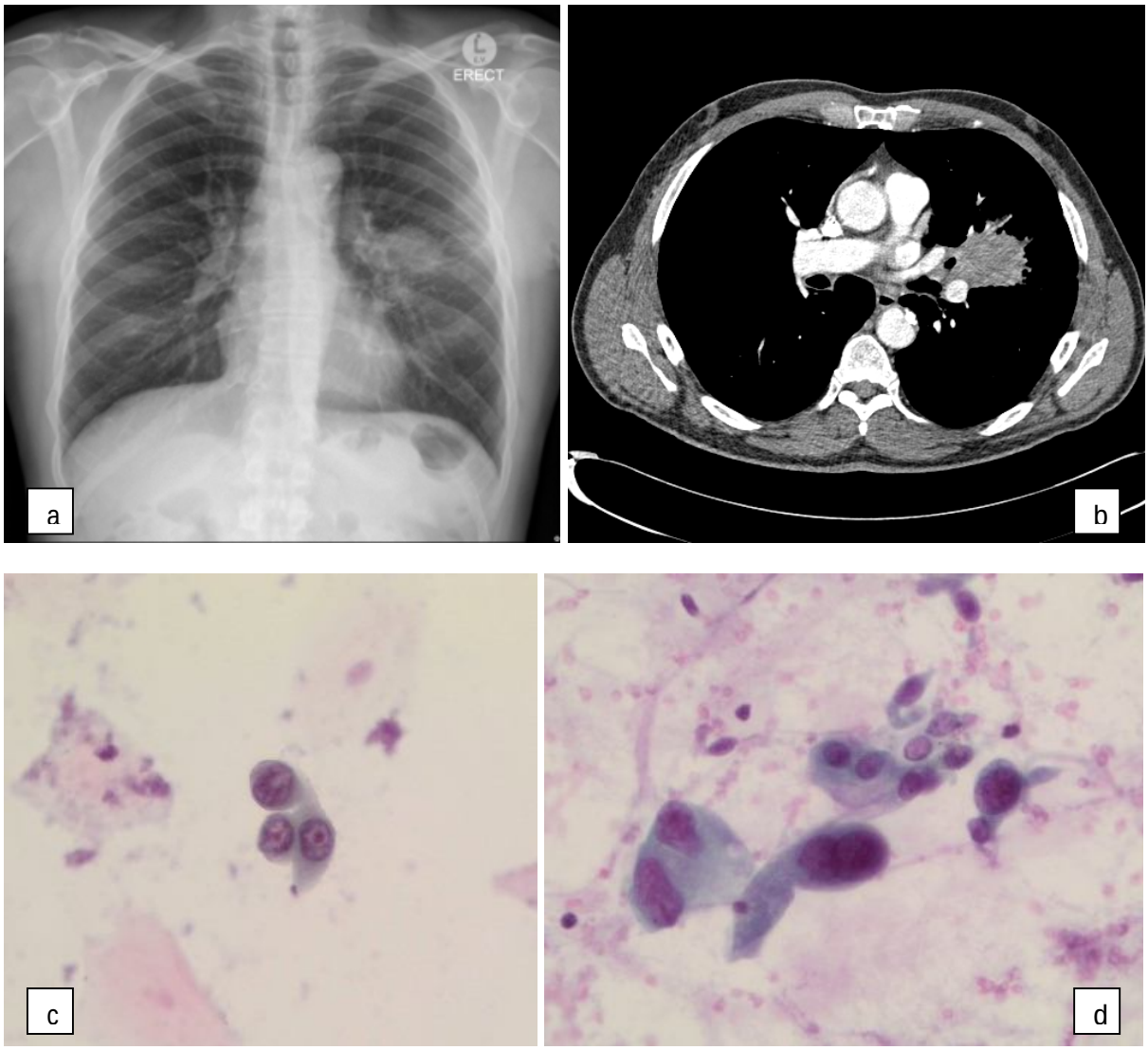

Figure 6.25: *Left hilar, spiculated tumour-nodal complex on chest X-ray (a) and CT (b). Sputum - malignant. Small cells scattered with clearing of the chromatin, thickened nuclear membranes and prominent nucleoli (c). TBNA - Adenocarcinoma (d).*

Case 89: 63 Male - TTNA of left lung mass protruding through chest wall in patient with weight loss and dyspnoea.

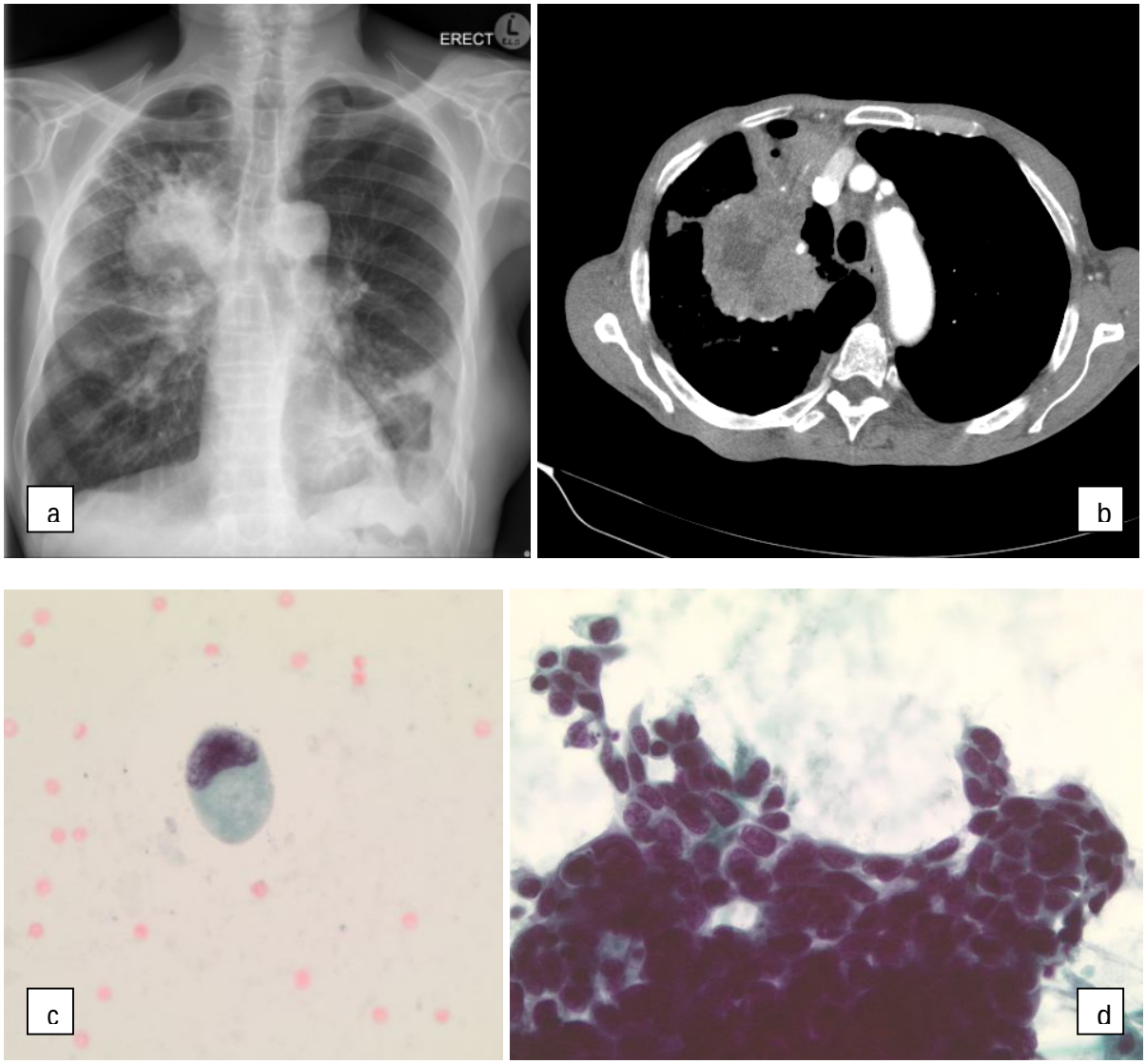

Figure 6.26: *Large, extensive, right upper lobe tumour with metastatic spread to the left rib cage and left sided hilar lymphadenopathy on chest X-ray (a) and CT (b). Sputum - malignant. Large, scattered cells with hyperchromasia and irregular nuclear membranes (c). TTNA - Squamous cell carcinoma with extreme hyperchromasia (d).*

Case 109: 76 Female – TTNA of large, left upper lobe mass in patient known with lobular carcinoma of the breast.

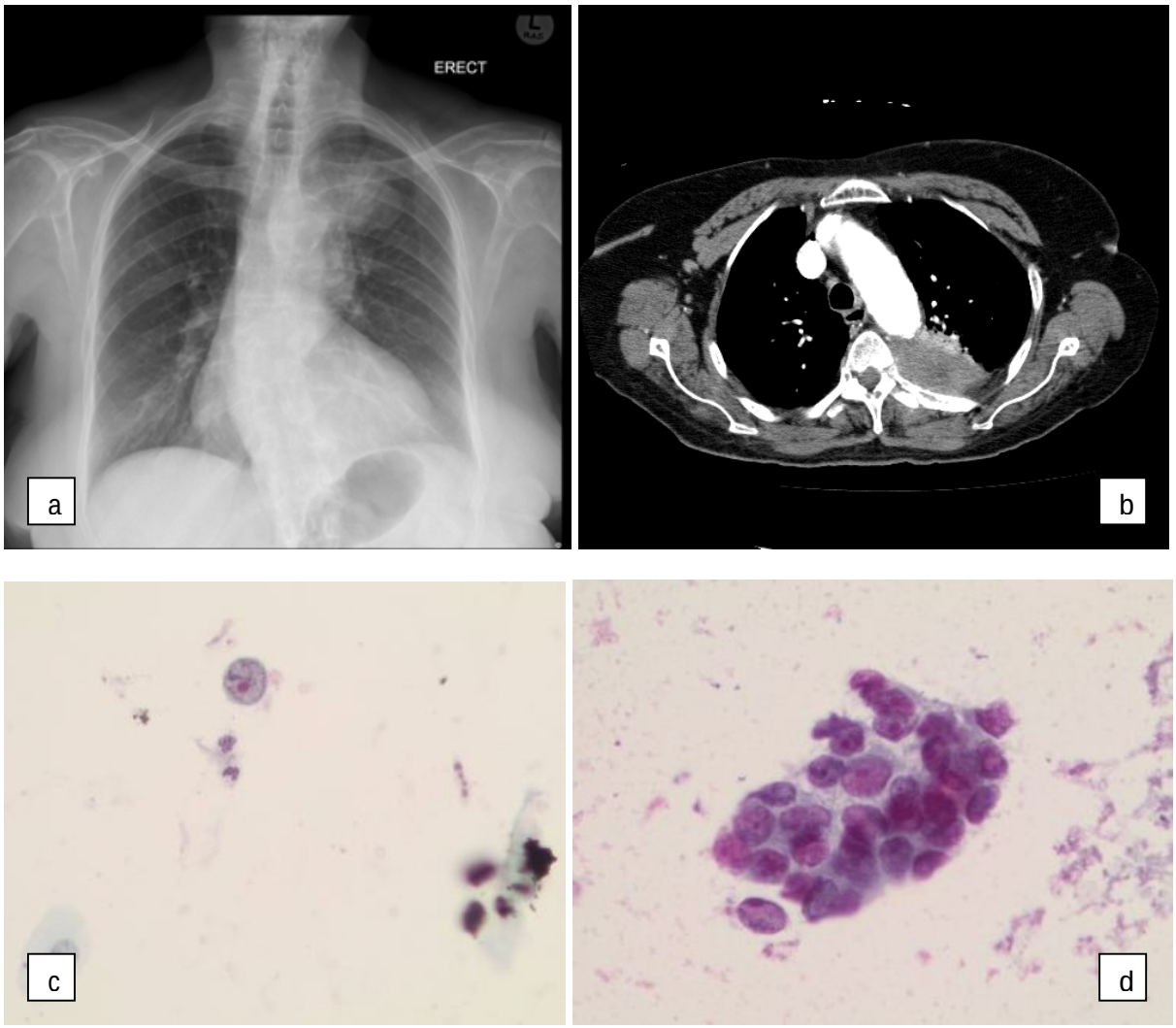

Figure 6.27: *Left upper lobe mass with infiltration of the chest wall and early rib destruction on chest X-ray (a) and CT (b). Sputum - malignant. Sparse cells present with extremely high N/C ratio, chromatin clearing with a thickened, irregular nuclear membrane and prominent nucleolus. These cells were missed with manual screening (c). TTNA - Large cell undifferentiated carcinoma (d). Immunocytochemistry: Progesterone (23) and oestrogen receptors (ER) negative (false negative cannot be excluded). PR and ER were strongly positive in the core needle breast biopsy. (images not included)*

Case 110: 48 Female - TTNA of right upper lobe lesion in patient with chronic cough, chest pain and weight loss.

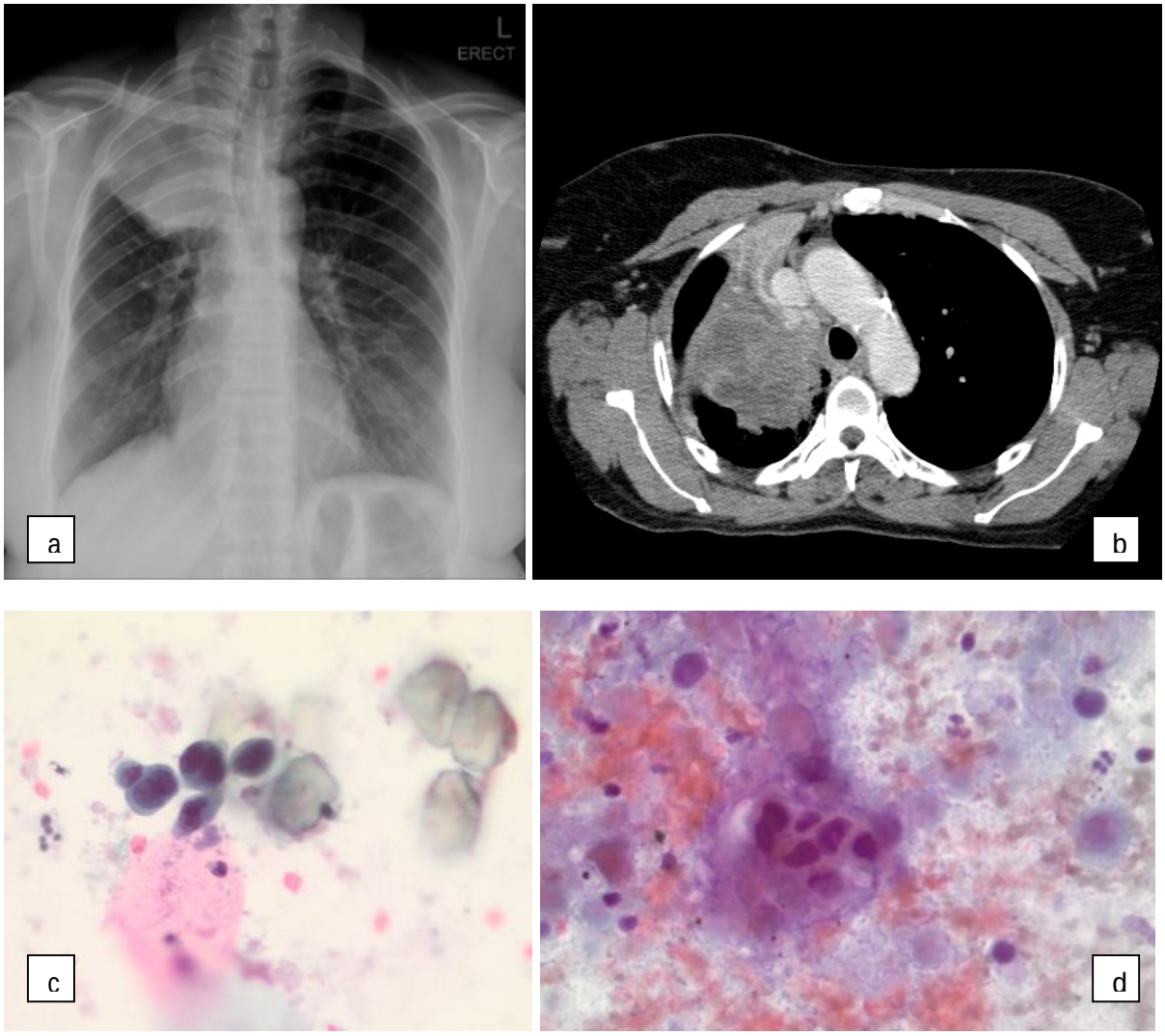

Figure 6.28: *Collapse consolidation of the right upper lobe and mediastinal lymphadenopathy on chest X-ray (a) and CT (b). Sputum - suspicious for malignancy. Sparse atypical cells present with prominent nucleoli and irregular nuclear membranes. These cells were missed with manual screening (c). TTNA - Non-small cell carcinoma, suggestive of adenocarcinoma with necrosis (d).*

Case 111: 68 Male – TTNA in patient with pain, weight loss and rib destruction.

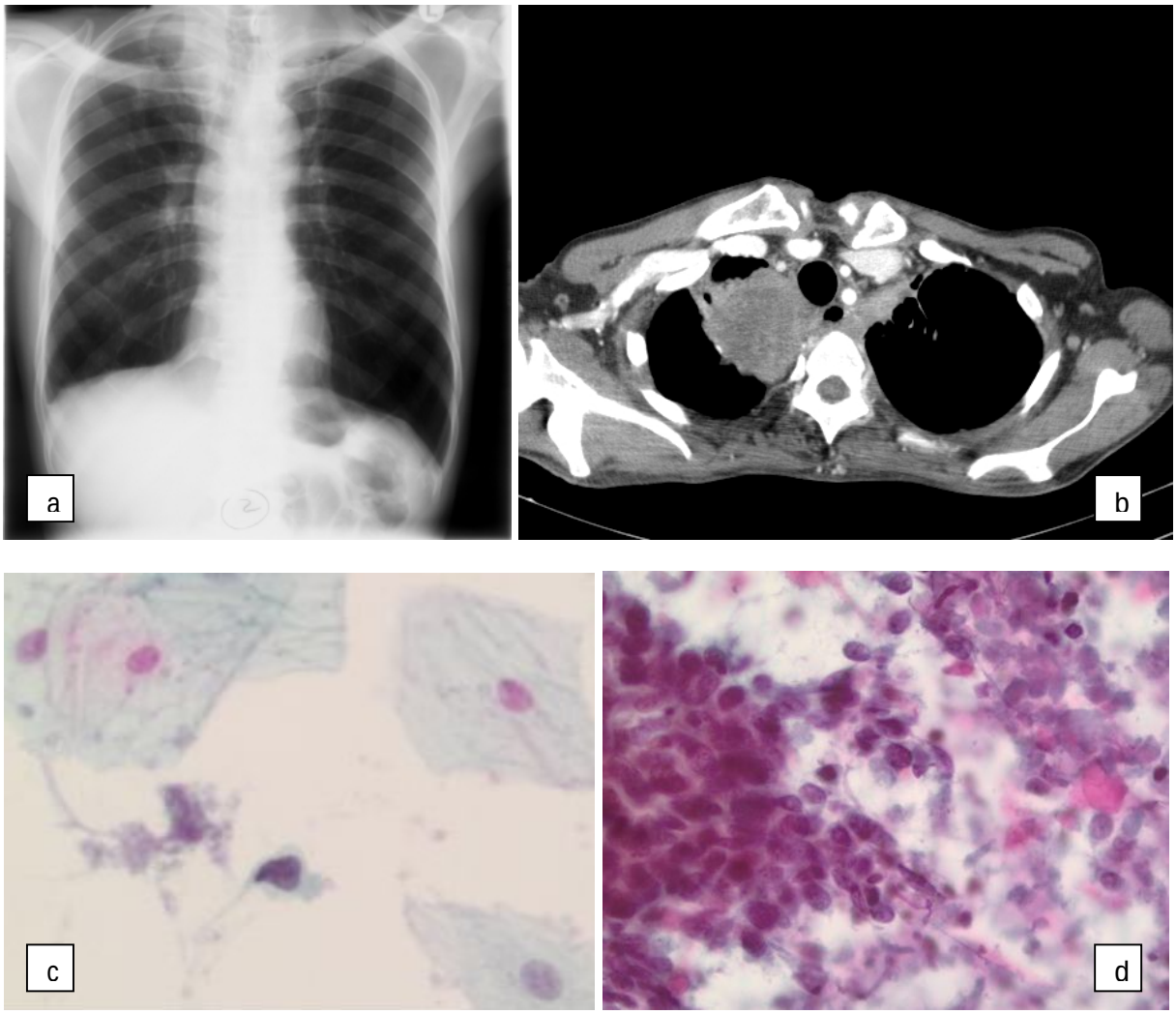

Figure 6.29: *Bilateral Pancoast tumours with destruction and erosion of vertebral bodies and ribs on chest X-ray (a) and CT (b). Sputum - suspicious for malignancy. Sparse spindled cells present with irregular nuclei (c). TTNA - Squamous cell carcinoma with keratinised necrotic debris (d).*

Case 112: 77 Male – TTNA of left upper lobe mass.

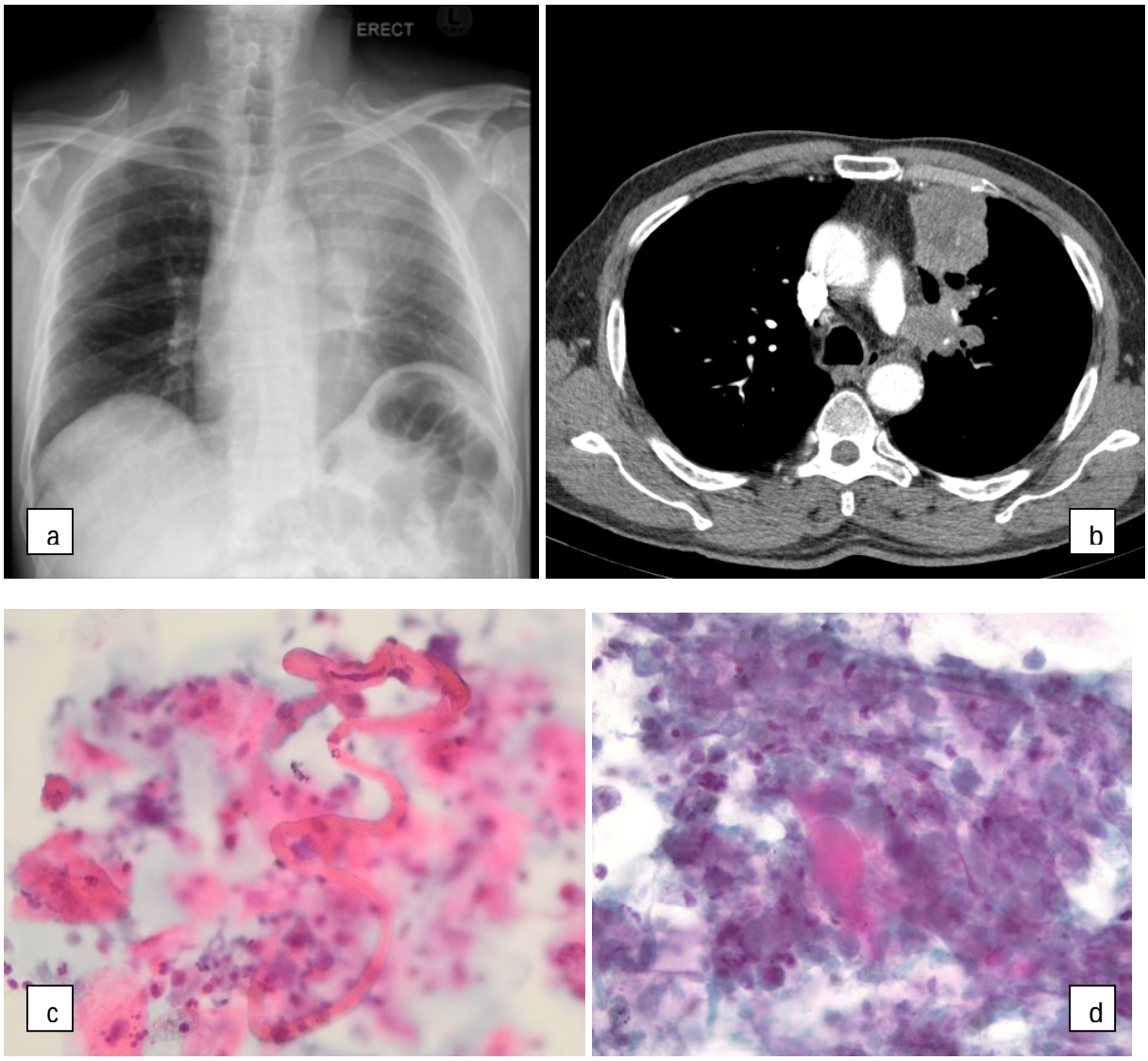

Figure 6.30: *Left upper lobe, peripheral mass abutting the chest wall on chest X-ray (a) and CT (b). Sputum - malignant. Cellular specimen with abundant bizarre, keratinised cells and elongated nuclei (c). TTNA - Squamous cell carcinoma with abundance of keratinised necrosis (d).*

Case 113: 75 Male – TTNA in patient with haemoptysis, wasting and grossly abnormal chest X-ray.

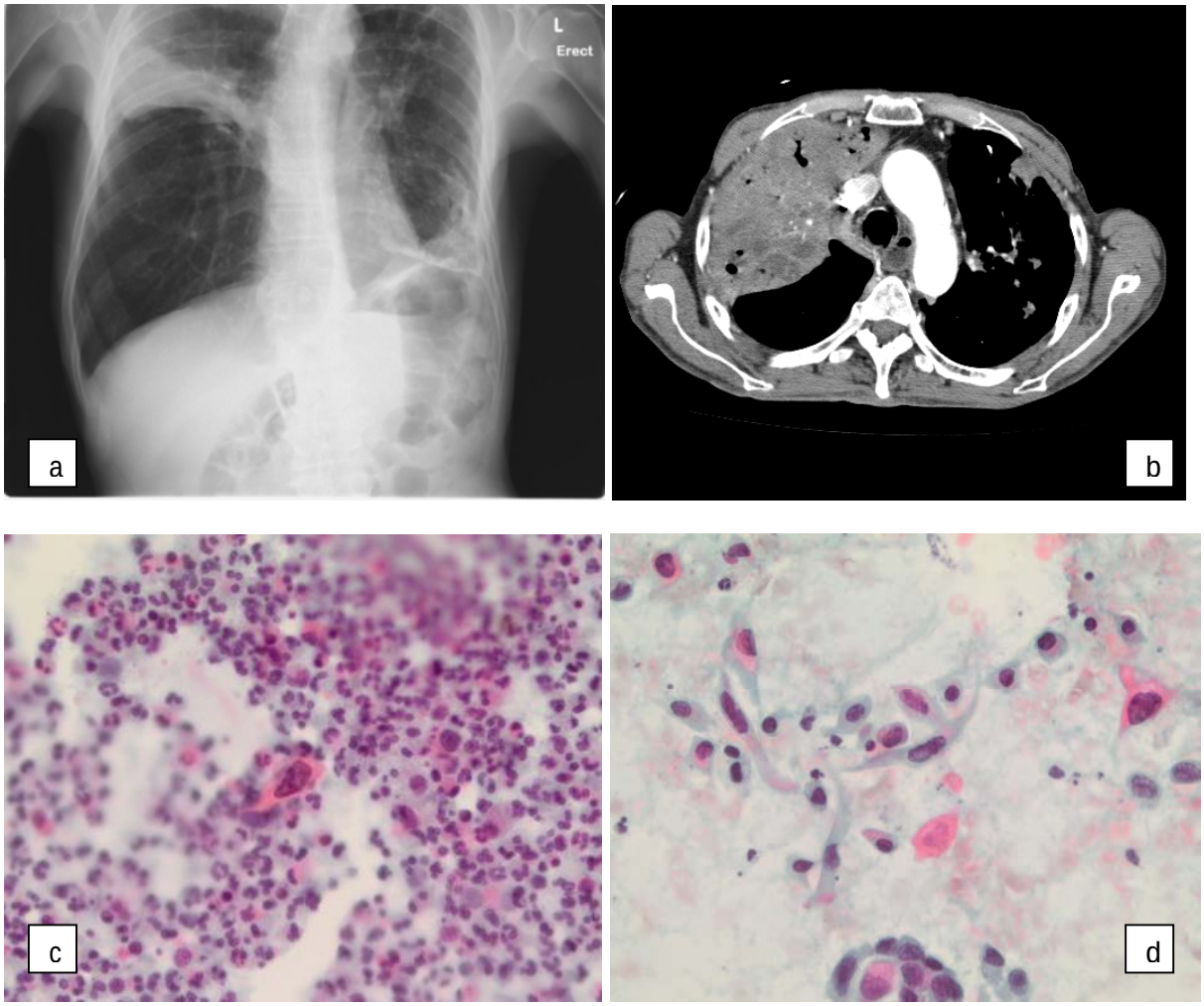

Figure 6.31: *Complete collapse of the right upper lobe with a mass-nodal-complex in the right hilum on chest X-ray (a) and CT (b). Sputum - malignant. Scattered malignant cells covered by a severe inflammatory exudate. Spindled shaped cells with marked nuclear irregularities. These cells were missed with manual screening (c). TTNA - Squamous cell carcinoma with spindled shapes and a necrotic background (d).*

Case 116: 58 Male – TBNA in patient with haemoptysis, loss of weight and chest pain.

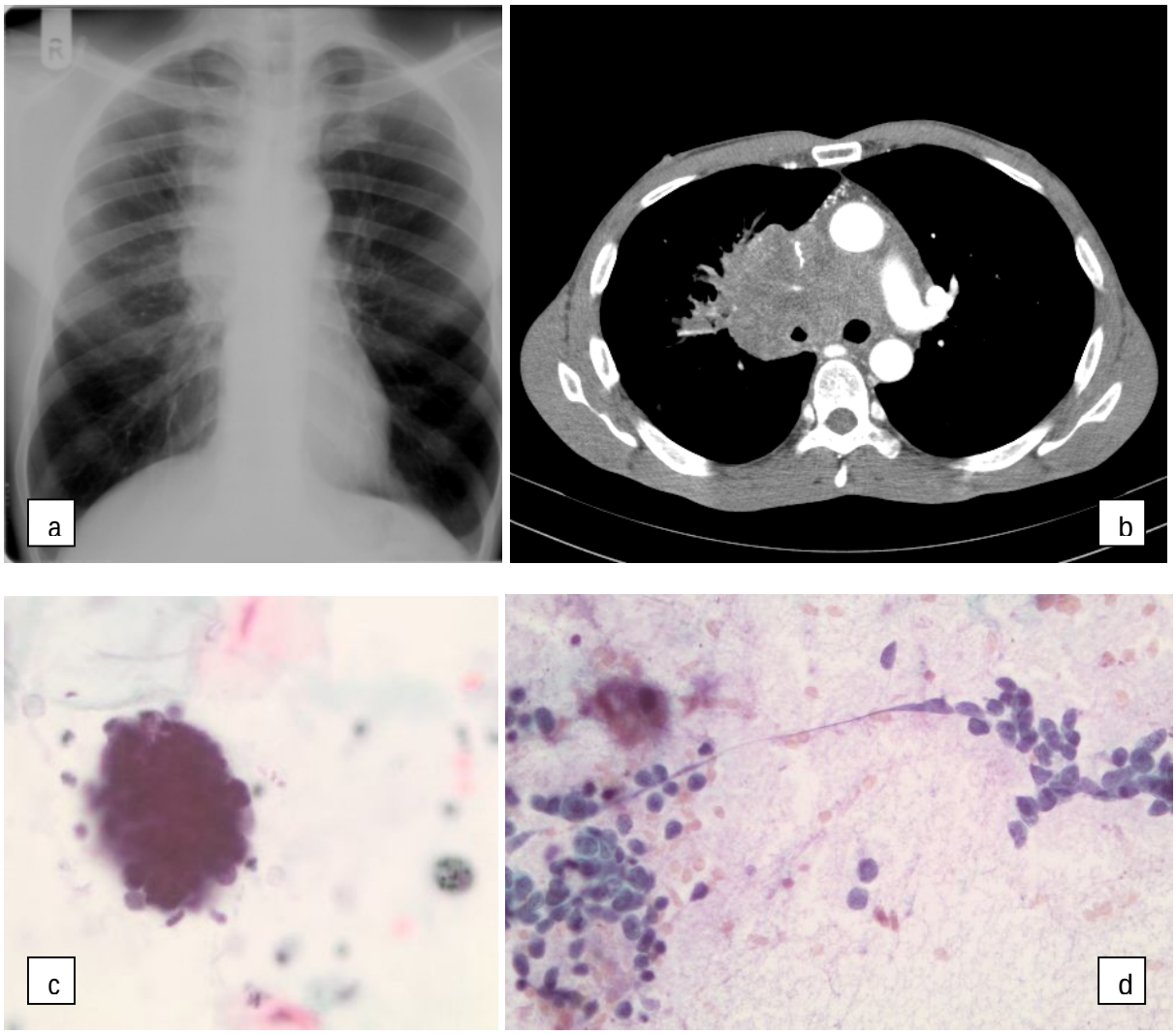

Figure 6.32: *Large mass-nodal-complex involving the mediastinum and right hilar area on chest X-ray (a) and CT (b). Sputum - malignant. Scant cellularity of small cells in moulded groups with extremely high N/C ratio's. These cells were missed with manual screening (c). TBNA - Small cell carcinoma with characteristic smear artefact and cells with high N/C ratio (d).*

Case 121: 55 Female - TTNA of right lung mass in patient presenting with a spontaneous pneumothorax.

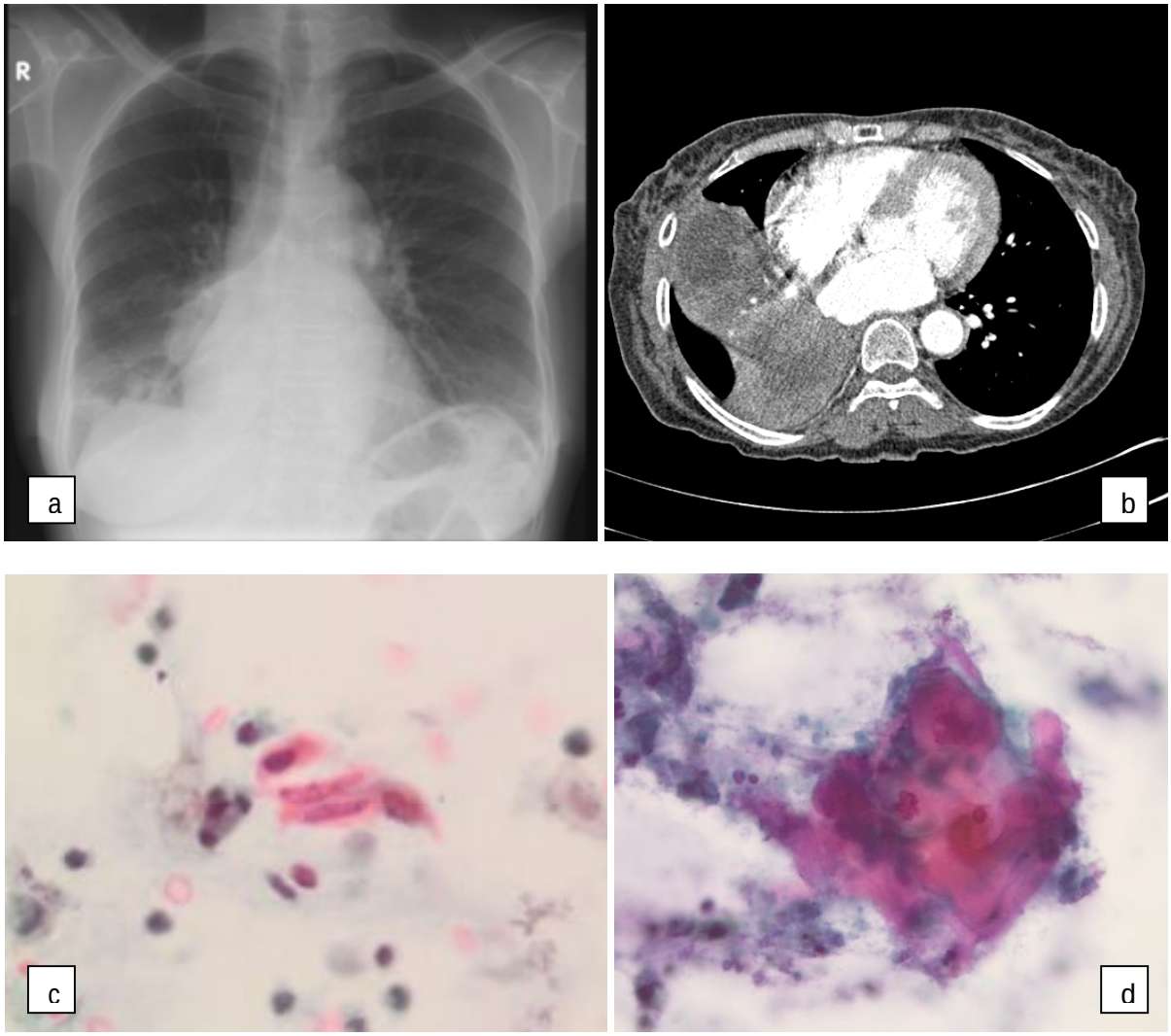

Figure 6.33: *Mass in right lower lobe, occlusion of the bronchus and a mass-nodal-complex on the right on chest X-ray (a) and CT (b). Sputum - suspicious for malignancy. Sparse spindled cells present with elongated nuclei and dense cytoplasm (c). TTNA - Non-small cell carcinoma, squamous cell carcinoma with necrosis (d).*

Case 125: 51 Male – TBNA in patient complaining of pleuritic-like chest pain.

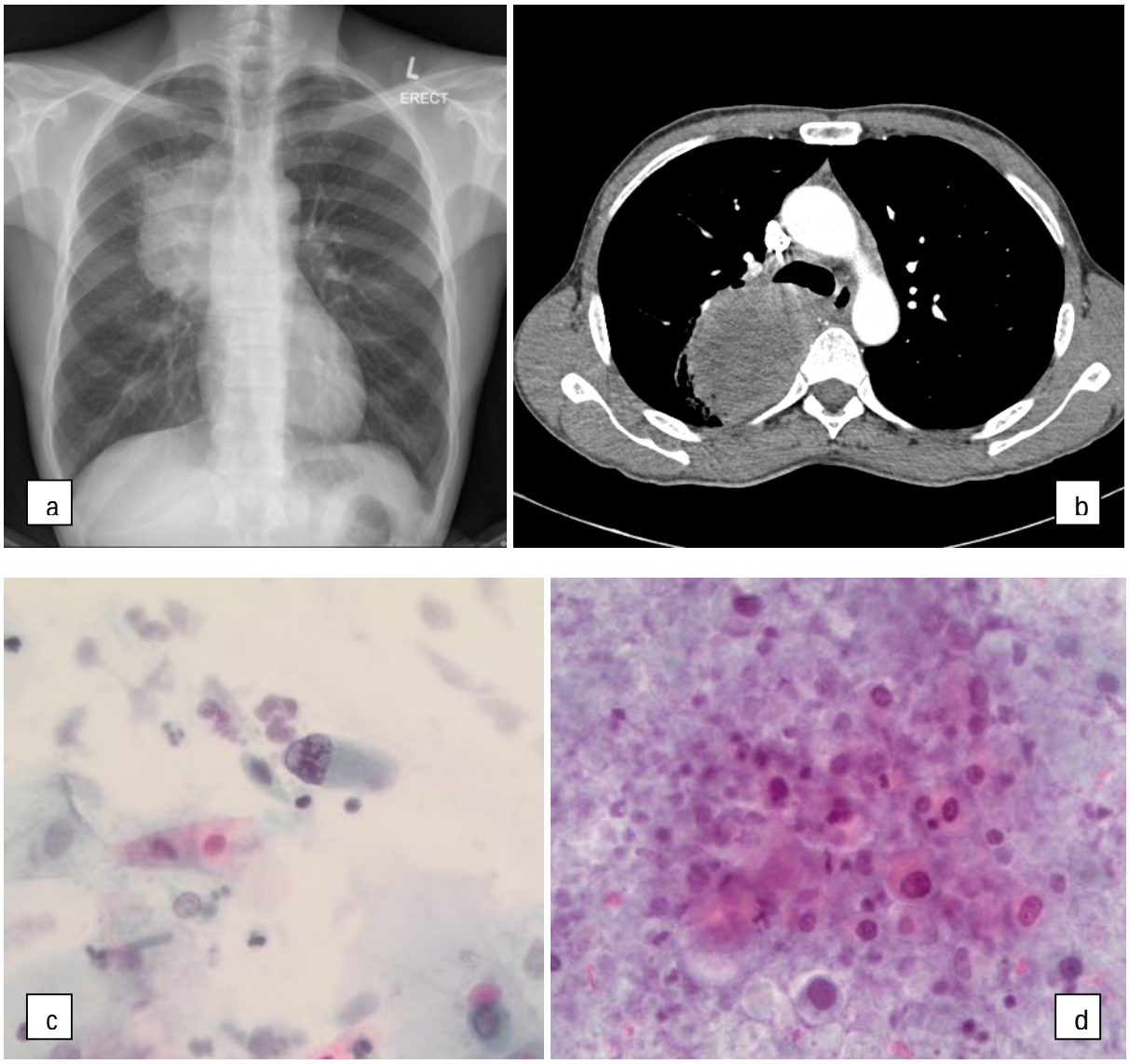

Figure 6.34: *Solid mass lesion in the apex of the right lower lobe, sharing a broad surface area with the adjacent pleura on chest X-ray (a) and CT (b). Sputum - malignant. Scattered malignant cells with extremely irregular nuclear membranes and eccentric nuclei (c). TBNA - Squamous cell carcinoma with necrosis (d).*

Case 127: 52 Male – TBNA in HIV positive patient with history of pulmonary tuberculosis and vertebral body collapse.

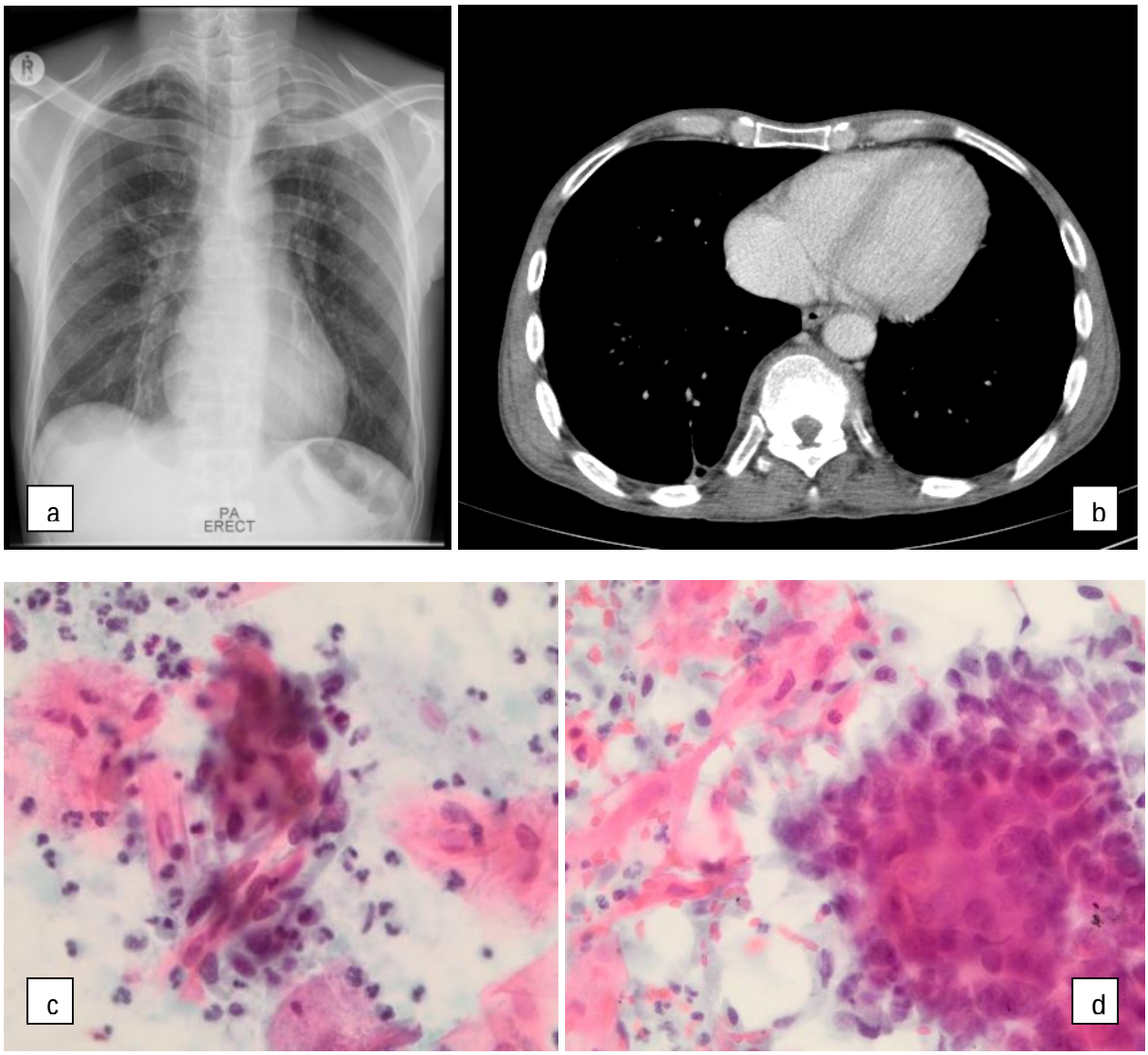

Figure 6.35: *Left, apical mass adjacent to the mediastinum with infiltration of vertebrae and lymphadenopathy on chest X-ray (a) and CT (b). Sputum - malignant. Spindled cells with elongated nuclei and keratinised cytoplasm (c). TBNA - Squamous cell carcinoma with a keratinising and non-keratinising component (d).*

Case 128: 66 Female - TBNA in patient with chronic obstructive pulmonary disease and possible primary bronchus carcinoma.

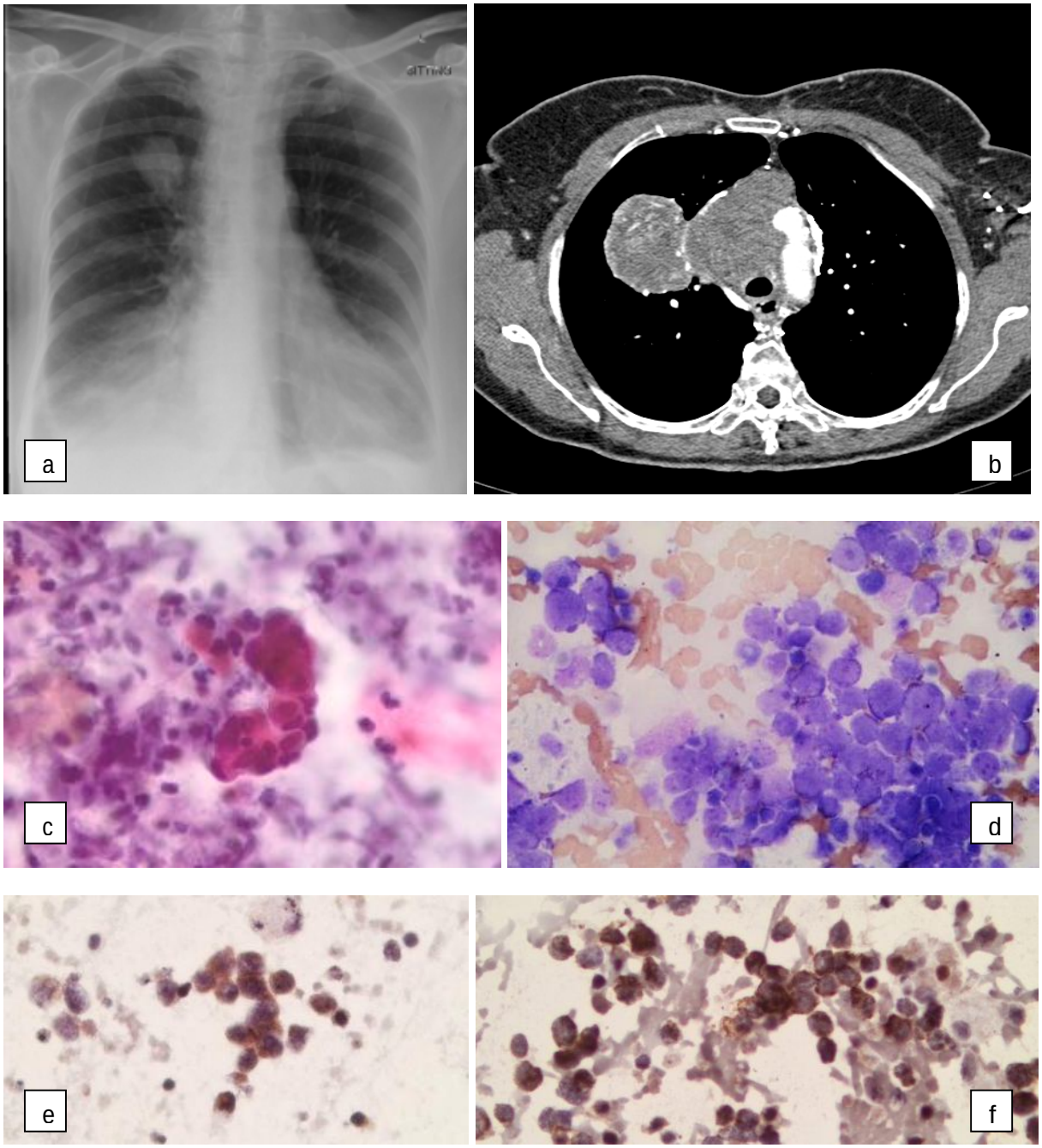

Figure 6.36: *Well-defined right upper lobe mass with hilar adenopathy on chest X-ray (a) and CT (b). Sputum - suspicious of malignancy. Small groups of small, moulded cells with high N/C ratio. These cells were missed with manual and automated screening (c). TBNA - High N/C ratio cells with moulding characteristic of small cell carcinoma (d) MGG. Immunocytochemistry profile supports a neuroendocrine carcinoma with Synaptophysin positive (e) and MNF positive (f).*

Case 129: 58 Male - TTNA of posterior lung tumour in patient with haemoptysis and pleural effusion.

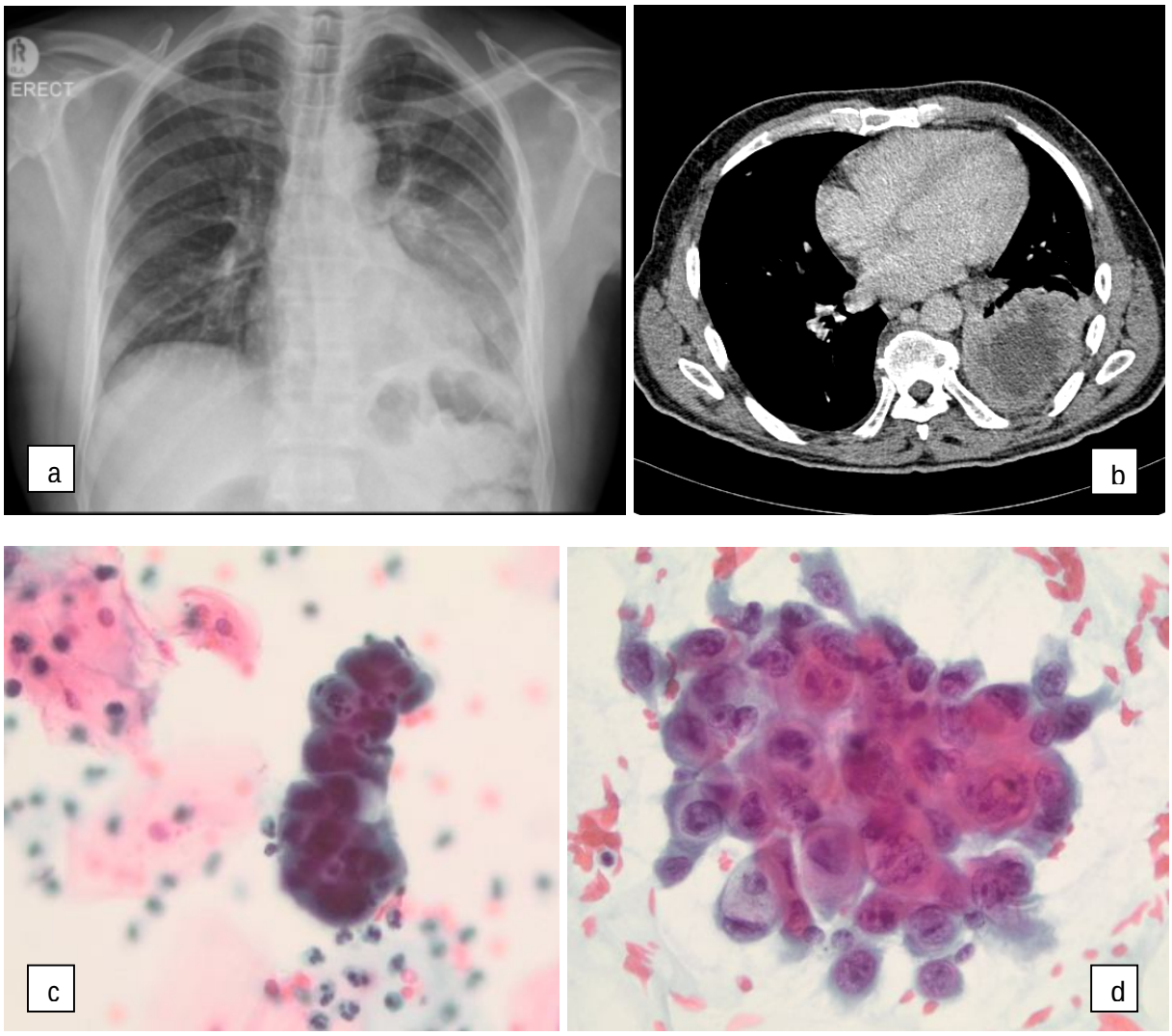

Figure 6.37: *Mass in left hilum and throughout left upper lobe with associated collapse of the left lower lobe on chest X-ray (a) and CT (b). Sputum - malignant. Balls of cells present with vacuolated cytoplasm suggestive of adenocarcinoma (c). TTNA - Non-small cell carcinoma, favour a squamous cell carcinoma or adenosquamous carcinoma (d).*

Case 133: 56 Female - 200ml brown coloured pleural fluid received for processing.

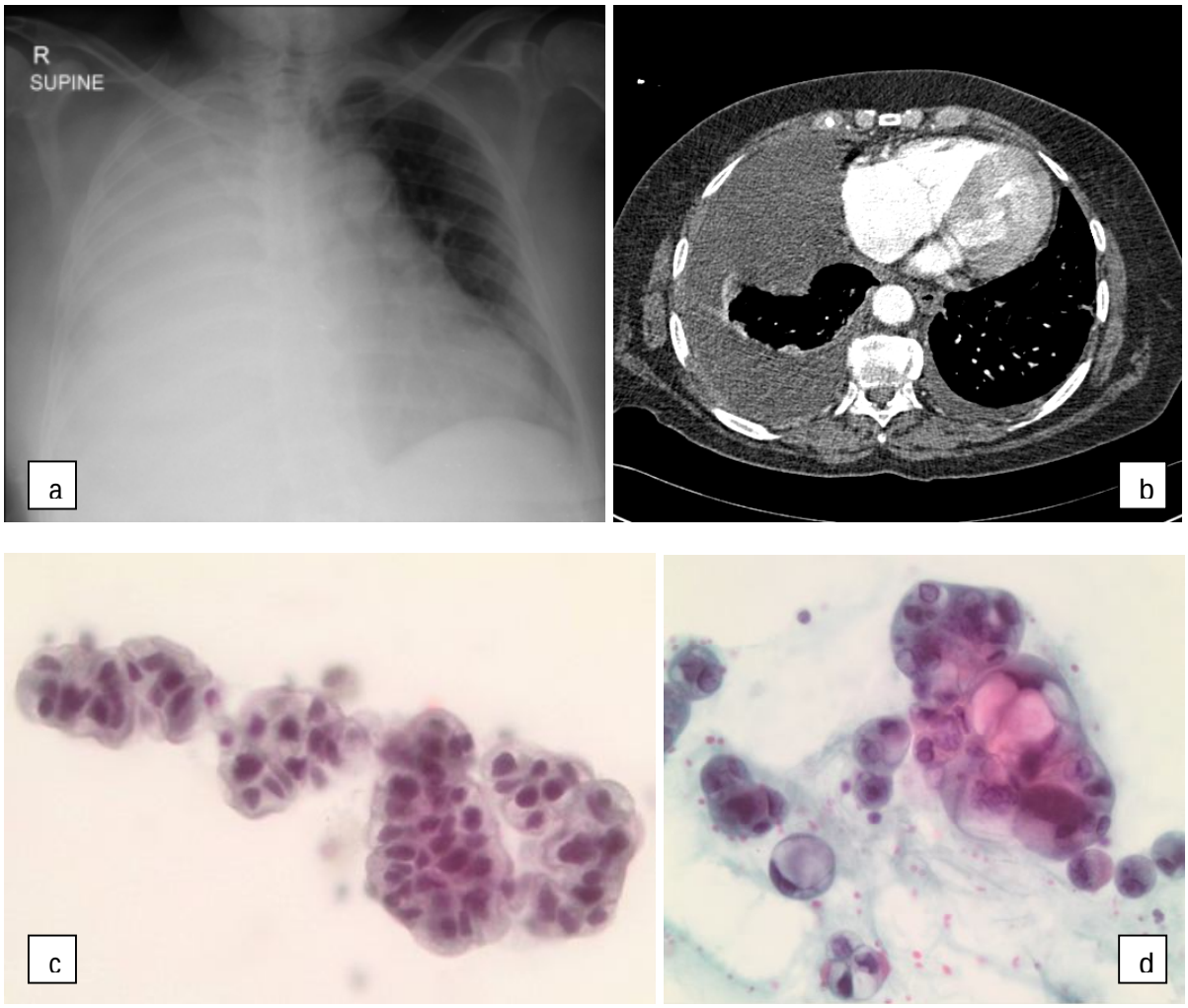

Figure 6.38: *Involved mediastinal lymph nodes with large right sided pleural effusion on chest X-ray (a) and CT (b). Sputum - malignant. Cells seen in balls with abundant, clear, vacuolated cytoplasm (c). Pleural effusion - Metastatic adenocarcinoma (d).*

Case135: 42 Female - TTNA of a mass in the right lung extending to the pleura and massive pleural effusion.

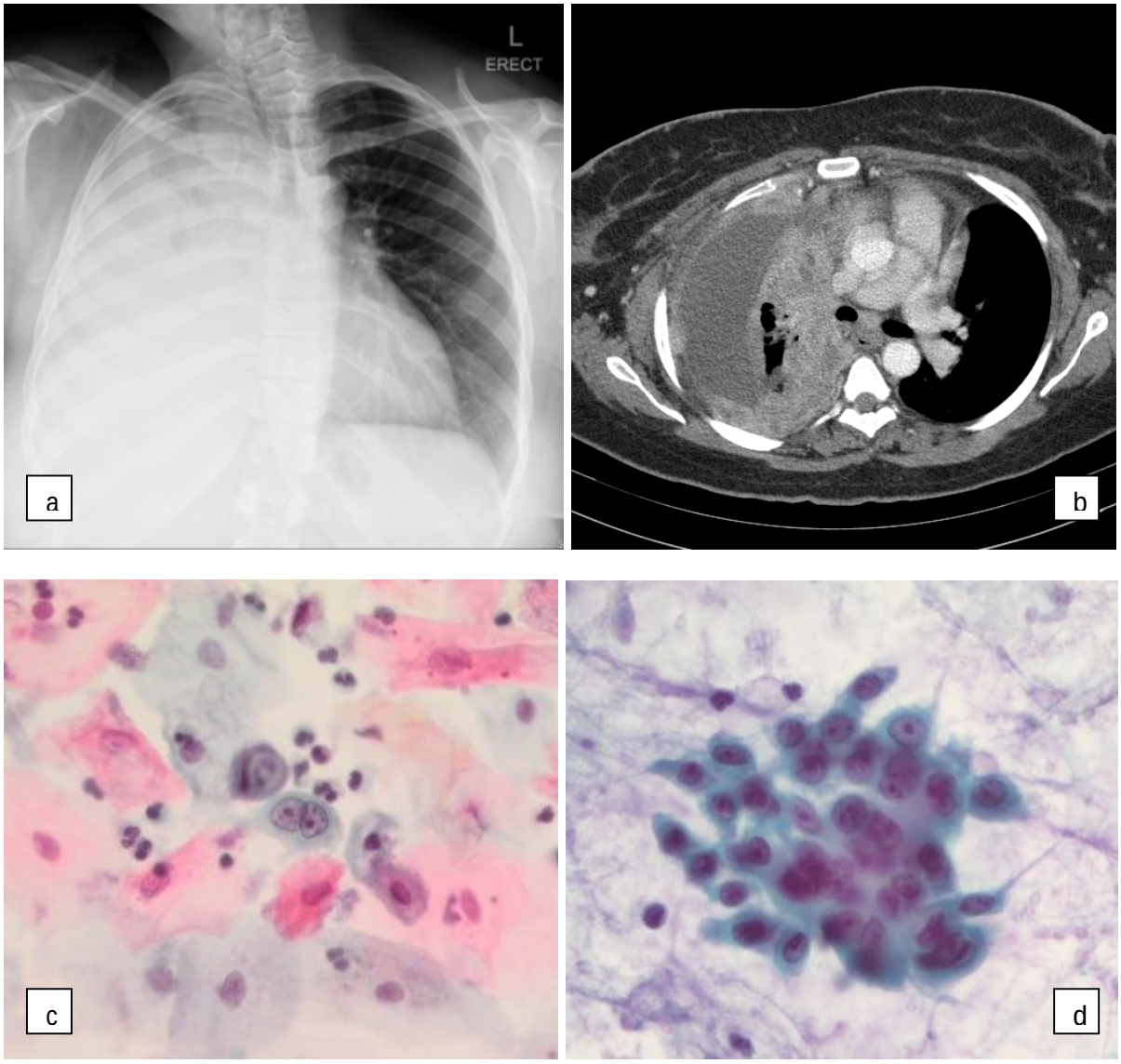

Figure 6.39: *Right lung collapse with ipsilateral pulmonary nodules on chest X-ray (a) and CT (b). Sputum - malignant. Cells had prominent nucleoli with marked clumping and clearing of chromatin. Cytoplasm appeared dense (c). TTNA - Squamous cell carcinoma, non-keratinising (d).*

Case 138: 56 Female – TTNA in patient known with bronchogenic carcinoma and in need of new staging CT.

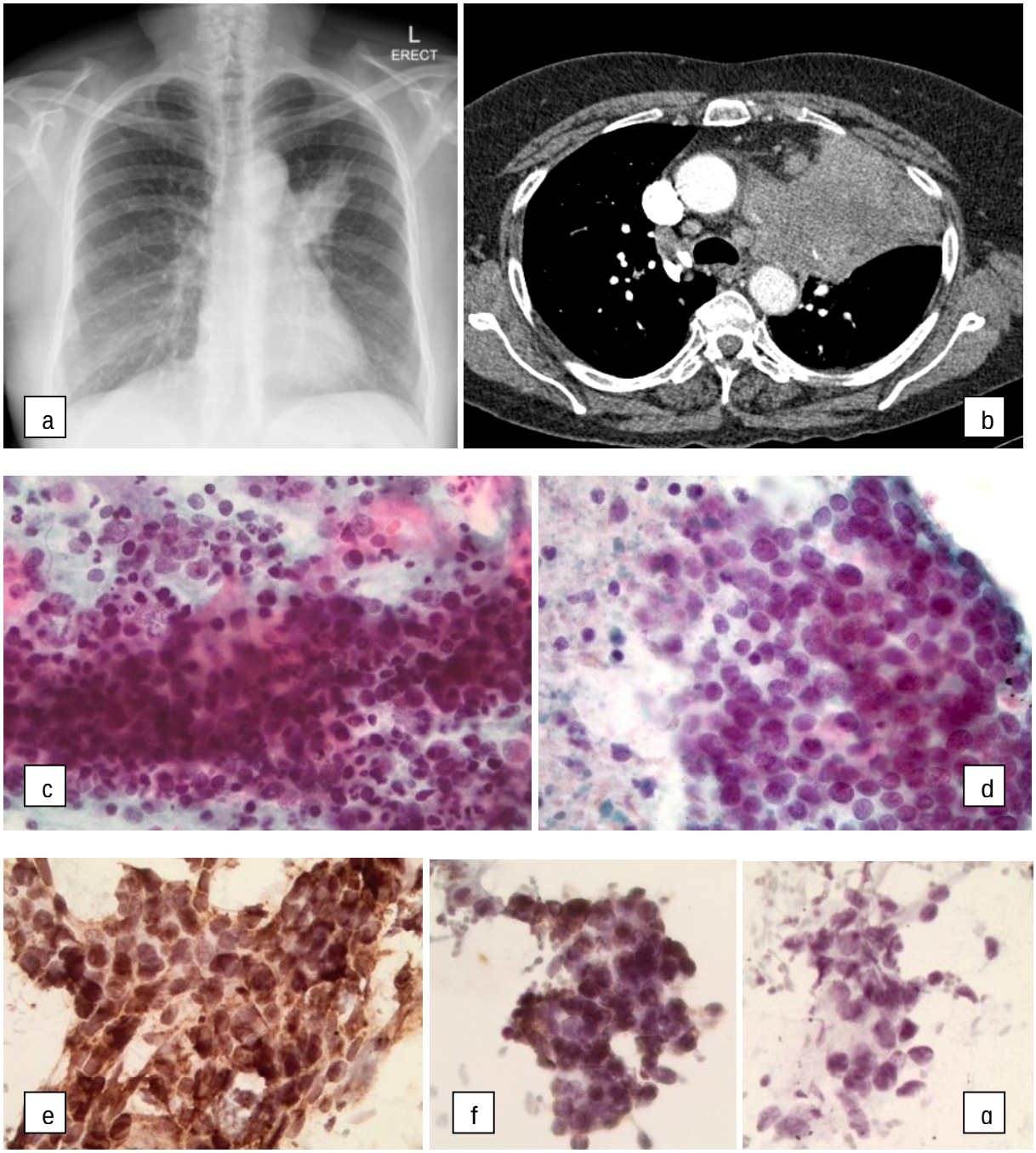

Figure 6.40: *Large left perihilar mass with multiple pleural based and pulmonary nodules and lymphadenopathy on chest X-ray (a) and CT (b). Sputum - malignant. Small, poorly differentiated cells with high N/C ratio's (c). TTNA - Adenocarcinoma with neuroendocrine differentiation (d) confirmed with immunocytochemistry: strong BerEP4 positivity (e) and Synaptophysin positivity (f) while CK5 was negative (g).*

7. DISCUSSION

7.1 Patient demographics of study population

This prospective, laboratory-based comparative study was conducted on pre-bronchoscopy sputum submitted from adult patients attending the Division of Pulmonology at TBH for suspected bronchogenic carcinoma. Sputum from a total of 108 patients was included. The average age of the 91 patients with confirmed bronchogenic carcinoma was 59.2 (range 37 - 81), compared to the 17 patients with benign disease aged 60.6 (range 40 - 78). Gender distribution in patients with bronchogenic carcinoma was 29.7% females (n=27), and 70.3% males (n=64), compared to benign disease with 47.1% females (n=8), and 52.9% males (n=9).

7.2 **Patient data** (83)

The patient data included clinical, radiological and bronchoscopic characteristics.

7.2.1 Clinical characteristics

Among the 91 patients with confirmed bronchogenic carcinoma, the most frequently observed clinical symptoms included cough (n=78; 39.7% positive sputum) and loss of weight (n=73; 34.3% positive sputum). The presence of weight loss was negatively associated with positive sputum cytology (p=0.049). Only 25 patients with weight loss had positive sputum, while in total 82.0% of patients (n=73) with weight loss had carcinoma confirmed. 24.4% of the patients reported a history of tuberculosis (n=22; 45.5% positive sputum) and almost all were smokers or ex-smokers (n=83; 34.4% positive sputum) with a mean of 33 pack-years. 7 Patients had bronchogenic carcinoma confirmed but denied to have smoked before (57.1% positive sputum).

None of the symptoms above were associated with positive sputum cytology, neither were general tumour symptoms such as shortness of breath (n=60; 41.7% positive sputum) and various paraneoplastic syndromes (n=4; 0% positive sputum). Surprisingly, none of the clinical manifestations generally associated with a central tumour location such as hoarseness (n=23; 39.1% positive sputum), haemoptysis (n=29; 48.3% positive sputum) or superior vena cava syndrome (n=12; 41.7% positive sputum) predicted the sputum cytology outcome. Neither of the symptoms generally associated with a peripheral tumour such as chest pain (n=51; 43.1% positive sputum) or Pancoast syndrome (n=5; 60.0% positive sputum) predicted a positive sputum result.

While 14 patients reported haemoptysis, only 7 of the sputum samples were macroscopically blood stained. All these macroscopically blood stained sputum had bronchogenic carcinoma confirmed, while 5 of these patients (71.4%) had positive sputum cytology. This data correlates with results from Risse et al. reporting a strong association between macroscopic bloody sputum and the presence of malignant cells in sputum.(11)

7.2.2 Radiological characteristics

From the literature review, expected radiological results associated with positive sputum included central tumours and tumours communicating with the airways. (33, 34, 38) Of the 50 patients with central malignant lesions, 44% had positive sputum (n=22) compared to the 38 patients with peripheral malignant lesions of which 31.6% had positive sputum (n=12). These results correlate with Neumann et al. that reported more frequent positive sputum in central lesions, but with relatively small differences compared to peripheral lesions. (30)

The most frequent site for malignant tumours were right upper lobe (n=41; 34.2% positive sputum) masses. Surprisingly, left upper lobe tumours had the highest proportion of positive sputum (n=22; 45.5% positive sputum). We anticipated that right upper lobe tumours would be more likely to exfoliate diagnostic cells with its proximity to the central airways. None of these specified sites, however, was significantly associated with positive sputum.

Tumour size characteristics showed no significant association with positive sputum. Many of the tumours were more than 7cm in diameter (N=34) and these showed less positive sputum results (29.4%) compared to the smaller tumours (43.2% positive sputum). This data correlates with results discussed by Pilotti et al. They stated that when tumours are too large, they may obstruct bronchi or produce an abundance of necrosis.(63)

Spiculated tumours were negatively associated with positive sputum. Only 32.4% of these patients had positive sputum (n=22), while in total 84.0% of patients with spiculated lesions had carcinoma confirmed. The presence of a round mass (n=10; 70.0% positive sputum) was positively associated with sputum cytology.

Neither associated pleural effusion (n=20; 35.0% positive sputum) nor metastasis (n=60; 40.0% positive sputum) showed increased sputum sensitivity.

7.2.3 Bronchoscopic characteristics

Bronchoscopy related findings significantly associated with positive sputum were the presence of an endobronchial tumour (n=17; 47.1% positive sputum) and airway obstruction or stenosis (n=11; 63.6% positive sputum). Only a few of the patients had exophytic (n=4; 50.0% positive sputum) or ulcerated lesions (n=2; 0% positive sputum) and showed no significant association with positive sputum as expected. This can also be attributed to the small sample with these tumour characteristics.

7.3 Comparing manual screening to automated screening

Manual screening was performed by various cytotechnologists (screener: 0-15 years experience; checker: ≥10 years experience) employed at the NHLS Cytology Unit situated in TBH. They were blinded from all patient data and automated screening results. Automated screening was performed by the FP, and the FOV's interpreted by one cytotechnologist (principal investigator with 10 years experience). She was also blinded to all the patient data and manual screening result.

7.3.1 Efficiency of manual compared to automated screening

Automated screening was significantly more time efficient ($p<0.001$) resulting in improved TAT. There was a 93.8% reduction in fields to be reviewed, resulting in an overall 83.1% reduction in the screening time spent per case by a cytotechnologist. The minimum time spent on manual screening and the maximum time spent of FP screening did not even overlap.

7.3.2 Sensitivity of manual compared to automated screening

The automated screening results (94.3%) were more sensitive than manual screening (74.3%). This is surprising and encouraging. No statistical significant difference exists between the two screening methods.

The FP has been designed for cervical cytology, and although the cells found in sputum and Pap smears are rather similar, there are still some differences. These include the presence of:

- alveolar macrophages usually with an abundance of carbon ingested mimicking atypia / malignancy
- more food particles including vegetable cells
- malignant cells usually smaller due to degeneration as a result of prior, natural exfoliation
- abnormal cells usually more sparse than on Pap smears.

Manual sputum screening is extremely difficult and requires concentration, thoroughness and is extremely time consuming. For these reasons automated screening is well suited to reduce the fields to be looked at (as described in 7.3.1), allowing screeners to spend more time on potential abnormal fields.

This study obtained a good sensitivity with automated screening. This allowed us to dismiss pessimistic predictions that the presence of alveolar macrophages with abundant carbon pigment could interfere with automated screening. Macrophages may mimic enlarged, hyperchromatic, malignant nuclei and it was contemplated that these cells could be misinterpreted by the FP.

There are no data available at this laboratory for the sensitivity of routine sputum cytology. There is no reference point and specimens are currently not correlated with the various cytology tests or pathology. NHLS at TBH drains a large area, and not all these patients are referred to TBH for a diagnostic procedure.

7.3.3 Specificity of automated screening

As discussed in the literature review, the FP acts as a triage mechanism. A provisional diagnosis is recorded by the cytotechnologist who will determine if slides will be rapid reviewed if benign, or re-screened if abnormal. Only 1 case (from 46 triaged for re-screen) had a provisional diagnosis of “atypical, NOS” which was eventually reported as “benign” due to tuberculosis. This resulted in a specificity of 97.8% for FP screening.

7.4 Macroscopic examination of sputum samples

Before preparation, sputum samples were weighed and the macroscopic appearance was noted.

7.4.1 Weight of sputum samples

Most of the positive sputum samples obtained, weighed between 1 and 3 grams (n=45; 48.9% positive sputum). The positive results did not show an increase proportional to the sputum weight increase. Most of the false negative sputum samples weighed less than 1 gram (n=26; 66.7% negative sputum).

Results seem to indicate a trend with significant differences (p=0.005) in results obtained in the first two groups (0-1g and 1-3g) between the categories. Additional testing with more cases in the >5g group would be ideal to confirm this trend.

7.4.2 Macroscopic appearance

Most of the sputum samples (n=93; 31.2% positive sputum) were noted as macroscopically white mucus. 7 Cases were macroscopically bloody and all of these (100%) had malignancy confirmed on FNA. 5 of these had positive sputum (71.4%).

Statistically, no significant association or trend could be established ($p > 0.05$), since there are too few cases in the watery, purulent and bloody categories.

7.5 Inter-observer variability

3 of the authors independently, and partially blinded, reviewed the abnormal sputum cases identified by manual and automated screening. These were categorised as stipulated in 5.6. No inter-observer agreement was established (0.02 to -0.009 Cohen Kappa). When individual results were compared to the consensus result, at least some agreement was obtained varying from 0.45 (fair) to 0.27 (slight). The low Cohen Kappa values and wide confidence intervals which include the point zero, confirm the difficulty of sputum diagnosis, poor reproducibility and consensus between cytologists.

7.6 Automation processing performance

The overall performance of the FP on sputum samples was better than expected with 90.0% of cases (n=108) qualifying for FOV review. Expectations were based on the pilot study which was retrospective and utilised fairly old cases. Only 41.7% of those cases qualified after automated screening. Reasons for these poor results were attributed to fact that the slides were old resulting in staining deterioration and air bubble formation.

7.6.1 Initial automation success

Initial automation success was achieved in 70.8% of cases (n=85). This indicates that both slides of the sputum were qualified after the first attempt of automated screening, and amenable to FOV review. This is a good result even if compared to Pap smear automation, since:

- 2 slides were processed instead of the usual 1 slide for a Pap smear. If only one of the slides succeeded, it was removed from this category
- The FP experienced complications throughout the study period due to a defective image system, increasing process review percentage
- Average automation success for Pap smears during the study period was 85.3%
- This instrument was designed for Pap smears and not yet adjusted for sputum which may improve results

7.6.2 Process review cases

10.8% of cases (n=13) did not qualify for FOV review after initial FP processing. The process review comments included dirty coverslip, poor cellularity or too thick smears. Slides were cleaned and reloaded into the FP and subsequently succeeded automated screening.

Only 8.3% of the cases (n=10) had to be either reloaded more than once or even re-prepared. Most of the process review comments were due to poor cellularity. These cases eventually succeeded automated screening.

7.6.3 Failed automation

10.0% of the cases (n=12) failed automation even after several attempts including re-preparation. Sputum volumes were small and most of the process review comments were due to poor cellularity. These 12 cases had to be excluded from the study since successful automation was the most important inclusion criteria.

3 of these cases contained abnormal cells and did not qualify for FOV review due to “scant cellularity”. Often positive cases contain numerous malignant cells, but with insufficient reference cells according to minimum automation criteria. When the FP rejects these cases they appear on the print set under “qualified slides”, but with review. These cases warrant attention and therefore the FP forces cytotechnologists to manually screen the case as part of its inbuilt quality assurance.

7.6.4 Process review status

When slides do not qualify for FOV review, reasons are provided on the print set. In this study, 54% of process reviews’ were attributed to poor cellularity. This could be ascribed to the small volume sputum samples that were obtained (average 1.97g). Other reasons included sub-optimal staining (20%), thick smears (14%) and dirty coverslips (12%).

7.7 Inadequate sputum

Besides the 12 cases that failed automation mainly due to scant cellularity (see 7.6.3), a further 16 cases (14.8%) in the study (n=108) had a final sputum diagnosis of “Inadequate” due to reasons other than failed automation. These included absent or too few alveolar macrophages (n=12) and more than 75% of cells obscured by an inflammatory exudate (n=4).

7.7.1 Inadequate amount of alveolar macrophages

At this laboratory it is practise to count at least 7 carbon-laden macrophages per slide to render the sputum adequate. This minimum criterion was adopted from the Monograph in Respiratory Cytology published in 1979.(67) Current literature does not require an exact number for adequacy, but rather states “an abundance of alveolar macrophages should be present.”(64) The presence of alveolar macrophages indicates that the sputum is representative of the lower respiratory system. When only saliva is present, it is considered inadequate.

All the sputum cases (n=12; 100%) with absent or sparse alveolar macrophages had negative sputum results, but with subsequent carcinoma confirmed on FNA (i.e. false negative results). These specimens consisted of saliva only. It is therefore recommended to ask for an early morning, deeply coughed, repeat sputum.

7.7.2 Cells obscured by inflammation (>75%)

It is important to note that the presence of inflammatory exudate does not exclude malignancy. Patients with bronchogenic carcinoma may have concurrent obstructive pneumonia and this may be evident on the slide as an inflammatory background. When most epithelial cells are obscured by inflammation, it is recommended to ask for repeat sputum after treating the infection. This will reduce the risk of missing abnormal cells obscured by inflammation.

7.8 Sputum with inflammatory exudate

Marked inflammatory exudate was noted when > 50% of cells were covered by inflammation (predominantly neutrophils). 40.0% of all the patients with confirmed carcinoma, had markedly inflammatory sputum (n=36). This could contribute to the low sputum sensitivity. Marked inflammatory exudate in the background of scant malignancy may lead to false negative results. Diagnostic epithelial cells may be obscured by inflammatory cells or cytologists may decide that the sputum merely represents an inflammatory condition and screen slides less diligently. This is a potential and significant diagnostic pitfall for cytologists.

17 of the positive sputum (n=35) had marked inflammatory backgrounds, indicating that 48.6% of these patients' possibly had concurrent obstructive pneumonia with bronchogenic carcinoma.

7.9 Final diagnostic procedure

This section discusses the various methods used to confirm the final diagnoses of the patient. Originally this study was designed to use the TBNA/TTNA results as the control or gold standard, however occasionally these procedures were abandoned. Pulmonary FNA reported on by an experienced cytopathologist is accurate for subtyping carcinoma and is now an acceptable, and at some institutions the preferred diagnostic modality.(22-25) Treatment may be planned on these results and molecular analysis can be done, thus not requiring more invasive procedures.(26)

FNA of a palpable lymph node or mass, pleural tap or biopsy was intermittently performed in this cohort. These patients were also included in the study. As expected, TBNA (n=45, 41.7% of total cohort) and TTNA (n=46, 42.6% of total cohort) were the two most common diagnostic procedures used to confirm the final diagnosis.

A significant association between the diagnostic procedure and sputum cytology was obtained. While comparing TBNA and TTNA, there is a significant difference between the positive and negative sputum results. When patients had a TTNA as diagnostic procedure, they were more likely to have positive sputum cytology.

7.9.1. Transbronchial fine needle aspiration

While TBNA's are usually the procedure of choice in central tumours, only 28.9% (n=11) of sputum was positive in patients with bronchogenic carcinoma. Previous researchers have shown that central lesions exfoliate diagnostic cells more readily in sputum, but these results had no significant association (44.0%, p>0.05).(34, 38)

7.9.2. Transthoracic fine needle aspiration

TTNA results were higher than logically expected since this procedure is only performed when a peripheral lesion is present. 43.2% of these patients (n=19) had positive sputum. According to literature, peripheral lesions exfoliate cells less frequently in sputum.(20, 33, 34, 65)

When patients are amenable to a TTNA, this does not exclude a concurrent central tumour as well. 21 patients with bronchogenic carcinoma in this cohort (47.7%) with TTNA as the diagnostic procedure also had central lesions recorded. A tumour abutting the chest wall is also a marker of advanced disease. TTNA is only performed for diagnostic purposes, and not for staging purposes as with TBNA.

7.9.3 Other miscellaneous diagnostic procedures

2 patients had a malignant diagnosis confirmed with bronchial brushings (28.6%). 1 of these patients had positive sputum.

Bronchial and pleural biopsies were used in 5 patients to confirm the final diagnosis. Only 2 patients (40.0%) had carcinoma with no positive sputum.

FNA (other than lung) was used in 4 cases to confirm carcinoma:

- 1 Patient had various metastatic lesions including chest wall, back and breast masses with a positive sputum
- 1 cervical lymph node with a positive sputum
- 1 supraclavicular lymph node with a positive sputum
- 1 metastatic liver lesion with a false negative sputum

Pleural fluid was obtained in 1 patient. This patient also had positive sputum.

7.10 Final diagnosis of study population

91 patients had a final diagnosis of carcinoma (84.3%), while 17 patients in this cohort had a benign diagnosis (15.7%) documented. These benign cases were incorporated as negative controls to test the sputum specificity.

7.10.1 Malignant cases

The three most common carcinomas in this cohort were diagnosed as adenocarcinoma (n=33; 36.3% of all malignant cases), squamous cell carcinoma (n=30; 33.0%) and small cell carcinoma (n=15; 16.5%) using diagnostic procedures described in 7.9. The incidence of adenocarcinoma in this study is not as high as described by Nanguzgambo. (55.4%) from the same institution, nevertheless it is still the most common primary bronchogenic carcinoma in this cohort.(49)

The other carcinomas diagnosed included large cell undifferentiated carcinoma (n=3; 3.3%), adenosquamous cell carcinoma (n=2; 2.2%), non-small cell carcinoma NOS (n=4; 4.4%), poorly differentiated carcinoma (n=3; 3.3%) and one plasmacytoma (n=1; 1.1%).

In 13 cytology specimens and 2 biopsies, immunocytochemistry was requested to confirm the diagnosis. This was most frequently used to confirm neuroendocrine origin (n=4), epithelial origin (n=4) and a biphasic nature (n=2). In effect only 16.5% of the malignant cases needed immunocytochemistry. The diagnosis was based on morphology, enabling costs to be kept to a minimum.

7.10.2 Resection rate of cohort

At the same institution, Nanguzgambo reported that only 4.4% of lung tumours were resectable in a large consecutive series of cases representative of those included in the present study. The reasons for this low rate included the fact that patients presented with advanced disease at the time of diagnosis.(49) With the high burden of tuberculosis in the Western Cape (18), patients are often initially treated for tuberculosis when they present with a pulmonary lesion or symptoms of pulmonary disease. Patients only return after medication does not clear up symptoms, allowing for further tumour progression.

In this cohort, 7 patients (7.7% of patients with bronchogenic carcinoma) were possible candidates for surgery, i.e. stage IIIA or less. Of these 7, only 1 patient had positive sputum. Contributing factors used to confirm if patients are candidates for surgery includes: general performance status of the patient, patients' choice to undergo surgery and other co-morbidities. These factors were not further investigated in this study.

7.10.3 Benign cases

Patients that are referred to the Division of Pulmonology for a diagnostic procedure, are suspected of having bronchogenic carcinoma. This however does not exclude the possibility of a benign condition or disease as the final diagnosis.

Mycobacterium tuberculosis (18) was diagnosed and confirmed with culture in 4 patients (23.5% of all benign cases). 3 patients were diagnosed with granulomatous inflammation, but with negative TB cultures (17.6%). 1 patient had acute inflammation (5.9%) with *Streptococcus pneumoniae* confirmed on culture.

In 8 patients no malignancy was found, and a diagnosis of "benign, NOS" was rendered (47.1%). Only benign bronchial cells were present on cytology with no pathogens identified or marked inflammation. TB cultures were also negative after 42 days. In 1 TBNA, atypical bronchial cells were seen but with extremely scant cellularity. This patient had no follow-up procedure performed.

7.11 Sensitivity and specificity of sputum

7.11.1 Sputum sensitivity

Since this study recruited high risk patients, it was anticipated that a fairly high sensitivity could be obtained from the sputum. True positive sputum was established in 35 (8=suspicious, 27=malignant) of the 91 patients with bronchogenic carcinoma. This equates to a sensitivity of 38.5%. This result is within those described in the literature where the range is very wide.(35)

The low sensitivity of sputum cytology in screening poorly selected populations is the reason for its virtual extinction in developed countries. In South Africa and other developing countries, sputum cytology can continue to play a role in the work-up for bronchogenic carcinoma. In decentralised services typically found in such settings, patients receive initial medical assessment at their local clinic or hospital and sputum cytology can be requested as a first investigation when bronchogenic carcinoma is suspected. Patients then have to travel long distances to receive specialised diagnostic procedures and care in poorly resourced tertiary institutions. (83)

7.11.2 Investigation of the false negative rate

This study found a false negative rate of 51.9%. When these results are further categorised, 15 cases (26.8% of the false negative sputum) were deemed inadequate. 12 cases had absent or too few alveolar macrophages present and consisted predominantly of saliva. These cases were clearly not well representative of the lower respiratory tract, and would have been recommended to repeat. 3 Cases had extremely inflammatory exudates present masking more than 75% of the epithelial cells and was subsequently not deemed adequate. These cases were also recommended for repeat sputum.

When a diagnosis of “atypical, NOS” is rendered, atypical cells are present but the potential/significance is uncertain. 12 cases (21.4% of the false negative sputum) contained only a few atypical cells and the decision between inflammatory/reactive changes and suspicious for malignancy was not possible. These cases were also recommended for repeat sputum.

In conclusion, these 27 (48.2% of the false negative sputum) cases described would have been repeated in the clinical setting and the repeat sputum might have revealed carcinoma.

The cases of concern are the 29 cases (51.8% of the false negative sputum) that were diagnosed as benign and adequate. These cases were deemed truly false negative sputum accounting for 26.9% of the total cohort. True false negative result was reported when manual screening, FP screening and re-screen had a consensus benign diagnosis. From a practical point of view, no clinician will declare a patient initially suspected of bronchogenic carcinoma free of such suspicion based on a negative sputum cytology result alone and should proceed to more invasive investigations.

7.11.3 False negative FP results

2 cases were missed with automated screening (no atypical cells in FOV’s). These included one adenocarcinoma and one small cell carcinoma. Retrospectively, smears showed very sparse atypical cells and in both cases relatively small cells with high N/C ratio’s. One of the cases (adenocarcinoma) only had a single group of cells present towards the edge of the coverslip.

7.11.4 Sputum specificity

17 benign cases were included as negative controls to test the specificity. 100% sputum specificity was achieved since no false positive diagnosis was made. This is an important factor and indicates that a patient with positive sputum needs specialised care and urgent follow-up, as patients are very likely to have bronchogenic carcinoma.

7.11.5 Sputum sensitivity in histological subtypes

Squamous cell carcinoma showed a significant association with positive sputum ($n=18$, $p<0.001$) resulting in 60.0% sensitivity. Neumann et al. also reported that squamous cell carcinoma was more often identified in sputum compared to the other histological subtypes. They obtained an overall sensitivity of 62.3% in a category named “pre-malignant or worse” cases, while only 34.8% sensitivity in “positive for cancer cells” specimens.

Adenocarcinoma was only present in 10 cases (30.3%). The results are comparable to data from Neumann et al. who reported 36.1% sensitivity in spontaneously obtained sputum.(30)

Small cell carcinoma had the lowest sensitivity of all the histological subtypes with only 2 cases (13.3%) identified on sputum. This data does not correlate with studies in the literature. (8, 11, 30, 53) Assessment of this low sensitivity recognised that:

- Malignant cells are usually degenerate in sputum with obscured nuclear detail and fragmentation
- Cells are typically small (1-2x size of lymphocyte) due to poor preservation and may be missed
- Diagnostic cells are usually sparse and may occur singly or in small aggregates
- Cells may resemble lymphocytes and often lack moulding
- Cells are often dispersed in Saccomanno preparations

7.11.6 Prediction of histological subtype in malignant cases on sputum

Recent advances in conventional and targeted chemotherapy have necessitated that cytopathologists refine their diagnosis. The distinction between squamous cell carcinoma and adenocarcinoma is important for predictive, prognostic and treatment reasons.(87) The positive sputum cases in this cohort reported correctly on the specific histological subtype in 15 squamous cell carcinomas (83.3%), 4 adenocarcinomas (40.0%), and 2 small cell carcinomas (100%).

These results are not adequate to commence treatment and indicate that alternative diagnostic procedures are essential. In this institution, as in most of Africa and the developing world, specialised chemotherapeutic agents are not available in the public health sector and a diagnosis of non-small cell carcinoma is still sufficient. This is unlikely to change in the near future due to the high cost involved with modern palliative treatment.

7.12 Predictors of positive sputum

6 factors were identified that were significantly associated with positive sputum:

- Endobronchial tumour
- Partial airway obstruction / stenosis
- Round mass
- Spiculated mass
- Loss of weight
- Squamous cell carcinoma

Data from all 6 factors were available in 39 cases of which 9 (23.1%) had positive sputum cytology. Receiver operating characteristic (ROC) analysis revealed the best diagnostic cut-off was the presence of ≥4 of the parameters that would predict positive sputum with a sensitivity of 56% and specificity of 83%.(83)

7.13 Quantity of alveolar macrophages

Results from this study indicate that the number of alveolar macrophages is not as vital in sputum sensitivity as hypothesised. True positive and false negative sputum had most samples in the category of “abundance of macrophages”. These results do not correlate with Greenberg who noted that sputum adequacy is directly proportional to the number of alveolar macrophages. (66)

A significant association could not be established between the quantity of alveolar macrophages and an improved sputum cytology result. This study was unable to confirm what is reported in the literature or reasoning due to too few samples collected in this cohort for statistical calculations and significance.

Disease representation did however improve with sputum adequacy in this cohort. Results indicate that 85.3% of true positive (n=29) and 65.5% of false negative (n=36) sputum samples were in the category of “abundance of macrophages”.

This high false negative rate in well represented sputum cannot be explained, and will have to be integrated with co-factors. It must be accepted that not all bronchogenic carcinoma patients have cells detectable in sputum. Risse et al. also showed that the mere presence of alveolar macrophages does not accurately predict pathology of the lung. (11) They found that sputum cases with low numbers of alveolar macrophages often have false negative results.

This study had 16 cases (29.1%) with low numbers of alveolar macrophages, and 36 cases (65.5%) with numerous alveolar macrophages in the false negative group. All of the cases without alveolar macrophages (n=9) had false negative results.

7.14. Limitations and disadvantages of sputum automation

Literature advocates that 3 consecutive, early morning sputum samples should be collected to improve the sensitivity and identification of bronchogenic carcinoma.(8) Unfortunately, it was not practical in this study. One spontaneous sputum sample was collected just before the planned, diagnostic procedure. It was already difficult to coordinate with patients often arriving late for their procedure. This was not optimal, and probably played an enormous role in the low sputum sensitivity. The sample size of this cohort was also too small, hampering the statistical calculations and interpretations.

Cytologists knew when they were screening cases from this study, which might have created bias. Although they might have anticipated the presence of malignant cells (since these sputum samples were from high risk patients), not all the patients attending the Division of Pulmonology for a diagnostic procedure will have bronchogenic carcinoma. As shown in the results, 15.7% of patients did have benign conditions. Although this was a high risk population, false positive diagnosis would have negatively affected the specificity. With 100% specificity obtained in this study, it indicated that cytologists did not over diagnose these sputum cases. You should always be on high level of alertness, no matter the population you are screening or clinical information you are provided with.

Furthermore, the principal investigator was involved with manual checking and re-screening of cases, FP screening and the final diagnosis or consensus process. Although she had indirect access to results of the various arms, the FNA results was only obtained and incorporated once all the sputum cases were concluded.

As shown with the high inter observer variability, the review of sputum samples at the GS Review Station could result in misinterpretations of the FOV's and may lead to false negative results. The FP instruments and service plan options are expensive. This is however already in place at the NHLS labs in South Africa for cervical screening. Training is also necessitated to be able to review cases at the FP (same as for Pap smears). Training is conducted in-house for 2 hours. Staff is able to start reviewing slides straight away which are re-evaluated by the trainer until she deems them competent. This generally takes 2 days. Only qualified cytotechnologists with an internal quality control (IQC) for diagnostic missed lesions less than 2% and 2 years post qualification should be allowed to review FP screened cases.

8. CONCLUSION

Sputum cytology remains an important part of the screening programme for bronchogenic carcinoma in the public health sector of South Africa. South Africa has countless rural areas, patients have transport difficulties and limited access to hospitals with costly bronchoscopy units. In health care settings with limited resources, attempts to improve the quality and adequacy of sputum (larger volume with more alveolar macrophages), and to improve cytology screening and interpretation should be undertaken. For these reasons automation of sputum screening was investigated to ascertain if cytology sputum sensitivity could be improved.

Sputum sensitivity of 38.5% in patients with bronchogenic carcinoma was obtained in this cohort, with a specificity of 100%. This was the first prospective evaluation of a novel technology for the analysis of sputum samples. Benefit from this study was apparent in the laboratory where automated screening was shown to be more time efficient than manual screening, resulting in 83.1% reduction in the screening time spent per case by a cytotechnologist. This indicated a significantly improved TAT, while decreasing cytotechnologist fatigue. Improved sensitivity was obtained when compared to manual screening, but this difference was not statistically significant due to the small sample size, providing no significant increased benefit to the patient. Automated screening identified 94.3% of the positive sputum cases, while manual screening identified 74.3%.

Although the number of alveolar macrophages is not as vital in sputum sensitivity as hypothesised, it continues to play an important role. All of the cases without alveolar macrophages had false negative results. Results indicate that 85.3% of true positive sputum and 65.5% of false negative sputum samples fell in the category “abundance of macrophages”. Disease representation did however improve with sputum adequacy, but showed no statistical significant association.

It can therefore be concluded that a better exporated sputum with a larger volume/weight and more abundant alveolar macrophages, in combination with automated screening for the detection of malignant cells can improve the sputum result and decrease the false negative rate of sputum cytology.

Sputum with a marked inflammatory response remains a potential and significant diagnostic dilemma for cytologists. 48.6% of patients with positive sputum had a marked inflammatory exudate, indicating possible concurrent obstructive pneumonia. This may result in false negative results since diagnostic cells may be obscured by inflammatory cells or cytologists may misinterpret cellular changes or screen slides less diligently anticipating a benign result.

Adenocarcinoma was the most common bronchogenic carcinoma diagnosed in this cohort (36.3% of malignant cases). Sputum cytology showed significant sensitivity for squamous cell carcinoma (60.0% true positive sputum; $p<0.001$), while small cell carcinoma (13.3% true positive sputum) had poor sensitivity.

The only clinical, radiological and bronchoscopic findings significantly associated with positive sputum included endobronchial tumour ($p=0.045$), partial airway obstruction / stenosis ($p=0.007$) and round mass ($p=0.039$). Factors with negative association included spiculated lesions ($p=0.026$) and weight loss ($p=0.049$).

Recommendations from this study include adopting automated sputum screening. After successful automation and FOV review, slides can be re-screened by a senior cytotechnologist. This will save a cytotechnologist from initially screening the sputum samples. The FP can also be employed as a quality control tool for sputum screening. The manufacturer of the FP may however not endorse this idea since developed countries do not received large quantities of sputum samples. In South Africa, however, this will be a useful extension to the usage of existing FP systems.

Automated screening is also currently under investigation at this institution on liquid based cytology of oral and laryngeal brush specimens as an outcome of this study.

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Appendix A:

Ethical Approval

23 June 2010

MAILED

Ms G Neethling
Cytology lab, NHLS
10th Floor, Western Side
Tygerberg Hospital

Dear Ms Neethling

Automated Sputum Screening using the BD FocalPoint Slide profiler: Correlation with Transbrochial and Transthoracic Needle Aspirates.

ETHICS REFERENCE NO: N10/04/135

RE : APPROVAL

A panel of the Health Research Ethics Committee reviewed this project on 11 May 2010; the above project was approved on condition that further information is submitted.

This information was supplied and the project was finally approved on 23 June 2010 for a period of one year from this date. This project is therefore now registered and you can proceed with the work.

Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report (obtainable on the website of our Division: www.sun.ac.za/rds should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.
Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239
The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

Approval Date: 23 June 2010

Expiry Date: 23 June 2011

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352

Yours faithfully

MS CARLI SAGER
RESEARCH DEVELOPMENT AND SUPPORT
Tel: +27 21 938 9140 / E-mail: carlis@sun.ac.za
Fax: +27 21 931 3352

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Ethics Letter

25-Mar-2013

Ethics Reference #: N10/04/135
Title: Automated Sputum Screening using the BD FocalPoint Slide profiler: Correlation with Transbrochial and Transthoracic Needle Aspirates.

Dear Mrs Greta NEETHLING,

At a meeting of the Health Research Ethics Committee that was held on 20 March 2013, the progress report for the abovementioned project has been approved and the study has been granted an extension for a period of one year from this date.

Please remember to submit progress reports in good time for annual renewal in the standard HREC format.

Approval Date: 20 March 2013 Expiry Date: 20 March 2014

If you have any queries or need further help, please contact the REC Office 0219389207.

Sincerely,

REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

Appendix B:

Cytology Request Form

Pre-bronchoscopy Sputum

** Attention lab: Do not prepare.
Please inform C. Cook or G. Neethling

Cytology Laboratory
C 10C
Tygerberg Hospital
Tel: (021) 938 4202
(021) 938 4200

FOR LAB LABEL

SPECIMEN RECEPTION

Sputum Sample Number:	Ethics Reference no: N10/04/135
Clinician's name and signature:	Date of specimen collection:

SPECIMEN PREPARATION

Type of specimen: TA1 – sputum	Location request: X5085 Account no: ZSTU0420	Preparation date:
Origin of specimen: T32 – Lung	Specimen weight:	Prepared by:

LABORATORY FINDINGS – Diagnostic categories

Categories	Screener	Checker	FocalPoint	Adequacy (per square cm)	FNA result
I Benign II Atypical, III Suspicious IV Malignant V Inadequate	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode.....	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode.....	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode: GK2	☐ 0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ > 21	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode:.....
Type of atypia/ malignancy	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other	N/A	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other .....

Correlation notes:

– ***Normal***: No abnormal cells present and adequate. Inflammation or reactive changes may be present. Include benign squamous metaplasia: cells are round seen in cobble-stone arranged fragments. Cells have a well defined cell border and small to medium sized, smooth, central nuclei. Nuclear:cytoplasmic (N/C ratio fairly low.

– ***Atypical***: Only a few atypical cells present, uncertain potential. Cells have some pleomorphism with enlarged nuclei and coarsely granular chromatin. The cytoplasm is decreased and may exhibit keratinisation.

– ***Suspicious***: Highly atypical cells present, suspicious of malignancy. Adequacy is not important. Criteria are quantitatively or qualitatively not enough for a diagnosis of malignancy. Cells are pleomorphic with enlarged nuclei, irregular membranes and coarsely granular chromatin. The cytoplasm is decreased and may exhibit keratinisation or vacuolisation.

– ***Malignant***: Malignant cells present. Adequacy not important. Cellular, with marked pleomorphism and a variable N/C ratio. Nuclear chromatin tends to be regularly distributed, coarsely granular and hyperchromatic. Pearls and keratinisation may be present, balls and other glandular arrangements, or even indiscriminate sheeting and moulding.

– ***Inadequate***: This category is composed of specimens where no or little cellular material is obtained, the material is artifactually distorted by blood, inflammation or poor preservation such that a diagnosis cannot be rendered. Also included is sputa not representative of "deep lung" (none, or too few alveolar macrophages present).

CYTOLOGY REPORT: FOR LABORATORY USE ONLY

RECEIVED Date D D M M Y Y
Prepared by
Time H
Volume M L

NUMBER OF SLIDES PAP MGG
SPECIAL STAINS: DATE

Macroscopic

URGENT RESULTS CONVEYED TELEPHONICALLY / FAXED
TO:
BY: DATE: / / TIME: H

MALIGNANCY (1 = Absent, 2 = Present, 3 = Uncertain) 1 2 3

DIAGNOSIS & ADDITIONAL FINDINGS

PATHOGENS P P P

ENDOCERVICAL COMPONENT Y N N/A

ADEQUACY OF SMEAR V

COMMENTS

FREE TEXT (prints on report)

RECOMMENDATIONS A A A

FOLLOW UP DATE D D M M Y Y
LETTER NUMBER

DIAG CODE STAFF CODE

EXAMINED BY
RAPID RESCREENED BY
CHECKED BY
PATHOLOGIST

ICD10 - CODE

OPINIONS (DIAG CODE / STAFF CODE)

RE-EVALUATION:
SLIDE NO.
SLIDE NO.

QC CODE

WORKSHOP CODE

FOLLOW UP Y LETTER Y IN MONTHS

COMMENTS

EXAMINED BY:
REPORT DATE: / /
RAPID RESCREENED BY:
CHECKED BY:
PATHOLOGIST:

T
T
T
T
T

Appendix C:

Data Collection Sheet

Patient's Name: _____

Folder Number: _____

Date of the collection/procedure: _____

Diagnostic yield of Sputum using the FocalPoint^{tm} Slide Profiler in patients with a pulmonary mass lesion.

Investigators (for the Lung Unit): Maurizio Bernasconi(Phone 072 8280 448) and Annari Du Plessis (0827868361).

1.Checklist (everything done?):

- Informed consent signed [ ]
- CT chest on PACS? Y [ ], N [ ]
  If not, please please fill the posterior page (radiological features a well)!!
- Copy of white card (after the procedure [ ]

2. Sputum collection: Sputum Sample Number _____

Collect **3 non-induced sputum** in the provided bottle before the planned procedure.

Patient able to produce a valid sputum? Y [ ], N [ ], if not, no further information are needed (but keep this sheet for statistical purpose)

The sputum bottle will be collected by the cytologist attending the theater and brought to the cytological laboratory!

3. Pulmonologist evaluation prior the procedure:

Likelihood of cancer (subjective pretest probability of cancer, visual analogue scale, please tick)?

very unlikely (0%) [----|----|----|----|----|----|----|----|----|----] (100 %) **very likely**

4. Diagnostic procedure performed:

Date of the procedure: ____/____/20____

[ ] Bronchoscopy:

Vocal cord paralysis? Y [ ], N [ ].

Endobronchial tumor? Y [ ], N [ ]. If yes continue below:

-- Characteristics of the tumor? 1. Ulcerated [ ], 2. Exophytic [ ], 3. Mucosal thickening [ ]

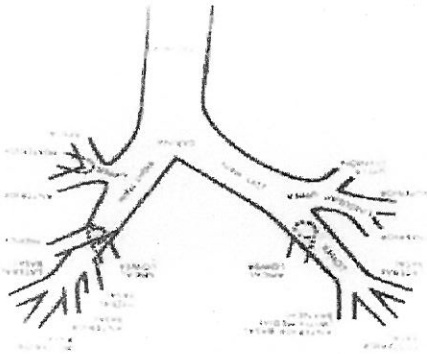
-- Localisation? RUL [ ], ML [ ], RLL [ ], LUL [ ], Lingula [ ], LLL[ ]. Please drawn on the draft (see example).

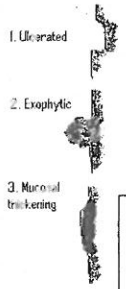
-- Complete occlusion of an ostium? Y [ ], N [ ]

-- Stenosisof the airways?

_____

[ ] TTNA [ ] Tru-Cut Biopsy [ ] Thoracentesis [ ] CT guided FNA [ ] FNA other localistion, which? _____





For the more motivated ..... please fill although the posterior site (prior the procedure)! Thanks!!!

This page can although be completed retrospectively by the investigators .....

Patient's Name: _____

Folder Number: _____

1. Patient's Characteristics and clinical features:

Prev. malignancy? Y [ ], N [ ] which? _____.

Prev. pulm. TB? Y [ ], N [ ].

Smoking: never [ ], active [ ], former [ ]. Total ____P/Y.

Cough? [ ], chronic ( ), new ( ) since ___d/w/m

Pleuritic chest pain? Y [ ] N [ ]; since ___d/w/m.

Weight loss? mild ( ), important ( ), if poss. ___kg in ___d/w/m

Any increasing dyspnea? Y [ ], N [ ].

Hemoptysis Y[ ], N [ ]; since ___d/w/m.

Hoarseness? Y [ ], N [ ]; since ___d/w/m

Superior vena cava syndrome? Y [ ], N [ ].

Pancoast's syndrome? Y [ ], N [ ]; since ___d/w/m

Neurologic symptoms? Y [ ], N [ ]; since ___d/w/m

others:_____

Paraneoplastic phenomena? Y [ ], N [ ]., since ___d/w/m.

2. Radiological features (on contrasted Chest/upper Abdomen CT scan):

Lesion localisation: RUL [ ], ML [ ], RLL [ ], LUL [ ], LLL [ ] (more than 1 possible). **Biggest Ø Size** = _____mm.

Lesion characteristics? round [ ], calcified [ ], spiculated [ ], cavitating [ ], aerobronchogramm in the lesion [ ],endobronchial lesion [ ].

Atelectasis? Y [ ], N [ ]. **Pleura effusion?** Y [ ], N [ ]. **Distant metastasis?** Y [ ], N [ ], where?_____

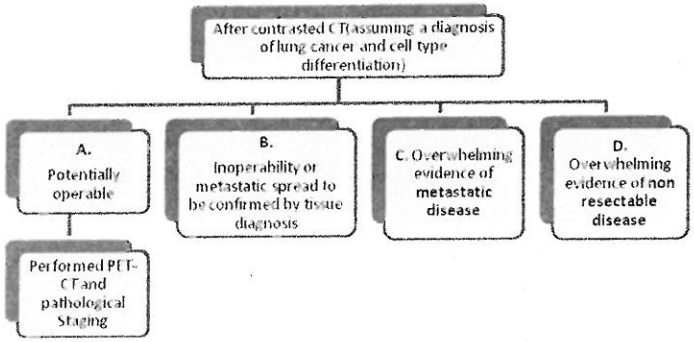
3. After the points 1 and 2 (previous diagnostic procedure!!):

A. Do imaging (CT) alone present overwhelming evidence of **non resectable disease(T4)**? Y [ ], N [ ]

B. Do imaging (CT) alone present overwhelming evidence of **metastatic disease?** Y [ ], N, [ ]

Please take into account that overwhelming evidence of metastatic disease means a clear M1b stage or a cytological proven malign pleura effusion (M1a).

C. Please circle the appropriate clinical situation after the CT scan assuming a diagnosis of lung cancer (including a definitive cell type differentiation).



Diagnostic procedure performed (in order to reach a diagnosis/staging):

[ ] Bronchoscopy: [ ] TBNA: number of passes? ____, [ ] brush, [ ] TBB, [ ] EBB, [ ] BW

Result of ROSE: _____

[ ] TTNA, number of passes? ____ , Result of ROSE: _____

[ ] Tru-Cut Biopsy

[ ] FNA other localisation, number of passes? _____

[ ] Thoracentesis **[ ] previous sputum** **[ ] CT guided FNA** **[ ] others,** _____

Appendix D:

Consent Form (English, Afrikaans & Xhosa)

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:
Automated Sputum Screening using the BD FocalPoint^{tm} Slide Profiler: Correlation with Transbronchial and Transthoracic Needle Aspirates.

REFERENCE NUMBER: N10/04/135

PRINCIPAL INVESTIGATOR: Ms Greta S Neethling

ADDRESS: Anatomical Pathology
Tygerberg Hospital

CONTACT NUMBER: 0846683836 or 0219385356

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- This study will only be conducted at Tygerberg Hospital's bronchoscopy unit. A total of 200 participants will be recruited.
- The aim of this project is to determine if sputums (lung mucous) would be able to be screened by a machine, and whether the sputum result and the bronchoscopy (procedure that you are here for today) result is the same. This method might improve sputum screening and make it faster too.
- The sputum that you would submit will be prepared, and screened by a machine to try and detect any abnormal cells. This result will be correlated with the result of the bronchoscopy.

Why have you been invited to participate?

- All patients attending the lung unit for a bronchoscopy, would be asked to give a sputum sample before the procedure.

What will your responsibilities be?

- None

Will you benefit from taking part in this research?

- None

Are there in risks involved in your taking part in this research?

- None. It is safe to produce a sputum.

Who will have access to your medical records?

- The information collected will be treated as confidential and protected. If it is used in a publication or a thesis, your identity will remain anonymous. It is only 5 researchers that will have access to your identity, and upon arrival in the lab your name will be removed from your sample and a number will be put on instead. This number will be used from then onwards.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

- You will not suffer any injury.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study and there will be no costs involved for you, if you do take part.

Is there any thing else that you should know or do?

- You can contact Greta Neethling at 021 9385356 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ..... agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ..... on (*date*) ..... 2005.

....

Signature of participant **Signature of witness**

Declaration by investigator

I, Greta Neethling, declare that:

- I explained the information in this document to .....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ..... on (*date*) ..... 2005.

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I (*name*) ..... declare that:

- I assisted the investigator (*name*) ..... to explain the information in this document to (*name of participant*) ..... using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ..... on (*date*) .....

....

Signature of interpreter **Signature of witness**

DEELNEMERINLIGTINGSBLAD EN -TOESTEMMINGSVORM

TITEL VAN DIE NAVORSINGSPROJEK:
Geautomatiseerde sputum ontleding deur gebruik te maak van die BD FocalPoint^{tm} Slide Profiler. 'n Korrelasie met TT/TB naald aspirasies.

VERWYSINGSNOMMER: N10/04/135

HOOFNAVORSER: Ms Greta Neethling

ADRES: Anatomiese Patologie, Tygerberg Hospitaal.

KONTAKNOMMER: 0846683836 of 021 9385356

U word genooi om deel te neem aan 'n navorsingsprojek. Lees asseblief hierdie inligtingsblad op u tyd deur aangesien die detail van die navorsingsprojek daarin verduidelik word. Indien daar enige deel van die navorsingsprojek is wat u nie ten volle verstaan nie, is u welkom om die navorsingspersoneel of dokter daaroor uit te vra. Dit is baie belangrik dat u ten volle moet verstaan wat die navorsingsprojek behels en hoe u daarby betrokke kan wees. U deelname is ook **volkome vrywillig** en dit staan u vry om deelname te weier. U sal op geen wyse hoegenaamd negatief beïnvloed word indien u sou weier om deel te neem nie. U mag ook te eniger tyd aan die navorsingsprojek onttrek, selfs al het u ingestem om deel te neem.

Hierdie navorsingsprojek is deur die Gesondheids Navorsing Etiese Kommitee van die Universiteit Stellenbosch **goedgekeur en sal uitgevoer word volgens die etiese riglyne en beginsels van die Internasionale Verklaring van Helsinki en die Etiese Riglyne vir Navorsing van die Mediese Navorsingsraad (MNR).**

Wat behels hierdie navorsingsprojek?

- *Die studie sal slegs by Tyegrberg hospitaal se long eenheid plaasvind. 200 Patiente sal ingesluit word.*
- *Die studie gaan probeer bepaal of sputum (long slym) deur 'n nuwe masjien geontleed kan word, en of die resultaat dieselfde is as die van die naald aspirasie. Hierdie nuwe metode kan die ontleding beter en vinniger maak.*
- *Die sputum wat u sal gee, sal voorberei word en deur die masjien ontleed word om enige moontlike abnormale selle te identifiseer.*

Waarom is u genooi om deel te neem?

- *Al die patiente wat die long eenheid bywoon vir 'n naald aspirasie, word vriendelik versoek om ook 'n sputum monster te produseer vir die studie.*

Wat sal u verantwoordelikhede wees?

- *Geen.*

Sal u voordeel trek deur deel te neem aan hierdie navorsingsprojek?

- *Geen*

Is daar enige risiko's verbonde aan u deelname aan hierdie navorsingsprojek?

- *Geen, dit is heeltemal veilig om slym uit te hoes.*

Wie sal toegang hê tot u mediese rekords?

- *Die informasie van die studie is heeltemal konfidentieel. Indien dit in 'n publikasie gebruik gaan word, sal u anoniem bly. Wanneer die monster in die lab arriveer, kry dit 'n nommer wat dan voortaan gebruik sal word i.p.v u naam en van.*

Wat sal gebeur in die onwaarskynlike geval van 'n besering wat mag voorkom as gevolg van u deelname aan hierdie navorsingsprojek?

- *U sal geen besering op doen nie.*

Sal u betaal word vir deelname aan die navorsingsprojek en is daar enige koste verbonde aan deelname?

U sal nie betaal word vir deelname aan die navorsingsprojek nie, deelname aan die navorsingsprojek sal u niks kos nie.

Is daar enigiets anders wat u moet weet of doen?

- U kan Ms Greta Neethling kontak by tel 021 9385356 indien u enige verdere vrae het of enige probleme ondervind.
- U kan die Gesondheids Navorsing Etiese Kommitee kontak by 021-938 9207 indien u enige bekommernis of klagte het wat nie bevredigend deur u studiedokter hanteer is nie.
- U sal 'n afskrif van hierdie inligtings- en toestemmingsvorm ontvang vir u eie rekords.

Verklaring deur deelnemer

Met die ondertekening van hierdie dokument onderneem ek, ..., om deel te neem aan 'n navorsingsprojek getiteld (*Titel van navorsingsprojek*).

Ek verklaar dat:

- Ek hierdie inligtings- en toestemmingsvorm gelees het of aan my laat voorlees het en dat dit in 'n taal geskryf is waarin ek vaardig en gemaklik mee is.
- Ek geleentheid gehad het om vrae te stel en dat al my vrae bevredigend beantwoord is.

- Ek verstaan dat deelname aan hierdie navorsingsprojek **vrywillig** is en dat daar geen druk op my geplaas is om deel te neem nie.
- Ek te eniger tyd aan die navorsingsprojek mag onttrek en dat ek nie op enige wyse daardeur benadeel sal word nie.
- Ek gevra mag word om van die navorsingsprojek te onttrek voordat dit afgehandel is indien die studiedokter of navorser van oordeel is dat dit in my beste belang is, of indien ek nie die ooreengekome navorsingsplan volg nie.

Geteken te *(plek)* ..... op *(datum)* ..... 2005.

....

Handtekening van deelnemer **Handtekening van getuie**

Verklaring deur navorser

Ek *(naam)* ..... verklaar dat:

- Ek die inligting in hierdie dokument verduidelik het aan .....
- Ek hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek tevrede is dat hy/sy al die aspekte van die navorsingsprojek soos hierbo bespreek, voldoende verstaan.
- Ek 'n tolk gebruik het/nie 'n tolk gebruik het nie. *(Indien 'n tolk gebruik is, moet die tolk die onderstaande verklaring teken.)*

Geteken te *(plek)* ..... op *(datum)* ..... 2005.

....

Handtekening van navorder **Handtekening van getuie**

Verklaring deur tolk

Ek *(naam)* ..... verklaar dat:

- Ek die navorser *(naam)* ..... bygestaan het om die inligting in hierdie dokument in Afrikaans/Xhosa aan *(naam van deelnemer)* ..... te verduidelik.

- Ons hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek 'n feitelik korrekte weergawe oorgedra het van wat aan my vertel is.
- Ek tevrede is dat die deelnemer die inhoud van hierdie dokument ten volle verstaan en dat al sy/haar vrae bevredigend beantwoord is.

Geteken te *(plek)* ..... op *(datum)* ..... 2005.

....

Handtekening van tolk **Handtekening van getuie**

INCWADANA ENGOLWAZI NGOMTHATHI-NXAXHEBA KUNYE NEFOMU YEMVUMELWANO

ISIHLOKO SEPROJEKTHI YOPHANDO:

Kukuxilongwa kwezikhohlela ngokusebenzisa umatshini omtsha obizwa ngokuba yi"BD Focal point slide profiler'
Ukujonga unxibelelwana phakathi kweziphumo zesikhohlela kwakunye neTransbronchial FNA (uphayiphu omncinci onenaliti othi ufakwe empumleni okanye emlonyeni ukuya kwimibhobho emincinci engaphakathi kwiziphunga ukuze kufumaneke izakha mzimba zesifo somhlaza); ne Transthoracic FNA (inaliti encinci ethi ifakwe apho kukhona iqhuma khona, lingaba sesifubeni ngandle okanye ngaphakathi; okanye libe semqolo ukuze kufumaneke izakhamzimba zezifo somhlaza.

INOMBOLO YONXULUMANO: N10/04/135

UMPHANDI OYINTLOKO: Ms Greta Neethling

IDILESI: Anatomical Pathology, Tygerberg Hospital

INOMBOLO YOQHAGAMSHELWANO: 0846683836, 0219385356

Uyamenywa ukuba athathe inxaxheba kwiprojekthi yophando. You are being invited to take part in a research project. Nceda thatha ixesha lokufunda ulwazi oluvezwe apha, oluzakuthi luchaze iinkcukacha zale projekthi. Nceda buza nayiphina imibuzo emalunga nayiphina indawo ongayiqondiyo ngokupheleleyo kubasebenzi besi sifundo okanye kugqirha. Kubaluleke kakhulu ukuba waniliseke ngokupheleleyo yinto yokuba ucacelwe kakuhle ukuba yintoni ebangwa sesi sifundo kwaye ungabandakanyeka njani. Kwakhona, ukuthatha kwakho inxaxheba **kungentando yakho ngokupheleleyo** kwaye ukhululekile ukuba ungarhoxa ekuthatheni inxaxheba. Ukuba uthi hayi, oku akusayi kuchaphazela ukungavumi kwakho nangayiphina indlela. Ukwakhululekile ukuba uyeke kwesi sifundo naninina, nkqu nokokuba uyavuma ukuthatha inxaxheba ekuqaleni.

Olu phando luvunywe ziinqobo ezisesikweni **iikomiti yesebe lezempilo yoPhando Lomntu kwiYunivesithi yaseStellenbosch** kwaye luzakwenziwa ngokwemigaqo esesikweni lophando elamkelekileyo kwiSaziso sehlabathi sika-Helsinki, iMigaqo eLungileyo yoMzantsi Afrika yokuSebenza eKliniki kunye neBhunga lezoPhando ngamaYeza (MRC) iMigaqo yeNqobo yezoPhando.

Simalunga nantoni esi sifundo sophando?
Oluphando luyakuba luqhutywelwa kwisibhedlele saseTygerberg, kwicandelo lezifo sesifuba.
Malunga namakhulu amabini ezigulana ziyakuthi zicelwe ukuba zithabathe inxaxheba koluphando.
Iinjongo zoluphando kukujonga nokuphanda ukuba singakwazi ukuxilonga isikhohlela ngalomatshini umtsha kusini na kwaye singakwazi ukungqamanisa iziphumo zesikhola kwakunye nezenaliti encinci ethi ifakwe eziphungeni ukuze kutsalwe izakha mzimba zesifo somhlaza. Ie nkqubo yoluphando iyakuthi iphucule ukuxilongwa kwezikhohlela ze neziphumo zifumaneke ngokukhawulezileyo.
Lo matshini omtsha uyakuthi uphucule izinga lokuxilongwa kwezikhohlela kwaye iziphuma ziyakufumaneka ngokukhawuleza

Isikhohlela siya kuthi sithunyelwe elaboratri apho siyakuthi silungiselelwe uvavanyo ze sixilongwe ngalomatshini umtsha ngethemba lokuba lomatshini uyakuthi ukwazi ukuziqwalasela nokuzibona ezizakhamzimba zesifo somhlaza kwisikhohlela.
Iziphumo zesikhohlela ziyakuthi zingqanyaniswe/zifaniswe kwakunye neziphumo zenaliti encinci ebithe yafakwa esifubeni.

Kutheni umenyiwe ukuba uthathe inxaxheba?
Zonke izigulana ezisesibhedlele kwicandelo lezifo zesifuba eziya kwibroncoscopy, ziyakuthi zicelwe ukuba zinikezele ngesikhokhela phambi kokuba zenziwe ibroncoscopy.
Ngoluphando sifuna ukujonga nokuqwalasela ukuba lo matshini uyakuthi ukwazi ukuxilonga isikhohlela.

Luyakuba yintoni uxanduva lwakho?

Alukho uxanduva oyakuthi ube nalo

Ingaba uza kuzuza ekuthatheni inxaxheba kolu phando?

Ukuba umatshini uthe wazibona izakha mzimba zesifo somhlaza kwisikhohlela sakho awusayi kuba sayenza ibroncoscopy; kwaye ukuba kwibroncoscopy izakhamzimba abathe bazitsala ngenaliti azoneli ukukhupha iziphumo eziqinisekileyo; iziphumo zesikhokhela ziyakuba luncedo ukuze ufumane unyango olukhawulezileyo.

Ingaba zikho iingozi ezibandakanyekayo ekuthatheni kwakho inxaxheba kolu phando?
Hayi azikho, kukhuselekile ukukhupha isikhohlela

Ukuba awuvumi ukuthatha inxaxheba, loluphi olunye unyango onalo?

Ingxelo ngeziphumo zakho ziyakuba yimfihlelo kwaye zikhuseleke.ukuba zithe zasetyenzisa njengemizekelo kubhalo lwencwadi iincukacha zakho (umzekelo; igama, idilesi) aziyikusetyenziswa.
Ngabaqhubi boluphando abahlanu abayakuthi bakwazi ukufikelela kwinkcukacha zakho, kwaye xa ufika elaboratri kuyathi kusetyenziswa inombolo eyakusetyenziswa njengegama lakho.

Ngubani uza kufumana ingxelo yakho yamayeza?

Hayi, awusayi kuhlawulwa ngokuthabatha inxaxheba kwaye akukho zindleko zibandakanya wena xa uthe wathabatha inxaxheba.

Kuza kwenzeka ntoni kwimeko yesiganeko esingalindekanga sokwenzakala ngenxa yokuthatha kwakho inxaxheba kwesi sifundo sophando?

- *Cacisa imiba enxulumene nentlawulo ye-inshorensi ukuba ikhona. Ukuba kukho iindawo ezibandakanyekayo ezithengisa amayeza ingaba intuthuzelo izakuhamba ngokwemigaqo ye-ABPI? (Imigaqo yentuthuzelo enxulumene nokwenzakala kuphando lweManyano yaseBrithane yezoRhwebo lwamaYeza ethathwa njengekwizinga lehlabathi legolide). Ukuba ewe, nceda bandakanya iinkcukacha apha. Ukuba hayi, cacisa ke ukuba yeyiphi intuthuzelo eyakubakhona kwaye phantsi kweziphi iimeko..*

Ingaba uza kuhlawulwa ngokuthatha inxaxheba kwesi sifundo kwaye ingaba kukho iindleko ezibandakanyekayo?

Hayi awusayi kuhlawulwa ngokuthatha inxaxheba kwesi sifundo kodwa isithuthi sakho neendleko zokutya ziza kuhlawulelwa kundwendwelo ngalunye lwesifundo. Akusayi kubakho zindleko ezibandakanyelwa wena, ukuba uthatha inxaxheba.

Ingaba ikho enye into ekumele uyazi okanye uyenze?

- Ungaqhgamshelana Greta Neethling kule inombolo yomnxeba 0219385356 ukuba unemibuzo engaphaya okanye uhlangabezana neengxaki.
- Ungaqhagamshelana neKomiti yoPhando Lomntu kwa-021-938 9207 ukuba unenkxalabo okanye izikhalazo ezingasonjululwanga kakuhle ngugqirha wakho wesifundo.
- Uza kufumana ikopi yolu lwazi kunye nefomu yemvumelwano ukwenzela iingxelo zakho.

Isifungo somthathi-nxaxheba

Ngokuytyikitya ngezantsi, Mna ..... ndiyavuma ukuthatha inxaxheba kwisifundo sophando semfuzo esibizwa ngokuba *(faka ishloko sesifundo)*.

Ndazisa ukuba:

- Ndilufundile okanye ndalufunda olu lwazi kunye nefomu yemvumelwano kwaye ibhalwe ngolwimi endiliciko nendikhululekileyo kulo
- Bendinalo ithuba lokuba ndibuze imibuzo kwaye yonke imibuzo yam iphendulwe ngokwanelisayo.
- Ndiyakuqonda ukuba ukuthatha inxaxheba kolu phando kube **kukuzithandela kwam** kwaye andikhange ndinyanzelwe ukuba ndithathe inxaxheba.
- Ndingakhetha ukusishiya isifundo naninina kwaye andisayi kohlwaywa okanye uqal' ugwetywe nangayiphi indlela.
- Usenokucelwa ukuba usishiye isifundo phambi kokuba siphele, ukuba ugqirha wesifundo okanye umphandi ukubona kuyinzuzo kuwe, okanye ukuba andisilandeli isicwangciso sesifundo, ekuvunyelenwe ngaso.

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2005.

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Umtyikityo womthathi-nxaxheba **Umtyikityo wengqina**

Isifungo somphandi

Mna *(igama)* Greta Neethling ndiyafunga ukuba:

- Ndilucacisile ulwazi olu kweli xwebhu ku-.....

- Ndimkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.
- Ndiyaneliseka kukuba uyakuqonda ngokwanelisayo konke okumalunga nophando okuxoxwe ngasentla.
- Ndisebenzise/andisebenzisanga toliki. (*Ukuba itoliki isetyenzisiwe kumele ityikitye isaziso ngezantsi.*

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2005.

....

Umtyikityo womphandi **Umtyikityo wengqina**

Isifungo setoliki

Mna *(igama)* ..... ndazisa ukuba:

- Ndicende umphandi (*igama*) ..... Ekucaciseni ulwazi olu lapha kweli xwebhu ku-(*igama lomthathi-nxaxheba*) ..... ndisebenzisa ulwimi lwesiAfrikaans/lwesiXhosa.
- Simkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.
- Ndimxelele eyona nto iyiyo malunga nokunxulumene nam.
- Ndiyaneliseka kukuba umthathinkxaxheba ukuqonda ngokupheleleyo okuqulathwe loluxwebhu lwemvumelwano eyazisiweyo kwaye nemibuzo yakhe yonke iphendulwe ngokwanelisayo.

Kutyikitywe e-(*indawo*) ..... ngo-(*usuku*) ..... 2005.

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Umtyikityo wetoliki **Umtyikityo wengqina**

Appendix E:

Manufacturer addresses

1. Shandon MUCOLEXX™ Mucoliquefying Preservative

   Thermo Scientific
   171 Industry Drive
   Pittsburgh
   PA 15275
   USA
   www.thermo.com/shandon

2. IKA ULTRA-TURRAX® T25: S25N – 18G (Saccomanno homogeniser)

   Janke & Kunkel GmbH & Co. KG
   IKA® Laboratory Technology
   Str. 10
   79219 Staufen
   Germany
   www.ika.com

3. BD FocalPoint™ Slide Profiler

   Becton, Dickinson and Company
   1 Becton Drive
   Franklin Lakes
   NJ USA 07417
   www.bd.com

Appendix F:

Proposed IASLC/ERS Classification for Cytology

APPENDIX F:

Proposed IASLC/ATS/ERS Classification for Bronchogenic Carcinoma (Cytology)

2004 WHO Classification	SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	*Morphologic adenocarcinoma patterns clearly present:* Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification) Comment: If pure lepidic growth – mention an invasive component cannot be excluded in this small specimen
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloid pattern
Signet ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
No 2004 WHO counterpart – most will be solid adenocarcinomas	*Morphologic adenocarcinoma patterns not present (supported by special stains):* Non-small cell carcinoma, favor adenocarcinoma
SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	*Morphologic squamous cell patterns clearly present:* Squamous cell carcinoma
No 2004 WHO counterpart	*Morphologic squamous cell patterns not present (supported by stains):* Non-small cell carcinoma, favor squamous cell carcinoma
SMALL CELL CARCINOMA	Small cell carcinoma
LARGE CELL CARCINOMA	Non-small cell carcinoma, not otherwise specified (NOS)
Large cell neuroendocrine carcinoma (LCNEC)	Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNEC
Large cell carcinoma with NE morphology (LCNEM)	Non-small cell carcinoma with NE morphology (negative NE markers) – see comment Comment: This is a non-small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.
ADENOSQUAMOUS CARCINOMA	*Morphologic squamous cell and adenocarcinoma patterns present:* Non-small cell carcinoma, with squamous cell and adenocarcinoma patterns Comment: this could represent adenosquamous carcinoma.
No counterpart in 2004 WHO classification	*Morphologic squamous cell or adenocarcinoma patterns not present but immunostains favor separate glandular and adenocarcinoma components* Non-small cell carcinoma, NOS, (specify the results of the immunohistochemical stains and the interpretation) Comment: this could represent adenosquamous carcinoma.
Sarcomatoid carcinoma	Poorly differentiated NSCLC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)

IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society; WHO, World Health Organization. NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; TTF, thyroid transcription factor.

Appendix G:

Morphologic Criteria Lists used in FocalPoint Algorithms

Appendix G:

Comprehensive Morphologic Criteria Lists used in FocalPoint Algorithms

Cell type	Nuclear area (μm2)	Nuclear area (pixels)	Cell area (μm2)	Cell area (pixels)	Relative nuclear area (%)
Intermed Sq	23–49	76–162	995–2145	3289–7091	1.5–7.5
Endometrial	32–41	106–136	55–70[a]	182–231	59[a]
Sq Metapl (P)	44–57	145–188	176–226	582–747	22–27
Sq Metapl (I)	38–63	126–208	272–365	899–1207	14–19
Sq Metapl (M)	42–61	139–202	588–697	1944–2304	7 +/–
Endometrial hyperplasia	35–56	116–185	65–123	215–407	46–54
Atypical endometrial hyperplasia	42–63	139–208	84–149	278–492	42–50
Endometrial Adenoca (I)	60	198	132	436	45
Endometrial Adenoca (II)	67	221	151	499	44
Endometrial stromal cell	38–54	127–178	45–72	149–238	75–84
Endocervical	41–61	136–202	148–228 (cols)	489–754	26–35
Atrophic squamous	50	165	325	1074	15
SC CIS	60–80	198–264	95	314	63–84
Sq CA, small cell type	52–78	172–258	132–206[b]	436–681[b]	35–45
AISMT	60–70	198–231	100–175	330–578	40–60
Atypical immature metapl repair	59	195	270	892	21
ASCUS	75–125	248–413	250–450	826–1488	30–35
Atypical mature Sq metapl repair	75	248	270	892	28
AIS	34–102	112–337	67–219	221–724	37–59
ICIS	80–120	264–397	189	625	42–63
K Sq CA	49–105	162–347	168–382	555–1263	28–29
NK Sq CA	58–118	192–390	187–325	618–1074	32–36
Endocervical Adenoca (I–III)	73–97	241–321	167–201	552–664	44–50
Endometrial Adenoca (III–IV)	85–92	281–304	176–198	582–654	46–48
Atypical squamous repair	75	250	350	1157	21
Endocervical atypia (reactive)	100–200	330–661	300–600	992–1983	33
Atypical endocervical repair	144–201	476–664	350–530	1157–1752	39
Dysplasia (M)	121–191	400–631	386–601	1276–1987	31
Dysplasia (K)	126–210	416–694	637–1453	2106–4803	16
Dysplasia (NK)	146–210	482–694	1063–1465	3514–4843	14
Endocervical Adenoca (IV)	165	545	314	1038	53
LC CIS	150–200	496–661	435	1438	38
Acute radiation	140–175	463–578	2000–6000	6612–19,835	3 +/–
ExtraUT	Depends	Depends	Depends	Depends	Depends
Sarcomas & other rare lesions	Depends	Depends	Depends	Depends	Depends

Intermed: intermediate; Sq: squamous; Metapl: metaplastic; P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; SC: small cell; CIS: carcinoma in situ; CA: carcinoma; AISMT: atypical cells of immature metaplastic type; ASCUS: atypical squamous cells of undetermined significance; AIS: adenocarcinoma in situ; ICIS: intermediate carcinoma in situ; K: keratinizing; NK: nonkeratinizing; LC: large cell; Extraut: extrauterine.

[a] Cytoplasmic boundaries intact.

[b] Frequent nuclear distortion.

Cell type	Nuclear hyperchromasia	Chromatin particles	Chromatin distribution	Micronucleoli	Macronucleoli	Irregular nucleoli
Intermed Sq		Fine	Even			
Endometrial		Fine	Even			
Sq Metapl (P)		Fine	Even			
Sq Metapl (I)		Fine	Even			
Sq Metapl (M)		Fine	Even			
Endometrial hyperplasia		Fine	Even			
Atypical endometrial hyperplasia	+	Fine	Even	25%		
Endometrial Adenoca (I)	+	Fine	Uneven	70%	3%	
Endometrial Adenoca (II)	++	Fine	Uneven	86%	11%	
Endometrial stromal cell		Fine	Even	+		
Endocervical		Fine	Even	100%		
Atrophic squamous		Fine	Even			
SC CIS	++++	Fine to coarse	Even			
Sq CA, small cell type	++++	Fine to coarse	Uneven	20%		
AISMT	++	Fine	Even			
Atypical immature metapl. repair	+	Fine	Even	16%	80%	
ASCUS	+	Fine	Even			
Atypical mature Sq Metapl repair	+	Fine	Even	8%	92%	
AIS	+++	Coarse	Even			
ICIS	+–4+	Fine to coarse	Even			
K Sq CA	++++	Opaque	Even to uneven		5%	
NK Sq CA	++	Fine to coarse	Uneven		24%	
Endocervical Adenoca (I–III)	+++	Fine	Uneven	38–87%	13–62%	1–5%
Endometrial Adenoca (III–IV)	+++	Fine	Uneven	55–65%	36–45%	
Atypical squamous repair	++	Fine	Even	5%	95%	
Endocervical atypia (reactive)		Fine	Even		100%	
Atypical endocervical repair	++	Fine	Even	2%	98%	
Dysplasia (M)	+++	Fine to clumped	Even			
Dysplasia (K)	++++	Opaque	Even			
Dysplasia (NK)	+	Fine	Even			
Endocervical Adenoca (IV)	+++	Fine to coarse	Uneven	16%	84%	35%
LC CIS	+	Fine	Even			
Acute radiation		Fine	Even			
ExtraUT	++	Fine to clumped	Uneven	Yes	Yes	+
Sarcomas & other rare lesions	+	Fine	Uneven	Yes	Yes	+

Intermed: intermediate; Sq: squamous; Metapl: metaplastic; P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; SC: small cell; CIS: carcinoma in situ; CA: carcinoma; AISMT: atypical cells of immature metaplastic type; ASCUS: atypical squamous cell of undetermined significance; AIS: adenocarcinoma in situ; ICIS: intermediate carcinoma in situ; K: keratinizing; NIC: nonkeratinizing; LC: large cell; Extraut: extrauterine.

Cell type	Cell shape	Cytoplasmic texture	Cell borders	Cell arrangement	Tumor diathesis	Multiple nucleoli
Intermed Sq	Polygonal	Homogeneous	Yes	Isolated		
Endometrial	Round	Finely vacuolated	No	Isolated/cluster		
Sq Metapl (P)	Round/oval	Homogeneous	No	Isolated		
Sq Metapl (I)	Round/oval	Primitive	Yes	Isolated		
Sq Metapl (M)	Small ploygonal	Homogeneous	Yes	Isolated		
Endometrial hyperplasia	Round	Vacuolated	No	Cluster		
Atypical endometrial hyperplasia	Round	Vacuolated	No	Cluster		
Endometrial Adenoca (I)	Round	Vacuolated	No	Isolated/cluster	Serous	8%
Endometrial Adenoca (II)	Round	Vacuolated	No	Isolated/cluster	Serous	34%
Endometrial stromal cell	Round	Vacuolated	Yes	Isolated		
Endocervical	Columnar	Granular	Yes	Isolated/sheets		Frequent
Atrophic squamous	Round/oval	Homogeneous	Yes	Isolated		
SC CIS	Oval	Primitive	No	Syncytial		
Sq CA, small cell type	Oval	Primitive	No	Syncytial	Tumor	
AISMT	Round	Homogeneous	Yes	Isolated		
Atypical immature Metapl. repair	Columnar/oval	Homogeneous	Yes	Sheets		
ASCUS	Polygonal/round/oval	Homogeneous	Yes	Isolated		
Atypical Mature Sq Metapl repair	Round/oval	Homogeneous	Yes	Sheets		15%
AIS	Columnar	Granular	Yes	Isolated/sheets		
ICIS		Primitive	No	Isolated/syncytial		
K Sq CA	Pleomorphic	Homogeneous	Yes	Isolated	Clean	
NK Sq CA		Primitive	No	Isolated/Syncytial	Tumor	"+/−"
Endocervical Adenoca (I–III)	Columnar	Granular	No (25–77%)	Cluster	Tumor	1–5%
Endometrial Adenoca (III–IV)	Round	Vacuolated	No	Cluster	Serous	
Atypical squamous repair	Round/oval	Homogeneous	Yes/no			22%
Endocervical atypia (reactive)	Columnar/round/oval	Vacuolated	Yes	Sheets		
Atypical endocervical repair	Polygonal	Homogeneous	Yes/no	Sheets		32%
Dysplasia (M)	Round/oval	Homogeneous	Yes	Isolated		
Dysplasia (K)	Pleomorphic	Homogeneous	Yes	Isolated		
Dysplasia (NK)	Polygonal	Homogeneous	Yes	Isolated		
Endocervical Adenoca (IV)	Side/side	Granular	No	Isolated/side/side	Tumor	35%
LC CIS		Primitive	No	Isolated/syncytial		
Acute radiation	Round/oval/polygonal	Variable	Yes	Isolated		
ExtraUT	Variable	Variable	Yes/no	Cluster	Clean	?
Sarcomas & other rare lesions	Variable	Variable	Yes/no	Isolated	Clean/tumor	?

Intermed: intermediate; Sq: squamous; Metapl: metaplastic; P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; SC: small cell; CIS: carcinoma in situ; CA: carcinoma; AISMT: atypical cells of immature metaplastic type; of invasive squamous type; ASCUS: atypical squamous cells of undetermined AIS: adenocarcinoma in situ; ICIS: intermediate carcinoma in situ; K: keratinizing; NK: nonkeratinizing; LC: large cell; Extraut: extrauterine.

	Chromatin particles	Chromatin distribution	Cell shape	Cyto texture	Cell borders	Cell arrangement
Intermediate squamous	Fine	Even	Polygonal	Homogeneous	T	Isolated
Endometrial	Fine	Even	Round	Finely vacuolated	F	Isolated/cluster
Squamous metaplastic (P)	Fine	Even	Round/oval	Homogeneous	F	Isolated
Squamous metaplastic (I)	Fine	Even	Round/oval	Primitive	T	Isolated
Squamous metaplastic (M)	Fine	Even	Small polygonal	Homogeneous	T	Isolated
Atypical endometrial hyperplasia	Fine	Even	Round	Vacuolated	F	Cluster
Endometrial Adenoca	Fine	Uneven	Round	Vacuolated	F	Isolated/cluster
Endocervical	Fine	Even	Columnar	Granular	T	Isolated/sheets
Atrophic squamous	Fine	Even	Round/oval	Homogeneous	T	Isolated
Squamous atypical repair	Fine	Even	Round/oval	Homogeneous	T	Sheets
ASCUS	Fine/oval	Even	Polyg/round	Homogeneous	T	Isolated
Metaplastic dysplasia	Fine/clumped	Even	Round/oval	Homogeneous	T	Isolated
Intermediate CIS	Fine/coarse	Even	None	Primitive	F	Isolated/syncytial
Large cell CIS	Fine	Even	None	Primitive	T	Isolated/syncytial
Nonkeratinizing sq CA	Fine/coarse	Uneven	None	Primitive	F	Isolated/syncytial
Keratinizing dysplasia	Opaque	Even	Pleomorphic	Homogeneous	T	Isolated
Keratinizing sq carcinoma	Opaque	Even/uneven	Pleomorphic	Homogeneous	T	Isolated
Small cell CIS	Fine/coarse	Even	Oval	Primitive	F	Syncytial
Small cell sq carcinoma	Fine/coarse	Uneven	Oval	Primitive	F	Syncytial
Atypical immature squamous metaplastic type	Fine	Even	Round	Homogeneous	T	Isolated
Endocervical atypia	Fine	Even	Columnar/oval	Homogeneous	T	Sheets
Adenocarcinoma in situ	Coarse	Even	Columnar	Granular	T	Isolated/sheets
Endocarvical adenoca	Fine	Uneven	Columnar	Granular	F	Cluster

P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; ASCUS: atypical squamous cells of undetermined significance; CIS: carcinoma in situ; sq: squamous; CA: carcinoma; T: true; F: false.

Appendix H:

Papanicolaou Staining Method (regressive)

Reagent	Duration
95% Ethanol	10 minutes
80% Ethanol	30 seconds
50% Ethanol	30 seconds
Running Water	30 seconds
Harris Haematoxylin (Merck)	3 - 5 minutes
Running Water	30 seconds
0.5% Hydrochloric Acid	2 seconds
Running Water	30 seconds
1.0% Lithium Carbonate	3 minutes
Running Water	30 seconds
95% Ethanol	30 seconds
Orange II (Merck)	3 minutes
95% Ethanol	30 seconds
EA 50 (Merck)	3 minutes 30 seconds
95% Ethanol	30 seconds
95% Ethanol	30 seconds
100% Ethanol	30 seconds
100% Ethanol	30 seconds
100% Ethanol	30 seconds
Xylene	30 seconds
Xylene	30 seconds
Xylene	30 seconds

Appendix I

Validation of the FP for Conventional Cervical Cytology Smears at NHLS,TBH

RETROSPECTIVE VALIDATION OF THE FOCALPOINT SLIDE PROFILER AUTOMATED SCREENING INSTRUMENT IN CYTOLOGY

AIM:

To establish historical validation of the FocalPoint Slide Profiler Screening Instrument serial no: 0355 by using the data generated by the technologist screening and interpreting the 15 fields of view of conventional gynaecological slides at the GS Review station.

METHOD:

Data from slides run from January 2010 - June 2010 was assessed in order to establish whether this instrument has been proven fit for its intended use in the Cytology Laboratory at Tygerberg Hospital. The data used was the laboratory's "Diagnostic screening Internal Quality Control", the labs'**acceptable range for IQC of missed abnormalities: less than 1.7%** and the recorded "missed abnormalities" by the FocalPoint. The missed abnormalities were not present in the 15 fields of view interpreted by the FocalPoint. The slides were screened on the Standard Process Mode by the FocalPoint and reviewed by a qualified and competent technologist at the GS Review Station.

EVALUATION OF DATA COLLECTED:

Total number of slides: **9922**
Number of unacceptable "missed abnormalities": **20**
This was an error rate of: **0.2%** this rate was acceptable as it was less than 10%.
The mean **IQC** score was within the acceptable range for each run assessed.

Specimen Measurement Issues	Problem stated
STC 3002353	ASC-US
STC 3003264	LSIL
STC 3007510	LSIL
STC 3010195	LSIL
STC 3010801	LSIL
STC 3011160	ASC-US
STC 3015872	ASCUS
STC 3016751	ASCUS
STC 3018412	ASCUS
STC 3023390	ASC-US
STC 3024075	LSIL
STC 3023834	LSIL
STC 3027441	LSIL
STC 3030271	ASC-US
STC 3029807	ASC-US
STC 3030335	LSIL
STC 3030040	LSIL
STC 3030948	HSIL
STC 3031338	LSIL
STC 3032081	LSIL

The slides where a "missed abnormality" was not present in the 15 fields of view but picked up during rapid review were re-screened in all of the above instances. This instrument was placed in the Cytology Department July 2008 and has performed well and is reliable. The instrument is serviced annually.

SUMMARY:

All slides with a normal diagnosis interpreted at the GS Review Station are manually rapid reviewed by a qualified and competent technologist, while all abnormal slides are re-screened by a checker technologist. If slides were found to be unacceptable for re-screening a repeat would be requested.
On assessing the above data it was proven that this instrument has performed well and is fit for the intended purpose in this laboratory on **14/2/2011.**

Data assessed by: M. Hill **Signature:** M Hill **Date:** 14/2/2011

Validation approved by: L van Vuuren Laboratory Manager

Signature: L van Vuuren **Date:** 14/2/2011

PROTOCOL FOR VALIDATION/VERIFICATION OF METHODS/INSTRUMENTS/REAGENTS

Instrument/Method/Reagent: Focal Point Slide Profiler

Performed by: Mauritz Hill

Type: New ☐ Retrospective ☒

Quantitative ☐ Qualitative/Semi qualitative ☐

No. of Samples selected: 10 ☐ 20 ☐ 40 ☐ 100 ☐ more than 100

Samples type: Patient samples ☒ IQC ☐ EQC ☐

Acceptance criteria quantitative:

Manufacturers specifications ☐
Biological variation ☐
CLIA ☐
RCPA ☐
Other.....

Acceptance criteria qualitative:

Cohen's Kappa agreement test
McNemar test for Symetry
Sensitivity/positive agreement/PVP ☐
Specificity/negative agreement/PVN ☐
Literature review ☒
Other .....

Experiments run:

Replication within run (imprecision) ☐
Replication between run (imprecision) ☐
Alternate Comparison ☐
Recovery of known values ☒
Linearity ☐
Reference range verification ☐

Current IQC practice Daily ☒ Weekly ☐ Monthly ☐ New Lot. No./Kit ☐

Special environmental conditions: Yes ☐ No ☐ N/A ☒

.....

Equipment used: Focal Point Slide Profiler
GS Reviewstation
Olympus microscopes

Reviewed and Accepted by: C van Wyk

Date 15/3/2011

Reference: GPL1530, EP Evaluator™ by David G Rhoads

IQC 2010 (Gynae)

QC	MISSED														MIS-DIAGNOSED								
	T'S	CANDIDA	OTHER PATHOG	ECC	EMC	ASCUS	HPV	LSIL N>I	HSIL N>J	SQ CA	AD CA	RAD	OTHER	MISSED TOTAL	OVER DIAG 1°	OVER DIAG 2	UNDER NOW LSIL E/F>I	UNDER NOW HSIL/CA F/I>J	over/under Total	CASES SCREENED	% MISSED	% over/under
MONTHLY																						
JANUARY	14	22	2	20	5	21	0	30	5	0	0	0	1	**120**	19	9	13	2	43	**3354**	3.58%	
FEBRUARY	17	18	1	26	8	27	5	24	1	0	0	0	0	**127**	26	11	2	7	46	**4110**	3.09%	
MARCH	16	22	0	17	11	23	0	33	5	0	0	0	1	**128**	23	6	6	8	43	**5172**	2.47%	
APRIL	10	15	3	13	3	19	0	6	1	0	0	0	0	**70**	6	7	1	2	16	**1718**	4.07%	
MAY	26	15	3	22	11	15	0	36	3	0	0	0	0	**131**	32	12	3	4	51	**2204**	5.94%	
JUNE	12	26	1	12	6	9	0	30	3	1	0	0	0	**100**	25	5	5	9	44	**3152**	3.17%	
JULY	13	8	2	7	3	8	1	27	4	0	0	0	0	**73**	17	3	3	4	27	**2508**	2.91%	
AUGUST	10	14	0	31	5	16	0	40	4	0	0	0	0	**120**	9	2	2	9	22	**2766**	4.34%	
SEPTEMBER	3	25	4	18	4	11	1	18	4	0	0	0	0	**88**	7	3	2	6	18	**4008**	2.20%	
OCTOBER	19	32	2	27	4	19	0	33	3	0	0	0	0	**139**	16	8	6	4	34	**1944**	7.15%	
NOVEMBER	22	22	3	42	13	20	0	60	6	0	0	0	0	**188**	13	3	11	18	45	**5367**	3.50%	
DECEMBER	19	45	3	28	10	37	1	53	5	0	0	0	0	**201**	26	8	5	12	51	**3885**	5.17%	
TOTAL	181	264	24	263	83	225	8	390	44	1	0	0	2	**1485**	219	77	59	85	440	**40188**	3.70%	
% on Total abnormals						33.7			42					**668**						**40188**		
% on Total cases						0.6	%		196.4%					1.7	%				1.1	%		
									Sil vs Ascus													

15/3/2011

23 June 2010

MAILED

Ms G Neethling
Cytology lab, NHLS
10th Floor, Western Side
Tygerberg Hospital

Dear Ms Neethling

Automated Sputum Screening using the BD FocalPoint Slide profiler: Correlation with Transbrochial and Transthoracic Needle Aspirates.

ETHICS REFERENCE NO: N10/04/135

RE : APPROVAL

A panel of the Health Research Ethics Committee reviewed this project on 11 May 2010; the above project was approved on condition that further information is submitted.

This information was supplied and the project was finally approved on 23 June 2010 for a period of one year from this date. This project is therefore now registered and you can proceed with the work.

Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report (obtainable on the website of our Division: www.sun.ac.za/rds should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit.
Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372
Institutional Review Board (IRB) Number: IRB0005239
The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

Approval Date: 23 June 2010

Expiry Date: 23 June 2011

Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences

Verbind tot Optimale Gesondheid · Committed to Optimal Health
Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
Posbus/PO Box 19063 · Tygerberg 7505 · Suid-Afrika/South Africa
Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352

Yours faithfully

MS CARLI SAGER
RESEARCH DEVELOPMENT AND SUPPORT
Tel: +27 21 938 9140 / E-mail: carlis@sun.ac.za
Fax: +27 21 931 3352

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Ethics Letter

25-Mar-2013

Ethics Reference #: N10/04/135
Title: Automated Sputum Screening using the BD FocalPoint Slide profiler: Correlation with Transbrochial and Transthoracic Needle Aspirates.

Dear Mrs Greta NEETHLING,

At a meeting of the Health Research Ethics Committee that was held on 20 March 2013, the progress report for the abovementioned project has been approved and the study has been granted an extension for a period of one year from this date.

Please remember to submit progress reports in good time for annual renewal in the standard HREC format.

Approval Date: 20 March 2013 Expiry Date: 20 March 2014

If you have any queries or need further help, please contact the REC Office 0219389207.

Sincerely,

REC Coordinator
Mertrude Davids
Health Research Ethics Committee 2

Pre-bronchoscopy Sputum

** Attention lab: Do not prepare.
Please inform C. Cook or G. Neethling

Cytology Laboratory
C 10C
Tygerberg Hospital
Tel: (021) 938 4202
(021) 938 4200

FOR LAB LABEL

SPECIMEN RECEPTION

Sputum Sample Number:	Ethics Reference no: N10/04/135
Clinician's name and signature:	Date of specimen collection:

SPECIMEN PREPARATION

Type of specimen: TA1 – sputum	Location request: X5085 Account no: ZSTU0420	Preparation date:
Origin of specimen: T32 – Lung	Specimen weight:	Prepared by:

LABORATORY FINDINGS – Diagnostic categories

Categories	Screener	Checker	FocalPoint	Adequacy (per square cm)	FNA result
I Benign II Atypical, III Suspicious IV Malignant V Inadequate	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode.....	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode.....	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode: GK2	☐ 0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ > 21	☐ I ☐ II ☐ III ☐ IV ☐ V StaffCode:.....
Type of atypia/ malignancy	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other	N/A	☐ squamous ☐ adeno ☐ small cell ☐ large cell ☐ mixed ☐ other .....

Correlation notes:

– ***Normal***: No abnormal cells present and adequate. Inflammation or reactive changes may be present. Include benign squamous metaplasia: cells are roun d seen in cobble-stone arranged fragments. Cells have a well defined cell border and small to medium sized, smooth, central nuclei. Nuclear:cytoplasmic (N/C atio fairly low.

– ***Atypical***: Only a few atypical cells present, uncertain potential. Cells have some pleomorphism with enlarged nuclei and coarsely granular chromatin. Th ytoplasm is decreased and may exhibit keratinisation.

– ***Suspicious***: Highly atypical cells present, suspicious of malignancy. Adequacy is not important. Criteria are quantitatively or qualitatively not enough for a agnosis of malignancy. Cells are pleomorphic with enlarged nuclei, irregular membranes and coarsely granular chromatin. The cytoplasm is decreased and ay exhibit keratinisation or vacuolisation.

– ***Malignant***: Malignant cells present. Adequacy not important. Cellular, with marked pleomorphism and a variable N/C ratio. Nuclear chromatin tends to b regularly distributed, coarsely granular and hyperchromatic. Pearls and keratinisation may be present, balls and other glandular arrangements, or even india ling and moulding.

– ***Inadequate***: This category is composed of specimens where no or little cellular material is obtained, the material is artifactually distorted by bloo nflammation or poor preservation such that a diagnosis cannot be rendered. Also included is sputa not representative of "deep lung" (none, or too few alveol acrophages present).

CYTOLOGY REPORT: FOR LABORATORY USE ONLY

RECEIVED Date D D M M Y Y
Prepared by
Time H
Volume M L

NUMBER OF SLIDES PAP MGG
SPECIAL STAINS: DATE

Macroscopic

URGENT RESULTS CONVEYED TELEPHONICALLY / FAXED
TO:
BY: DATE: / / TIME: H

MALIGNANCY 1 = Absent 2 = Present 3 = Uncertain — 1 2 3

DIAGNOSIS & ADDITIONAL FINDINGS

PATHOGENS — P / P / P

ENDOCERVICAL COMPONENT — Y N N/A

ADEQUACY OF SMEAR — V

COMMENTS

FREE TEXT (prints on report)

RECOMMENDATIONS — A / A / A

FOLLOW UP DATE D D M M Y Y
LETTER NUMBER

DIAG CODE STAFF CODE
EXAMINED BY
RAPID RESCREENED BY
CHECKED BY
PATHOLOGIST

ICD10 - CODE

OPINIONS — DIAG CODE STAFF CODE

RE-EVALUATION:
SLIDE NO.
SLIDE NO.

QC CODE

WORKSHOP CODE

FOLLOW UP Y LETTER Y IN MONTHS

COMMENTS

EXAMINED BY:
REPORT DATE: / /
RAPID RESCREENED BY:
CHECKED BY:
PATHOLOGIST:

T
T
T
T
T

Patient's Name: _____

Folder Number: _____

Date of the collection/procedure: _____

Diagnostic yield of Sputum using the FocalPoint^{tm} Slide Profiler in patients with a pulmonary mass lesion.

Investigators (for the Lung Unit): Maurizio Bernasconi(Phone 072 8280 448) and Annari Du Plessis (0827868361).

1.Checklist (everything done?):

- Informed consent signed [ ]
- CT chest on PACS? Y [ ], N [ ]
  If not, please please fill the posterior page (radiological features a well)!!
- Copy of white card (after the procedure [ ]

2. Sputum collection: Sputum Sample Number _____

Collect **3 non-induced sputum** in the provided bottle before the planned procedure.

Patient able to produce a valid sputum? Y [ ], N [ ], if not, no further information are needed (but keep this sheet for statistical purpose)

The sputum bottle will be collected by the cytologist attending the theater and brought to the cytological laboratory!

3. Pulmonologist evaluation prior the procedure:

Likelihood of cancer (subjective pretest probability of cancer, visual analogue scale, please tick)?

very unlikely (0%) [----|----|----|----|----|----|----|----|----|----] (100 %) **very likely**

4. Diagnostic procedure performed:

Date of the procedure: ____/____/20____

[ ] Bronchoscopy:

- Vocal cord paralysis? Y [ ], N [ ].

- Endobronchial tumor? Y [ ], N [ ]. If yes continue below:

--- Characteristics of the tumor? 1. Ulcerated [ ], 2. Exophytic [ ], 3. Mucosal thickening [ ]

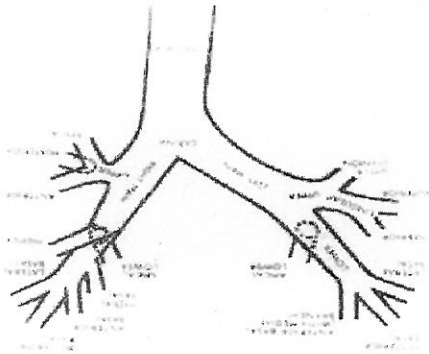
--- Localisation? RUL [ ], ML [ ], RLL [ ], LUL [ ], Lingula [ ], LLL[ ]. Please drawn on the draft (see example).

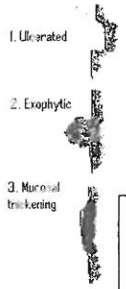
--- Complete occlusion of an ostium? Y [ ], N [ ]

--- Stenosisof the airways?

_____

[ ] TTNA [ ] Tru-Cut Biopsy [ ] Thoracentesis [ ] CT guided FNA [ ] FNA other localistion, which? _____





For the more motivated ..... please fill although the posterior site (prior the procedure)! Thanks!!!

This page can although be completed retrospectively by the investigators .....

Patient's Name: _____

Folder Number: _____

1. Patient's Characteristics and clinical features:

Prev. malignancy? Y [ ], N [ ] which? _____.

Prev. pulm. TB? Y [ ], N [ ].

Smoking: never [ ], active [ ], former [ ]. Total ____P/Y.

Cough? [ ], chronic ( ), new ( ) since ___d/w/m

Pleuritic chest pain? Y [ ] N [ ]; since ___d/w/m.

Weight loss? mild ( ), important ( ), if poss. ___kg in ___d/w/m

Any increasing dyspnea? Y [ ], N [ ].

Hemoptysis Y[ ], N [ ]; since ___d/w/m.

Hoarseness? Y [ ], N [ ]; since ___d/w/m

Superior vena cava syndrome? Y [ ], N [ ].

Pancoast's syndrome? Y [ ], N [ ]; since ___d/w/m

Neurologic symptoms? Y [ ], N [ ]; since ___d/w/m

others:_____

Paraneoplastic phenomena? Y [ ], N [ ]., since ___d/w/m.

2. Radiological features (on contrasted Chest/upper Abdomen CT scan):

Lesion localisation: RUL [ ], ML [ ], RLL [ ], LUL [ ], LLL [ ] (more than 1 possible). **Biggest Ø Size** = _____mm.

Lesion characteristics? round [ ], calcified [ ], spiculated [ ], cavitating [ ], aerobronchogramm in the lesion [ ],endobronchial lesion [ ].

Atelectasis? Y [ ], N [ ]. **Pleura effusion?** Y [ ], N [ ]. **Distant metastasis?** Y [ ], N [ ], where?_____

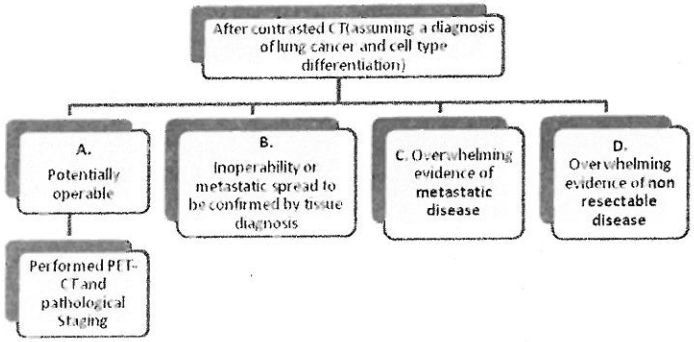
3. After the points 1 and 2 (previous diagnostic procedure!!):

A. Do imaging (CT) alone present overwhelming evidence of **non resectable disease(T4)**? Y [ ], N [ ]

B. Do imaging (CT) alone present overwhelming evidence of **metastatic disease?** Y [ ], N, [ ]

Please take into account that overwhelming evidence of metastatic disease means a clear M1b stage or a cytological proven malign pleura effusion (M1a).

C. Please circle the appropriate clinical situation after the CT scan assuming a diagnosis of lung cancer (including a definitive cell type differentiation).



Diagnostic procedure performed (in order to reach a diagnosis/staging):

[ ] Bronchoscopy: [ ] TBNA: number of passes? ____, [ ] brush, [ ] TBB, [ ] EBB, [ ] BW

Result of ROSE: _____

[ ] TTNA, number of passes? ____ , Result of ROSE: _____

[ ] Tru-Cut Biopsy

[ ] FNA other localisation, number of passes? _____

[ ] Thoracentesis **[ ] previous sputum** **[ ] CT guided FNA** **[ ] others,** _____

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:
Automated Sputum Screening using the BD FocalPoint^{tm} Slide Profiler: Correlation with Transbronchial and Transthoracic Needle Aspirates.

REFERENCE NUMBER: N10/04/135

PRINCIPAL INVESTIGATOR: Ms Greta S Neethling

ADDRESS: Anatomical Pathology
Tygerberg Hospital

CONTACT NUMBER: 0846683836 or 0219385356

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Committee at Stellenbosch University** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- This study will only be conducted at Tygerberg Hospital's bronchoscopy unit. A total of 200 participants will be recruited.
- The aim of this project is to determine if sputums (lung mucous) would be able to be screened by a machine, and whether the sputum result and the bronchoscopy (procedure that you are here for today) result is the same. This method might improve sputum screening and make it faster too.
- The sputum that you would submit will be prepared, and screened by a machine to try and detect any abnormal cells. This result will be correlated with the result of the bronchoscopy.

Why have you been invited to participate?

- All patients attending the lung unit for a bronchoscopy, would be asked to give a sputum sample before the procedure.

What will your responsibilities be?

- None

Will you benefit from taking part in this research?

- None

Are there in risks involved in your taking part in this research?

- None. It is safe to produce a sputum.

Who will have access to your medical records?

- The information collected will be treated as confidential and protected. If it is used in a publication or a thesis, your identity will remain anonymous. It is only 5 researchers that will have access to your identity, and upon arrival in the lab your name will be removed from your sample and a number will be put on instead. This number will be used from then onwards.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

- You will not suffer any injury.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study and there will be no costs involved for you, if you do take part.

Is there any thing else that you should know or do?

- You can contact Greta Neethling at 021 9385356 if you have any further queries or encounter any problems.
- You can contact the Committee for Human Research at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study doctor.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ..... agree to take part in a research study entitled *(insert title of study)*.

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) ..... on (*date*) ..... 2005.

....

Signature of participant **Signature of witness**

Declaration by investigator

I, Greta Neethling, declare that:

- I explained the information in this document to .....
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (*If a interpreter is used then the interpreter must sign the declaration below.*

Signed at (*place*) ..... on (*date*) ..... 2005.

....

Signature of investigator **Signature of witness**

Declaration by interpreter

I (*name*) ..... declare that:

- I assisted the investigator (*name*) ..... to explain the information in this document to (*name of participant*) ..... using the language medium of Afrikaans/Xhosa.

- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) ..... on (*date*) .....

....

Signature of interpreter **Signature of witness**

DEELNEMERINLIGTINGSBLAD EN -TOESTEMMINGSVORM

TITEL VAN DIE NAVORSINGSPROJEK:
Geautomatiseerde sputum ontleding deur gebruik te maak van die BD FocalPoint^{tm} Slide Profiler. 'n Korrelasie met TT/TB naald aspirasies.

VERWYSINGSNOMMER: N10/04/135

HOOFNAVORSER: Ms Greta Neethling

ADRES: Anatomiese Patologie, Tygerberg Hospitaal.

KONTAKNOMMER: 0846683836 of 021 9385356

U word genooi om deel te neem aan 'n navorsingsprojek. Lees asseblief hierdie inligtingsblad op u tyd deur aangesien die detail van die navorsingsprojek daarin verduidelik word. Indien daar enige deel van die navorsingsprojek is wat u nie ten volle verstaan nie, is u welkom om die navorsingspersoneel of dokter daaroor uit te vra. Dit is baie belangrik dat u ten volle moet verstaan wat die navorsingsprojek behels en hoe u daarby betrokke kan wees. U deelname is ook **volkome vrywillig** en dit staan u vry om deelname te weier. U sal op geen wyse hoegenaamd negatief beïnvloed word indien u sou weier om deel te neem nie. U mag ook te eniger tyd aan die navorsingsprojek onttrek, selfs al het u ingestem om deel te neem.

Hierdie navorsingsprojek is deur die Gesondheids Navorsing Etiese Kommitee van die Universiteit Stellenbosch **goedgekeur en sal uitgevoer word volgens die etiese riglyne en beginsels van die Internasionale Verklaring van Helsinki en die Etiese Riglyne vir Navorsing van die Mediese Navorsingsraad (MNR).**

Wat behels hierdie navorsingsprojek?

- *Die studie sal slegs by Tyegrberg hospitaal se long eenheid plaasvind. 200 Patiente sal ingesluit word.*
- *Die studie gaan probeer bepaal of sputum (long slym) deur 'n nuwe masjien geontleed kan word, en of die resultaat dieselfde is as die van die naald aspirasie. Hierdie nuwe metode kan die ontleding beter en vinniger maak.*
- *Die sputum wat u sal gee, sal voorberei word en deur die masjien ontleed word om enige moontlike abnormale selle te identifiseer.*

Waarom is u genooi om deel te neem?

- *Al die patiente wat die long eenheid bywoon vir 'n naald aspirasie, word vriendelik versoek om ook 'n sputum monster te produseer vir die studie.*

Wat sal u verantwoordelikhede wees?

- *Geen.*

Sal u voordeel trek deur deel te neem aan hierdie navorsingsprojek?

- *Geen*

Is daar enige risiko's verbonde aan u deelname aan hierdie navorsingsprojek?

- *Geen, dit is heeltemal veilig om slym uit te hoes.*

Wie sal toegang hê tot u mediese rekords?

- *Die informasie van die studie is heeltemal konfidentieel. Indien dit in 'n publikasie gebruik gaan word, sal u anoniem bly. Wanneer die monster in die lab arriveer, kry dit 'n nommer wat dan voortaan gebruik sal word i.p.v u naam en van.*

Wat sal gebeur in die onwaarskynlike geval van 'n besering wat mag voorkom as gevolg van u deelname aan hierdie navorsingsprojek?

- *U sal geen besering op doen nie.*

Sal u betaal word vir deelname aan die navorsingsprojek en is daar enige koste verbonde aan deelname?

U sal nie betaal word vir deelname aan die navorsingsprojek nie, deelname aan die navorsingsprojek sal u niks kos nie.

Is daar enigiets anders wat u moet weet of doen?

- U kan Ms Greta Neethling kontak by tel 021 9385356 indien u enige verdere vrae het of enige probleme ondervind.
- U kan die Gesondheids Navorsing Etiese Kommitee kontak by 021-938 9207 indien u enige bekommernis of klagte het wat nie bevredigend deur u studiedokter hanteer is nie.
- U sal 'n afskrif van hierdie inligtings- en toestemmingsvorm ontvang vir u eie rekords.

Verklaring deur deelnemer

Met die ondertekening van hierdie dokument onderneem ek, ..., om deel te neem aan 'n navorsingsprojek getiteld (*Titel van navorsingsprojek*).

Ek verklaar dat:

- Ek hierdie inligtings- en toestemmingsvorm gelees het of aan my laat voorlees het en dat dit in 'n taal geskryf is waarin ek vaardig en gemaklik mee is.
- Ek geleentheid gehad het om vrae te stel en dat al my vrae bevredigend beantwoord is.

- Ek verstaan dat deelname aan hierdie navorsingsprojek **vrywillig** is en dat daar geen druk op my geplaas is om deel te neem nie.
- Ek te eniger tyd aan die navorsingsprojek mag onttrek en dat ek nie op enige wyse daardeur benadeel sal word nie.
- Ek gevra mag word om van die navorsingsprojek te onttrek voordat dit afgehandel is indien die studiedokter of navorser van oordeel is dat dit in my beste belang is, of indien ek nie die ooreengekome navorsingsplan volg nie.

Geteken te *(plek)* ..... op *(datum)* ..... 2005.

....

Handtekening van deelnemer **Handtekening van getuie**

Verklaring deur navorser

Ek *(naam)* ..... verklaar dat:

- Ek die inligting in hierdie dokument verduidelik het aan .....
- Ek hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek tevrede is dat hy/sy al die aspekte van die navorsingsprojek soos hierbo bespreek, voldoende verstaan.
- Ek 'n tolk gebruik het/nie 'n tolk gebruik het nie. *(Indien 'n tolk gebruik is, moet die tolk die onderstaande verklaring teken.)*

Geteken te *(plek)* ..... op *(datum)* ..... 2005.

....

Handtekening van navorder **Handtekening van getuie**

Verklaring deur tolk

Ek *(naam)* ..... verklaar dat:

- Ek die navorser *(naam)* ..... bygestaan het om die inligting in hierdie dokument in Afrikaans/Xhosa aan *(naam van deelnemer)* ..... te verduidelik.

- Ons hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek 'n feitelik korrekte weergawe oorgedra het van wat aan my vertel is.
- Ek tevrede is dat die deelnemer die inhoud van hierdie dokument ten volle verstaan en dat al sy/haar vrae bevredigend beantwoord is.

Geteken te *(plek)* ..... op *(datum)* ..... 2005.

....

Handtekening van tolk **Handtekening van getuie**

INCWADANA ENGOLWAZI NGOMTHATHI-NXAXHEBA KUNYE NEFOMU YEMVUMELWANO

ISIHLOKO SEPROJEKTHI YOPHANDO:

Kukuxilongwa kwezikhohlela ngokusebenzisa umatshini omtsha obizwa ngokuba yi"BD Focal point slide profiler'
Ukujonga unxibelelwana phakathi kweziphumo zesikhohlela kwakunye neTransbronchial FNA (uphayiphu omncinci onenaliti othi ufakwe empumleni okanye emlonyeni ukuya kwimibhobho emincinci engaphakathi kwiziphunga ukuze kufumaneke izakha mzimba zesifo somhlaza); ne Transthoracic FNA (inaliti encinci ethi ifakwe apho kukhona iqhuma khona, lingaba sesifubeni ngandle okanye ngaphakathi; okanye libe semqolo ukuze kufumaneke izakhamzimba zezifo somhlaza.

INOMBOLO YONXULUMANO: N10/04/135

UMPHANDI OYINTLOKO: Ms Greta Neethling

IDILESI: Anatomical Pathology, Tygerberg Hospital

INOMBOLO YOQHAGAMSHELWANO: 0846683836, 0219385356

Uyamenywa ukuba athathe inxaxheba kwiprojekthi yophando. You are being invited to take part in a research project. Nceda thatha ixesha lokufunda ulwazi oluvezwe apha, oluzakuthi luchaze iinkcukacha zale projekthi. Nceda buza nayiphina imibuzo emalunga nayiphina indawo ongayiqondiyo ngokupheleleyo kubasebenzi besi sifundo okanye kugqirha. Kubaluleke kakhulu ukuba waniliseke ngokupheleleyo yinto yokuba ucacelwe kakuhle ukuba yintoni ebangwa sesi sifundo kwaye ungabandakanyeka njani. Kwakhona, ukuthatha kwakho inxaxheba **kungentando yakho ngokupheleleyo** kwaye ukhululekile ukuba ungarhoxa ekuthatheni inxaxheba. Ukuba uthi hayi, oku akusayi kuchaphazela ukungavumi kwakho nangayiphina indlela. Ukwakhululekile ukuba uyeke kwesi sifundo naninina, nkqu nokokuba uyavuma ukuthatha inxaxheba ekuqaleni.

Olu phando luvunywe ziinqobo ezisesikweni **iikomiti yesebe lezempilo yoPhando Lomntu kwiYunivesithi yaseStellenbosch** kwaye luzakwenziwa ngokwemigaqo esesikweni lophando elamkelekileyo kwiSaziso sehlabathi sika-Helsinki, iMigaqo eLungileyo yoMzantsi Afrika yokuSebenza eKliniki kunye neBhunga lezoPhando ngamaYeza (MRC) iMigaqo yeNqobo yezoPhando.

Simalunga nantoni esi sifundo sophando?
Oluphando luyakuba luqhutywelwa kwisibhedlele saseTygerberg, kwicandelo lezifo sesifuba.
Malunga namakhulu amabini ezigulana ziyakuthi zicelwe ukuba zithabathe inxaxheba koluphando.
Iinjongo zoluphando kukujonga nokuphanda ukuba singakwazi ukuxilonga isikhohlela ngalomatshini umtsha kusini na kwaye singakwazi ukungqamanisa iziphumo zesikhola kwakunye nezenaliti encinci ethi ifakwe eziphungeni ukuze kutsalwe izakha mzimba zesifo somhlaza. Ie nkqubo yoluphando iyakuthi iphucule ukuxilongwa kwezikhohlela ze neziphumo zifumaneke ngokukhawulezileyo.
Lo matshini omtsha uyakuthi uphucule izinga lokuxilongwa kwezikhohlela kwaye iziphuma ziyakufumaneka ngokukhawuleza

Isikhohlela siya kuthi sithunyelwe elaboratri apho siyakuthi silungiselelwe uvavanyo ze sixilongwe ngalomatshini umtsha ngethemba lokuba lomatshini uyakuthi ukwazi ukuziqwalasela nokuzibona ezizakhamzimba zesifo somhlaza kwisikhohlela.
Iziphumo zesikhohlela ziyakuthi zingqanyaniswe/zifaniswe kwakunye neziphumo zenaliti encinci ebithe yafakwa esifubeni.

Kutheni umenyiwe ukuba uthathe inxaxheba?
Zonke izigulana ezisesibhedlele kwicandelo lezifo zesifuba eziya kwibroncoscopy, ziyakuthi zicelwe ukuba zinikezele ngesikhokhela phambi kokuba zenziwe ibroncoscopy.
Ngoluphando sifuna ukujonga nokuqwalasela ukuba lo matshini uyakuthi ukwazi ukuxilonga isikhohlela.

Luyakuba yintoni uxanduva lwakho?

Alukho uxanduva oyakuthi ube nalo

Ingaba uza kuzuza ekuthatheni inxaxheba kolu phando?

Ukuba umatshini uthe wazibona izakha mzimba zesifo somhlaza kwisikhohlela sakho awusayi kuba sayenza ibroncoscopy; kwaye ukuba kwibroncoscopy izakhamzimba abathe bazitsala ngenaliti azoneli ukukhupha iziphumo eziqinisekileyo; iziphumo zesikhokhela ziyakuba luncedo ukuze ufumane unyango olukhawulezileyo.

Ingaba zikho iingozi ezibandakanyekayo ekuthatheni kwakho inxaxheba kolu phando?
Hayi azikho, kukhuselekile ukukhupha isikhohlela

Ukuba awuvumi ukuthatha inxaxheba, loluphi olunye unyango onalo?

Ingxelo ngeziphumo zakho ziyakuba yimfihlelo kwaye zikhuseleke.ukuba zithe zasetyenzisa njengemizekelo kubhalo lwencwadi iincukacha zakho (umzekelo; igama, idilesi) aziyikusetyenziswa.
Ngabaqhubi boluphando abahlanu abayakuthi bakwazi ukufikelela kwinkcukacha zakho, kwaye xa ufika elaboratri kuyathi kusetyenziswa inombolo eyakusetyenziswa njengegama lakho.

Ngubani uza kufumana ingxelo yakho yamayeza?

Hayi, awusayi kuhlawulwa ngokuthabatha inxaxheba kwaye akukho zindleko zibandakanya wena xa uthe wathabatha inxaxheba.

Kuza kwenzeka ntoni kwimeko yesiganeko esingalindekanga sokwenzakala ngenxa yokuthatha kwakho inxaxheba kwesi sifundo sophando?

- *Cacisa imiba enxulumene nentlawulo ye-inshorensi ukuba ikhona. Ukuba kukho iindawo ezibandakanyekayo ezithengisa amayeza ingaba intuthuzelo izakuhamba ngokwemigaqo ye-ABPI? (Imigaqo yentuthuzelo enxulumene nokwenzakala kuphando lweManyano yaseBrithane yezoRhwebo lwamaYeza ethathwa njengekwizinga lehlabathi legolide). Ukuba ewe, nceda bandakanya iinkcukacha apha. Ukuba hayi, cacisa ke ukuba yeyiphi intuthuzelo eyakubakhona kwaye phantsi kweziphi iimeko..*

Ingaba uza kuhlawulwa ngokuthatha inxaxheba kwesi sifundo kwaye ingaba kukho iindleko ezibandakanyekayo?

Hayi awusayi kuhlawulwa ngokuthatha inxaxheba kwesi sifundo kodwa isithuthi sakho neendleko zokutya ziza kuhlawulelwa kundwendwelo ngalunye lwesifundo. Akusayi kubakho zindleko ezibandakanyelwa wena, ukuba uthatha inxaxheba.

Ingaba ikho enye into ekumele uyazi okanye uyenze?

- Ungaqhgamshelana Greta Neethling kule inombolo yomnxeba 0219385356 ukuba unemibuzo engaphaya okanye uhlangabezana neengxaki.
- Ungaqhagamshelana neKomiti yoPhando Lomntu kwa-021-938 9207 ukuba unenkxalabo okanye izikhalazo ezingasonjululwanga kakuhle ngugqirha wakho wesifundo.
- Uza kufumana ikopi yolu lwazi kunye nefomu yemvumelwano ukwenzela iingxelo zakho.

Isifungo somthathi-nxaxheba

Ngokuytyikitya ngezantsi, Mna ..... ndiyavuma ukuthatha inxaxheba kwisifundo sophando semfuzo esibizwa ngokuba *(faka ishloko sesifundo)*.

Ndazisa ukuba:

- Ndilufundile okanye ndalufunda olu lwazi kunye nefomu yemvumelwano kwaye ibhalwe ngolwimi endiliciko nendikhululekileyo kulo
- Bendinalo ithuba lokuba ndibuze imibuzo kwaye yonke imibuzo yam iphendulwe ngokwanelisayo.
- Ndiyakuqonda ukuba ukuthatha inxaxheba kolu phando kube **kukuzithandela kwam** kwaye andikhange ndinyanzelwe ukuba ndithathe inxaxheba.
- Ndingakhetha ukusishiya isifundo naninina kwaye andisayi kohlwaywa okanye uqal' ugwetywe nangayiphi indlela.
- Usenokucelwa ukuba usishiye isifundo phambi kokuba siphele, ukuba ugqirha wesifundo okanye umphandi ukubona kuyinzuzo kuwe, okanye ukuba andisilandeli isicwangciso sesifundo, ekuvunyelenwe ngaso.

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2005.

....

Umtyikityo womthathi-nxaxheba **Umtyikityo wengqina**

Isifungo somphandi

Mna *(igama)* Greta Neethling ndiyafunga ukuba:

- Ndilucacisile ulwazi olu kweli xwebhu ku-.....

- Ndimkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.
- Ndiyaneliseka kukuba uyakuqonda ngokwanelisayo konke okumalunga nophando okuxoxwe ngasentla.
- Ndisebenzise/andisebenzisanga toliki. (*Ukuba itoliki isetyenzisiwe kumele ityikitye isaziso ngezantsi.*

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2005.

....

Umtyikityo womphandi **Umtyikityo wengqina**

Isifungo setoliki

Mna *(igama)* ..... ndazisa ukuba:

- Ndicende umphandi (*igama*) ..... Ekucaciseni ulwazi olu lapha kweli xwebhu ku-(*igama lomthathi-nxaxheba*) ..... ndisebenzisa ulwimi lwesiAfrikaans/lwesiXhosa.
- Simkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.
- Ndimxelele eyona nto iyiyo malunga nokunxulumene nam.
- Ndiyaneliseka kukuba umthathinkxaxheba ukuqonda ngokupheleleyo okuqulathwe loluxwebhu lwemvumelwano eyazisiweyo kwaye nemibuzo yakhe yonke iphendulwe ngokwanelisayo.

Kutyikitywe e-(*indawo*) ..... ngo-(*usuku*) ..... 2005.

....

Umtyikityo wetoliki **Umtyikityo wengqina**

APPENDIX F:

Proposed IASLC/ATS/ERS Classification for Bronchogenic Carcinoma (Cytology)

2004 WHO Classification	SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	*Morphologic adenocarcinoma patterns clearly present:* Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification) Comment: If pure lepidic growth – mention an invasive component cannot be excluded in this small specimen
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloid pattern
Signet ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
No 2004 WHO counterpart – most will be solid adenocarcinomas	*Morphologic adenocarcinoma patterns not present (supported by special stains):* Non-small cell carcinoma, favor adenocarcinoma
SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	*Morphologic squamous cell patterns clearly present:* Squamous cell carcinoma
No 2004 WHO counterpart	*Morphologic squamous cell patterns not present (supported by stains):* Non-small cell carcinoma, favor squamous cell carcinoma
SMALL CELL CARCINOMA	Small cell carcinoma
LARGE CELL CARCINOMA	Non-small cell carcinoma, not otherwise specified (NOS)
Large cell neuroendocrine carcinoma (LCNEC)	Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNEC
Large cell carcinoma with NE morphology (LCNEM)	Non-small cell carcinoma with NE morphology (negative NE markers) – see comment Comment: This is a non-small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.
ADENOSQUAMOUS CARCINOMA	*Morphologic squamous cell and adenocarcinoma patterns present:* Non-small cell carcinoma, with squamous cell and adenocarcinoma patterns Comment: this could represent adenosquamous carcinoma.
No counterpart in 2004 WHO classification	*Morphologic squamous cell or adenocarcinoma patterns not present but immunostains favor separate glandular and adenocarcinoma components* Non-small cell carcinoma, NOS, (specify the results of the immunohistochemical stains and the interpretation) Comment: this could represent adenosquamous carcinoma.
Sarcomatoid carcinoma	Poorly differentiated NSCLC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)

IASLC, International Association for the Study of Lung Cancer; ATS, American Thoracic Society; ERS, European Respiratory Society; WHO, World Health Organization. NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; TTF, thyroid transcription factor.

Appendix G:

Comprehensive Morphologic Criteria Lists used in FocalPoint Algorithms

Cell type	Nuclear area (μm2)	Nuclear area (pixels)	Cell area (μm2)	Cell area (pixels)	Relative nuclear area (%)
Intermed Sq	23–49	76–162	995–2145	3289–7091	1.5–7.5
Endometrial	32–41	106–136	55–70[a]	182–231	59[a]
Sq Metapl (P)	44–57	145–188	176–226	582–747	22–27
Sq Metapl (I)	38–63	126–208	272–365	899–1207	14–19
Sq Metapl (M)	42–61	139–202	588–697	1944–2304	7 +/–
Endometrial hyperplasia	35–56	116–185	65–123	215–407	46–54
Atypical endometrial hyperplasia	42–63	139–208	84–149	278–492	42–50
Endometrial Adenoca (I)	60	198	132	436	45
Endometrial Adenoca (II)	67	221	151	499	44
Endometrial stromal cell	38–54	127–178	45–72	149–238	75–84
Endocervical	41–61	136–202	148–228 (cols)	489–754	26–35
Atrophic squamous	50	165	325	1074	15
SC CIS	60–80	198–264	95	314	63–84
Sq CA, small cell type	52–78	172–258	132–206[b]	436–681[b]	35–45
AISMT	60–70	198–231	100–175	330–578	40–60
Atypical immature metapl repair	59	195	270	892	21
ASCUS	75–125	248–413	250–450	826–1488	30–35
Atypical mature Sq metapl repair	75	248	270	892	28
AIS	34–102	112–337	67–219	221–724	37–59
ICIS	80–120	264–397	189	625	42–63
K Sq CA	49–105	162–347	168–382	555–1263	28–29
NK Sq CA	58–118	192–390	187–325	618–1074	32–36
Endocervical Adenoca (I–III)	73–97	241–321	167–201	552–664	44–50
Endometrial Adenoca (III–IV)	85–92	281–304	176–198	582–654	46–48
Atypical squamous repair	75	250	350	1157	21
Endocervical atypia (reactive)	100–200	330–661	300–600	992–1983	33
Atypical endocervical repair	144–201	476–664	350–530	1157–1752	39
Dysplasia (M)	121–191	400–631	386–601	1276–1987	31
Dysplasia (K)	126–210	416–694	637–1453	2106–4803	16
Dysplasia (NK)	146–210	482–694	1063–1465	3514–4843	14
Endocervical Adenoca (IV)	165	545	314	1038	53
LC CIS	150–200	496–661	435	1438	38
Acute radiation	140–175	463–578	2000–6000	6612–19,835	3 +/–
ExtraUT	Depends	Depends	Depends	Depends	Depends
Sarcomas & other rare lesions	Depends	Depends	Depends	Depends	Depends

Intermed: intermediate; Sq: squamous; Metapl: metaplastic; P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; SC: small cell; CIS: carcinoma in situ; CA: carcinoma; AISMT: atypical cells of immature metaplastic type; ASCUS: atypical squamous cells of undetermined significance; AIS: adenocarcinoma in situ; ICIS: intermediate carcinoma in situ; K: keratinizing; NK: nonkeratinizing; LC: large cell; Extraut: extrauterine.

[a] Cytoplasmic boundaries intact.

[b] Frequent nuclear distortion.

Cell type	Nuclear hyperchromasia	Chromatin particles	Chromatin distribution	Micronucleoli	Macronucleoli	Irregular nucleoli
Intermed Sq		Fine	Even			
Endometrial		Fine	Even			
Sq Metapl (P)		Fine	Even			
Sq Metapl (I)		Fine	Even			
Sq Metapl (M)		Fine	Even			
Endometrial hyperplasia		Fine	Even			
Atypical endometrial hyperplasia	+	Fine	Even	25%		
Endometrial Adenoca (I)	+	Fine	Uneven	70%	3%	
Endometrial Adenoca (II)	++	Fine	Uneven	86%	11%	
Endometrial stromal cell		Fine	Even	+		
Endocervical		Fine	Even	100%		
Atrophic squamous		Fine	Even			
SC CIS	++++	Fine to coarse	Even			
Sq CA, small cell type	++++	Fine to coarse	Uneven	20%		
AISMT	++	Fine	Even			
Atypical immature metapl. repair	+	Fine	Even	16%	80%	
ASCUS	+	Fine	Even			
Atypical mature Sq Metapl repair	+	Fine	Even	8%	92%	
AIS	+++	Coarse	Even			
ICIS	+–4+	Fine to coarse	Even			
K Sq CA	++++	Opaque	Even to uneven		5%	
NK Sq CA	++	Fine to coarse	Uneven		24%	
Endocervical Adenoca (I–III)	+++	Fine	Uneven	38–87%	13–62%	1–5%
Endometrial Adenoca (III–IV)	+++	Fine	Uneven	55–65%	36–45%	
Atypical squamous repair	++	Fine	Even	5%	95%	
Endocervical atypia (reactive)		Fine	Even		100%	
Atypical endocervical repair	++	Fine	Even	2%	98%	
Dysplasia (M)	+++	Fine to clumped	Even			
Dysplasia (K)	++++	Opaque	Even			
Dysplasia (NK)	+	Fine	Even			
Endocervical Adenoca (IV)	+++	Fine to coarse	Uneven	16%	84%	35%
LC CIS	+	Fine	Even			
Acute radiation		Fine	Even			
ExtraUT	++	Fine to clumped	Uneven	Yes	Yes	+
Sarcomas & other rare lesions	+	Fine	Uneven	Yes	Yes	+

Intermed: intermediate; Sq: squamous; Metapl: metaplastic; P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; SC: small cell; CIS: carcinoma in situ; CA: carcinoma; AISMT: atypical cells of immature metaplastic type; ASCUS: atypical squamous cell of undetermined significance; AIS: adenocarcinoma in situ; ICIS: intermediate carcinoma in situ; K: keratinizing; NIC: nonkeratinizing; LC: large cell; Extraut: extrauterine.

Cell type	Cell shape	Cytoplasmic texture	Cell borders	Cell arrangement	Tumor diathesis	Multiple nucleoli
Intermed Sq	Polygonal	Homogeneous	Yes	Isolated		
Endometrial	Round	Finely vacuolated	No	Isolated/cluster		
Sq Metapl (P)	Round/oval	Homogeneous	No	Isolated		
Sq Metapl (I)	Round/oval	Primitive	Yes	Isolated		
Sq Metapl (M)	Small ploygonal	Homogeneous	Yes	Isolated		
Endometrial hyperplasia	Round	Vacuolated	No	Cluster		
Atypical endometrial hyperplasia	Round	Vacuolated	No	Cluster		
Endometrial Adenoca (I)	Round	Vacuolated	No	Isolated/cluster	Serous	8%
Endometrial Adenoca (II)	Round	Vacuolated	No	Isolated/cluster	Serous	34%
Endometrial stromal cell	Round	Vacuolated	Yes	Isolated		
Endocervical	Columnar	Granular	Yes	Isolated/sheets		Frequent
Atrophic squamous	Round/oval	Homogeneous	Yes	Isolated		
SC CIS	Oval	Primitive	No	Syncytial		
Sq CA, small cell type	Oval	Primitive	No	Syncytial	Tumor	
AISMT	Round	Homogeneous	Yes	Isolated		
Atypical immature Metapl. repair	Columnar/oval	Homogeneous	Yes	Sheets		
ASCUS	Polygonal/round/oval	Homogeneous	Yes	Isolated		
Atypical Mature Sq Metapl repair	Round/oval	Homogeneous	Yes	Sheets		15%
AIS	Columnar	Granular	Yes	Isolated/sheets		
ICIS		Primitive	No	Isolated/syncytial		
K Sq CA	Pleomorphic	Homogeneous	Yes	Isolated	Clean	
NK Sq CA		Primitive	No	Isolated/Syncytial	Tumor	"+/−"
Endocervical Adenoca (I–III)	Columnar	Granular	No (25–77%)	Cluster	Tumor	1–5%
Endometrial Adenoca (III–IV)	Round	Vacuolated	No	Cluster	Serous	
Atypical squamous repair	Round/oval	Homogeneous	Yes/no			22%
Endocervical atypia (reactive)	Columnar/round/oval	Vacuolated	Yes	Sheets		
Atypical endocervical repair	Polygonal	Homogeneous	Yes/no	Sheets		32%
Dysplasia (M)	Round/oval	Homogeneous	Yes	Isolated		
Dysplasia (K)	Pleomorphic	Homogeneous	Yes	Isolated		
Dysplasia (NK)	Polygonal	Homogeneous	Yes	Isolated		
Endocervical Adenoca (IV)	Side/side	Granular	No	Isolated/side/side	Tumor	35%
LC CIS		Primitive	No	Isolated/syncytial		
Acute radiation	Round/oval/polygonal	Variable	Yes	Isolated		
ExtraUT	Variable	Variable	Yes/no	Cluster	Clean	?
Sarcomas & other rare lesions	Variable	Variable	Yes/no	Isolated	Clean/tumor	?

Intermed: intermediate; Sq: squamous; Metapl: metaplastic; P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; SC: small cell; CIS: carcinoma in situ; CA: carcinoma; AISMT: atypical cells of immature metaplastic type; of invasive squamous type; ASCUS: atypical squamous cells of undetermined AIS: adenocarcinoma in situ; ICIS: intermediate carcinoma in situ; K: keratinizing; NK: nonkeratinizing; LC: large cell; Extraut: extrauterine.

	Chromatin particles	Chromatin distribution	Cell shape	Cyto texture	Cell borders	Cell arrangement
Intermediate squamous	Fine	Even	Polygonal	Homogeneous	T	Isolated
Endometrial	Fine	Even	Round	Finely vacuolated	F	Isolated/cluster
Squamous metaplastic (P)	Fine	Even	Round/oval	Homogeneous	F	Isolated
Squamous metaplastic (I)	Fine	Even	Round/oval	Primitive	T	Isolated
Squamous metaplastic (M)	Fine	Even	Small polygonal	Homogeneous	T	Isolated
Atypical endometrial hyperplasia	Fine	Even	Round	Vacuolated	F	Cluster
Endometrial Adenoca	Fine	Uneven	Round	Vacuolated	F	Isolated/cluster
Endocervical	Fine	Even	Columnar	Granular	T	Isolated/sheets
Atrophic squamous	Fine	Even	Round/oval	Homogeneous	T	Isolated
Squamous atypical repair	Fine	Even	Round/oval	Homogeneous	T	Sheets
ASCUS	Fine/oval	Even	Polyg/round	Homogeneous	T	Isolated
Metaplastic dysplasia	Fine/clumped	Even	Round/oval	Homogeneous	T	Isolated
Intermediate CIS	Fine/coarse	Even	None	Primitive	F	Isolated/syncytial
Large cell CIS	Fine	Even	None	Primitive	T	Isolated/syncytial
Nonkeratinizing sq CA	Fine/coarse	Uneven	None	Primitive	F	Isolated/syncytial
Keratinizing dysplasia	Opaque	Even	Pleomorphic	Homogeneous	T	Isolated
Keratinizing sq carcinoma	Opaque	Even/uneven	Pleomorphic	Homogeneous	T	Isolated
Small cell CIS	Fine/coarse	Even	Oval	Primitive	F	Syncytial
Small cell sq carcinoma	Fine/coarse	Uneven	Oval	Primitive	F	Syncytial
Atypical immature squamous metaplastic type	Fine	Even	Round	Homogeneous	T	Isolated
Endocervical atypia	Fine	Even	Columnar/oval	Homogeneous	T	Sheets
Adenocarcinoma in situ	Coarse	Even	Columnar	Granular	T	Isolated/sheets
Endocarvical adenoca	Fine	Uneven	Columnar	Granular	F	Cluster

P: primitive; I: immature; M: mature; Adenoca: adenocarcinoma; ASCUS: atypical squamous cells of undetermined significance; CIS: carcinoma in situ; sq: squamous; CA: carcinoma; T: true; F: false.

RETROSPECTIVE VALIDATION OF THE FOCALPOINT SLIDE PROFILER AUTOMATED SCREENING INSTRUMENT IN CYTOLOGY

AIM:

To establish historical validation of the FocalPoint Slide Profiler Screening Instrument serial no: 0355 by using the data generated by the technologist screening and interpreting the 15 fields of view of conventional gynaecological slides at the GS Review station.

METHOD:

Data from slides run from January 2010 - June 2010 was assessed in order to establish whether this instrument has been proven fit for its intended use in the Cytology Laboratory at Tygerberg Hospital. The data used was the laboratory's "Diagnostic screening Internal Quality Control", the labs'**acceptable range for IQC of missed abnormalities: less than 1.7%** and the recorded "missed abnormalities" by the FocalPoint. The missed abnormalities were not present in the 15 fields of view interpreted by the FocalPoint. The slides were screened on the Standard Process Mode by the FocalPoint and reviewed by a qualified and competent technologist at the GS Review Station.

EVALUATION OF DATA COLLECTED:

Total number of slides: **9922**
Number of unacceptable "missed abnormalities": **20**
This was an error rate of: **0.2%** this rate was acceptable as it was less than 10%.
The mean **IQC** score was within the acceptable range for each run assessed.

Specimen Measurement Issues	Problem stated
STC 3002353	ASC-US
STC 3003264	LSIL
STC 3007510	LSIL
STC 3010195	LSIL
STC 3010801	LSIL
STC 3011160	ASC-US
STC 3015872	ASCUS
STC 3016751	ASCUS
STC 3018412	ASCUS
STC 3023390	ASC-US
STC 3024075	LSIL
STC 3023834	LSIL
STC 3027441	LSIL
STC 3030271	ASC-US
STC 3029807	ASC-US
STC 3030335	LSIL
STC 3030040	LSIL
STC 3030948	HSIL
STC 3031338	LSIL
STC 3032081	LSIL

The slides where a "missed abnormality" was not present in the 15 fields of view but picked up during rapid review were re-screened in all of the above instances. This instrument was placed in the Cytology Department July 2008 and has performed well and is reliable. The instrument is serviced annually.

SUMMARY:

All slides with a normal diagnosis interpreted at the GS Review Station are manually rapid reviewed by a qualified and competent technologist, while all abnormal slides are re-screened by a checker technologist. If slides were found to be unacceptable for re-screening a repeat would be requested.
On assessing the above data it was proven that this instrument has performed well and is fit for the intended purpose in this laboratory on **14/2/2011.**

Data assessed by: M. Hill **Signature:** M Hill **Date:** 14/2/2011

Validation approved by: L van Vuuren Laboratory Manager

Signature: L van Vuuren **Date:** 14/2/2011

PROTOCOL FOR VALIDATION/VERIFICATION OF METHODS/INSTRUMENTS/REAGENTS

Instrument/Method/Reagent: Focal Point Slide Profiler

Performed by: Mauritz Hill

Type: New ☐ Retrospective ☒

Quantitative ☐ Qualitative/Semi qualitative ☐

No. of Samples selected: 10 ☐ 20 ☐ 40 ☐ 100 ☐ more than 100

Samples type: Patient samples ☒ IQC ☐ EQC ☐

Acceptance criteria quantitative:

Manufacturers specifications ☐
Biological variation ☐
CLIA ☐
RCPA ☐
Other.....

Acceptance criteria qualitative:

Cohen's Kappa agreement test
McNemar test for Symetry
Sensitivity/positive agreement/PVP ☐
Specificity/negative agreement/PVN ☐
Literature review ☒
Other .....

Experiments run:

Replication within run (imprecision) ☐
Replication between run (imprecision) ☐
Alternate Comparison ☐
Recovery of known values ☒
Linearity ☐
Reference range verification ☐

Current IQC practice Daily ☒ Weekly ☐ Monthly ☐ New Lot. No./Kit ☐

Special environmental conditions: Yes ☐ No ☐ N/A ☒

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Equipment used: Focal Point Slide Profiler
GS Reviewstation
Olympus microscopes

Reviewed and Accepted by: C van Wyk

Date 15/3/2011

Reference: GPL1530, EP Evaluator™ by David G Rhoads

IQC 2010 (Gynae)

QC	MISSED														MIS-DIAGNOSED								
	T'S	CANDIDA	OTHER PATHOG	ECC	EMC	ASCUS	HPV	LSIL N>I	HSIL N>J	SQ CA	AD CA	RAD	OTHER	MISSED TOTAL	OVER DIAG 1°	OVER DIAG 2	UNDER NOW LSIL E/F>I	UNDER NOW HSIL/CA F/I>J	over/under Total	CASES SCREENED	% MISSED	% over/under
MONTHLY																						
JANUARY	14	22	2	20	5	21	0	30	5	0	0	0	1	**120**	19	9	13	2	43	**3354**	3.58%	
FEBRUARY	17	18	1	26	8	27	5	24	1	0	0	0	0	**127**	26	11	2	7	46	**4110**	3.09%	
MARCH	16	22	0	17	11	23	0	33	5	0	0	0	1	**128**	23	6	6	8	43	**5172**	2.47%	
APRIL	10	15	3	13	3	19	0	6	1	0	0	0	0	**70**	6	7	1	2	16	**1718**	4.07%	
MAY	26	15	3	22	11	15	0	36	3	0	0	0	0	**131**	32	12	3	4	51	**2204**	5.94%	
JUNE	12	26	1	12	6	9	0	30	3	1	0	0	0	**100**	25	5	5	9	44	**3152**	3.17%	
JULY	13	8	2	7	3	8	1	27	4	0	0	0	0	**73**	17	3	3	4	27	**2508**	2.91%	
AUGUST	10	14	0	31	5	16	0	40	4	0	0	0	0	**120**	9	2	2	9	22	**2766**	4.34%	
SEPTEMBER	3	25	4	18	4	11	1	18	4	0	0	0	0	**88**	7	3	2	6	18	**4008**	2.20%	
OCTOBER	19	32	2	27	4	19	0	33	3	0	0	0	0	**139**	16	8	6	4	34	**1944**	7.15%	
NOVEMBER	22	22	3	42	13	20	0	60	6	0	0	0	0	**188**	13	3	11	18	45	**5367**	3.50%	
DECEMBER	19	45	3	28	10	37	1	53	5	0	0	0	0	**201**	26	8	5	12	51	**3885**	5.17%	
TOTAL	181	264	24	263	83	225	8	390	44	1	0	0	2	**1485**	219	77	59	85	440	**40188**	3.70%	
% on Total abnormals						33.7			42					**668**						**40188**		
% on Total cases						0.6	%		196.4%					1.7	%				1.1	%		
									Sil vs Ascus													

15/3/2011