Comparative Analysis of the Diagnostic Accuracy of Cell Block Technique to Conventional Smears in Minimally Invasive Procedures of Pulmonary Lesions

DISSERTATION SUBMITTED TO

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

in partial fulfilment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

TIRUNELVELI MEDICAL COLLEGE HOSPITAL

TIRUNELVELI

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CERTIFICATE

This is to certify that this Dissertation entitled "**COMPARATIVE ANALYSIS OF THE DIAGNOSTIC ACCURACY OF CELL BLOCK TECHNIQUE TO CONVENTIONAL SMEARS IN MINIMALLY INVASIVE PROCEDURES OF PULMONARY LESIONS**" is the bonafide original work of **Dr.S.SARANYAA**, during the period of her Post graduate study from 2012 – 2015, under my guidance and supervision, in the Department of Pathology Tirunelveli Medical College & Hospital, Tirunelveli, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University will be held in April 2015.

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I hereby certify that this dissertation entitled **"COMPARATIVE ANALYSIS OF THE DIAGNOSTIC ACCURACY OF CELL BLOCK TECHNIQUE TO CONVENTIONAL SMEARS IN MINIMALLY INVASIVE PROCEDURES OF PULMONARY LESIONS"** is a record of work done by **Dr. S.SARANYAA,** in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during her postgraduate degree course period from 2012- 2015. This work has not formed the basis for previous award of any degree.

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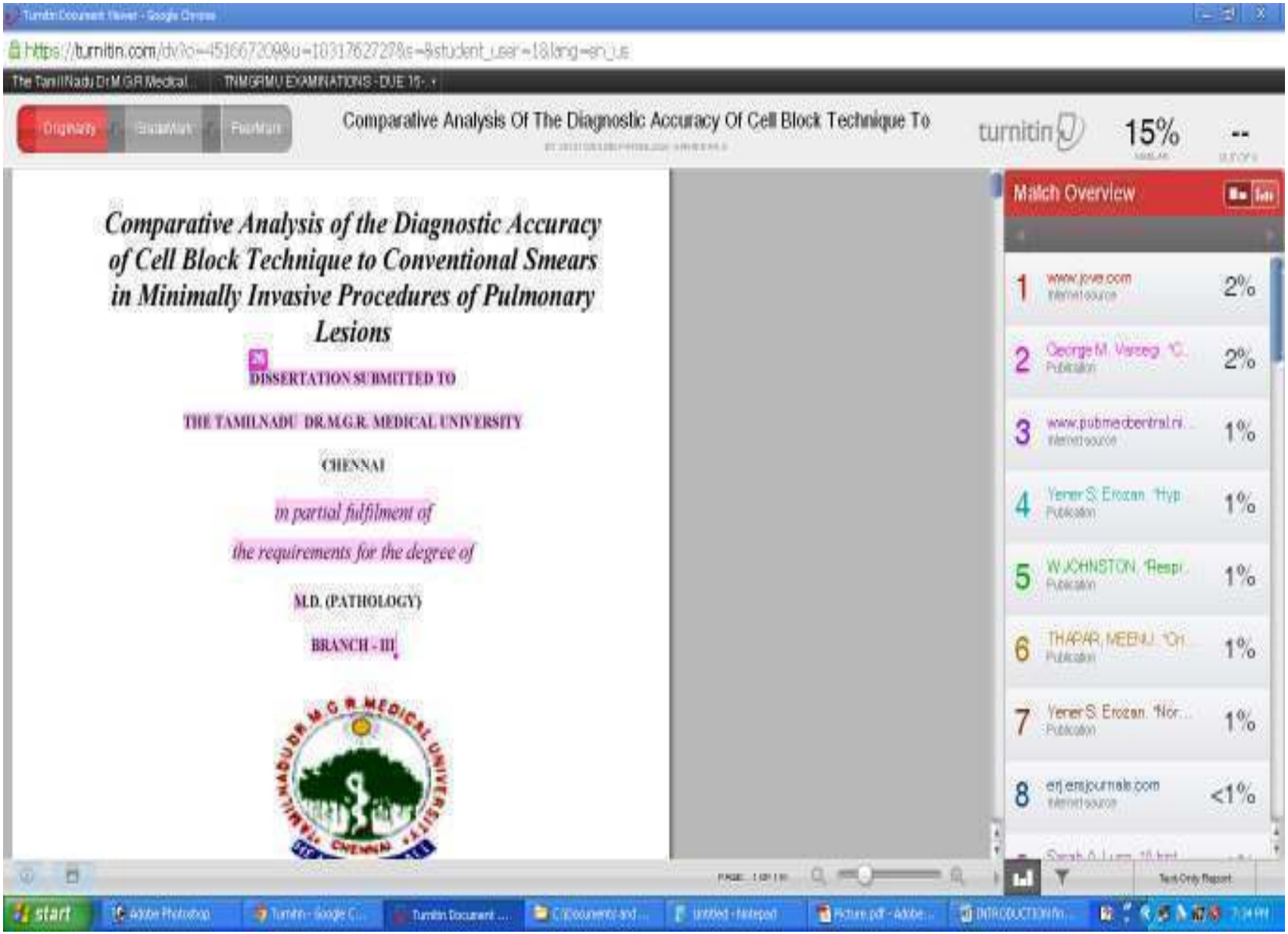
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Dr. S.SARANYAA

ABBREVIATION

CS	:	CONVENTIONAL SMEAR
CB	:	CELL BLOCK
FNAC	:	FINE NEEDLE ASPIRATION CYTOLOGY
IHC	:	IMMUNOHISTOCHEMISTRY
ER	:	ESTROGEN RECEPTOR
TTF-1	:	THYROID TRANSCRIPTION FACTOR -1
CK	:	CYTOKERATIN
WHO	:	WORLD HEALTH ORGANISATION

CONTENTS

Comparative Analysis of the Diagnostic Accuracy of Cell Block Technique to Conventional Smears in Minimally Invasive Procedures of Pulmonary Lesions.

Abstract:

Cell blocks prepared from residual tissues fluids and fine needle aspirations can be useful adjuncts to smears for establishing a more definitive cytopathologic diagnosis. Conventional smear cytology, however carefully prepared, leaves behind residue that is not further investigated but that might contain valuable diagnostic material. The cell block technique employs the retrieval of small tissue fragments from the residual sample which are processed to from a paraffin block. Multiple sections can be obtained from this block. This allows for immunostains and other studies to be performed similar to the paraffin sections produced in histopathology. They can be particularly useful for categorization of tumors on cytology specimens that may not be possible from smears themselves.

Aim:

Preparation of cell blocks from fluids (pleural fluid, bronchial washings) and residual FNAC material. To compare the diagnostic accuracy of cell block technique with conventional smears. To use immunohistochemistry on cell block for subtyping tumors and in cases suspicious for malignancy.

Methods:

In this prospective study, 100 samples which include 50 pleural effusion samples, 35 bronchial wash samples and 15 imaging guided FNAC

samples were subjected to make both conventional smear and cell block. Cell blocks were made by plasma thromboplastin method. Both the techniques were compared based on the cellularity, architecture, quality and diagnosis. Immunohistochemistry was performed in cell blocks to confirm and subtype malignant tumors.

Results:

Abundant cellularity was seen in 31% of the cases by cell block and only 10% of the cases had abundant cellularity by conventional smear. Excellent architecture equivalent to histology was seen in 15% of the cases by cell block and this was not seen in conventional smear. By conventional smear, benign, suspicious of malignancy, malignant and non-diagnostic cases were 66%, 8%, 15%, 11% respectively. By cell block 71% benign, 24% malignant, 5% non-diagnostic. Combining both conventional smear and cell block, the diagnostic yield of malignancy increased by 9%. Immunohistochemistry was performed in 18% of the cases to confirm and subtype the malignancy.

Conclusion:

Cell block is a very good adjunct to conventional smear study in the better yield of cellularity and architecture with an advantage to do immunohistochemistry, leading to better diagnosis of malignancy in the fluids and residual FNAC samples of pulmonary lesions. Ideally cell block technique should be used in routine practice for cytological diagnosis.

Key words: conventional smear, cell block, immunohistochemistry.

INTRODUCTION

Cell block (CB) technique was first described by Bahrenberg in 1896.This is an old method for evaluation of body cavity fluids. The cell block technique employs the retrieval of cells or small tissue fragments from any body fluid including pleural fluid, bronchial wash and imaging guided fine needle aspiration cytology (FNAC) specimens.

Cytology of pulmonary lesions provide valuable diagnostic information by non or minimally invasive procedures[1]. Direct sampling methods like bronchoscopy and fine needle aspiration (FNA) increases the ability to diagnose pulmonary diseases by cytology[2]. Pleural fluid cytology is one of the commonly performed investigations in the diagnosis of malignant lesions, staging and prognosis.

The cytodiagnosis by conventional smears (CS) have got some drawbacks due to overcrowding of cells, cell loss leading to less cellularity and different laboratory processing methods[3]. To overcome these drawbacks cell block technique was employed.Cell blocks from fluid specimenscan be prepared by using agar or plasma thrombin method, the cellbutton formed is formalin fixed and processed routinely like histopathological specimens.

The main advantages of cell block techniques are preservation of tissue architecture and possibility to obtain multiple sections from the same material for special stains and immunohistochemistry[4]. The material preserved by cell block also improves the diagnostic accuracy.

There is an increasing need for additional diagnostic techniques such as immunohistochemistry (IHC), to define a specific cell lineage on cytology and FNAC specimens[5,6]. Immunohistochemistry is a highly effective ancillary tool that can be used on cell block to distinguish adenocarcinoma, squamous cell carcinoma and small cell carcinoma.Cell block increases accuracy, reproducibility and minimizes the rate of unclassified carcinomas.

ADVANTAGES OF CELL BLOCK[7]:

1. The method is simple, reproducible and readily adaptable in routine hospitallaboratory.
2. It bridges the gap between cytology and histology.
3. There is adequate cellularity, cell aggregates and microscopic tissue fragments are easily recoverable.
4. Preservation of architectural pattern like cell balls, papillae and three dimensional clusters.
5. Concentration of cellular material in one small area that can be evaluated at aglance with all cells lying in the same focal plane of microscope.
6. Delineation of nucleus and cytoplasmic details.
7. Intact cell membranes and crisp chromatin details.
8. Cell block sections are suitable for histochemical stains and IHC.

Hence the present study was undertaken to assess the utility and diagnostic accuracy of cell-block technique in the diagnosis of pulmonary lesions by minimally invasive diagnostic procedures like bronchial washings, pleural fluid samples and image guided fine needle aspiration cytology of lung mass.

AIM& OBJECTIVES

1) Preparation of cell blocks from fluids (pleural fluid, bronchial washings) and residual FNAC material from pulmonary lesions.
2) To compare the diagnostic accuracy of cell block technique with conventional smears.
3) To use immunohistochemistry on cell block for subtyping tumors and in cases suspicious for malignancy.

REVIEW OF LITERATURE

Bennet in 1848 gave an account on tumor cells in effusion fluids in his publication that led to the development of cytopathological diagnosis of body cavity fluids[8]. In 1867, Luke and Klebs gave a description of malignant cells in effusion[9]. Kanhouwa et al, correlated cytopathology and histopathology in the typing of lung carcinoma. He showed a correlation of cytopathology and histopathology of 77.5%[10] in his study.

The cytological examination of the fluids and effusions has increasingly gained acceptance in clinical medicine, with the surge of minimally invasive procedures to such an extent that a positive diagnosis is often considered the definitive test and obviates explorative surgery. It is important not only in the diagnosis of malignant lesions, but also help in staging and prognosis. Lack of morphological details of the representative cells contributes to considerable difficulties in making conclusive diagnosis on conventional smears.

Cell blocks prepared from residual tissue fluids can be used as an adjunct to smear, for establishing a more definitive cytopathological diagnosis. The technique is simple, safe, cost-effective and reproducible even in resource limited settings[11]. The use of cell blocks is being increasingly advocated in the diagnostic work-up of patients. The routine use of this technique remains confined to a limited number of centers.

HISTORIC EVOLUTION OF CELL BLOCK TECHNIQUE:

A variety of cell block techniques have been in use for over a century. Since Bahrenburg and Mandlebaum described their technique of embedding and sectioning cellular sediments in 1896, there have been a number of reports concerning the formed elements in serous effusions[8].

In1917, malignant tumors were diagnosed in paraffin sections of centrifuged exudates to make specimens more readily interpretable even by histopathologists. Karnanchowand Bouin, modified and developed plasma embedding "cell block" technique[12].

Zemansky in 1928, established the definite arrangement of the cells as acini and papillae or of aggregates of abnormal cells to be of malignant nature.Chapman and Whalen, Schlesinger and others set up similar criteria for the diagnosis of malignant tumor cells in serous effusions[13].

THE ADVANTAGES OF THE CELL BLOCK PROCEDURE:

- Less cellular dispersal, which permits easier microscopic observation than do traditional smears[14].
- Less difficulty in interpretation as background shows no excess blood on microscopic observation.
- Recognition of histological patterns of diseases that sometimes cannot be identified reliably in conventional smears[14].
- Possible to study multiple sections by routine and special staining.

- They allow for the evaluation of ancillary studies such as immunocytochemistry, in-situ hybridization tests (FISH/CISH) and in-situ polymerase chain reaction (PCR)[15].
- Possibility of storing slides for retrospective studies.

ANATOMY AND HISTOLOGY:

The pleura is a thin, glistening, slippery serous membrane lining the thoracic wall and diaphragm, as the parietal pleura. It is then reflected onto the lung as the visceral pleura.The pleural cavity is a potential space between the two layers, containing a thin film of fluid.

On light microscopy,the pleura is typically divided into a mesothelial layer, a thin submesothelial connective tissue layer, a superficial elastic layer, a loose sub pleural connective tissue layer anda deep fibroelastic layer[16].

The major portions of the upper and lower respiratory tract are lined by a pseudostratified and ciliated columnar epithelium. The morphology of benign cellular components of respiratory tract material has been well described in the literature by Farber and associates, Woolner and McDonald, Koss and Frost and associates[17-19].

The components of respiratory specimens (bronchial aspirates or brushings and FNAs) may be divided into epithelial cells (squamous, ciliated columnar epithelium and goblet cells), macrophages, leukocytes, inanimate components and organisms.

NORMAL CYTOLOGY:

EPITHELIAL COMPONENTS:

Ciliated Bronchial Columnar Cells[20]*:*

Ciliated columnar cells are uniform, columnar or prismatic shape, ending in a tail and forms monolayered tissue fragments. The cells are columnar with basally placed nucleus, one or more nucleoli and a fine granular chromatin. The cell clusters on en-face gives a honeycomb appearance. Ciliated columnar cells are usually seen in bronchoscopic brush lavage, aspirate, transbronchial or tracheal fine needle aspirations.

Goblet Cells:

These cells are seen as mucus producing cells in the bronchial epithelium. Cells have single or multiple vacuoles filled with mucus within the cells.These cells are seen in bronchial brushings of patients affected with chronic bronchitis and bronchiectasis and not very commonly encountered in the washings[21].

Alveolar Epithelium:

These cells are normally not seen in the cytologic material. In reactive conditions they may appear in bronchoalveolar lavages (BAL) and fine needle aspirations but mostly misinterpreted as alveolar macrophages. Reactive alveolar cells are singly scattered with central nucleus, small nucleoli and vacuolated cytoplasm. These cells are differentiated from macrophages by the absence of any phagocytosed material within the cells.

Mesothelial cells in pleural fluid:

Mesothelial cellsare sheets of polygonal cells that are usually separated from each other by clear gaps or “windows”.The cells are oval or spherical in shape, about 20 μm in diameter with eosinophilic cytoplasm and round to oval centrally placed nucleus with conspicuous nucleoli.Reactive mesothelium can appear atypical and be misinterpreted as neoplastic. The origin of these cells from the mesothelial surface is best documented in cell blocks that show the linear nature of the clusters cut “on edge”.The outer edges of such clusters are usually composed of rows of cells showing smooth borders or “scalloping”.

Non-Epithelial Cellular Elements[22]:

Non-epithelial inflammatory cells like macrophages, lymphocytes, neutrophils and eosinophils may be seen even in the samples from normal individuals. The type of cells present in the sample varies depending on the method of sampling, processing and history of smoking in the patient. Large number of inflammatory cells predominantly pulmonary macrophages and neutrophils are seen in bronchoalveolar lavage (BAL) specimens of smokers affected by chronic bronchitis. The type of the inflammatory cell, its number and distribution may vary depending on the nearby neoplasm or a reaction to the injury.

Macrophages and Giant Cells:

The cells may have a single nucleus or may be multinucleated.The nucleus is oval or kidney shaped with small nucleoli and granular fine chromatin. Macrophages are often multinucleated; the nuclei within each multinucleated cells are of similar size and morphology.They account for 60–90% of the cells in the pulmonary specimens and are very commonly seen in BAL specimens. Inflammatory conditions such as pneumonia, granulomas, bronchitis and malignant tumors with extensive necrosis have abundant macrophages.

The cytoplasm of the macrophages may contain intrinsic and extrinsic elements. Hemosiderin, blood cells, lipofuscin and lipid are few intrinsic elements seen within the cytoplasm of the alveolar macrophages. In smokers, tan brown granules are seen which should be differentiated from siderophages. The granules in the siderophages are finer and stain positive for iron.

Extrinsic elements like carbon particles, silica and asbestos fibers may be seen within the macrophages. Multinucleated giant cells are seen as a reaction to fungal or mycobacterial infection.

Siderophages:

The hemosiderin granules seen within the siderophages are golden brown in colour and are refractile.Hemosiderin in the siderophages are positive for Prussian blue stain and this helps to differentiate it from

lipofuscin and melanin.Siderophages are seen in Wegener granulomatosis, idiopathic pulmonary hemosiderosis, hemorrhage, congestive heart failure and infarcts.

Polymorphonuclear Leukocytes:

Acute inflammatory cells, like neutrophils are seen in many pathological conditions or may be even as contaminants from the oral cavity. Neutrophils are seen more commonly in the malignant neoplasms with a necrotic background and also a variety of conditions like abscess, acute bronchitis and bacterial pneumonia.

Lymphocytes:

Small mature lymphocytes can commonly be seen in the specimens procured by bronchial brushings, bronchial lavage or bronchoalveolar lavage.Abundant lymphocytes are seen in granulomatous lesions caused by tuberculosis, many viral infections, hypersensitivity pneumonitis and drug induced reactions.

Any inflammatory condition associated with abundant lymphocytes may mimic small cell carcinoma or leukemia/lymphoma of the lungs. The mature lymphocytes in the inflammatory conditions are smaller than the neoplastic cells in small cell carcinoma. Pleomorphism of the neoplastic cells, necrosis and nuclear molding is seen in the small cell carcinoma, which is absent in the chronic inflammation. These two conditions can also

be differentiated by neuroendocrine markers, which show positivity in small cell carcinoma.

Eosinophils:

These inflammatory cells are not normally seen in the pulmonary specimens. In normal individuals, less than 1% of the eosinophils may be seen in BAL samples. They appear usually after antigenic stimulation such as in bronchial asthma. Abundant eosinophils are seen in conditions like bronchial asthma, hypersensitivity pneumonitis and in eosinophilic pneumonia. Infection with parasites and fungus also cause antigenic stimulation leading to increase in eosinophils.

NON-CELLULAR ELEMENTS:

Curschmann Spirals:

These are mucous casts that form corkscrew like spirals with a central core. From the axis of the core, filamentous structures radiate perpendicularly.With Papanicolaou stain, it stains pale cyanophilic or eosinophilic and stains black with silver.

Sputum of the heavy smokers commonly contains Curschmann spirals in the medium-sized to small bronchioles. They can also be seen in patients with chronic bronchitis, bronchial asthma and other obstructive lung disease[23].

Charcot–Leyden Crystals:

Cytoplasmic granules of the eosinophils condense to from needle-shaped or rhomboidal structures of variable sizes.In many conditions with abundant eosinophils, Charcot–Leyden crystals can be seen.Bronchial asthma and allergic pneumonitis are few conditions associated with these crystals. With Papanicolaou these crystals have well defined refractile edges and they stain green or red.

REACTIVE CHANGES:

Squamous metaplasia and atypia:

Exposure to chemical, physical or biologic agents causes sustained injury to the respiratory tract columnar epithelium. This leads to squamous metaplasia of the respiratory epithelium. These cells appear as miniature keratinized squamous cells.The metaplastic cells are oval or elliptical shaped with bright cyanophilic or orangeophilic cytoplasm and pyknotic uniform nucleus when stained with Papanicolaou. Atypia may be prominent in cases of chronic inflammation, tuberculosis, abscesses, etc., which can mimic carcinoma. Squamous metaplastic cells are most commonly seen in smokers and patients with chronic infections like fungal infections, usual interstitial pneumonia (UIP) and organizing pneumonia. These cells should be mentioned in the cytopathology report as it can also undergo dysplastic changes and later may develop into squamous cell carcinoma[24].

Reactive bronchial cells:

Reactive changes in bronchial cells are usually associated with hyperplasia of the bronchial epithelium and it appears as cellular cohesive sheets.The individual cells are enlarged and have poorly defined cell borders. The reactive cells have oval to round nuclei and prominent nucleoli.Some cells show variation in the size of the nucleus. The nucleus contains fine, bland granular, evenly distributed chromatin. The prominent nucleoli, which is uniform in shape and size present in the nucleus of the reactive cells is an important characteristic feature. These cells are seen associated with many conditions including pneumonia (Viral pneumonias), bronchitis, instrumentation, bronchial asthma, bronchiectasis, chemotherapeutic agents, exposure to toxins and radiation.

Goblet cell hyperplasia:

Goblet cells undergo hyperplasia due to chronic inflammation. Hyperplastic goblet cells are seen associated with bronchial asthma, chronic bronchitis and bronchiectasis. They may be seen as clusters or singly dispersed cells in the procured cytologic material. Particularly in bronchial brushings they are seen as clusters. The individual cells are enlarged with single large or multiple smaller mucinous vacuoles with a basally located flattened nucleus.

Reactive Terminal Bronchial/Alveolar Epithelium:

The reactive alveolar epithelial cells form small clusters and papillary arrangement of the cells may be seen in type II pneumocytes surrounded by abundant macrophages. The individual cells are smaller in size with enlarged nucleus. Viral pneumonias, interstitial lung disease, infarction, organizing pneumonia, asbestosis and some drug-mediated changes induce the macrophages and type II pneumocytes to proliferate. Proliferating type II pneumocytes have cytoplasmic vacuoles and may form papillary clusters which mimics acinar, papillary or bronchiolo-alveolar type of well differentiated adenocarcinoma.

Reactive macrophages may have large nuclei with prominent nucleoli and cytoplasmic vacuoles. It is difficult to differentiate it from adenocarcinoma. The presence of a spectrum that encompasses normal and atypical macrophages with similar nuclear shape and chromatin characteristics, speaks against adenocarcinoma.

Reserve Cell Hyperplasia:

Bronchial reserve cells are located close to the basement membrane of the mucosa between the basal parts of ciliated columnar cells. These cells are small round to polygonal multipotent cells that undergoes hyperplasia with exposure to smoke and chemical irritants. They are seen in specimens procured by brushing or washing of large bronchi, forming small cohesive clusters or tissue fragments rather than isolated cells. These cells are small,

round to polygonal with scant cyanophilic cytoplasm and round to oval nuclei that have a uniformly distributed bland chromatin. Reserve cells clusters are associated with few ciliated bronchial cells which can be differentiated from the reserve cells by its abundant cytoplasm. Some metaplastic squamous cells can also be observed around the rim of some of the larger tissue fragments. Small cell carcinoma is the most important differential diagnosis and it is differentiated from reserve cell clusters by large amount of isolated cells in a necrotic background.

Creola Bodies:

Creola bodies are sheets or thick 3-D tissue fragments of reactive bronchial cells. These are exfoliated papillary fragments of reactive bronchial cells that may be seen in the patients with chronic bronchitis, particularly due to asthma. The fragments are also often seen in other reactive or inflammatory lesions of the bronchial mucosa. The clusters of bronchial mucosa are partially covered by ciliated respiratory epithelium. Because of the thickness of the tissue fragment, accurate visualization of details of the crowded nuclei is difficult. When visualized around the edge of the fragment, the nuclei are bland with evenly distributed chromatin, smooth nuclear outline and uniform nuclear membrane. Small nucleoli may be present. Identification of cilia is facilitated by partial closure of the microscope's condenser.

THERAPY-INDUCED CHANGES:

Radiation Therapy:

Radiation therapy given for metastatic and primary breast carcinomas are the most common cause for the exposure of the lungs to radiation[25]. The respiratory epithelium undergoes changes like cytomegaly with nuclear enlargement and degenerative changes both in the nucleus and cytoplasm. The individual cells are bizarre shaped, amphophilic cytoplasm with vacuoles and debris and smudged nuclear chromatin.

Chemotherapy:

Alveolar pneumocytes and the respiratory columnar epithelium are usually affected by chemotherapy. The changes seen in the columnar cells are similar to that of the changes caused by radiation therapy. Some drugs, such as bleomycin affects the squamous cells and causes atypia, while many other drugs causes alveolar hyperplasia[26].

RESPIRATORY INFECTIONS:

Mycobacterial Infections:

Tuberculosis caused by M. tuberculosis or M. avium intracellulare is a common mycobacterial disease worldwide[27,28]. Emerging drug resistant strains complicates the treatment of the disease. Cytologic smear shows abundant lymphocytes and histiocytes with eosinophilic necrotic material. Inflammatory cells are absent in immunocompromised individuals.

Occasionally Langhans giant cells are also seen. In chronic cases, the cells lining the tuberculous cavity show atypia.

In immunosuppressed and elderly patients with upper and lower respiratory tract infections many other species of bacteria like legionella, actinomycosis and nocardiosis can be seen as a superimposed infection.

Viral Infections:

Many viruses produce some similar cytologic changes, which can be commonly seen but nonspecific. Techniques like immunocytochemistry, DNA in-situ hybridization, culture or polymerase chain reaction (PCR) is needed to identify these viruses.

Herpes Simplex Infection[29]:

Cell clusters or isolated cells show cytopathic changes. The cells have nuclear inclusion which is well defined with a halo and condensed chromatin against the nuclear envelope. These cells are surrounded by acute inflammatory cells in a necrotic background.

Cytomegalovirus Infection:

In immunocompromised patients, cytomegalovirus infection can be commonly seen. The virus affects pneumocytes, macrophages, bronchial epithelial and endothelial cells. The cells show marked enlargement with both cytoplasmic and nuclear inclusions, surrounded by a very scant inflammatory cells. The cytoplasmic inclusions are cyanophilic, small and

variable in size. Nuclear inclusions are mostly single, described as owl eye inclusion with a large halo[30].

Mycotic Infections:

Respiratory organs particularly in immunosuppressed individuals (AIDS and transplant patients) are commonly affected by mycotic infections. Cytologic examination and special fungal stains such as Gomori methenamine silver (GMS) or per-iodic acid Schiff (PAS) can be used in the detection of the infections. Microbiologic testing such as cultureand PCR can also be used in the diagnosis of the infection.

Candidiasis:

These organisms can be in the form of pseudohyphal filaments, irregular and10–15μm in diameter or Yeast buds which are oval in shape and 2–4μm in diameter. They cause opportunistic infections in immunocompromised patients. It evokes an acute inflammatory response of neutrophils.

Aspergillosis:

In immunosuppressed individuals, many pulmonary lesions are produced by the inhalation of the spores of Aspergillus spp. The spectrum of lesions include localized mycetoma (aspergilloma), diffuse invasive aspergillosis, abscesses, eosinophilic pneumonia or allergic bronchopulmonary aspergillosis (ABPA)[31]. Aspergillus is a thin septate

hyphae with a uniform branching pattern at 45◦ and is 3–6 μm in diameter. Calcium oxalate crystals, eosinophils and lamellated mucus are seen.

Zygomycosis (Phycomycosis):

These fungal hyphae are non-septate, broad with a variable diameter of 6-50μm and branching pattern at 90◦. These fungi are more commonly seen in paranasal sinuses in immunocompromised patients.

Histoplasmosis:

This infection is symptomatic in immunocompromised patients, which is otherwise mostly asymptomatic. This infection is acquired by the inhalation of the soil infected by bird droppings. The organism is 2–4μm in diameter in size with a thin capsule which is positive for PAS and GMS. The organism is located intracellularly within the macrophages and the neutrophils. Extracellular organisms trigger a granulomatous reaction.

NEOPLASTIC PULMONARY LESIONS:

Lung cancer is the most common non-cutaneous malignancy reported worldwide and 80–85% of it is caused by smoking[32]. Asbestos exposure, radiation exposure, nickel, arsenic, cadmium, beryllium and vinyl chloride are other factors which are attributed to cause lung cancer.

Classification of lung cancer:

Most lung cancers arise from the lining epithelium of the bronchioles and bronchi, but few tumors also arise from the alveolar lining epithelium.

These tumors are commonly described as bronchogenic carcinomas. They may be classified into the following main groups:

- Carcinomas exhibiting predominantly squamous differentiation, classified as squamous or epidermoid carcinomas.
- Carcinomas forming glandular patterns, mimicking bronchi or alveoli, classified as bronchogenic adenocarcinomas of various types or as bronchioloalveolar carcinomas.
- Carcinomas composed of undifferentiated small cells, resembling the basal or reserve cells of the bronchial epithelium, forming the group of small cell carcinomas.
- Carcinomas composed of undifferentiated or poorly differentiated large cells, some of which may exhibit glandular or squamous differentiation or even endocrine features.
- Rare types of carcinomas including tumors with endocrine features.

Although a more detailed classification was proposed for the World Health Organization by an expert pathology panel of the International Association for the study of Lung Cancer (Travis et al, 1999), the simple classification shown here is adequate for cytological diagnosis[33].

CLASSIFICATION OF PRIMARY LUNG CARCINOMAS AND RELATED TUMORS[33]

Squamous carcinoma

Keratinizing (well-differentiated)

Poorly differentiated (epidermoid)

Large-cell undifferentiated carcinoma*

Small-cell undifferentiated carcinoma

Oat cell carcinoma

Intermediate cell type

Adenocarcinoma

Adenocarcinoma of central bronchial origin

"Acinar" carcinoma

Solid carcinoma with mucin formation

Papillary carcinoma

Bronchioloalveolar carcinoma

Adenosquamous carcinoma

Mucoepidermoid carcinoma

Spindle and giant-cell carcinoma

Neuroendocrine tumors

Carcinoid

Atypical carcinoid (well-differentiated neuroendocrine carcinoma)

Large-cell carcinoma with endocrine differentiation

Rare carcinomas

Squamous cell carcinoma:

Squamous cell carcinoma mostly occurs centrally, arising in the segmental, lobar, or main stem bronchi. This type of tumor can also present with extensive areas of necrosis with a central cavity.

Cytologicaly, these tumors are characterized mainly by singly scattered cells and loose clusters. The individual cells show marked pleomorphism with well-defined borders, hyperchromatic nucleus with irregularly dispersed chromatin. Bizarre shaped cells like tadpole or spindle shaped cells, caused due to cytoplasmic thinning are also seen. Intense hyaline appearance of the cytoplasm of the cells is an indicator of keratinization. Small tissue fragments can be seen in FNAC and bronchial specimens[20].In poorly differentiated tumors, the tissue fragments gives a crab like appearance with very few cells showing keratinization.

Histologically, the well-differentiated keratinizing squamous cancers are composed of sheets of cells attempting to form squamous epithelium, often with abundant keratin formation and keratin pearls. Central keratinization and necrosis is characteristic, particularly in larger tumors[33].

Adenocarcinoma:

Histological features in adenocarcinomas, the cells are arranged in glandular, papillary or bronchioloalveolar pattern with large amount of mucin in the background.

Cytologically, the exfoliated malignant cells are large, usually round or polygonal, occasionally columnar and are found singly, scattered or in clusters with a three-dimensional papillary or spherical configuration with tumor cells superimposed upon each other. Cytoplasm of the well-preserved cells is moderate in amount, often finely vacuolated, faintly staining, usually basophilic with round to ovoid vesicular nucleus that is placed eccentrically with a macronucleoli[34-36].

Bronchoalveolar carcinoma:

These tumors arise in bronchiolar or alveolar epithelium of peripheral lung tissue and may present as a localized mass or masses in lung parenchyma. The proliferating tumor cells are uniform and orderly in appearance and utilize the alveolar framework for support so that initially the basic architecture of the lung remains well preserved, so-called lepidic spread. Tumor cells often form papillary projections into the alveolar space.

Cytologically, the neoplastic cells are arranged in ball-like three dimentional cell clusters or papillary structures[37]. Cells have round to oval uniform nucleus with granular chromatin and inconspicuous nucleoli.

Small cell carcinoma:

Histologically, small-cell (oat cell) carcinoma is composed of sheets of small, round, ovoid or spindle cells that characteristically seem separated from each other.

Cytologically, the cells are mostly arranged in loose clusters with very few singly scattered cells. Cells have very scanty cytoplasm, round to irregular shaped nucleus with hyperchromatic dense chromatin. The cells are very small equal to the one and half times the size of a small lymphocyte. The tumor cells show very prominent intercellular molding and necrosis[38].

Large cell carcinoma:

Histologically, the large-cell undifferentiated bronchogenic carcinomas are composed of broad, diffusely infiltrating sheets of usually moderate size tumor cells with moderate to abundant cytoplasm .They are without substantial squamous or glandular differentiation, although they may exhibit focal features of squamous cancer or adenocarcinoma, sometimes side by side.

Cytologically, the cells are arranged as syncytial groupings and single cells.The individual cells are large with ill-defined cell borders, round to lobulated nucleus with hyperchromatic and irregularly dispersed chromatin with prominent nucleoli.

Adenosquamous carcinoma:

Adenosquamous carcinoma is defined as a carcinoma with both squamous and adenocarcinomatous areas. The minor component should account for atleast 10% of the whole tumor.

PLEURAL EFFUSION:

The pleural effusion associated with benign disorders mostly has a nonspecific cytologic picture.

Acute pleuritis:

Bacterial infection causing pneumonia also causes secondary infection of the pleura leading to acute pleuritis and later pleural empyema. The pleural fluid becomes creamy, purulent pale yellow with foul smell. Cytologic smears shows very high cellularity and predominantly composed of polymorphonuclear leukocytes. Metastatic malignancy may also present with abundant acute inflammatory cells which sometimes masks the malignant cells. So a careful search for the malignant cells should be done in suspected cases of malignancy. Special stains can also be used to demonstrate the bacteria in case of infection.

Eosinophilic Effusions:

In a pleural effusion if the eosinophil count is 10% or more of the nucleated cells it is considered to be eosinophilic effusion. Between 5% and 16% of exudative effusions are eosinophilic effusions[39].On cell blocks when stained with eosin and hematoxylin, the granules of the eosinophils are stained brightly eosinophilic.Pneumothorax and hemothorax produced due to procedures like thoracenteses are the common cause for eosinophilic effusion[40].Pulmonary infarction,drug reactions,Churg-Strauss syndrome and

parasitic infections are other causes which leads to the eosinophilic effusion[41].

Lymphocytic Effusions:

In pleural effusion, the presence of lymphocytes is a nonspecific finding, but they are commonly present. Cytologic smears from the pleural effusion are usually highly cellular and show abundant dispersed lymphocytes and few mesothelial cells and histiocytes[42]. The most common cause of lymphocytic effusion is tuberculosis and malignancy. Lymphocytic effusion is also caused by the obstruction of the lymphatics by the malignancy without spreading to the pleural surfaces or the pleural malignancy produces only lymphocytic response without shedding the malignant cells into the effusion[46]. The mere absence of malignant cells in the lymphocytic effusion does not rule out malignancy.

Rheumatoid pleuritis:

Rheumatoid pleuritis is caused in less than 5% of patients with joint disease.Pleuritis in a patient with rheumatoid arthritis develops either before or after the appearance of the joint disease or develops along with the joint disease[44, 45].

Malignant pleural effusions[47]:

Are seen in association with

1) Primary Tumors of Mesothelium (Mesothelioma)
2) Metastatic tumors

Malignant Mesotheliomas-

It is most commonly due to occupational exposure to asbestos and carries bad prognosis. Histologically it is composed of large malignant cells with abundant eosinophilic cytoplasm and large but pale vesicular nuclei forming glandular andtubular structures, often separated from each other by connective tissue septa.Papillary excresevences are common[47].

Metastatic Tumors-

Lung tumors in males and breast tumors in females are the malignant diseasesmost commonly responsible for malignant pleural effusion. Besides the lung and pleura, the primary common sites of malignancy in males were the gastrointestinal tract, liver and pancreas. In females, the breast, lung, ovary, pancreas, gastrointestinal tract and uterus were in descending order of frequency[48].

General characteristics of tumor cells in effusions that may help in identifying nature and site of primary lesions:

Recognition of the cells by size:

The size of tumor cells may vary gently according to tumor type. To determine the size of a suspect cell, a comparison must be made with identifiable cell types suchas erythrocytes, lymphocytes or mesothelial cells.

In large cell types, cells are significantly larger than normal mesothelial cells.Some mesotheliomas, metastatic carcinomas of various types, malignant melanomas and sarcomas belong to these groups.

In small cell type, tumor cells are much smaller than mesothelial cells. Lymphomas, Neuroblastomas, Wilm's tumor and Oat cell carcinoma belong to thisgroup.

Medium sized cells are approximately same in size to mesothelial cells. Carcinomas of mammary, Lung, Gastric, Pancreas, or Prostatic origin may have this presentation.

Cell Aggregates:

Malignant tumors, principally adenocarcinoma of various primary origin form three-dimensional cell aggregates or gland like structure with a central lumen.

Cell Products and cytoplasmic Features:

Products of metabolic activity of cells, such as mucus, melanin pigment, psammoma bodies, cytoplasmic cross striations and intracytoplasmic glandular inclusions(Target cell, Bulls eye cells) help in identifying the primary site of tumor.

Nuclear features:

Most of malignant cells in fluids have enlarged nuclei and increased nuclear cytoplasmic ratio.Nuclei are usually round to oval with smooth borders. Occasionally nuclei are irregular or indented in lymphomas. Large, irregularly shaped, single or multiple nucleoli are frequently observed in cancer cells. Presence of mitotic figure is a presumptive evidence of cancer.

Abnormal or multiple mitotic figures are more reliable identifiers of cancer cells in effusion.

Nuclear Cytoplasmic Inclusion:

Sharply demarcated clear areas within the nucleus correspond to cytoplasmic invaginations. It is seen in cells of metastatic melanoma, thyroid cancers and pulmonary adenocarcinoma.

Multiple Sex Chromatin (Barr) Bodies:

In female patients, two or more sex chromatin bodies in the same nucleus are virtually diagnostic of cancer, because they document the presence of an abnormal chromosomal complement. This observation is particularly helpful in the diagnosis of some cases of mammary carcinoma[47].

TECHNIQUES TO OBTAIN SAMPLES:

Bronchoscopy:

Gustav Killian developed the rigid bronchoscope in nineteenth century, which laid the foundation for visualizing the mucosal surfaces of the bronchi and sampling of tissue and evaluation of cellularity. Walloch summarized the important advances of flexible catheters passed into the bronchi under fluoroscopy and the development of the flexible fiberoptic bronchoscope[49].

The main bronchus divides into second-order (lobar) bronchi, which divides further into third-order (segmental) bronchi. The part of the lung supplying the third-order bronchus is a bronchopulmonary segment.

Bronchial Washings:

Bronchial wash cytology is a widely accepted safe, simple and minimally invasive technique to evaluate cell morphology. Bronchoscopic washing, brushing and fine needle aspirations may complement tissue biopsies in the diagnosis of lung cancer[17, 50]. The bronchial washing is a safer technique and the malignant cells can be readily recognized and typed[33]. It is a valuable investigation in situations where biopsy procedure cannot be attempted due to high risk of hemorrhage or in more peripheral sites.

Bronchial washings by bronchoscopy are performed to detect and characterize, ill-defined premalignant or malignant lesions and for the identifying microbiologic pathogens. Specimens are obtained with a suction apparatus that aspirates secretion. Washings are collected by instilling 3–5mL of a balanced salt solution and re-aspiration of the material. Smears are prepared by centrifugation or membrane filtration. The collected samples are centrifuged and the residual cellbutton is embedded in paraffin for histologic sectioning.To be considered as adequate bronchial brushing or lavage, abundant ciliated columnar cells must be present. In patients with pulmonary malignancies, alveolar hemorrhage, interstitial lung disease and pneumonia, BAL and washings are done.

Bronchial Brushings:

With flexible fiberoptic bronchoscope, a suspected lesion may be visualized and brush cytology material can be examined. Similar technique

as for preparation of cell block from the bronchial washings is used for bronchial brushings also.

Fine-Needle Aspiration:

Direct tissue sampling for the diagnosis of peripheral pulmonary lesions is essential in most patients for decisions regarding treatment and can be accomplished by fine needle aspiration cytology (FNAC). FNAC can be performed via the airway (endobronchial/transbronchial FNAC) or chest wall (CT-guided percutaneous FNAC). Transbronchial FNAC is useful for the diagnosis of primary pulmonary lesions that lie beneath the bronchial surface[51].

The respiratory tract cytology is obtained by radiologic imaging guided precise visualization and localization of the lung mass and sampling of such visualized lesions with a fine bore needle passed into them[52-54]. A syringe fitted with a fine needle is passed through the chest or bronchial wall into the pulmonary mass under the guidance of bronchoscopy, computed tomography or fluoroscopy. The aspirated specimen is subjected for analysis by conventional smear.

Indications and Contraindications:

Stitik and Heaston associates summarized the following indications[55-56]

1) Inoperable but suspected lung cancer.
2) Probable metastatic lesion due to a solitary pulmonary mass.

3) Malignant extra-pulmonary primary and a solitary pulmonary nodule.
4) Patient with suspected lung cancer, refusing exploratory thoracotomy.
5) Patient failing to respond to anti-tuberculous therapy.
6) A suspected infectious process, particularly in an immunocompromised patient.
7) A suspected superior sulcus tumor.
8) Multiple pulmonary mass lesions.
9) An undiagnosed pulmonary mass.
10) A patient who is suspected of having lung cancerand who has produced five consecutive earlymorning deep-cough specimens of sputum and one bronchial brushing or washing that have been negative for malignant tumor cells.

Contraindications for FNAC:

1) Debilitated or uncooperative patient orthose with uncontrollable cough.
2) Patients with bleeding diathesis, onanticoagulation therapy or suspected vascular lesion or pulmonary hypertension.
3) Patients with echinococcal cyst.

Complications:

Pneumothorax, hemothorax and hemoptysis.

Thoracentesis:

This is a technically simple and safe procedure, when done in a patient without bleeding tendencies or after correction of coagulopathy. For diagnostic studies, 50-100 ml of fluid is sufficient. It is done at the bedside with the patient sitting upright comfortably and the arms and head supported by an adjustable table.The upper border of moderate effusion is identified by stony dull percussion note and loss of tactile and vocal fremitus. Thoracentesis is performed in the interspace below this level. The skin is cleansed with iodophor or an antiseptic solution and the underlying tissues, including the periosteum of the rib is infiltrated with local anaesthetic. A 20 gauge needle is inserted 5-10 cm lateral to the spine above the superior border of lower rib in order to avoid the intercostal vessels or nerve injury. The needle is advanced with continuous gentle suction until the parietal pleural membrane is penetrated and adequate fluid is obtained. In case of minimal or loculated effusion the needle is inserted under ultrasound guidance[7].

CELL BLOCK TECHNIQUE:

Cell block technique or paraffin embedding of sediments of fluids is among the oldest methods of preparing material for microscopic examination. The method uses histologic techniques for processing and thus offers one major advantage: multiple sections of the material are processed for routine stains, such as hematoxylin and eosin, and for special stains that

may serve for immunocytochemistry and for identification of mucin, melanin, or other cell products, and identification of bacteria and fungi.

With the development of excellent cell preparation techniques, the cell block technique has been abandoned. This neglect is not justified and the residual material remaining aftercytologic preparations must be subjected to cell block technique. The residual material contains valuable evidence of tissue fragments for processing by cytologic techniques. Richardson et al (1955) have shown that additional diagnoses of cancer can be obtained in 5% of fluid specimens by cell block sections of residual material, supplementing the smear technique[57]. The additional benefit of cell block technique is the recognition of histologic patterns of disease that sometimes cannot be reliably identified in smears or filter preparations.

Aspiration biopsy material (FNA), sputum, effusions, urine sediment and material from the gastrointestinal tract are suitable for cell block processing, as all tissue fragments incidentally obtained during any other diagnostic cytologic procedure.

The best cellular details in cell blocks are obtained with Bouin's fixative or picric acid fixative. However, a more practical fixative is buffered formalin that allows a wide range of additional procedures.

Methods:

1. Fixed Sediment Method[17]:

1) Mix sediment or tissue fragments with the fixative. Fibrin clots can be wrung out and placed in fixative separately. Centrifuge this mixture.
2) Pour off supernatant and drain tube well by inverting the tube on a paper towel.
3) Carefully remove the packed sediment or fibrin clot from the test tube by means of a spatula and wrap it in lens paper. Place wrapped sediment in a carefully labeled tissue cassette.
4) Put tissue cassette into a jar of the same type of fixative used before. Process as tissue biopsy.

2. Bacterial Agar Method (3% Agar):

Steps 1 through 3 are the same as for the fixed sediment method.

4) If sediment becomes hard and packs well, gently remove it from the test tube with a spatula and place it on a paper towel with the conical side up.

5) Slice the sediment in half from the top to the bottom of the conical clot with a scalpel.

6) Place the cut side of the packed sediment in a small pool of melted agar that has been spread on a glass slide or in a Petri dish. Cover all exposed areas of the sediment with melted agar and let stand a few

minutes to harden. Care must be exercised to avoid bubbles in the agar.

7) Trim the excess agar from the sediment and slice the sediment in half from the top to the bottom of the conical clot with a scalpel and place it in a tissue cassette.

8) If sediment does not pack well or only a small amount is available after completion of steps 1 through 3, a few drops of melted agar should be added to the test tube and mixed thoroughly with sediment. After the agar hardens, gently remove the agar button from the test tube and place it in a tissue cassette.

Preparation of Agar:

The 3% agar is prepared by dissolving 3 g of bacterial agar in 100 ml of boiling water. The melted agar may be colored with a small amount of food coloring to ensure contrast with the paraffin. The dissolved agar should be poured into individual sterile glass tubes with a screw cap. Cap the tubes loosely until the agar cools and hardens. When the agar has cooled, tighten the caps and place the tubes in a refrigerator until ready for use. When it is needed, melt the agar in a 60°C water bath. Discard unused agar at the end of the day.

3. Simplified Cell Block Technique:

In 1988, Krogerus and Anderson[58] introduced a simple technique of cell block preparation from materials obtained from effusions, fine-needle

aspiration and brushings. The technique is unique in that, the procedure is carried out in the sample tube, ensuring minimal cell loss. No transfer of cells to a cassette is necessary, eliminating the need for wrapping paper, agar, or thrombin. The procedure is as follows:

- In a 50-ml plastic, conical centrifuge tube, fix cell sample with 50% alcohol for 1 hour.
- Spin sample at 300 g for 7 minutes and pour off supernatant.
- Re-suspend cell pellet in 3 ml of acetone for 10 minutes.
- Spin sample at 300 g for 10 minutes. Pour off acetone.
- Place tubes for 1 hour on a warm plate (not more than 60°C).
- Add melted paraffin to the dry, warm pellet.
- After paraffin has solidified, tap the bottom of the tube to remove block.
- Cut and process the conical end of the paraffin block as you would any tissue section.

4. Plasma-Thrombin Clot Method:

- Thoroughly mix a few drops of blood plasma obtained from blood bank with the fresh unfixed sediment. Plasma may be colored with a small amount of food coloring to ensure contrast with the paraffin. If the sample was prefixed with alcohol, the sediment must be washed several times with a balanced salt solution, since alcohol inhibits the clotting action of plasma and thrombin.

- Add the same number of drops of thrombin solution as of the pooled plasma and mix well. Thrombin is prepared by adding 5000 units(topical, 1 vial) with 10 ml of distilled water.
- This mixture will form a clot in 1 to 2 minutes if the reagents are fresh and not too cold. Place resulting clot in a cassette that has been lined with lens paper to prevent the clot from oozing through the holes.
- This clot is very soft and a spatula, instead of a forceps, is recommended for transfer to the embedding mold.

5. Compact Cell Block Technique:

Yang et al (1998)[59] described a technique that produces a compact cell block about 10% to 20% the size of conventional cell blocks. Cells are packed into a small area free of erythrocytes and extracellular protein, thereby reducing the overall time for screening and often the need for deeper cuts are eliminated.

- Pour off the supernatant after centrifugation of 40 cc of a well mixed aliquot of the sample.
- Mix the sediment with an equal volume of CytoRich Red.
- After 2 minutes, add 4 drops of plasma and 3 drops of thrombin (5,000 µl/10 ml).
- Gently agitate the mixture. When the clotting stops, the clot is slided onto the lens paper placed on top of paper towels.

- The lens paper is folded over the clot. Press and mold the clot flat and compact with a gloved fingertip. Wrap the compact clot tightly in lens paper and place in fixative.

6. Microwave Technique for Rapid Processing of Cell Block:

Since the early 1970s, microwaves have been used by histopathology laboratories to shorten fixation and processing times of tissue samples. In 1988, Kok et al[60] described a method in which cell blocks from fresh sputum can be prepared in 35 minutes. The method can be adapted for use with other types of specimens.Best results were obtained with a fixative consisting of 500 ml of 96% ethyl alcohol, 430 ml of distilled water, and 70 ml of polyethylene glycol.

- Place sputum in 40 ml of fixative in a microwave-safe jar.
- Microwave sample at 450 watts with the temperature set at 70°C. This usually takes 5 minutes.
- Place the sputum, which has become condensed and rubbery, into a tissue cassette. Put the cassette into 40 ml of absolute ethyl alcohol and microwave at 450 watts and 70°C. This usually takes 3 minutes; however, let the cassette sit in the microwave for another 2 minutes.
- Transfer cassette to 40 ml of Histoclear. Microwave at 450 watts or 80°C for 7 minutes.
- Embed the material, cool blocks, cut and mount sections.

- Sections can be de-paraffinized by placing them in Histoclear and microwaving them for 5 minutes at 700 watts and then stained by the method of choice.

7. Cell blocks from Millipore Filters:

Baloch et al (1999)[61] described a technique by which a portion of a Papanicolaoustained millipore filteris converted to a cell block for other stains or immunocytochemical analysis for specimens of limited cellularity.This technique produces hematoxylin and eosin (H&E) preparations with excellent morphology and antibody test results. In most cases, routine cell blocks with adequate background staining is not seen.The original cytologic preparation is preserved asonly half of the filter is used.

8. Shidhams protocol:

The use of cell block sections is a valuable ancillary tool for evaluation of non-gynecologic cytology. They enable the cytopathologist to study additional morphologic specimen detail including the architecture of the lesion. Most importantly, they allow for the evaluation of ancillary studies such as immunocytochemistry, in-situ hybridization tests (FISH/CISH) and in-situ polymerase chain reaction (PCR). Cell blocks have traditionally been applied to cytology of non-gynecologic specimens like fine needle aspiration biopsies and body fluid effusions.

Liquid based non-gynecologic specimens have many individual scattered cells. When the cellularity is less, the cell block sections are

difficult to achieve. The histotechnologist making sections of the block cannot identify the level of highest concentration of cells for sectioning and transferring it to the glass slides for analysis. Hence the cell block area with maximum cells may be missed, either by not cutting deep enough or cutting past the region. Current Shidham protocol eliminates these drawbacks. This protocol is standardized and reported for non-gynecologic specimens like FNA, brushings, effusion fluids, cyst contents etc., for improving the quality of material in cell blocks.

The following are the two critical features for preparing cell blocks from hypocellular specimens with scattered single loose cells by this protocol[62-66].

1) Step to concentrate the cells along a parallel plane to the cutting surface of the cell block.
2) Dark inclusion as AV-marker,serving like a beacon for two purposes:
   a) To visualize the site of cellular concentration, as dark colored beacon, exposed during cutting. The ability to identify a dark colored beaconprevents from cutting through the+ 8 level with most cells or not cutting too superficial into the level of highest concentration of cells.
   b) To serve as a locator reference point in serial cell block section on different slides. The beacon helps to locate particular cells or groups

of cells for assessment of a coordinate immunoreactivity pattern with the SCIP approach[67,68].

Protocol-

Sample Preparation:

1) The residual liquid based cytology (LBC) specimen is transferred to a flat bottomed glass tube (15mm diameter x 45mm). The glass tube is placed in a larger plastic carrier tube (28 x 85mm) for centrifugation. The glass bottomed tube is removed from the carrier tube and the supernatant is poured off.
2) The glass tube is capped (to prevent spillage of heating water in the next step) and placed inside acarrier plastic tube which is larger and flat bottomed.
3) The carrier plastic tube with the glass tube is then capped and placed for centrifugation (*with swiveling cups and not fixed angle cups so that the cells fall perpendicularly to the flat bottom of the glass tube*) at 1805 G (3000 rpms, rotor radius- 17cm) for five minutes.
4) The tubes are removedfrom the centrifuge vertically and the smaller glass tube is removed with forceps from the larger carrier plastic tube avoiding any disturbance to the sedimented pellet of cells.
5) The glass tube with specimen is uncapped and the supernatant is poured off without disturbing the flat layer of cells, sedimented at the bottom.

Inclusion of the reference coordinates, AV-marker and addition of gel:

1. A dark beacon *AV-marker* (about 2 mm X 2 mm size, flat surfaced, fragment of dark colored, sectionable material) which is added, acts as a signpost in the glass tube.
2. An aliquot of histogel (HG) is liquefied by melting it in a microwaveat medium power for 10 seconds.
3. 0.5 ml of molten HG is added to the tube and mixed with the sediment quickly and recapped (Proceed to the next step quickly without allowing the HG to begin solidifying).
4. 2.5 ml of warm (45° C) water is added to the carrier plastic tube.
5. The smaller capped glass tube is placed inside the plastic tube with warm water. (This step is necessary to keep the HG from solidifying during the next steps).
6. The carrier plastic tube is placed for centrifugation (*with swiveling cups and not fixed angle cups so that the cells fall perpendicularly to the flat bottom of the glass tube*), for five minutes at 1805 G (3000 rpms, rotor radius- 17cm). The centrifugation pushes the AV-marker and concentrates the cellsof the final paraffin embedded cell block into a layer closer to the cutting surface.
7. The tubes are removed vertically and gently from the centrifuge avoiding disturbance to the sedimented thin layer of cells at the bottom.

8. The larger plastic tube is uncapped and the smaller glass tube is removed vertically with a forceps without disturbing the sedimented layer of cells.
9. The small glass tube is refrigeratedfor 15 minutes in vertical position, to cool and solidify the HG.

Removal of the cell block as a button of gel with specimen for final processing:

1) The solidified HG disk, with the layer of concentrated/sediment specimen at the bottom is dislodged from the flat bottom glass tube by squirting 10% formalin through a 23 gauge needle with the syringe.
2) The needle is inserted along the side of the tube at the periphery of solidified HG disc with specimen.
3) The needle is rotated along the side of the tube while formalin is being slowly pushed in through the syringe. This results in the separation of the HG button along with dark colored beacon AV-marker and the concentrated specimen in it, from the flat bottom of the glass tube.
4) The cell block (gel button with specimen cells) is then placed in a labeled cassette and submitted for *tissue processing* to prepare paraffin embedded cell blocks.

Embedding and cutting of the specimen:

1. The disk is embedded in paraffin with the dark beacon marker side down as cutting surface.

2. The block is sectioned until the dark colored *AV-marker* as a beacon is exposed and clearly visible.
3. Three to four micron sections are cut from this level which should contain most of the singly scattered cells from the specimen.
4. The sections are collected on the glass slide for further staining, immunohistochemical staining or other tests as indicated. Generally for immunostaining, coated slides are used to prevent floating and loss of sections from the slides during the immunostaining steps.

9. Modified cell block technique:

Nathan et al in 2000 suggested a modified cell block technique by using Nathan alcohol formalin substitute (NAFS)[69]. After preparing smears, the needles and syringes utilized for fine-needle aspirates were rinsed in 10 mL of 50% ethanol in a specimen container. Any residual clot or tissue in the hub of needles was removed carefully in the laboratory with the aid of another needle and rinsed in 50% ethanol. At 4,000 rpm for 6 minutes,the material was centrifuged in a 10-mL centrifuge tube to create one or more cell pellets. The supernatant fluid is decanted and the deposit fixed in freshly prepared Nathan alcohol formalin substitute (NAFS) consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde. Since formalin oxidises to formic acid on exposure to air, forming acid hematin pigment artifacts, afresh working solution is desired.

Centrifuged deposits of effusions, clots, washings, and other fluids, following smear preparations, were fixed similarly for cell blocking. When centrifuged deposits were more than 0.2 mL thick, to facilitate adequate fixation, the deposit was detached carefully from the bottom of the centrifuge tube with the aid of a sharp-edged dipstick. If the centrifuged deposits were too thick, the material was divided into several tubes for multiple cell blocks before fixing in NAFS solution. The fixed cell pellets, at the end of fixation for 45 minutes, were re-centrifuged for 6 minutes at 4,000 rpm. These pellets should detach themselves or can be removed easily with a disposable Pasteur pipette following centrifugation. After wrapping the cell pellets in crayon paper and placing in a cassette it is stored in 80% ethanol until ready for processing in the automatic tissue processor.

10. Thromboplastin Plasma Cell Block (TP-CB) technique:

Kulkarni et al in 2009 used plasma thromboplastin for preparing cell block[70].After preparing conventional smears, the remaining fluid were centrifuged.In the case of aspirations, rinses of syringes and needles were centrifuged by collecting it in normal saline.The supernatant was carefully removed and the sedimentwas mixed with two drops of pooled plasma that was kept frozen and brought to room temperature before use.Subsequently, four drops of thromboplastin were added and mixed again. The thromboplastin used for the TP-CB was the same as the one used for the

thromboplastin test and it should be stored in the refrigerator between 2-8 C◦ and brought to room temperature before use.The tube was allowed to stand for 5 min. and the resultant clot was slid into a filter paper pre-moistened with formalin, wrapped and put in a cassette. The tissue cassette was then fixed in buffered formalin for at least 4 hrs. After-wards, the sample was processed as usual for histological techniques.

Principle antibodies currently used in diagnosis of lung and pleural tumor's-

Diagnosis and classification of lung and pleural neoplasms are complex due to diverse histopathology and tumor heterogeneity. A wide variety of primary neoplasms occur in the lung.Four major types make up 85% to 90% of primarylung neoplasms: adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and large-cell undifferentiatedcarcinoma[71].

Evaluation of pulmonary and pleural neoplasms requires determination of histopathologic type and differentiation, as well as assessment of probable site of origin. This process is currently based primarily on histopathologic features, immunohistochemistry (IHC) provides valuable additional information in several settings.

First, IHC can assist in diagnosis and classification of a neoplasm as a non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC), a distinction critically important for determining therapy.

Second, IHC can provide evidence to support the interpretation of a neoplasm with neuroendocrine differentiation. And finally, IHC can help the differential diagnosis between lung carcinomas and malignant mesotheliomas, between lung carcinomas and metastatic extra-pulmonary malignancies.

In the last decade, a broad spectrum of antibodies or immunohistochemical markers have been developed and used to resolve these differential diagnostic questions. A large number of immunohistochemical markers have recently become available to facilitate accurate diagnosis and classification of pulmonary and pleural neoplasms.

Most important generic immunomarkers of lung carcinomas are the following:

Cytokeratin 7 and cytokeratin 20:

Cytokeratin7 is a 54-kDa marker of simple epithelium found in the columnar and glandular epithelium of lung. In pulmonary pathology, antibodies to CK7/CK20 are most helpful in the differential diagnosis of pulmonary adenocarcinoma and metastatic colonic adenocarcinoma. Most lung adenocarcinoma are CK 7+/ CK 20-, while most metastatic colon adenocarcinoma in lung are CK 7-/ CK 20+[72].

Cytokeratin 5/6:

Cytokeratin 5 and 6 are high molecular weight, basic cytokeratins which correspond to keratins 58 and 56 kDa respectively. Cytokeratin 5/6 is most commonly used in the diagnosis of mesothelioma, where it stains tumour cellsand reactive mesothelium in a diffuse cytoplasmic fashion[73]. Most pulmonary adenocarcinomas do not express CK 5/6, although one study showed that 19% of them had weak or focal positive staining[74]. Antibodies to CK5/6 are best used in a panel of antibodies for the differential diagnosis of mesothelioma and pulmonary adenocarcinoma.

Calretinin:

Calretinin is a calcium binding protein with a molecular weight of 29kDa. Calretinin is consistently expressed in the normal and reactive mesothelial cell lining of serosal membranes. It stains in diffuse nuclear/ cytoplasmic pattern in formalin fixed paraffin embedded tissue sections. It is probably the most specific marker for mesothelial cells. The presence of calretinin is also a sensitive and specific indicator of normal and reactive mesothelial cells in effusion cytology. Anti calretinin antibody is a useful marker to distinguish mesothelioma from adenocarcinoma.

HBME-1:

HBME-1 was derived from human malignant epithelioid mesothelioma cells. It consists of antigens on the cell membrane of

mesothelial cells, both benign and malignant. So it shows membranous cell surface pattern in epithelioid mesotheliomas, while it is negative or shows cytoplasmic staining in adenocarcinoma. Its usefulness is limited by its low specificity.

Ber-EP4:

Antibody to Ber-EP4 shows a broad pattern of reactivity with human epithelial tissues, from simple epithelia to basal layers of stratified, non-keratinized squamous epithelium and epidermis. The staining pattern is membranous and used to distinguish adenocarcinoma from mesothelioma.it has very high sensitivity (94 – 100%) for lung adenocarcinomas, it also stains 9-18% of epithelioid mesotheliomas[75]. So the interpretation of staining result should always be done in combination with other antibodies.

B72.3:

The mouse monoclonal antibody to B72.3 recognizes a high molecular weight glycoprotein complex, TAG 72(tumor associated glycoprotein -72). This antibody works on formalin fixed, paraffin embedded tissues and cell blocks prepared from body fluids. It can be used in an antibody panel to distinguish adenocarcinomas from mesotheliomas. It has been shown to be positive in about 90% of pulmonary adenocarcinomas and in 0-14% of mesotheliomas[76].

Thyroid transcription factor -1(TTF-1):

Human TTF-1 is a single polypeptide of 371 aminoacids. It is expressed at the onset of lung and thyroid organogenesis and is essential for the normal development of these organs[77]. It is expressed in thyroid follicular epithelial cells, pulmonary type-II cells and clara cells which makes it a useful diagnostic epitope to identify adenocarcinomas. It is a useful marker in differential diagnosis of primary tumors of lung and thyroid versus metastases from other organs. It shows a nuclear staining pattern. TTF-1 is expressed in 90% of small cell carcinomas of the lung, 80-90% of pulmonary carcinoids and 70-100% of adenocarcinomas of lung.

Surfactant apoproteins A and B:

Antibodies to pulmonary epithelial cell specific surfactant apoproteins-A (SP-A) and B(SP-B) have been used to differentiate primary lung non-small cell carcinoma (in particular, adenocarcinoma) from extra pulmonary neoplasms. This stains about 50- 60% of both non-small cell carcinomas and adenocarcinoma of the lung[78].

NAPSIN A:

Napsin A is an aspartic proteinase involved in the maturation of surfactant protein-B. It is detected in the cytoplasm of type II pneumocytes and alveolar macrophages. It is a highly sensitive marker for pulmonary

adenocarcinomas (positive in about 80% of cases), and thus is a useful alternative of TTF-1[79].

ES1:

This antibody, which recognizes a variant form of CEA-related cell adhesion molecule-6, has been touted as a marker which stains selectively lung carcinoma in a more sensitive fashion than TTF-1. Obviously, this claim will need to be proven independently[80].

P63:

A study demonstrated by immunohistochemical methods showed that p53, p63, p73 expression are gradually increased in dysplastic squamous cells epithelium. Of these markers, p63 is of greatest potential interest as a marker for squamous cell carcinoma. This epitope is expressed in pulmonary epithelium and pulmonary squamous cell carcinoma, 95-100% of which show nuclear staining.

Immunohistochemical staining pattern in major histological subtypes of lung and pleural tumor's:

Squamous cell carcinoma:

Squamous cell carcinomas are immunoreactive to most epithelial markers, such as pancytokeratin, low molecular weight keratins, high molecular weight keratins and focally epithelial membrane antigen

(EMA).Some rare variants of squamous cell carcinoma that cause difficulties in diagnosis are papillary, clear cell, small cell and basaloid forms.

Staining with p63 and TTF-1 was shown to be helpful in differentiating small cell carcinoma from poorly differentiated squamous cell carcinoma[81]. Small cell carcinomas were negative for p63 and 87% of them were positive for TTF-1, whereas all poorly differentiated squamous cell carcinoma were positive for p63 and negative for TTF-1.

Adenocarcinoma:

Adenocarcinomas express low molecular weight keratin, in particular pulmonary adenocarcinomas are usually cytokeratin-7 positive and cytokeratin-20 negative except for bronchoalveolar carcinoma[82]. When high molecular weight keratins are present, the tumour may have a mixed adenosquamous phenotype. It also expresses EMA, CEA, Ber-EP4. In current practice, the most commonly used antibody to identify an adenocarcinoma as primary in lung is TTF-1(72- 96%)[83].

Bronchioloalveolar carcinoma:

The diagnosis of bronchioloalveolar carcinoma usually does not require immunohistochemical study because of its distinct alveolar growth pattern. Well differentiated mucinous or goblet cell bronchioloalveolar carcinomas, infrequently (0-20%) expresses TTF-1[82]. Nearly 90% of well differentiated mucinous bronchioloalveolar carcinomas express CK20. This

atypical immunohistochemical staining pattern can make the differential diagnosis with metastatic adenocarcinoma of colon difficult.

Small cell carcinoma:

Immunohistochemical stains are helpful to establish the diagnosis of small cell carcinoma, particularly in minute or crushed samples. It stains with antibodies to keratin, this is of great help to separate them from lymphoid cells and lymphomas. A classic finding is punctate cytoplasmic staining for keratin, but it is seen in only about 40% of cases. The most useful neuroendocrine markers for diagnosis are chromogranin-A and synaptophysin. Lyda and weiss et al[84], showed that 84% of small cell carcinomas stained for chromogranin-A and 58%stained for synaptophysin. Negative staining for neuroendocrine markers does not exclude the diagnosis of small cell carcinoma.

A variety of other markers reported in small cell carcinomas are CD56 or N-CAM, histidine decarboxylase, EMA, bombesin, gastrin releasing peptide and class III beta- tubulin[85]. CD-56 shows high percentage (94-100%) of small cell lung carcinomas. Tumour cells stain in a strong membranous pattern in the paraffin sections.

Large cell carcinoma:

All the large cell carcinoma stains for pancytokeratin and low and high molecular weight cytokeratin due to the epithelial nature of the

neoplasm. Vimentin is co-expressed in these tumors and almost negative for TTF-1[86]. Basaloidcarcinoma, a histological variant of large cell carcinoma preferentially expresses high molecular weight keratin (CK 5/6 and 14) but not lower molecular weight keratin. This variant can be differentiated from large cell neuroendocrine carcinoma as the later shows positivity for neuroendocrine markers.

Metastatic renal cell carcinoma is an important differential diagnosis for clear cell carcinoma variant of large cell carcinoma .This can be differentiated by the expression of CD-10 by most of the renal cell carcinoma.

In Large cell neuroendocrine carcinoma, the neoplastic cells are positive for neuroendocrine markers in a very patchy and weak pattern. The tumour cells also stain for keratin and CEA (100% each).

Adenosquamouscarcinoma:

There is no specific immunohistochemical marker for adenosquamous carcinoma. It expresses the antigens found in adenocarcinoma and squamous cell carcinoma of lung.The glandular component of the tumor is usually TTF-1 positive[86].

Sarcomatoid carcinoma:

This tumour refers to poorly differentiated non-small cell carcinomas that show marked variation in cell shape or size or that have a component of

sarcomatous or sarcoma like differentiation. Pleomorphic carcinoma is positive for cytokeratins in 75-100% of cases.EMA, CEA OR Ber –EP4 can also be present[87].

In spindle cell carcinoma, immunohistochemical detection of cytokeratin, CEA, EMA or other epithelial antigens in the spindle cells is required for the diagnosis. Keratin or epithelial membrane antigen is present, along with CEA and vimentin. In carcinosarcoma, the epithelial component stains positive for keratin (100%) and the stromal component shows positivity for S-100, desmin, actin, myoglobin based on the differentiation of the sarcomatous component.

Typical and atypical carcinoids:

Carcinoids express neuroendocrine cell markers, particularly chromogranin followed by synaptophysin and leu-7. These markers stain more intensely and diffusely than do other neuroendocrine tumors.Atypical carcinoids show slightly less extensive and intensive staining for these neuroendocrine markers than do carcinoids.TTF-1 can be used to distinguish pulmonary carcinoids and their metastases from metastatic intestinal and pancreatic carcinoids.

Carcinomas of salivary gland type:

Mucoepidermoid carcinoma rarely requires immunohistochemical staining for diagnosis. The tumour cells are immunoreactive for

pancytokeratin, mucicarmine or mucin stains for mucin containing cells and few cases show reactivity for CK7 and CA19-9.Adenoid cystic carcinoma shows strong epithelial reactivity for low molecular weight keratins, vimentins.

In Pleomorphic adenoma the epithelial component shows strong staining for low molecular weight keratins. The basal layer of ductular cells and many of the spindle and stellate cells lie in the myxoid matrix and stain for vimentin.

Malignant mesothelioma:

Immunohistochemistry places an important role in the differentiation between malignant epitheliod mesothelioma and adenocarcinoma. Mesothelial markers include calretinin, CK-5/6, WT-1, thrombomodulin, HMBE-1 and mesothelin. Adenocarcinoma markers include CEA, leu–M1, Ber-EP4, MOC-31, B72.3, TTF-1 and BG8. There is considerable variability in sensitivity and specificity of these markers.

Calretinin is highly sensitive marker for mesothelial lineage. It is present both in the cytoplasm and the nucleus of the mesothelial cells. Nuclear staining must be present for the diagnosis. The antibody stains 50-100% of the mesotheliomas but 0-70% of the adenocarcinomas.

CK-5/6 is highly sensitive and specific mesothelioma marker, staining in a cytoplasmic pattern. This marker is 55-100% of mesotheliomas and in 0-

21%in the adenocarcinomas. It can also be seen in squamous cell and transitional carcinoma.

Wilms tumour gene product (WT-1) is a nuclear protein expressed in the mesothelial cells. Nuclear staining is seen in 71-95% of malignant mesotheliomas and 0-22% in adenocarcinoma.

Thrombomodulin (CD 141) is less sensitive and specific than other markers. It shows membranous staining in 30-100% of the epithelial mesotheliomas and in 5-77% of adenocarcinoma.

HMBE-1 consists of antigens from the cell membrane of mesothelial cells. The antibody stains 57-100% of mesotheliomas.

TTF-1, a nuclear transcription factor is found in the adenocarcinoma originating from lung and thyroid. It is commonly expressed in the nuclei of 75% of adenocarcinoma of lung and 25% of the large cell carcinomas of the lung, but not in the mesotheliomas.

Carcinoembryonic antigen(CEA) is most sensitive and frequently positive marker in the adenocarcinoma of the lung and gastrointestinal tract. The staining pattern is cytoplasmic. Adenocarcinomas stain frequently (60-100%) and mesothelioma infrequently(0-21%).

MATERIALS AND METHODS

During the period from January 2013 to August 2014, 100 samples from minimally invasive procedures of pulmonary lesions (pleural fluid, bronchial washings, remnants of fine needle aspiration cytology) sent to the Department of Pathology, Tirunelveli Medical College and hospital were analyzed by conventional cytology smear study and cell block technique. Immunohistochemical staining was done for cases which were reported as malignant or suspicious for malignancy.

Clinical information of patients regarding age, sex and clinical diagnosis were recorded. Most sample fluid was processed immediately, but in small number of samples, when there is a delay the specimens were stored in refrigerator and processed later.

After receiving the samples of pleural fluid and bronchial washing, clots if present, were removed with a spatula by pressing the clot against the sides of the container and clot was cut into small fragments and was fixed in 10% buffered formalin.The fluid was divided in to two parts. One part of the fluid was taken and centrifuged at 2500rpm for 15 minutes and the sediment was smeared on a glass slide. The smears were fixed in 99.9% isopropyl alcohol for 20 minutes andwere stained with haematoxylin and eosin. The

other part was used for making cell block using plasma thromboplastin method described below.

Fine needle aspiration mostly percutaneous was done by multiple passes with 23G needle and the smears were made on a glass slide and stained with haematoxylin and eosin. Samples collected for the cell block were either from an additional dedicated needle aspiration and or needle rinse of the existing needle passes.The residual fine needle aspiration cytology material taken by rinsing the syringe and hub of the needle with 10% neutral buffered formalin and cell blocks were made as described below.

Cell block preparation:

The samples of pleural fluid, bronchial wash and FNAC remnants were centrifuged at 2500rpm for 15 minutes. After centrifuging, supernatant was carefully removed and discarded and the sediment was mixed with two drops of plasma that was kept frozen and brought to room temperature before use. If any clots were present in the sample, it was removed and processed as a routine histopathological specimen. In case of FNAC remnants, the rinses of syringes and needles were washed with normal saline before plasma is added.

Immediately, four drops of thromboplastin was added and mixed well. The thromboplastin was stored in refrigerator between 2 and 8°c and brought

to room temperature before use. The mixture was left undisturbed for few minutes until a clot was formed. If there was no clot formation, four more drops of thromboplastin was added until clot appeared. Then the clot was scooped out onto a filter paper and placed in a cassette. The tissue cassette was then fixed in 10% neutral buffered formalin for atleast 4 hrs. Afterwards it was processed along with routine histopathological specimens.

Cell blocks were made and tissue sections of 4-5 micron thickness were taken and stained with hematoxylin and eosin. Subsequently, these cell blocks were used to do immunohistochemistry whenever needed.

Procedure for immunohistochemistry:

1) From the selected cell blocks 3-4µm thickness sections were taken in a poly- lysine coated adhesive slides. The slides were incubated at 45°c for one hour.
2) Slides are then subjected to 2 changes of xylene for 5 minutes each for de-paraffinization. Then the slides are transferred to absolute alcohol for 5 minutes which is then followed by 80% and 70% alcohol for 5 minutes each to rehydrate the sections.
3) Sections are then placed in running tap water for 5 minutes and washed in distilled water.

4) Antigen retrieval was performed using pressure cooker in TRIS-EDTA buffer or citrate buffer depending on the primary antibody used. Sections are cooled and slides are washed in distilled water.
5) Endogenous peroxidase activity is removed by incubating the sections with enough drops of 3% peroxide block in a humidity chamber. Then the sections are washed in washed buffer. Then protein block is added for 20 minutes.
6) Primary antibody is then added to the section and incubated for 30 minutes, followed by that primary amplifier is added for 20 minutes and the sections are washed in wash buffer.
7) DAB chromogen (1ml DAB buffer + 1 drop of DAB chromogen) is then added over the section and incubated for 4 minutes and then washed with two changes of distilled water.
8) Counterstaining was done with Mayer's hematoxylin for 30 seconds and washed in running tap water.
9) Dehydration is done by 2 changes of 100% alcohol. Mounting is done by DPX mountant and observed under microscope.

Buffer preparation:

TRIS- EDTA buffer (pH 9.0):

TRIS – 6.05gm

EDTA – 0.744gm

Distilled water – 1000ml

TRIS wash buffer:

TRIS – 0.605gm

Sodium chloride – 8gm

1N Hcl – 4ml

Distilled water – 1000 ml.

Citrate buffer:

Citrate -1.92gm

Distilled water – 1000ml

Precautions:

1. All the buffers used should be prepared fresh and the Ph should be adjusted according to the preferred pH.
2. Humidity chamber should always be used to prevent drying during the staining procedure.
3. DAB chromogen should be handled and disposed carefully as it is a carcinogen.
4. Primary antibody and all the reagents used in the immunohistochemistry procedure should be stored in 4- 6°c
5. Glass wares used should be dry and clean.

Interpretation of conventional smears and cell block:

A comparison between the cellularity, morphologicalpreservation, architectural preservation and background was performed on both conventional smear and cell block based on the point scoring system described by Mair et al[68].

Criterion	Qualitative description	Point score
1)Volume of obscuringbackground blood or proteinaceous material	Large amount: Diagnosis greatly compromised	0
	Moderate amount: Diagnosis possible	1
	Minimal amount: Diagnosis easy	2
2)Amount of diagnosticcellular material present	Minimal or absent: Diagnosis not possible	0
	Sufficient for cytodiagnosis	1
	Abundant : Diagnosis simple	2
3) Degree of cellulardegeneration and cellular trauma.	Marked: Diagnosis impossible	0
	Moderate: Diagnosis possible	1
	Minimal: good preservation	2
4)Retention of appropriatearchitecture and cellular arrangement	Minimal to absent: non-diagnostic	0
	Moderate: some preservation eg: follicles, papillae,acini, synctia or single cell pattern.	1
	Excellent architectural display, closely reflectinghistology: diagnosis obvious	2

According to the criteria mentioned above, comments were rendered on the quality of the slides by qualitatively grouping them into three categories:

1) Diagnostically unsuitable (score 0-2)
2) Diagnostically adequate (score 3-6)
3) Diagnostically superior (score 7-8)

The conventional smears and cell block were reported under the diagnostic category as benign, suspicious, malignant and non-diagnostic.Combined evaluation of conventional smear and cell block was done and tabulation of cytomorphological characters was analyzed.

INCLUSION CRITERIA:

All samples of Pleural effusion, bronchial washings and guided fine needle aspiration cytology of lung masses received in clinical pathology.

EXCLUSION CRITERIA:

1) Samples processed after 48hrs
2) All other fluid specimens except pleural fluid and bronchial washings.

RESULTS & OBSERVATION

In this prospective study of 100 cases, 50 cases constitute pleural fluid, 35 were bronchial wash and 15 cases were residual material from Imaging guided fine needle aspiration cytology (FNAC) of lung.72 cases were male and 28 cases were female.

FIG 1:SAMPLE DISTRIBUTION OF THE STUDY

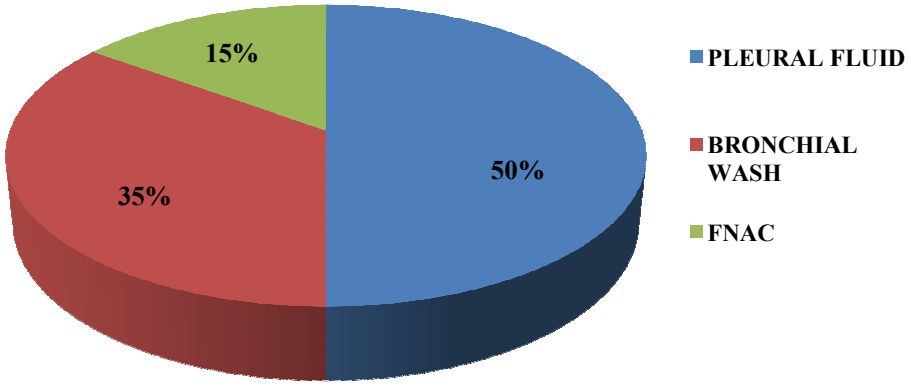
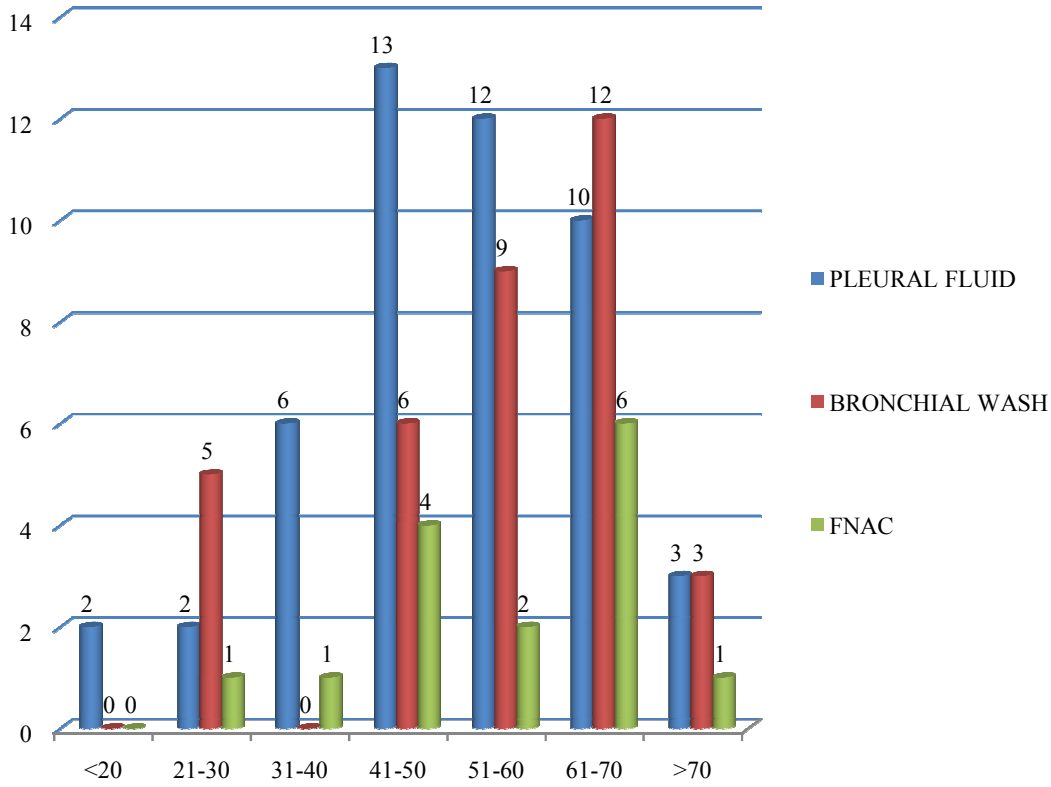


TABLE 1: AGE DISTRIBUTION OF SAMPLES:

AGE	PLEURAL FLUID		BRONCHIAL WASH		FNAC		TOTAL
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	
<20	2	0	0	0	0	0	2
21-30	2	0	5	0	0	1	8
31-40	4	2	0	0	1	0	7
41-50	5	8	5	1	4	0	23
51-60	6	6	7	2	2	0	23
61-70	6	4	10	2	6	0	28
>70	3	2	3	0	1	0	9
TOTAL	**28**	**22**	**30**	**5**	**14**	**1**	**100**

FIG 2: AGE DISTRIBUTION IN THE SAMPLES



The maximum number of samples was in the age group of 61-70 year (28%).Bronchial washings and FNAC samples were predominantly from the age group of 61-70 years, which accounts for 34.28% and 40% of samples respectively(From TABLE 1 & FIG 2).

I.ANALYSIS OF PLEURAL FLUID SAMPLES:

TABLE 2: AGE DISTRIBUTION:

AGE	MALE	FEMALE	TOTAL
<20	2	0	2
20-30	2	0	2
31-40	4	2	6
41-50	5	8	13
51-60	6	6	12
61-70	6	4	10
>70	3	2	5
TOTAL	**28**	**22**	**50**

Of the 50 pleural fluid samples, (Table 2) Maximum numbers of samples were in the age group of 41-50 years, which accounts for 26% of the pleural fluid samples. Males were predominantly in the age group of 51-70 years and females in the age group of 41-50 years (From TABLE 2 & FIG 3).

FIG 3: DISTRIBUTION OF AGE IN PLEURAL FLUID SAMPLES

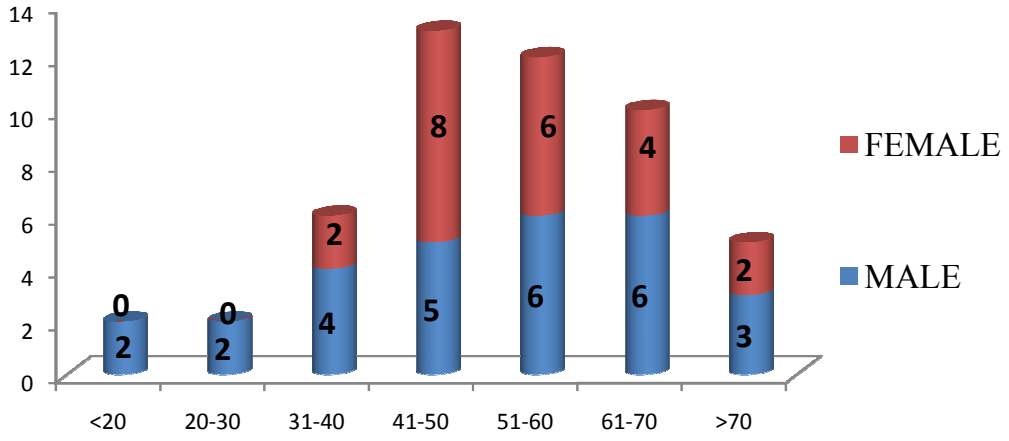


TABLE 3: COMPARISON OF QUALITY OF SMEAR AND CELL BLOCK IN PLEURAL EFFUSION

QUALITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Unsuitable	8	3	Pearson chi square 0.001
Adequate	39	30	
Superior	3	17	

The quality of smear analyzed conventionally was unsuitable in 8 cases (16%), adequate in 39 cases (78%) and superior in 3 cases (6%).

In cell block, 3 cases (6%) were unsuitable, 30 cases (60%) were adequate and 17 (34%) cases were superior(From TABLE 3 & FIG 4).

FIG 4:COMPARISON OF QUALITY OF CS AND CB IN PLEURAL EFFUSION

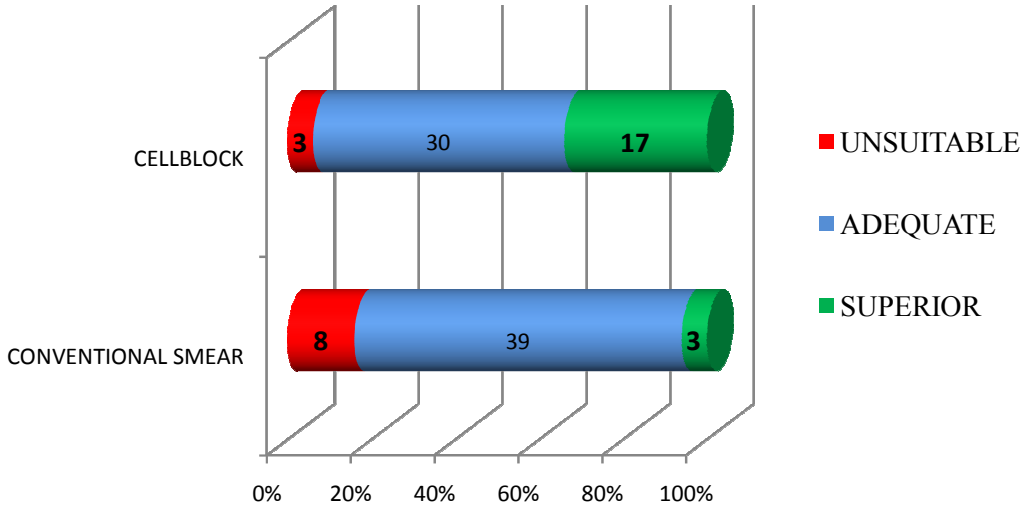


TABLE 4.COMPARISON OF CELLULARITY IN PLEURAL EFFUSION:

CELLULARITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	7	4	Pearson chi-square 0.006
Sufficient	36	24	
Abundant	7	22	

The cellularity in conventional smear showed minimal cellularity in 7 cases (14%), sufficient cellularity in 36 cases (72%) and abundant cellularity in 7 cases (14%).

The cellularity in cell block were minimal in 4 cases (8%), sufficient in 24 cases (48%) and abundant in 22 cases (44%) (From TABLE 4 & FIG 5).

FIG 5:COMPARISION OF CELLULARITY BETWEEN CS & CB IN PLEURAL EFFUSION

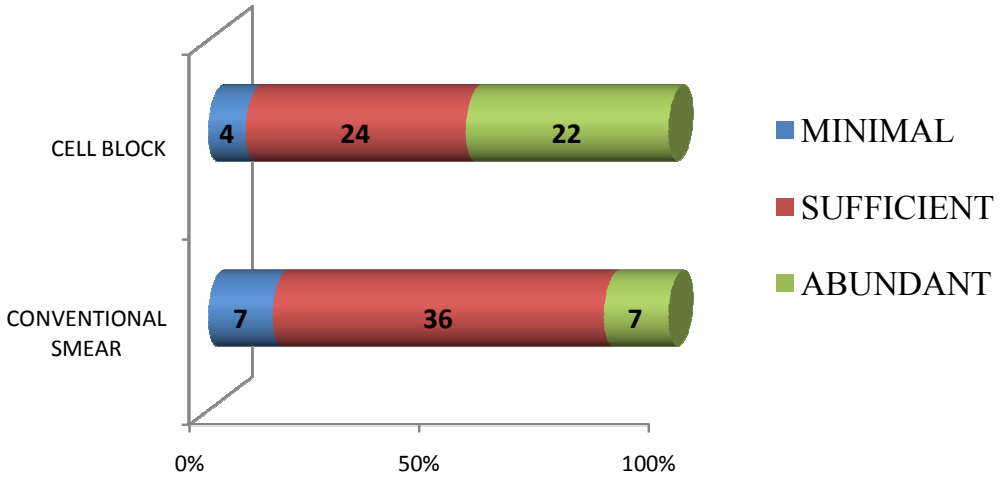


TABLE 5.COMPARISON OF ARCHITECTURE IN PLEURAL EFFUSION:

ARCHITECTURE	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	9	7	Pearson Chi-square 0.000
Moderate	41	39	
Excellent	0	4	

The architecture analysis in conventional smear showed scattered/ scant cells in 9 cases (18%), cellular arrangement (acini, papillae, cell balls, clusters) in 41 cases (82%) and excellent resemblance to histology in none.

Whereas, the architecture analysis in cell block showed scattered/ scant cells in 7 cases (14%), cellular arrangement (acini, papillae, cell balls, clusters) in 39 cases (78%) and excellent resemblance to histology in 4 cases (8%)(From TABLE 5 & FIG 6).

FIG 6:COMPARISON OF ARCHITECTURE BETWEEN CS AND CB IN PLEURAL EFFUSION

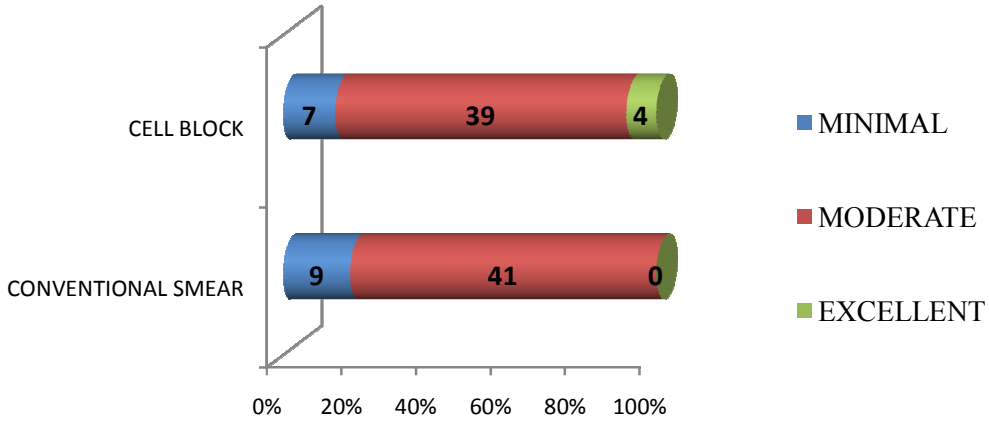


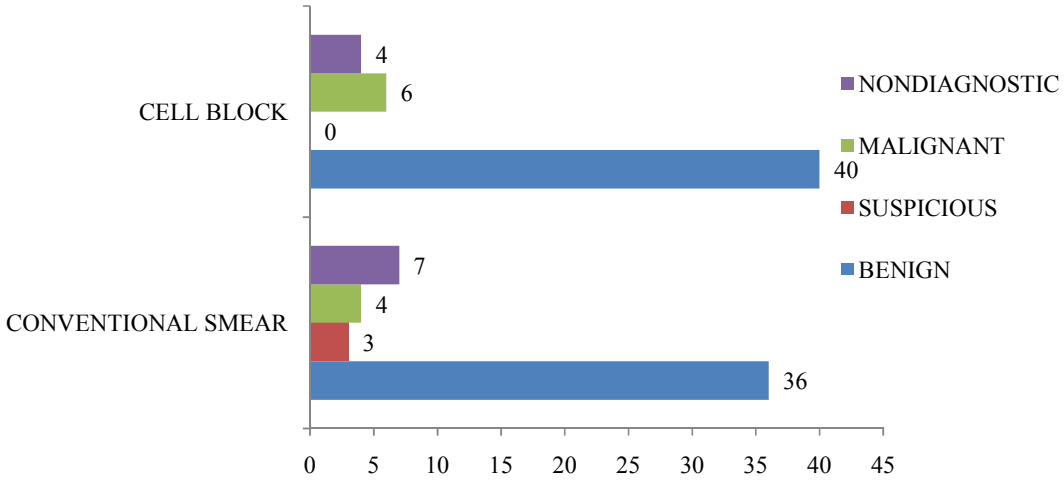
TABLE 6: COMPARISON OF DIAGNOSIS IN PLEURAL EFFUSION

DIAGNOSIS	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Benign	36	40	Pearson Chi-square 0.000
Suspicious	3	0	
Malignant	4	6	
Non-diagnostic	7	4	

By conventional smear the definite diagnosis of benign nature of pleural effusion was made in 36 cases (72%), malignant nature was made in 4 cases (8%). Suspicion of malignancy in effusion was made out in 3 cases (6%) and smear was non-diagnostic in 7 cases (14%).

In cell block, the benign nature was well defined in 40 cases (80%), malignant in 6 cases (12%), non-diagnostic in 4 cases (8%) and none was still suspicious (From TABLE 6 & FIG 7).

FIG 7: COMPARISON OF DIAGNOSIS BETWEEN CS & CB IN PLEURAL EFFUSION



PRIMARY SITES OF MALIGNANT PLEURAL EFFUSION:

In conventional smear, 4 malignant effusions were diagnosed which includes 1 case of breast malignancy, 1 from ovarian malignancy and 2 cases of metastatic adenocarcinoma.

By cell block, 6 malignant effusions were reported. Primary was already known for 3 cases which include 1 case of ovarian malignancy, 2 cases of breast malignancy. In 3 cases features were suggestive of adenocarcinoma which was further evaluated by immunohistochemistry.

II.ANALYSIS OF BRONCHIAL WASH SPECIMENS:

TABLE 7.AGE DISTRIBUTION:

AGE	MALE	FEMALE	TOTAL
20-30	5	0	5
31-40	0	0	0
41-50	5	1	6
51-60	7	2	9
61-70	10	2	12
>70	3	0	3
TOTAL	**30**	**5**	**35**

Maximum numbers of samples were in the age group of 61-70 years, which accounts for 34.28% of the pleural fluid samples. Males were predominantly in the age group of 61-70 years and females in the age group of 51-70 years (From TABLE 7 & FIG 8).

FIG 8: AGE DISTRIBUTION IN BRONCHIAL WASH SAMPLES

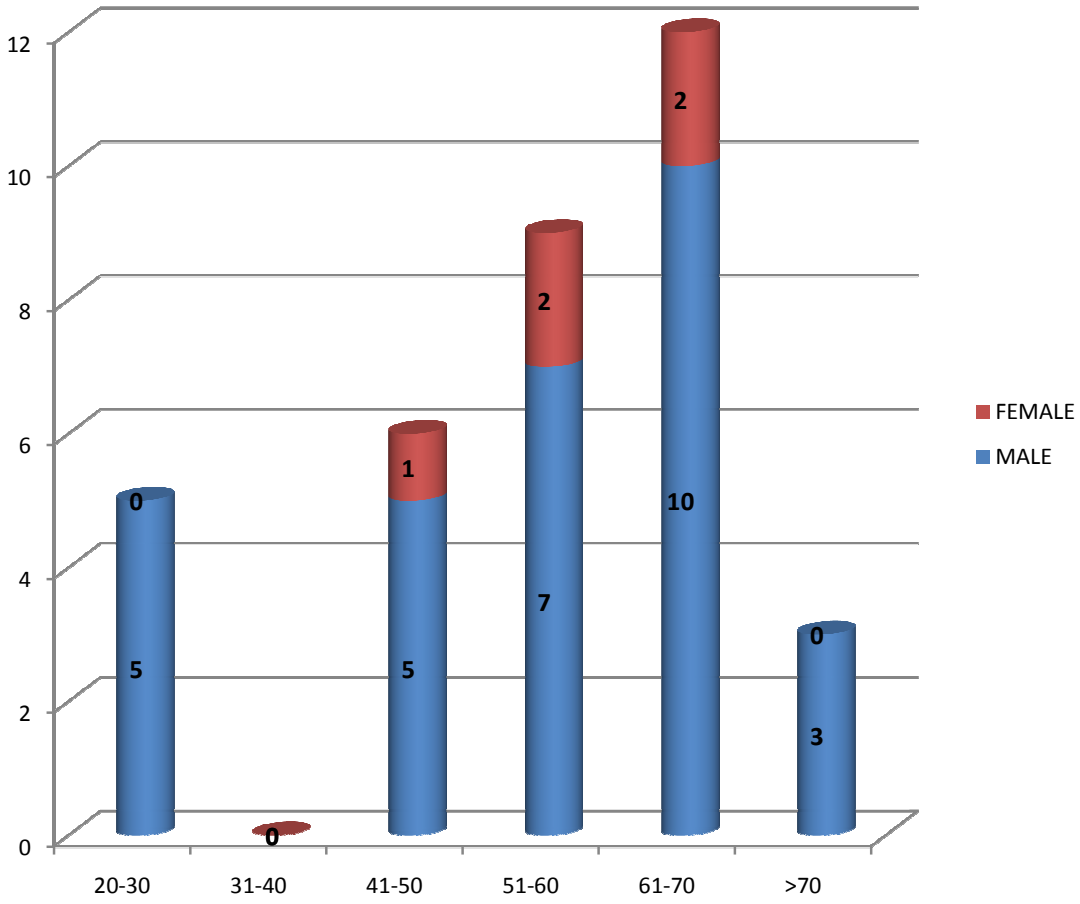


TABLE 8: COMPARISON OF QUALITY OF SMEAR AND CELL BLOCK IN BRONCHIAL WASH:

QUALITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Unsuitable	5	0	Pearson's chi-square 0.324
Adequate	30	30	
Superior	0	5	

The quality of smear determined by point scoring system by Mair et al in conventional smear was unsuitable in 5 cases (14.28%), adequate in 30 cases (85.71%) and superior in none.

In cell block, none were unsuitable, 30 cases (85.71%) were adequate and 5 (14.28%) cases were superior (From TABLE 8 & FIG 9).

FIG 9: COMPARISON OF QUALITY OF CS AND CB IN BRONCHIAL WASH

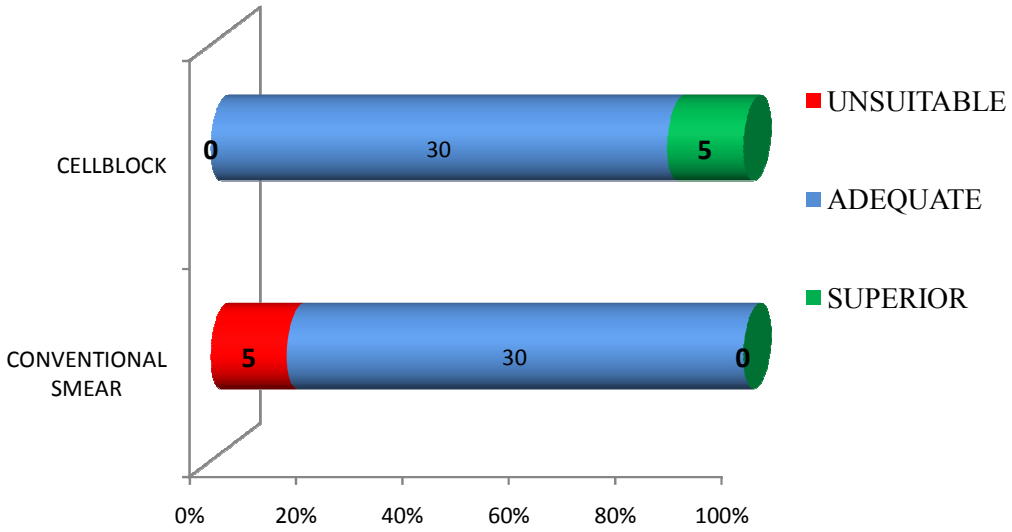


TABLE 9: COMPARISON OF CELLULARITY IN BRONCHIAL WASH:

CELLULARITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	8	1	Pearson Chi-square 0.119
Sufficient	24	28	
Abundant	3	6	

The cellularity in conventional smear showed minimal cellularity in 8 cases (22.85%), sufficient cellularity in 24 cases (68.57%) and abundant cellularity in 3 cases (8.57%).

The cellularity in cell block were minimal in 1 cases (2.85%), sufficient in 28 cases (80%) and abundant in 6 cases (17.14%)(From TABLE 9 & FIG 10).

FIG 10: COMPARISON OF CELLULARITY OF CS & CB IN BRONCHIAL WASH

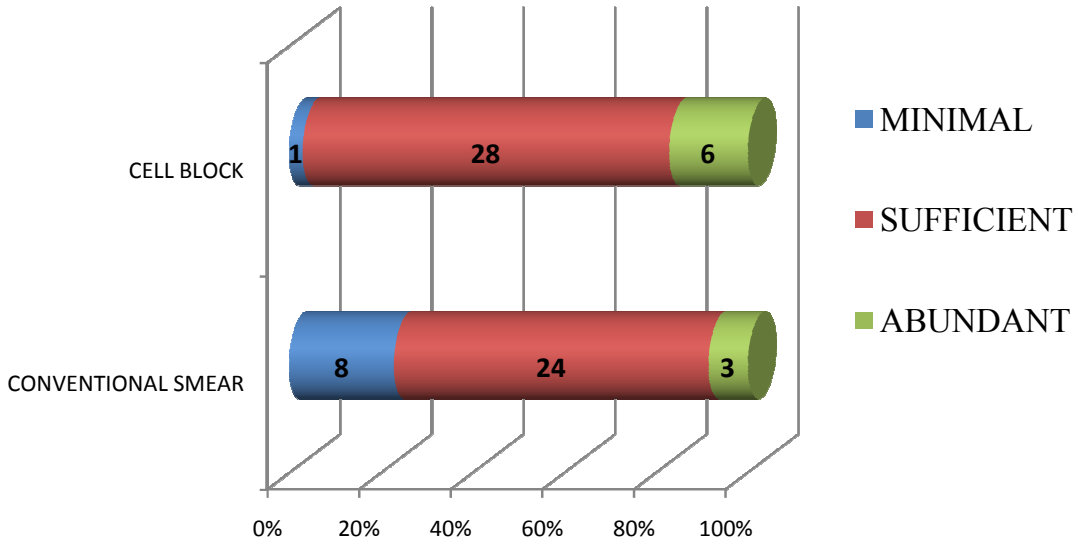


TABLE 10: COMPARISON OF ARCHITECTURE IN BRONCHIAL WASH:

ARCHITECTURE	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	9	1	Pearson chi square 0.364
Moderate	26	28	
Excellent	0	6	

The architecture analysis in conventional smear showed scattered/ scant cells in 8 cases (22.85%), cellular arrangement (acini, papillae, cell balls, clusters) in 24 cases (68.57%) and excellent resemblance to histology in 3 cases (8.57%).

Whereas, the architecture analysis in cell block showed scattered/ scant cells in 1 cases (2.85%), cellular arrangement (acini, papillae, cell balls, clusters) in 28 cases (80%) and excellent resemblance to histology in 6 cases (17.14%) (From TABLE 10 & FIG 11).

FIG 11:COMPARISON OF ARCHITECTURE IN CS & CB OF BRONCHIAL WASH

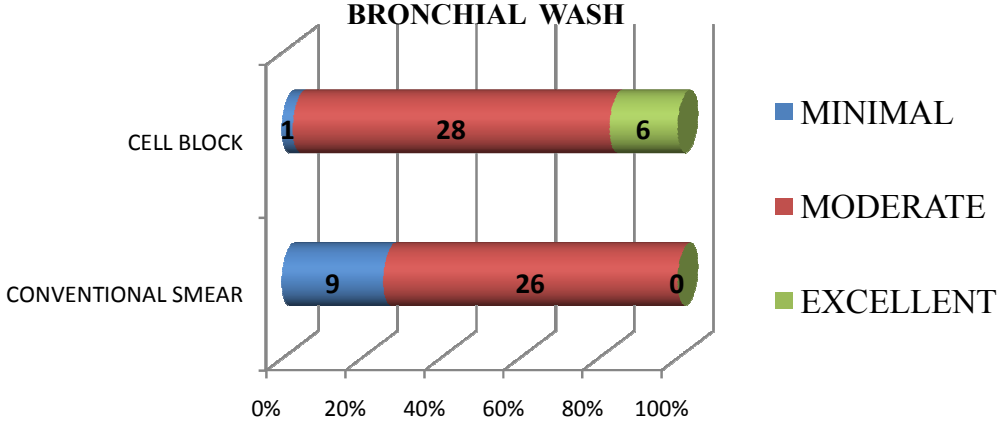


TABLE 11:COMPARISON OF DIAGNOSIS IN BRONCHIAL WASH:

DIAGNOSIS	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Benign	23	26	**Pearson Chi-square** 0.000
Suspicious	2	0	
Malignant	7	9	
Non-diagnostic	3	0	

By conventional smear the definite diagnosis of benign nature of bronchial wash was made in 23 cases (65.71%), malignant in 7 cases (20%). Suspicion of malignancy in bronchial wash was made out in 2 cases (5.71%) and smear was non-diagnostic in 3 cases (8.57%).

In cell block, the benign nature was well defined in 26 cases (74.28%), malignant in 9 cases (25.71%), non-diagnostic and suspicious in none (FromTABLE 11 & FIG 12).

FIG 12:COMARISON OF DIAGNOSIS BY CS & CB IN BRONCHIAL WASH

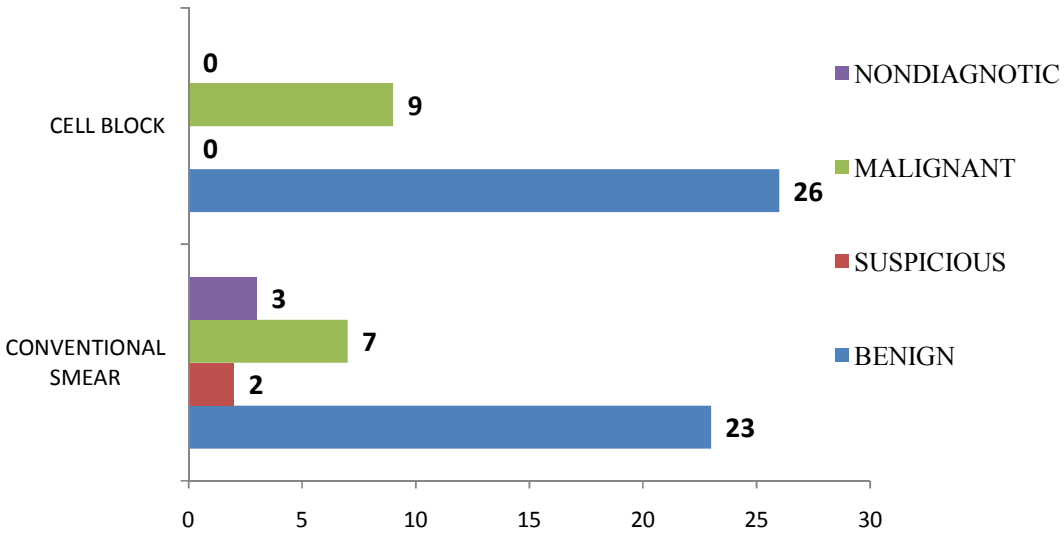


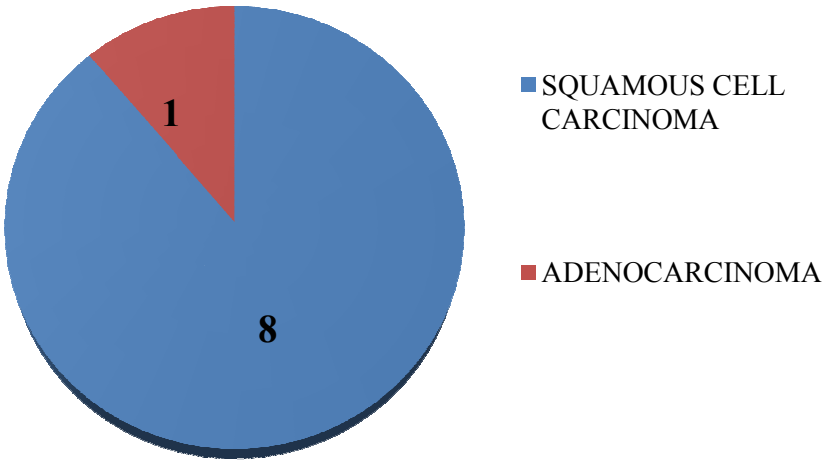
TABLE 12: MALIGNANCY BY CONVENTIONAL SMEAR AND CELL BLOCK IN BRONCHIAL WASH:

SUBTYPING OF MALIGNANCY	CONVENTIONAL SMEAR	CELL BLOCK
Squamous cell carcinoma	6	8
Adenocarcinoma	1	1
Small cell carcinoma	0	0
Large cell carcinoma	0	0
TOTAL	**7**	**9**

In conventional smear, 6 cases of squamous cell carcinoma and one case of adenocarcinoma was diagnosed.

By cell block technique, 8 cases of squamous cell carcinoma, one case of adenocarcinoma were diagnosed (From TABLE 12 & FIG 13).

FIG 13:SUBTYPING OF MALIGNANCY BY CELLBLOCK IN BRONCHIAL WASH



III.ANALYSIS OF FNAC SPECIMENS

TABLE 13: AGE DISTRIBUTION:

AGE	MALE	FEMALE	TOTAL
20-30	0	1	1
31-40	1	0	1
41-50	4	0	4
s51-60	2	0	2
61-70	6	0	6
>70	1	0	1
TOTAL	**14**	**1**	**15**

Maximum numbers of samples were in the age group of 61-70 years, which accounts for 40% of the FNAC samples. Males were predominantly in the age group of 61-70 years and females in the age group of 20-30 years (From TABLE 13 & FIG 14).

FIG 14:AGE DISTRIBUTION IN FNAC SAMPLES

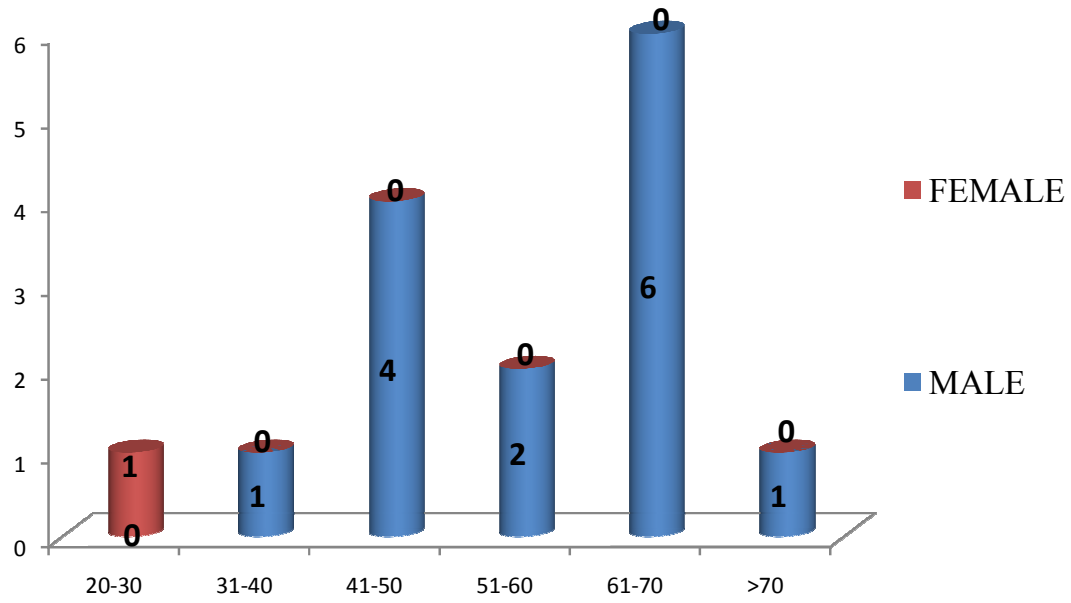


TABLE 14:COMPARISION OF QUALITY OF SMEAR& CELL BLOCK IN FNAC:

QUALITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Unsuitable	1	1	Pearson chi square 0.001
Adequate	14	8	
Superior	0	6	

The quality of smear analyzed conventionally was unsuitable in 1 case (6.66%), adequate in 14 cases (93.33%) and superior in none.

In cell block, 1(6.66%) case was unsuitable, 8 cases (53.33%) were adequate and 6 (40%) cases were superior (From TABLE 14 & FIG 15).

FIG 15:COMPARISION OF QUALITY OF CS & CB IN FNAC

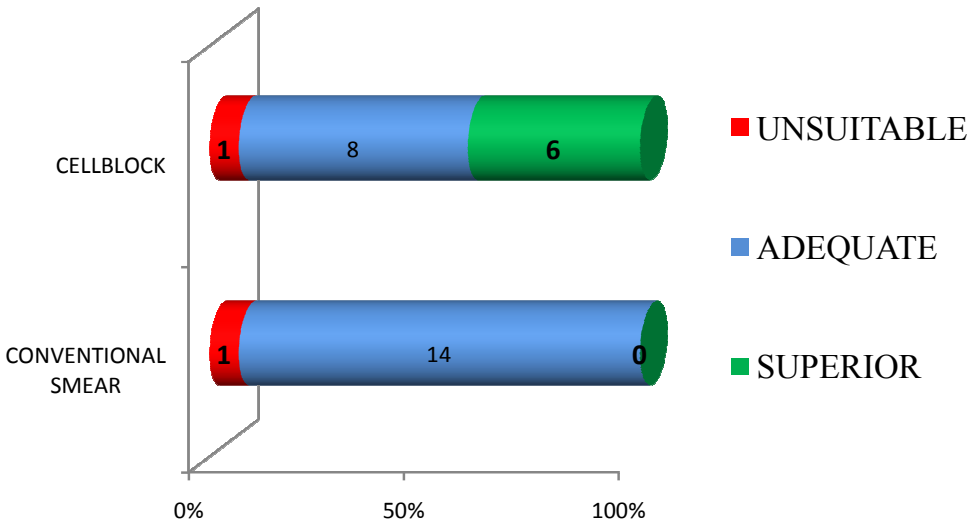


TABLE 15: COMPARISON OF CELLULARITY IN FNAC:

CELLULARITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	3	3	Pearson chi square 0.574
Sufficient	12	9	
Abundant	0	3	

The cellularity determined by in conventional smear showed minimal cellularity in 3 cases (20%), sufficient cellularity in 12 cases (80%) and abundant cellularity in none.

The cellularity in cell block were minimal in 3 cases (20%), sufficient in 9 cases (60%) and abundant in 3 cases (20%) (From TABLE 15 & FIG 16).

FIG 16:COMPARISION OF CELLULARITY IN CS & CB IN FNAC

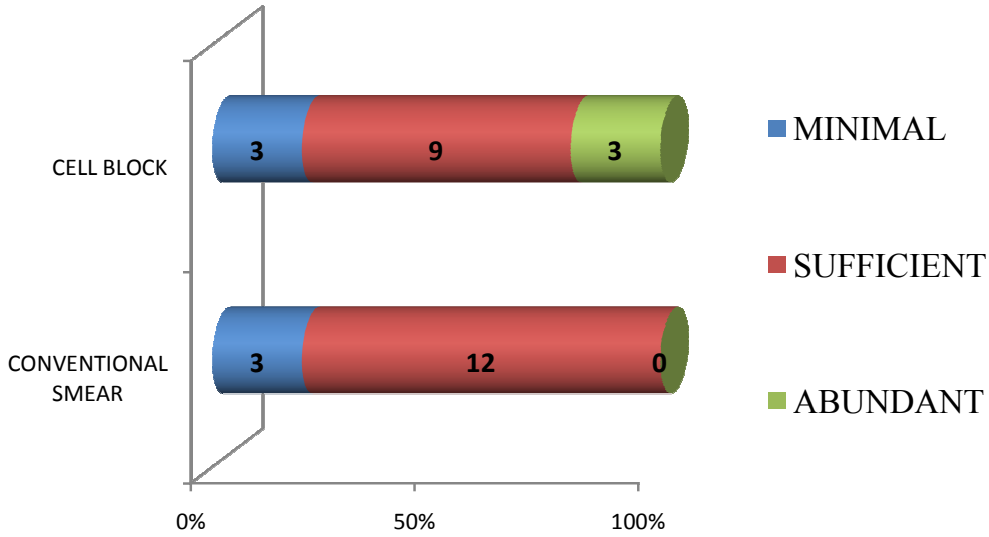


TABLE 16: COMPARISON OF ARCHITECTURE IN FNAC:

ARCHITECTURE	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	3	2	Pearson chi square 0.026
Moderate	11	8	
Excellent	1	5	

The architecture analysis in conventional smear showed scattered/ scant cells in 3 cases (20%), cellular arrangement (acini, papillae, cell balls, clusters) in 13 cases (73.33%) and excellent in none.

Whereas, the architecture analysis in cell block showed scattered/ scant cells in 2 cases (13.33%), cellular arrangement (acini, papillae, cell balls, clusters) in 8 cases (53.33%) and excellent resemblance to histology in 5 cases (33.33%).(From TABLE 16 & FIG 17).

FIG 17: COMPARISION OF ARCHITECTURE IN CS & CB IN FNAC

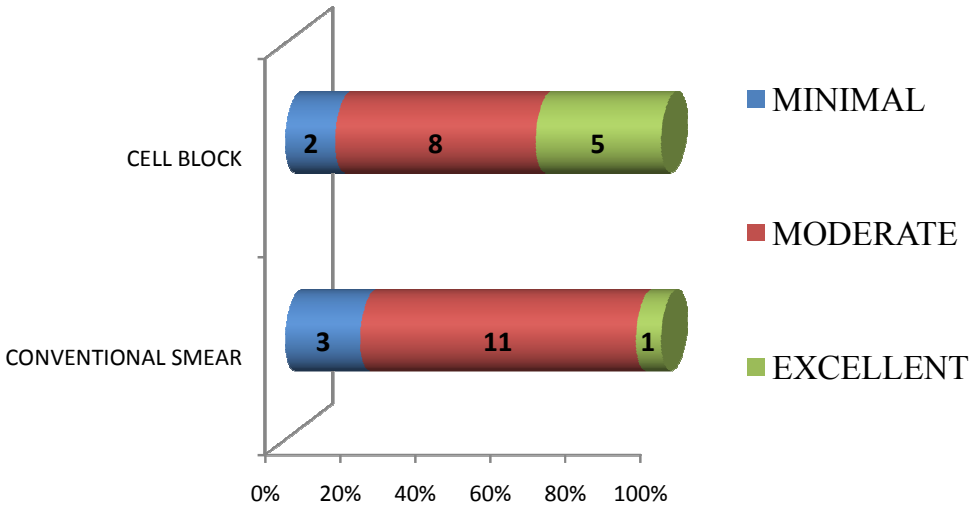


TABLE 17: COMPARISON OF DIAGNOSIS IN FNAC:

DIAGNOSIS	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Benign	7	5	Pearson Chi-square 0.001
Suspicious	3	0	
Malignant	4	9	
Non-diagnostic	1	1	

By conventional smear the definite diagnosis of benign nature of FNAC was made in 7 cases (46.66%), malignant nature was made in 4 cases (26.66%). Suspicion of malignancy in FNAC was made out in 3 cases (20%) and smear was non-diagnostic in 1 case (6.66%).In cell block, the benign nature was well defined in 5 cases (33.33%), malignant nature in 9 cases (60%), suspicious in none and inconclusive in 1 case (6.66%) (From TABLE 17 & FIG 18).

FIG 18:COMPARISON OF DIAGNOSIS BY CS & CB IN FNAC

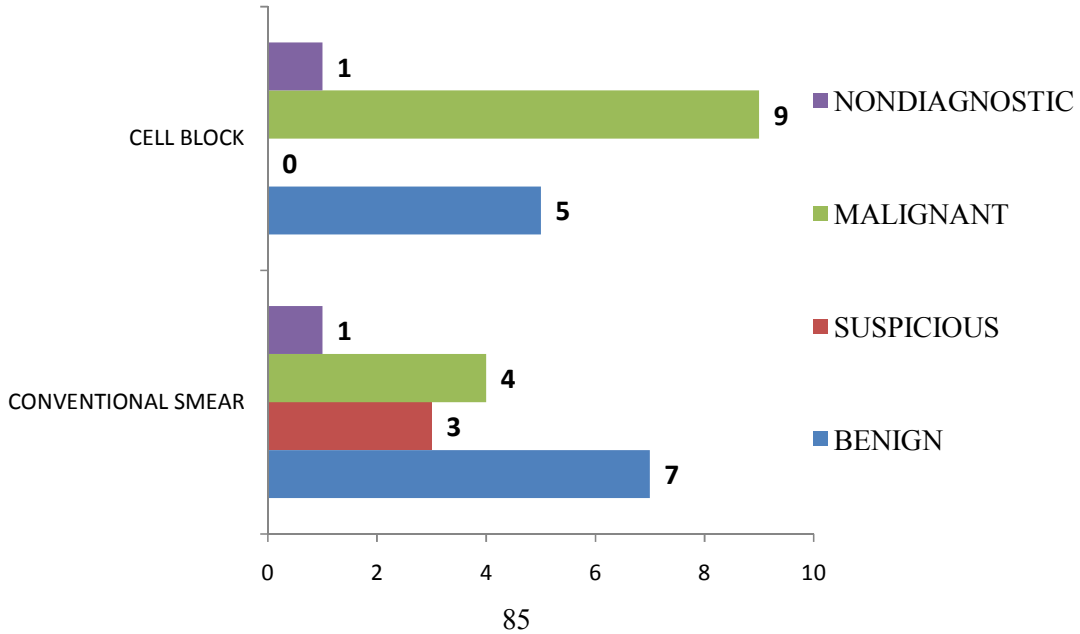
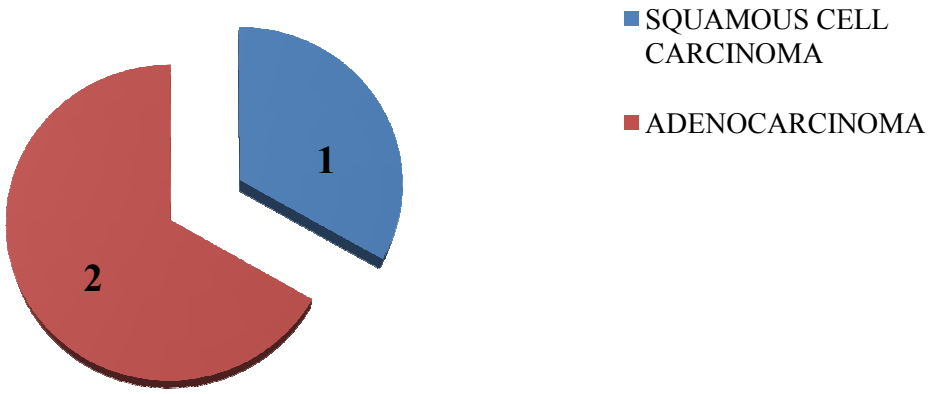


TABLE 18: SUSPICIOUS CYTOLOGY BY CONVENTIONAL SMEAR CONFIRMED TO BE MALIGNANT ON CELL BLOCK IN FNAC:

Squamous cell carcinoma	1
Adenocarcinoma	2
Small cell carcinoma	0
Total	3

FIG 19: SUSPICIOUS BY CS CONFIRMED TO BE MALIGNANT BY CB IN FNAC



Features characteristic of malignancy was observed by cell block in 3 cases which were suspicious on conventional smear. 1 was typical of squamous cell carcinoma and 2 were adenocarcinoma (From TABLE 18 & FIG 19).

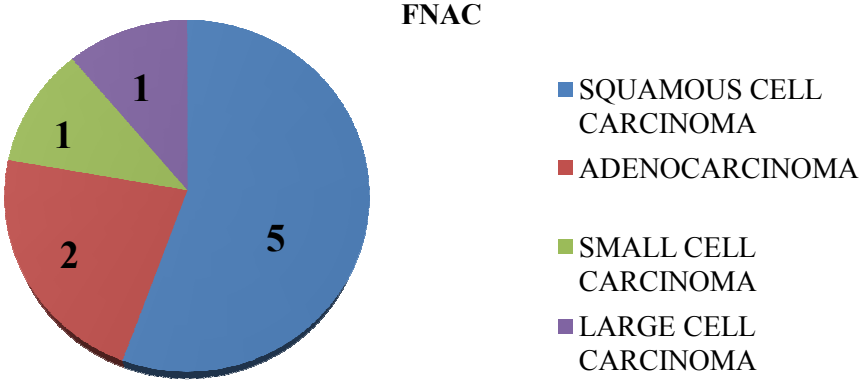
TABLE 19: SUBTYPING OF MALIGNANCY BY CONVENTIONAL SMEAR AND CELL BLOCK IN FNAC:

SUBTYPING OF MALIGNACY	CONVENTIONAL SMEAR	CELL BLOCK
Squamous cell carcinoma	2	5
Adenocarcinoma	0	2
Small cell carcinoma	1	1
Large cell carcinoma	1	1
TOTAL	**4**	**9**

Typical features of malignancy were observed by examination of conventional smear in 4 cases (26.6%) out of 15 cases examined. 2 cases were diagnostic of squamous cell carcinoma, 1 case of large cell carcinoma and 1 case of small cell carcinoma.

9 cases (60%) out of the 15 cases wereconfirmed to be malignant by cell block. 5 cases (33.3%) were squamous cell carcinoma, 2 cases (13.33%) were adenocarcinoma and 1 case (6.66%) was small cell carcinoma and 1 case (6.66%) of large cell carcinoma (From TABLE 19 & FIG 20).

FIG 20:SUBTYPING OF MALIGNANCY BY CELL BLOCK IN FNAC



IV.OVERALL ANALYSIS OF CONVENTIONAL SMEAR AND CELL BLOCK:

TABLE 20: OVERALL COMPARISON OF QUALITY OF SMEAR AND CELL BLOCK:

QUALITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
unsuitable	14	5	Pearson Chi-square 0.000
Adequate	83	67	
superior	3	28	

In the analysis of 100 conventional smears using point scoring system of Mair et al, 3 cases were diagnostically superior, 83 cases were adequate for diagnosis and 14 cases were inadequate.

Whereas by cell block, 28 cases were diagnostically superior equivalent to histopathology sections, 67 cases were adequate for diagnosis and 5 cases were inadequate in nature (From TABLE 20 & FIG 21).

FIG 21:OVERALL COMPARISON OF QUALITY OF CS & CB

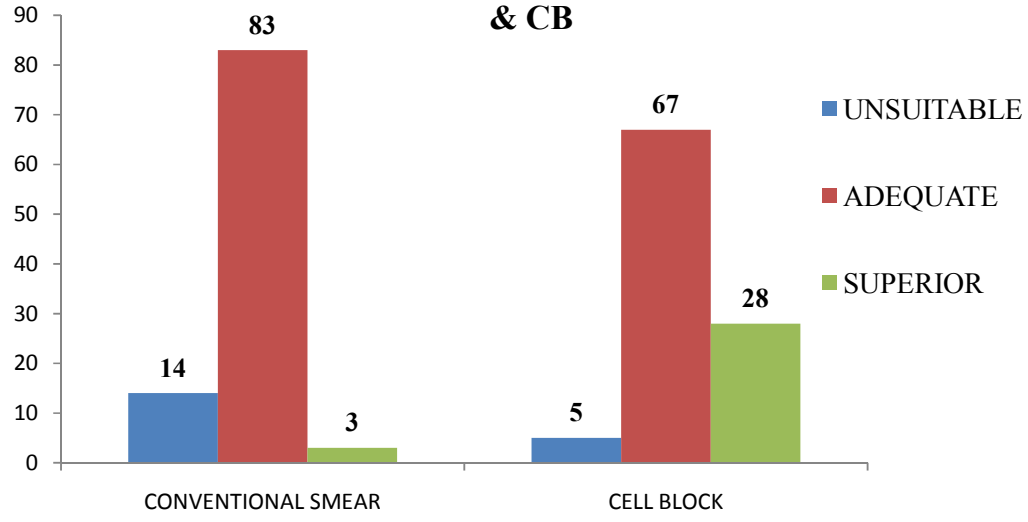
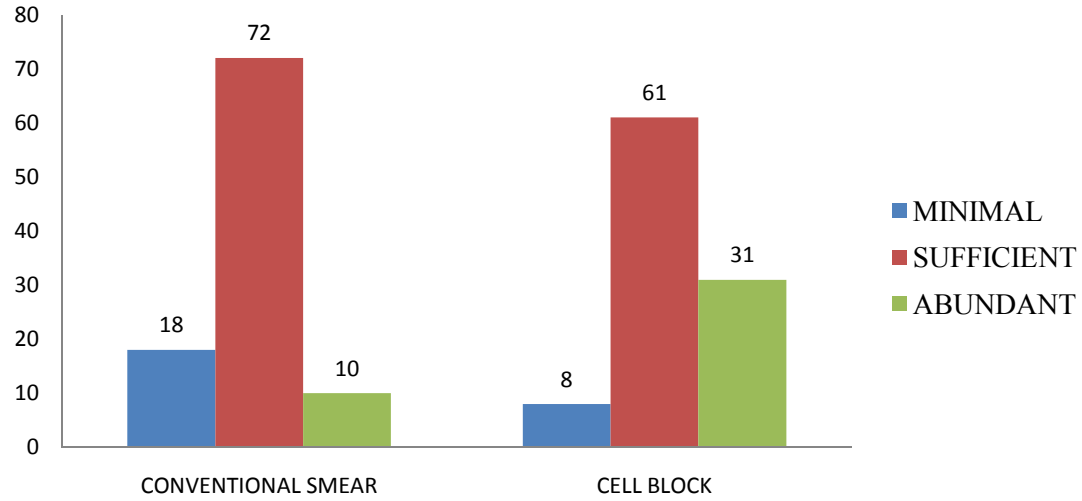


TABLE 21: CELLULARITY OF CONVENTIONAL SMEAR AND CELL BLOCK:

CELLULARITY	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	18	8	Pearson Chi-square 0.000
Sufficient	72	61	
Abundant	10	31	

FIG 22:OVERALL COMPARISON OF CELLULARITY IN CS & CB



In the overall analysis of 100 cases of conventional smear, 10 cases had abundant cellularity, 72 cases had sufficient cellularity and 18 cases had minimal cellularity.

Whereas by cell block, 31 cases had abundant cellularity, 61 cases had sufficient cellularity and 8 cases had minimal cellularity (From TABLE 21 & FIG 22).

TABLE 22: ARCHITECTURE ANALYSIS IN CONVENTIONAL SMEAR & CELL BLOCK:

ARCHITECTURE	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Minimal	20	10	Pearson Chi-square 0.000
Moderate	80	75	
Excellent	0	15	

Conventional smear analysis of 100 smears showed 80 cases had moderate architecture and 20 cases had minimal architecture.

Whereas by cell block, 15 cases had excellent architecture resembling histology, 75 cases had moderate architecture and 10 cases had minimal architecture (From TABLE 22 & FIG 23).

FIG 23:OVERALL COMPARISON OF ARCHITECTURE IN CS & CB

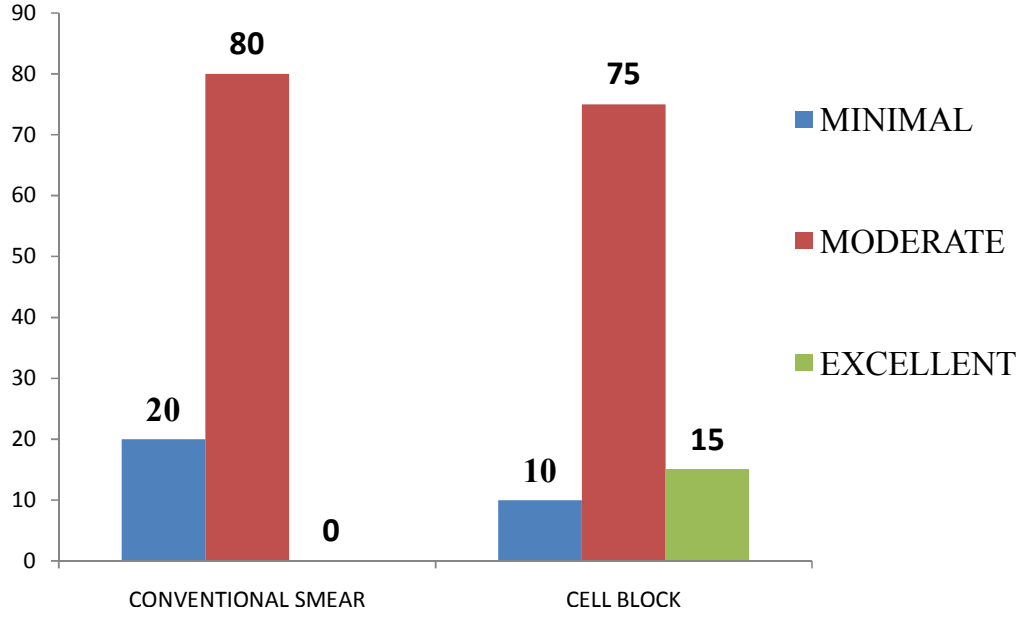


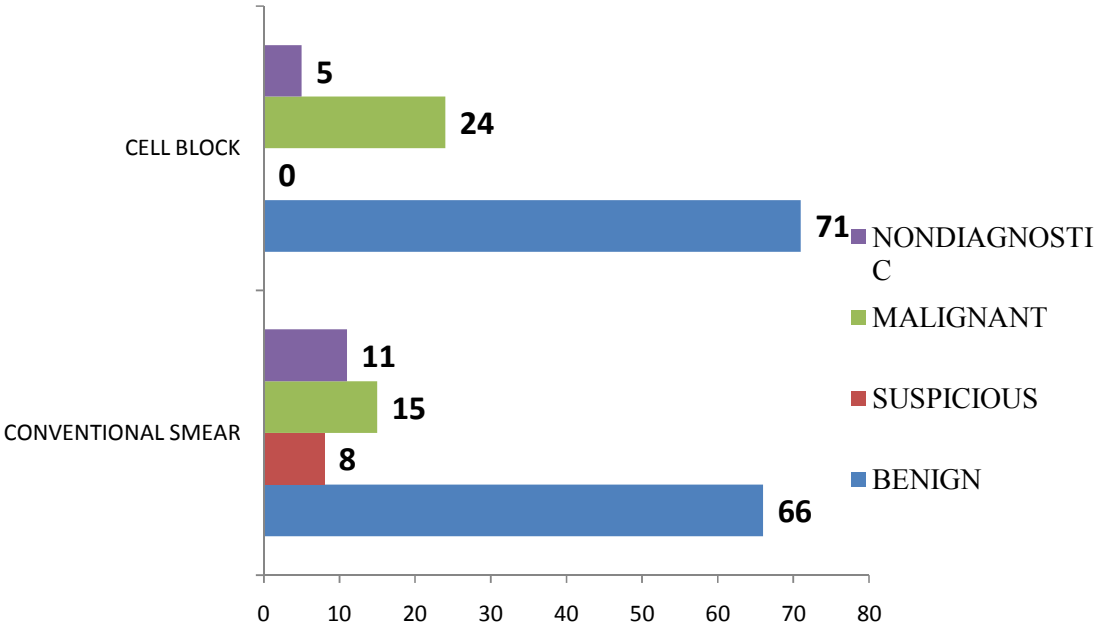
TABLE 23: OVERALL COMPARISON OF DIAGNOSIS:

DIAGNOSIS	CONVENTIONAL SMEAR	CELL BLOCK	INFERENCE
Benign	66	71	Pearson Chi-square 0.000
Suspicious	8	0	
Malignant	15	24	
Nondiagnostic	11	5	

By conventional smear 66 cases were benign, 15 were malignant, 8 cases were suspicious of malignancy and non-diagnostic in 11 cases.

By cell block 71 cases were benign, 24 cases were malignant, 5 cases were non-diagnostic and suspicious in none (From TABLE 23 & FIG 24).

FIG 24:COMPARISON OF DIAGNOSIS BY CS & CB



IMMUNOHISTOCHEMICAL ANALYSIS:

IMMUNOHISTOCHEMICAL ANALYSIS OF MALIGNANT PLEURAL EFFUSION:

Of the 6 cases of malignant pleural effusion, in 2 cases the primary was known to be breast carcinoma which showed positivity for estrogen receptor, negative for TTF-1 which confirmed the diagnosis of metastatic breast carcinomatous deposit. In one case of ovarian carcinoma, cell block of pleural fluid showed malignant cells which was confirmed by immunohistochemistry. 2 cases were positive for TTF-1, CK 7 and negative for calretinin and CK 20, confirming the diagnosis of adenocarcinoma of the lung. 1 case of metastasis from adenocarcinoma TTF-1, CK 7, CK 20 was negative but the morphology and the architecture of the cells gives a definitive diagnosis of adenocarcinoma.

TABLE 24: PRIMARY SITES OF MALIGNANT PLEURAL EFFUSION

S.NO	PRIMARY SITE	NO.OF CASES	PERCENTAGE
1	Breast	2	33.3%
2	Lung	2	33.3%
3	Ovary	1	16.6%
4	Unknown	1	16.6%
	Total	**6**	**100%**

IMMUNOTYPING OF MALIGNANCY CASES IN BRONCHIAL WASH:

Out of 9 malignant cases reported by cell blocks of the bronchial wash specimens, immunohistochemistry was done in 4 cases.3 cases (three squamous cell carcinomas), a confident cell type was established by morphology in the cell block and immunohistochemical staining was done (p63 and pancytokeratin positive) which was in agreement with the histological classification.

In one case of small cell carcinoma both conventional smear and cell block showed abundant cellularity with excellent morphological features but negative for all the immunohistochemical markers.

IMMUNOTYPING OF MALIGNANCY IN THE CELL BLOCKS OF FNAC:

There were 9 cases of malignancy diagnosed on cell block. Among these 9 cases, 4 cases had adequate material to perform immunohistochemistry. The panel of markers used are p63, TTF-1, synaptophysin, pancytokeratin.

In 2 cases where a confident cell type had been established by morphology in the cell block the immunohistochemical staining was in agreement (p63 and pancytokeratin positive) and correlated with the histological classification.

One case reported as poorly differentiated carcinoma by conventional smear was confirmed to be small cell carcinoma by immunohistochemistry oncell block which showed synaptophysin positivity pancytokeratin negativity and confirmed the diagnosis of small cell carcinoma.

In one case, where the morphological features were suggestive of poorly differentiated carcinoma, the immunohistochemical profile was done. The tumor cells were negative for TTF1, p63 and CK5/6, precluding any specific comment on probable cell type.

DISCREPANCIES OBSERVED IN DIAGNOSIS BETWEEN BOTH CS AND CB IN THIS STUDY:

TABLE 25: Discrepancies observed in pleural effusion:

CONVENTIONAL SMEAR				CELL BLOCK			
Benign	**Suspicious**	**Malignant**	**Non diagnostic**	**Benign**	**Suspicious**	**Malignant**	**Non diagnostic**
4	-	-	-	-	-	3	1
-	-	1	-	1	-	-	-
-	3	-	-	3	-	-	-
-	-	-	4	4	-	-	-

In the present study (from table 25), Of the 50 pleural effusion cases, 12 cases showed discrepancies in diagnosis between conventional smear and cell block.4 samples were diagnosed to be benign by conventional smear of which 3 were diagnosed to be malignant by cell block and 1 case was non-diagnostic.Of the 3 benign cases, 2 were diagnosed to be benign due to decreased cellularity in the smear, but in cell block the cellular yield was more with good preservation of morphology and architecture which led to the definite diagnosis of malignancy. By immunohistochemistry, they showed positivity for estrogen receptor and negative for TTF-1. In another case, the background was obscured by inflammatory cells and reactive mesothelial cells on conventional smear which was clear on cell block, leading to the diagnosis of malignancy.1 case was reported as malignant effusion on conventional smear showed very few malignant cells on cell block which was not adequate for diagnosis. Subsequently, immunohistochemistry also was negative for ER and TTF-1.

In 3 cases morphology was not well preserved in conventional smear and a few suspicious clusters were seen in cell block and were reported as suspicious of malignancy. Immunohistochemistry was done in the cell block. All the 3 cases showed positivity for calretinin and negative for TTF-1, CK and the cases were reported as reactive mesothelial hyperplasia. 1 case which

was considered benign by smear was non-diagnostic in cell block and 4 cases non-diagnostic in smear was diagnosed benign in cell block.

TABLE 26:DISCREPANCIES OBSERVED IN BRONCHIAL WASH:

CONVENTIONAL SMEAR				CELL BLOCK			
Benign	**Suspicious**	**Malignant**	**Non diagnostic**	**Benign**	**Suspicious**	**Malignant**	**Non diagnostic**
2	-	-	-	-	-	2	-
-	2	-	-	2	-	-	-
-	-	-	3	3	-	-	-

2 cases of bronchial washings, reported to be benign on conventional smear showed malignant clusters on cell block and was reported to be malignant (From table 26). Based on the morphological features in the cell block 1 case was reported as squamous cell carcinoma and the other case as large cell carcinoma. 2 cases of bronchial washings were suspicious on conventional smear confirmed to be metaplastic squamous cells on cell block due to increased cellularity and morphology. 3 cases which were non-diagnostic by conventional smear were reported as benign by cell block.

TABLE 27: DISCREPANCIES OBSERVED IN FNAC SAMPLES:

CONVENTIONAL SMEAR				CELL BLOCK			
Benign	**Suspicious**	**Malignant**	**Non diagnostic**	**Benign**	**Suspicious**	**Malignant**	**Non diagnostic**
2	-	-	-	-	-	2	-
-	3	-	-	-	-	3	-

2 cases of FNAC diagnosed as benign on conventional smear had malignant cells on cell block due to better cellular yield and morphology (From table 27). 3 cases of FNAC, suspicious on conventional smear due to atypical cells were confirmed to be malignant on cell block due to better cellular yield and morphological preservation.

TABLE 28: AGREEMENT MATRIX FOR CONVENTIONAL SMEAR AND CELL BLOCK:

	PLEURAL FLUID	**BRONCHIAL WASH**	**FNAC**
SMEAR POSITIVE, CELL BLOCK POSITIVE	3	7	4
SMEAR NEGATIVE, CELL BLOCK POSITIVE	3	2	2
SMEAR POSITIVE, CELLBLOCKNEGATIVE	1	0	0
SMEAR NEGATIVE, CELLBLOCK NEGATIVE	32	21	5

Sensitivity – 93.3%

Positive predictive value – 58.3%

Sensitivity – 93.3%

Negative predictive value – 98.68%

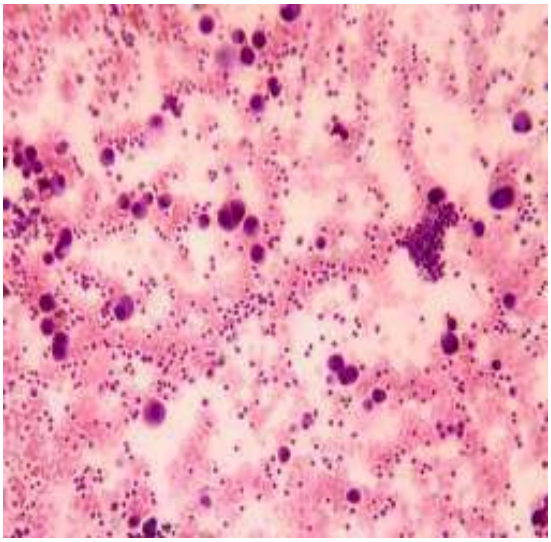


FIG 25:Conventional smear showing singly scattered tumour cells.
(H & E, 10x)

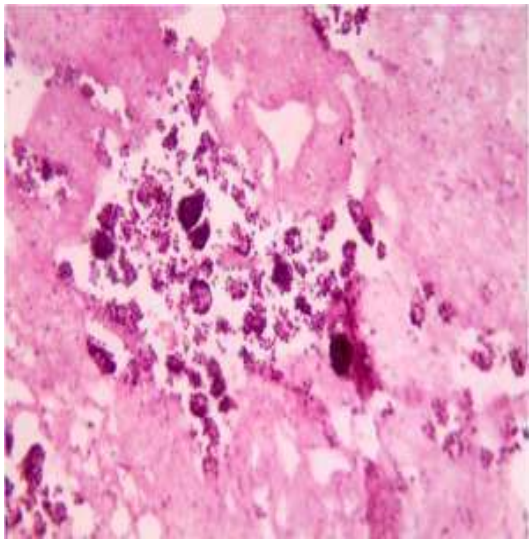


Fig 26:Cell block showing high cellularity in a localised area (H & E, 10x)

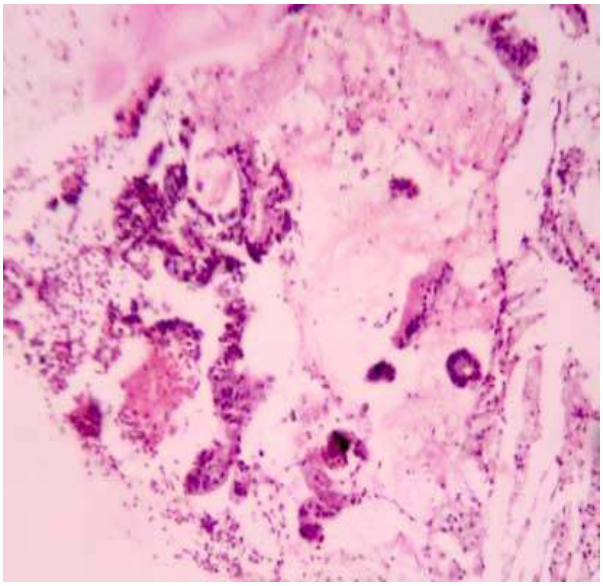


Fig 27: Cell block showing malignant cells arranged in acini and papillary pattern (H & E, 10x)

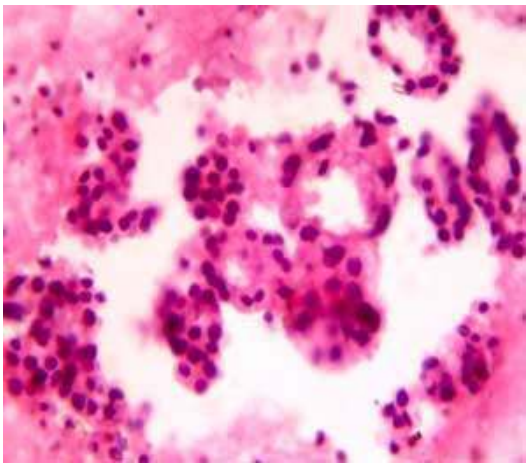


Fig 29: Photo micrograph showing mesothelial cells arranged in acini in cell block (H & E, 40 x)

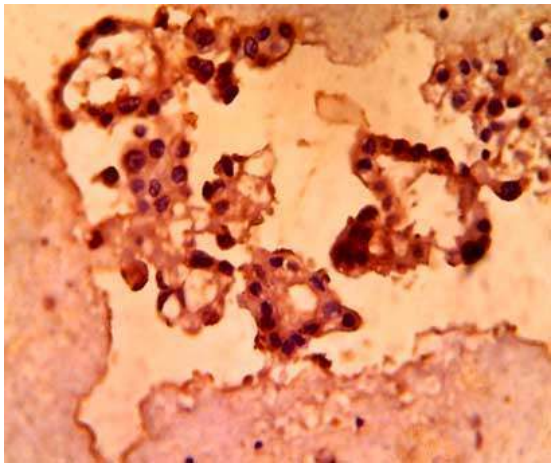


Fig 30: Mesothelial cells showing calretinin positivity in cell block (40 x)

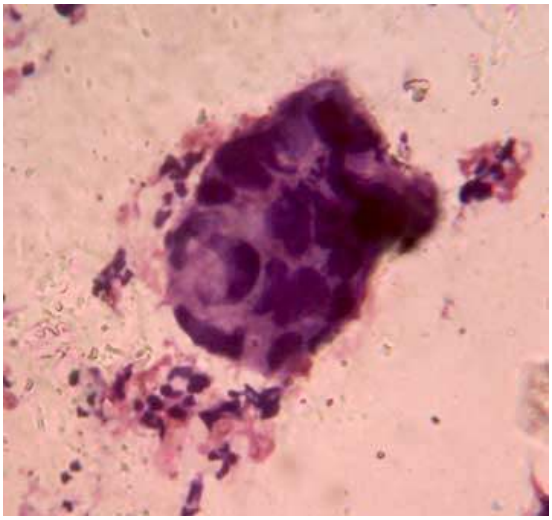


Fig 31: Conventional smear showing a cluster of suspicious cells (H& E, 40x)

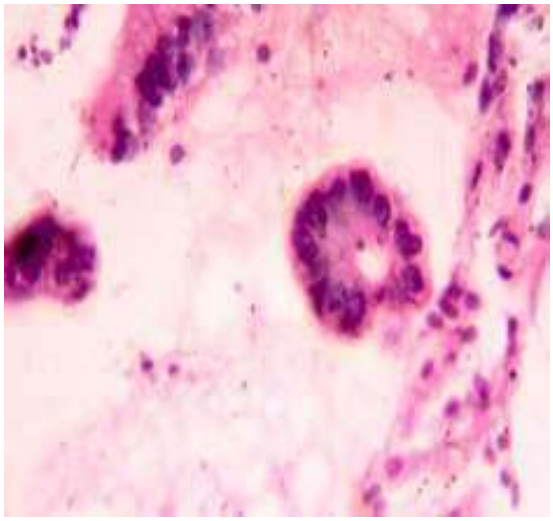


Fig32: Cell block showing malignant cells arranged in acini and with well preserved morphology in same case.

(H& E, 40x)

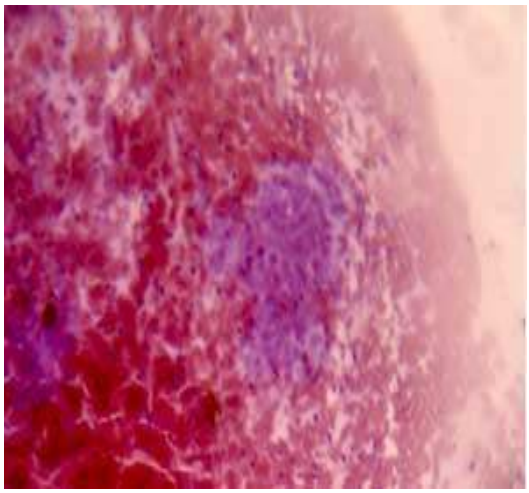


Fig 33: Conventional smear showing malignant cells obscured by hemorrhage in bronchial wash .(H & E, 10x)

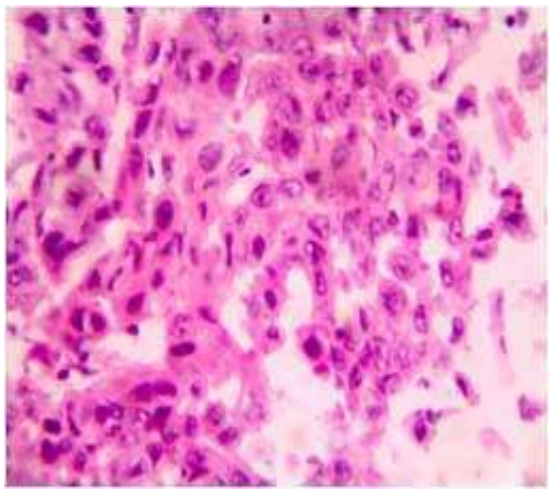


Fig 34: Cell block showing malignant squamous cells with well preserved morphology in bronchial wash of the same case. (H & e , 40 x)

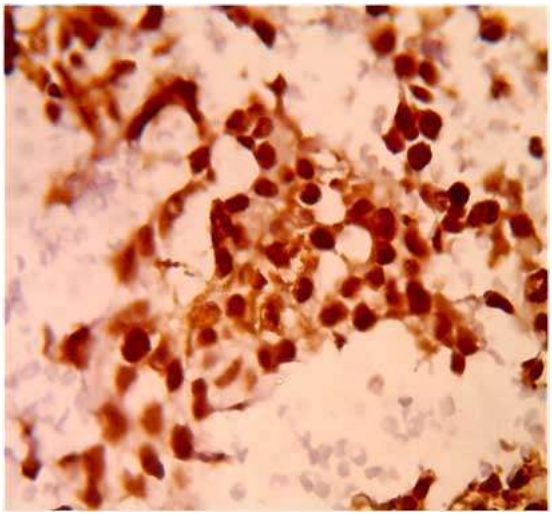


Fig 35: Malignant squamous cells showing nuclear positivity for p63in cell block (40 x)

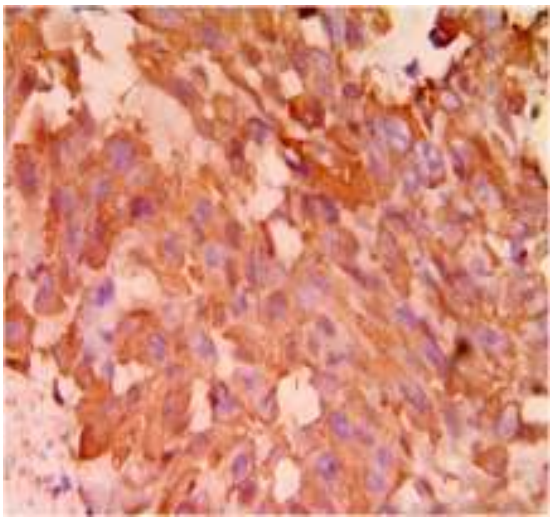


Fig 36: Cell block showing malignant squamous cells positive for pancytokeratin (40 x)

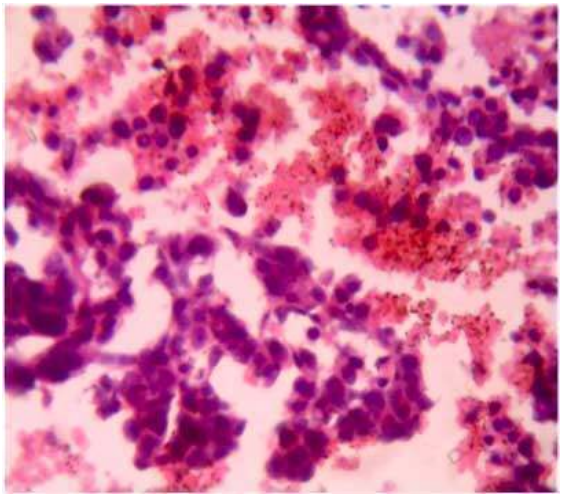


Fig 37: Cell block showing metastatic ovarian carcinoma cells in pleural effusion (H & E, 40x)

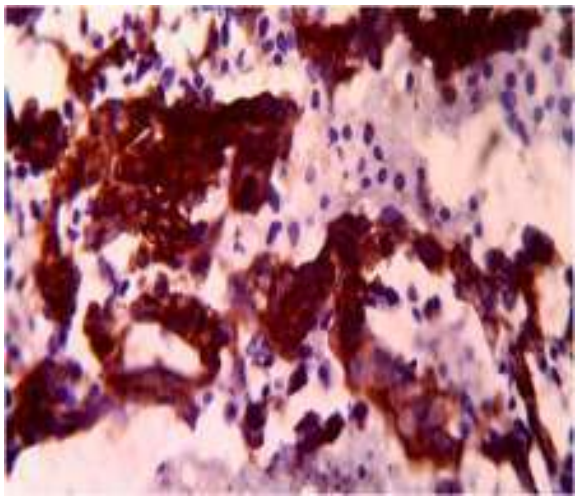


Fig 38: Cell block showing malignant cells positive for CK 7 in malignant pleural effusion (40 x)

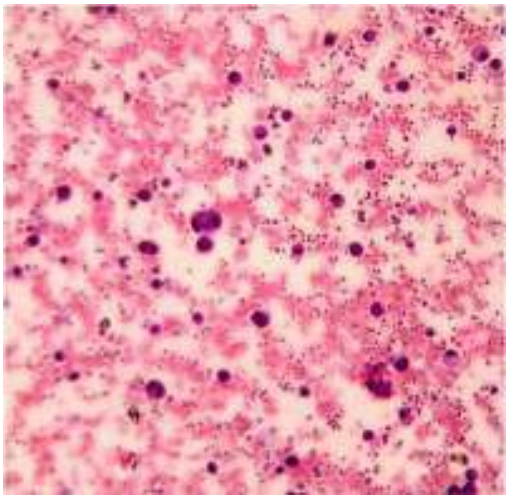


Fig 42: Conventional smear showing singly scattered malignant cells in pleural effusion (H & E, 10 x)

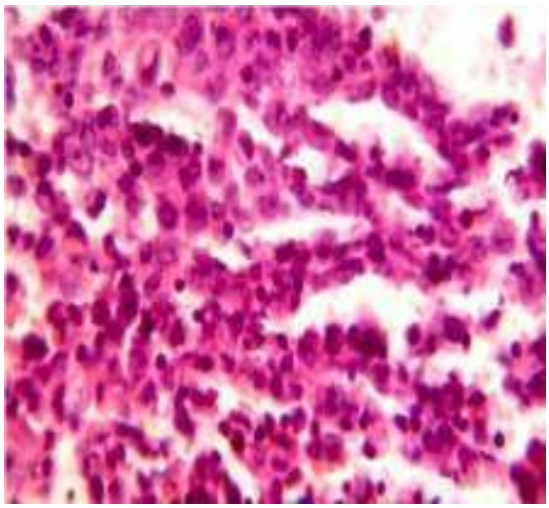


Fig 43: Cell block showing high cellularity and well preserved malignant cells in pleural effusion (H

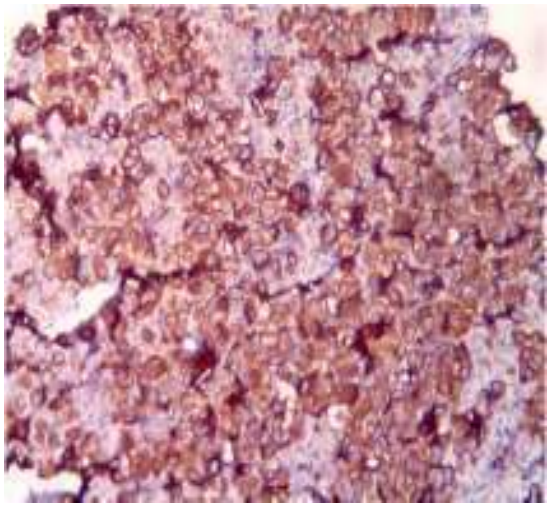


Fig : 44 Cell block showing Malignant cells positive for CK 7 in adenocarcinoma of lung Low Power (10x)

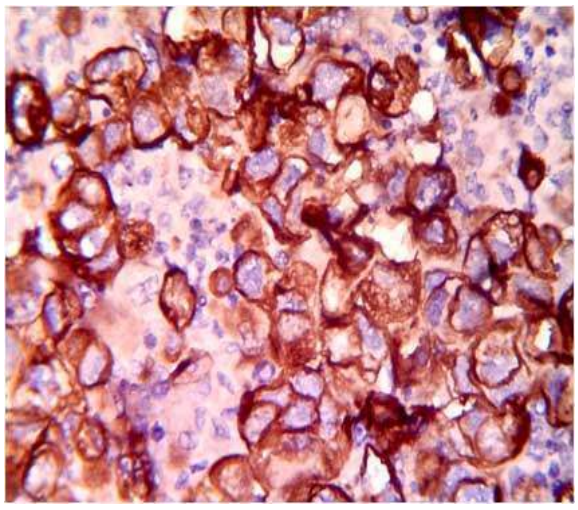


Fig : 45 Cell block showing Malignant cells positive for CK 7 in adenocarcinoma of lung High Power (40x)

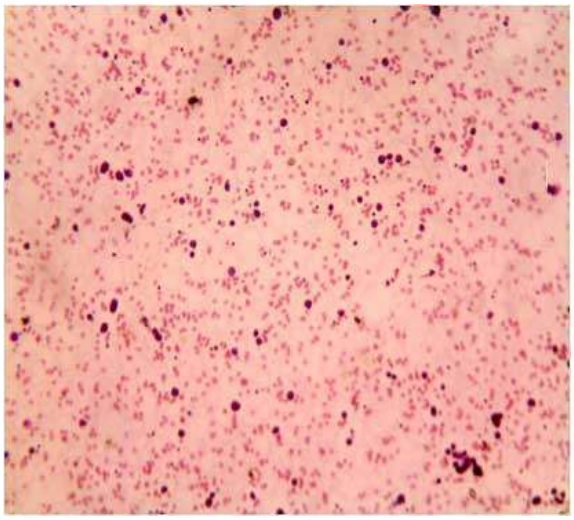


Fig 39: Conventional smear showing singly scattered malignant cells in pleural effusion (H & E, 10x)

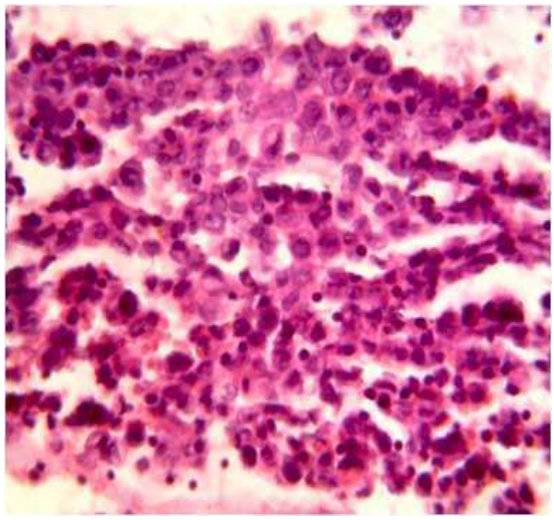


Fig 40: Cell block showing high cellularity of metastatic breast carcinoma cells in pleural effusion (H & E , 40 x)

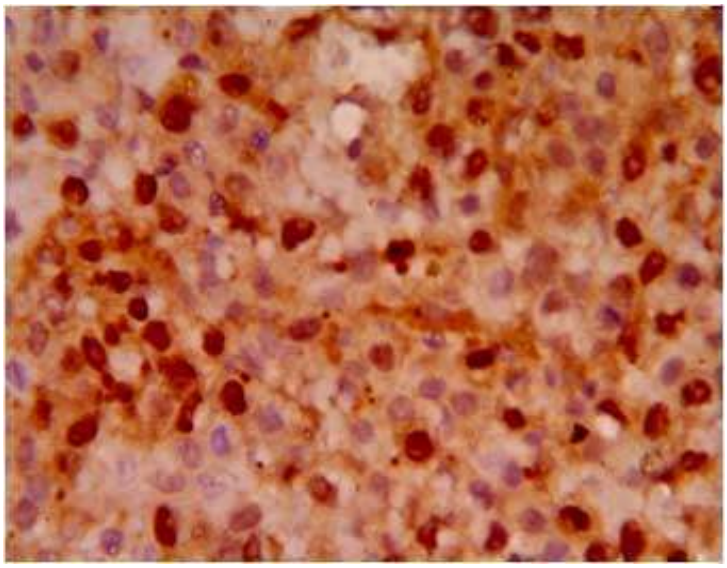


Fig 41: Cell block showing malignant cells in pleural effusion positive for estrogen receptor (40 x)

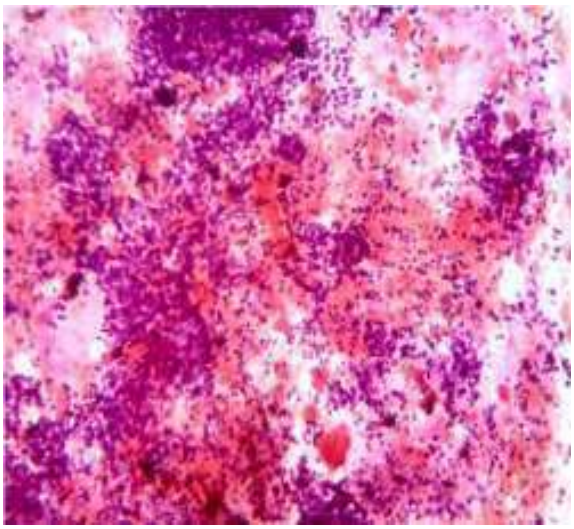


Fig46: Conventional smear showing malignant cells of small cell carcinoma in a dirty background in imaging guided FNAC (H & E, 40x)

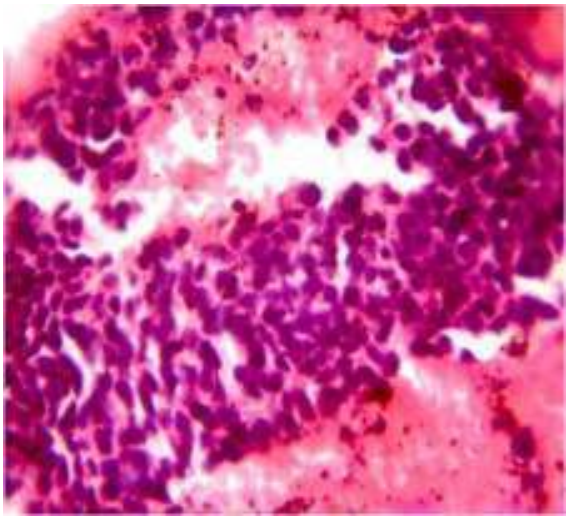


Fig 47: Cell block showing cluster of well preserved malignant cells in small cell carcinoma (H & E, 40x)

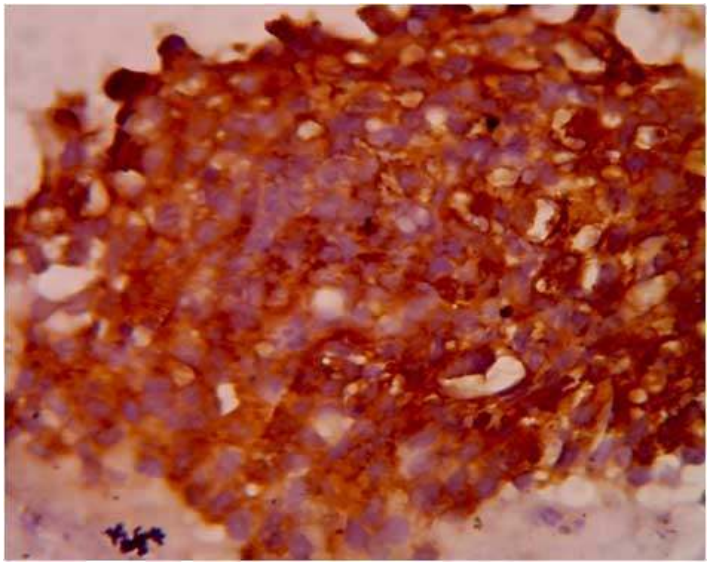


Fig 48: Cell block showing malignant cells of small cell carcinoma positive for synaptophysin – imaging guided FNAC (40x)

DISCUSSION

Cell-blocks work as an adjunct tool to conventional smears for establishing a definitive cytopathologic diagnosis. Several authors have reported the advantages of cell blocks in cytology which includes valuable diagnostic evidence that cannot be observed in smears[1, 3].

In this study, routine conventional smears and cell block from pleural fluid, bronchial wash and residual FNAC material from pulmonary lesions were compared for cellularity, architecture, cytological preservation and its diagnostic utility.

The objective of this study is to evaluate the utility of the routine use of cell block by assessing the concordance in diagnosis between conventional smear and cell block and the possibility to perform ancillary studies in cell blocks.

In this study,plasma thromboplastin method of cell block preparation is used to prepare cell blocks from pleural fluid, bronchial wash and residual FNAC samples. This is similar to the study done by Castro-Villabón D et al and Kulkarni et al which also used plasma thromboplastin method of cell block preparation[70,93].

Of the 100 cases, 50% of the sample was pleural effusion, 35% of bronchial wash and 15% of image guided FNAC from the pulmonary lesions.

Pleural fluid:

Of the 50 samples of the pleural fluid, the maximum numbers of samples were in the age group of 41-50 years accounting for 26%. Abundant cellularity was seen in 14% of the cases by conventional smear, while by cell block it was 44%. A study by Bista et al had 56.8% of the cases with abundant cellularity by cell block which is slightly higher than the present study[92].

Excellent architecture resembling histology by conventional smear was seen in none, but by cellblock it was seen in 8%. In the present study, cell block showed architecture with excellent resemblance to histology with glandular structures, papillary structures, three dimensional clusters and prominent signet ring cells, more reliably seen by the cell block method. The study done by Bhanvadia et al also states that, the benefit of cell block technique is the recognition of histologic patterns of disease that sometimes cannot be reliably identified in smears preparations but can be more reliably seen in cell block method[98].

In this study, conventional smear showed 84% of adequate smear, of which 6% were diagnostically superior. Whereas, by cell block method the adequacy of 94% was observed, of which 34% were diagnostically superior.

TABLE 29: COMPARISON OF QUALITY OF CELL BLOCK WITH OTHER STUDIES

QUALITY IN CELL BLOCK	SUPERIOR%	ADEQUATE%	UNSUITABLE%
THAPAR et al(2009)	67	21	12
NATHANI et al(2014)	25	55	20
PRESENT STUDY	34	60	6

In a study by Richa Nathani et al, 25% of the cases were diagnostically superior and the study by Thapar et al, had higher number of diagnostically superior cases accounting for 67%[94,89]. The percentage of diagnostically unsuitable cases by cell block is 6% which is very less when compared to the study by Richa Nathani et al which had 20% and Thapar et al which had 12% of diagnostically unsuitable cases[89].

The cell block preserves more cellular material from the sample, the statistical difference in cellularity, architecture and quality of smear between the two methods shows a 'p' value of 0.006, 0.000 and 0.001 respectively, which is very significant.

Of the 50 pleural effusion cases, the majority (80%) of the cases were benign effusion by cell block.In the present study, malignancy was

diagnosed in 8% of the cases by conventional smear and in 12% of cases by cell block.

TABLE 30: COMPARISON OF DIAGNOSIS BY CELL BLOCK WITH OTHER STUDIES

DIAGNOSIS BY CELL BLOCK	BENIGN %	SUSPICIOUS %	MALIGNANT %	NONDIAGNOSTIC %
Bhanvadia et al	78	0	22	0
RichaNathani et al	85	0	15	0
PRESENT STUDY	80	0	12	8

The 'p' value is 0.000 which shows a very significant difference between the two methods. Thus cell block yields higher malignancies which were missed by conventional smears.

In this study among the malignant effusion diagnosed by cell block, carcinoma of lung in males and metastatic effusion of carcinoma breast in females were commonest each accounting for 33.3%, followed by ovarian carcinoma (16.6%) and unknown primary (16.6%).Inkhan et al study, carcinoma of lung was the commonest site followed by carcinoma of ovary and carcinoma of GIT[90]. Similarly Murphy et al, study described that the commonest primary malignant lesions were in the breast followed by lung and ovary[91].

Bronchial wash:

Of 35 casesof bronchial washings maximum number of sample were in the age group of 61-70 years accounting for 34.28%.Abundant cellularity by conventional smear was seen in 8.57% of cases but by cell block it was 17.4%. Excellent architecture resembling histology by conventional smear was seen in 8.57% but by cellblock it was seen in 17.14%. The overall quality of smear by conventional method was superior in none of the sample but by cell block it was superior in 14.28%.Though cell block preserves more cellular material from the sample,the difference in cellularity, architecture and quality of smear between the two methods shows a 'p' value which is statistically insignificant.

Malignancy was diagnosed in 20% of the casesby conventional smear and in 25.71% of cases by cell block. Thus cell block has increased the diagnostic yield of malignancy by 5.7%.The 'p' value is 0.000 which shows a very significant difference between the two methods.

TABLE 31:Comparison of increase in yield of malignancy with other studies

	Flint et al(1993)[95]	Calabretto et al(1996)[96]	**Present study**
Increase in Malignancy yield	9%	6.5%	5.71%

Out of 35 cases, 20% of the cases were malignant which includes 88.8% (8/9) of squamous cell carcinoma and 11.1% (1/9) of adenocarcinoma. Immunohistochemistry was performed in 44.4% of malignant cases in which adequate material was available in the cell block and subtyping of the tumor was confirmed.

FNAC:

Of the 15cases of residual material from fine needle aspiration cytology(FNAC) done for pulmonary mass lesions, the maximum numbers of samples were in the age group of 61-70 years accounting for 40% of the samples.

By conventional smear none of the sample had abundant cellularity but by cell block 20% of the samples had abundant cellularity.Excellent architecture resembling histology by conventional smear was seen in 6.66%, but by cellblock it was seen in 33.33%. The quality of smear by conventional method was superior in none of the sample but by cell block it was superior in 40%. Though the cell block preserves more cellular material from the sample, the statistical difference in cellularity show a ‘p’ value of 0.574, which is statistically not significant. But the architectureand quality of smear show a ‘p’ value of 0.026 and 0.001 respectively, which is statistically significant.

In the 15 samples, a definitive diagnosis of malignancy was made by conventional smear in 26.66% and by cell block in 60%. In a study conducted by Nathan NA et al, 42.2% of the cases were diagnosed to be malignant by cell block[31].

TABLE 32: Comparison of diagnosis by cell block with other studies

Diagnosis by cell block	Benign	Suspicious	Malignant	Non-diagnostic
Nathan NA et al[31], 2000 **n=465**	6.2%	5.2%	42.2%	46.4%
Present study n=15	33.3%	0	60%	6.66%

The 'p' value is 0.001 which shows a very significant difference between the two methods. Thus cell block yields higher malignancies which were missed by conventional smears.

In this study, 55.5% of squamous cell carcinoma, 22.2% of adenocarcinoma and 11.1% of small cell carcinoma and large cell carcinoma each was diagnosed based on the morphological features of the malignant cells in the cell block. Immunohistochemistry was performed in 33.3% of the cases to confirm the subtyping of the malignancy.

In this study, squamous cell carcinoma is the most common subtype accounting for 72.2%. Sinard et al also has reported that squamous cell carcinoma (72.2%) is more common than adenocarcinoma (16.6%). W.A.H.Wallce et al states that, a slight bias towards squamous carcinoma

contributes to the overall accuracy of cell typing by morphology, as evidenced by keratinization- a reliable indicator of squamous differentiation[97]. In contrast, the identification of adenocarcinoma often requires architectural clues that are less often present in cytology samples.

In the present study of 100 cases, cell block showed abundant cellularity in 31% of the cases which is higher than that of the conventional smear which showed abundant cellularity in only 10% of the cases**.** In the study by Castro-Villabón D et al abundant cellularity in cell block was seen in 29.6% of the cases which is equal to our study. In the present study, cases with minimal cellularity were 8%. Castro-Villabón D et al had 37.4% of cases with minimal cellularity which is higher than that of the present study.

TABLE 33: Comparison of overall cellularity by cell block in the present study with other studies

CELLULARITY IN CELL BLOCK	ABUNDANT %	SUFFICIENT %	MINIMAL %
Castro-Villabón D et al	29.6	33	37.4
PRESENT STUDY	31	61	8

The quality of conventional smear and cell block was assessed by using the point scoring system by Mair et al. In this present study, conventional smear showed 86% of adequate smear, of which 3% were diagnostically superior. Whereas, by cell block method the adequacy of 95% was observed, of which 28% were diagnostically superior. The ‘p’ value of

cellularity, background and architectural difference between conventional smear and cell block is 0.000. The ‘p’ value of morphology is 0.001. Hence statistically, there is a highly significant difference in overall quality between conventional smear and cell block.

In the present study Of the 100 cases, 71% were benign and 24% were malignant. 76% of the cases had similar diagnosis both in conventional smear and cell block and discrepancies were seen in 16% of the cases.

TABLE 34: Comparison Of Diagnostic Concordance

Study	Diagnostic concordance (%)
Castro-Villabón D et al,2014	81.6
Kulkarni et al,2009	94
Present study	**76**

The diagnosis of malignancy by conventional smear was 15%, which increased to 24% with the cell block method. The additional yield of malignancy by this study is 9%. Thus cell block yields higher malignancies which were missed by conventional smears. The ‘p’ value by Pearsons Chi-square test is 0.000. Hence there ishighly significant difference in diagnosis between conventional smear and cell block.

Of the 16% of the cases with discrepancies includes 7 cases considered as benign by conventional smear were confirmed to be malignant by cell block.5 cases suspicious of malignancy and 1 cases considered as malignant by conventional smear were diagnosed to be benign by cell block.

3 cases suspicious of malignancy on conventional smear were diagnosed as malignant by cell block.

Immunocytochemistry studies were performed in 18% of cases. The markers used in order of frequency were: p63, TTF-1, calretinin, CK7, CK20, Synaptophysin, estrogen receptor, pancytokeratin. Of these immunohistochemistry was confirmatory in 86.6% of the cases.

SUMMARY

In this prospective study of 100 samples, 50 were pleural fluid, 35 were bronchial wash and 15 were residual material from imaging guided fine needle aspiration cytology (FNAC) of lung.These cases were evaluated by simultaneous use of smear and cell block technique. Immunohistochemistry was done on cell blocks whenever needed.

With each sample received conventional smear was made and the remaining sample was subjected for cell block preparation by plasma thromboplastin method. The comparison of the smear and cell block was made on the basis of cellularity, architecture, morphology and diagnosis. Abundant cellularity was seen in 31% of the cases by cell block and only 10% of the cases had abundant cellularity by conventional smear. Excellent architecture equivalent to histology was seen in 15% of the cases by cell block and this was not seen in conventional smear. Concordance in diagnosis between conventional smear and cell block was seen in 76% of the cases. Combining both conventional smear and cell block, the diagnostic yield of malignancy increased by 9%. Immunohistochemistry was performed in 18% of the cases to confirm and subtype the malignancy.

CONCLUSION

The cell block technique by plasma thromboplastin method is a simple cost effective technique and does not require any special training or instruments. This technique can be used in routine practice for cytological diagnosis. The cellularity and morphological features is better preserved in cell block method than conventional smear. By cell block method architectural pattern resembling that of histology is identified. Multiple sections can be obtained for immunohistochemistry to confirming and sub-type the malignancy. The accuracy of the diagnosis and yield of malignancy was increased when cell blocks are used along with the conventional smear method.Cell block is a very good adjunct to conventional smear study in the better yield of cellularity and architecture with an advantage to do immunohistochemistry, leading to better diagnosis of malignancy in the fluids and residual FNAC samples of pulmonary lesions.Ideally cell block technique should be used in routine practice for cytological diagnosis.

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ANNEXURE- I

PROFORMA

S.No:

Name: Age: Sex:

Hospital Ip/Op no:

Address of the patient:

Contact phone no:

Clinical details:

Investigations:

Clinical diagnosis:

Type of sample: Pleural Fluid/ Bronchial Wash/ Image Guided Fnac

Cytology no:

Conventional smear findings: Cellularity/ Architectural pattern/ Cytomorphology/ Background

Cell block findings: Cellularity/ Architectural pattern/ Cytomorphology/ Background

Immunohistochemistry (if needed):

Impression:

ANNEXURE II

CONSENT FORM

I have been informed in detail (verbal and written)from the doctor in our own language regarding the study. I hereby give consent to use the sample material for Dr. S.Saranyaa's thesis as a part of the MD degree curriculum. I have no objection to her publishing details of this study in medical journal after its completion. I understand that I have the liberty to withdraw from this study at any stage.

DATE:

Cytology NO:

Signature:

Name of the patient:

Addresss:

ஆராய்ச்சி தகவல் தாள்

திருநெல்வேலி அரசு பொது மருத்துவமனைக்கு வரும் நோயாளிகளுக்கு_____

_____பற்றிய ஆராய்ச்சி நடைபெற்று வருகிறது.

_____ எவ்வளவு பரவலாக இருக்கிறது என்பது பற்றியும் அதற்கான காரணங்கள் பற்றியும் அறிந்து கொள்வதே இந்த ஆராய்ச்சியின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்து கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போதோ அல்லது ஆராய்ச்சியின் முடிவிலோ தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

தேதி:

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு:

பெயர்:

வயது:

பால்:

தேதி:

உள்நோயாளி எண்:

ஆராய்ச்சி சேர்க்கை எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது. எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு எனது சம்மதத்தை தெரிவிக்கிறேன்.

இதற்கு தேவையான பரிசோதனைகளுக்கு நான் மனமார சம்மதிக்கிறேன்.

கையொப்பம்

PLEURAL FLUID

S.NO	PATH NO	NAME	AGE	SEX	IP/OP NO	CONVENTIONAL SMEAR					QUALITY	DIAGNOSIS	CELL BLOCK					QUALITY	DIAGNOSIS	
						C	B	M	A	S			C	B	M	A	S			
1	2426	petchiammal	60	F	50769	0	1	1	0	2	UNSUITABLE	nondiagnostic	2	1	2	1	6	ADEQUATE	benign	
2	2451	annathai	34	f	58799	1	2	2	1	6	ADEQUATE	benign	2	1	2	1	6	ADEQUATE	benign	
3	2658	velammal	60	f	59274	0	1	1	0	2	UNSUITABLE	nondiagnostic	0	1	1	0	2	UNSUITABLE	nondiagnostic	
4	2661	mohamed mydeen	50	m	63028	1	2	2	1	6	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
5	2825	ismail	62	m	66129	1	2	2	1	6	ADEQUATE	malignant	1	1	2	1	5	ADEQUATE	malignant	Mets adc
6	3011	veerapathiran	57	m	70284	0	1	1	0	2	UNSUITABLE	nondiagnostic	2	1	2	1	6	ADEQUATE	benign	
7	2858	saraswathi	48	f	67663	1	1	1	1	4	ADEQUATE	malignant	1	2	2	1	6	ADEQUATE	benign	
8	69	gnanasundravel	65	m	851	2	1	2	1	6	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
9	135	parvathyammal	64	f	2443	1	1	2	1	5	ADEQUATE	benign	2	2	2	2	8	SUPERIOR	malignant	mets breast
10	267	murugan	60	m	4729	1	1	1	1	4	ADEQUATE	suspicious	2	2	2	1	7	SUPERIOR	benign	
11	359	murugan	50	m	6489	2	1	2	1	6	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
12	415	shanmugam	75	m	6799	0	1	1	0	2	UNSUITABLE	nondiagnostic	1	2	2	1	6	ADEQUATE	benign	
13	430	vijay	42	m	7164	1	1	1	1	4	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
14	519	santhana kumar	29	m	9129	0	1	1	0	2	UNSUITABLE	benign	1	2	2	0	5	ADEQUATE	benign	
15	561	balakrishnan	16	m	10177	1	1	1	0	3	ADEQUATE	benign	1	1	1	0	3	ADEQUATE	benign	
16	597	anand	8	m	9667	2	2	2	1	7	SUPERIOR	benign	2	2	2	1	7	SUPERIOR	benign	
17	729	savariammal	32	f	13346	1	1	2	1	5	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
18	787	golusi beevi	74	f	14078	0	1	0	0	1	UNSUITABLE	nondiagnostic	1	1	2	0	3	ADEQUATE	benign	
19	905	mariammal	41	f	15707	0	1	1	0	2	UNSUITABLE	nondiagnostic	0	2	0	0	2	UNSUITABLE	nondiagnostic	
20	917	pattamuthu	50	f	15802	1	1	1	1	4	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
21	959	santhanam	60	m	16443	1	1	1	1	4	ADEQUATE	benign	0	1	1	0	2	UNSUITABLE	nondiagnostic	
22	1006	muthu	31	m	17530	1	1	2	0	4	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
23	1557	Ramuammal	60	f	26479	1	1	1	1	4	ADEQUATE	benign	1	2	1	1	5	ADEQUATE	malignant	mets adc
24	1560	kohila	48	f	26430	1	1	1	1	4	ADEQUATE	benign	1	2	0	1	4	ADEQUATE	benign	
25	1561	sudalai muthu	70	m	26907	1	0	2	1	4	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
26	1662	kuttythai	47	f	4588	1	1	1	1	4	ADEQUATE	benign	1	2	0	1	4	ADEQUATE	benign	
27	970	raj	34	m	16609	1	0	0	1	2	UNSUITABLE	nondiagnostic	0	2	0	0	2	UNSUITABLE	nondiagnostic	
28	916	isakki raj	65	m	25801	1	1	1	1	4	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
29	51	vasuki	63	f	4885	1	1	2	1	5	ADEQUATE	benign	2	2	2	2	8	SUPERIOR	malignant	mets breast
30	1584	sudalai kani	38	m	27004	1	1	1	1	4	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
31	65	nandha kumar	31	m	26785	2	2	2	1	7	SUPERIOR	benign	2	2	2	1	7	SUPERIOR	benign	
32	2499	vasantha	53	f	42470	2	1	2	1	6	ADEQUATE	malignant	2	2	2	2	8	SUPERIOR	malignant	mets adc
33	1610	nagamani	55	f	27774	1	1	2	1	5	ADEQUATE	suspicious	2	2	2	1	7	SUPERIOR	benign	
34	64	lakshmi	50	f	25684	1	2	2	1	6	ADEQUATE	benign	2	1	2	1	6	ADEQUATE	benign	
35	1848	raja mydeen	54	m	31962	1	1	1	1	4	ADEQUATE	benign	2	2	2	1	7	SUPERIOR	benign	
36	271	rajesh	55	m	35563	1	0	1	1	3	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
37	44	sankar ganesh	44	m	27566	1	2	1	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
38	72	prema	48	f	26972	1	1	2	1	5	ADEQUATE	suspicious	1	2	2	1	6	ADEQUATE	benign	
39	1042	nellaiappan	74	m	28086	1	2	2	1	6	ADEQUATE	benign	2	1	1	1	5	ADEQUATE	benign	
40	1135	sagunthala	45	f	26543	1	1	1	1	4	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
41	976	nirmala	72	f	26478	2	1	2	1	6	ADEQUATE	malignant	2	2	2	2	8	SUPERIOR	malignant	mets ovary
42	1111	dharmaraj	56	m	25689	1	1	1	1	4	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
43	8	mohamed eliyas	25	m	27549	1	0	1	1	3	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
44	45	kathiresan	73	m	32757	1	1	1	1	4	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
45	48	gowri	66	f	32646	2	2	2	1	7	SUPERIOR	benign	2	2	2	1	7	SUPERIOR	benign	
46	26	kannamal	62	f	36596	1	1	1	1	4	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
47	2718	ramalingam	48	m	37581	1	0	2	1	4	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
48	864	thilaga	55	f	32768	1	0	1	1	3	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
49	1119	chellaih	61	m	36793	1	1	2	1	5	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
50	2618	maruthan	66	m	35668	1	1	1	1	4	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	

C-Cellularity B-Background M-Morphology A-Architecture S-Score Mets-Metastasis adc-Adenocarcinoma

BRONCHIAL WASH

S.NO	PATH NO	NAME	AGE	SEX	IP/OP NO.	CONVENTIONAL SMEAR						DIAGNOSIS	CELL BLOCK						DIAGNOSIS	MALIGNAN
						C	B	M	A	S	QUALITY		C	B	M	A	S	QUALITY		
1	1	alaudeen	67	m	61835	1	2	2	1	6	ADEQUATE	malignant	2	2	2	2	8	SUPERIOR	malignant	scc
2	2817	thangavel	26	m	63420	1	1	2	1	5	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
3	2913	sankar	27	m	7841	1	2	2	1	6	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
4	200	vithiran	65	m	3304	1	2	2	1	6	ADEQUATE	malignant	1	1	2	1	5	ADEQUATE	malignant	scc
5	214	packiyanathan	63	m	2288	1	1	1	0	3	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
6	230	jeyaganthi	61	m	2925	0	1	1	1	3	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
7	290	ramar	60	m	3626	1	1	2	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
8	324	sudalaimadasamy	47	m	4915	0	1	1	0	2	UNSUITABLE	benign	1	2	2	1	6	ADEQUATE	benign	
9	2664	muniyandi	65	m	6409	0	1	2	0	3	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
10	370	behu	47	m	4707	1	1	1	1	4	ADEQUATE	benign	1	0	1	1	3	ADEQUATE	benign	
11	409	mahesh	25	m	6024	1	1	2	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
12	426	michael royappan	65	m	1644	2	1	2	1	6	ADEQUATE	malignant	2	2	2	2	8	SUPERIOR	malignant	scc
13	432	joseph	55	m	7583	1	1	2	1	5	ADEQUATE	benign	0	1	2	0	3	ADEQUATE	benign	
14	477	christy	67	f	1634	1	1	1	1	4	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
15	731	banumathy	59	f	1658	0	1	2	0	3	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
16	736	anand kumar	23	m	12112	1	2	2	1	6	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
17	801	clara	62	f	12534	1	1	2	1	5	ADEQUATE	benign	1	1	1	1	4	ADEQUATE	benign	
18	960	arumugam	48	m	16002	0	1	1	0	2	UNSUITABLE	nondiagnostic	1	1	1	1	4	ADEQUATE	benign	
19	973	kariguru	51	m	16422	1	1	2	1	5	ADEQUATE	malignant	1	1	2	1	5	ADEQUATE	malignant	scc
20	63	akbar ali	45	m	3802	1	2	2	1	6	ADEQUATE	malignant	2	2	2	2	8	SUPERIOR	malignant	scc
21	24	nelson	30	m	15856	1	1	2	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
22	1421	adaikalam	70	m	23577	1	1	2	1	5	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
23	1571	kajamoiden	80	m	26283	0	1	1	0	2	UNSUITABLE	benign	1	2	1	1	5	ADEQUATE	benign	
24	1594	mani	52	m	26073	1	1	1	1	4	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
25	1657	subramanian	60	m	27825	2	1	2	1	6	ADEQUATE	malignant	2	2	2	1	7	SUPERIOR	malignant	scc
26	1659	mani	69	m	27329	1	1	1	1	4	ADEQUATE	benign	2	1	2	1	6	ADEQUATE	benign	
27	1669	kuthalyan	53	m	25905	1	0	1	1	3	ADEQUATE	suspicious	2	1	2	1	6	ADEQUATE	benign	
28	1806	durairaj	60	m	4675	1	1	1	0	3	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	malignant	scc
29	1853	vargese	72	m	30357	0	1	1	0	2	UNSUITABLE	nondiagnostic	1	1	2	1	5	ADEQUATE	benign	
30	1898	palavesam	42	m	4823	1	0	2	1	3	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	malignant	scc
31	1942	masanam	71	m	4819	1	1	1	1	4	ADEQUATE	suspicious	1	2	2	1	6	ADEQUATE	benign	
32	1958	thotakani	60	f	33564	1	1	1	1	4	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
33	1966	kanthasamy	67	m	5145	1	2	1	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
34	1979	mokammal	45	f	34368	0	1	1	0	2	UNSUITABLE	nondiagnostic	1	1	2	1	6	ADEQUATE	benign	
35	2474	nijamudeen	61	m	42665	2	1	2	1	6	ADEQUATE	malignant	1	2	2	2	7	SUPERIOR	malignant	adc

C-Cellularity B-Background M-Morphology A-Architecture S-Score adc-Adenocarcinoma SCC -Squamous cell carcinoma

FNAC

						CONVENTIONAL SMEAR							CELL BLOCK							
s.no	path no	NAME	AGE	SEX	IP/OP NO	C	B	M	A	S	QUALITY	DIAGNOSIS	C	B	M	A	S	QUALITY	DIAGNOSIS	SUB TYPE
1	2820	nethaji	50	m	65245	1	2	2	1	6	ADEQUATE	malignant	2	2	2	1	7	SUPERIOR	malignant	lcc
2	3057	murugan	48	m	10360	1	2	2	1	6	ADEQUATE	suspicious	1	2	2	2	7	SUPERIOR	malignant	adc
3	3194	krishnamoorthy	55	m	10513	1	1	2	1	5	ADEQUATE	suspicious	1	2	2	1	6	ADEQUATE	malignant	adc
4	78	santhanam	72	m	633	1	1	2	1	5	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
5	103	shanmugaraj	48	m	1573	1	1	2	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	benign	
6	158	vitheeran	65	m	3304	0	2	2	1	5	ADEQUATE	suspicious	2	2	2	2	8	SUPERIOR	malignant	scc
7	219	jeyagandhi	61	m	2925	1	1	2	1	5	ADEQUATE	malignant	1	1	1	1	4	ADEQUATE	malignant	scc
8	400	mariappan	58	m	6595	1	1	2	1	5	ADEQUATE	malignant	2	2	2	2	8	SUPERIOR	malignant	smcc
9	634	vaithialingam	64	m	10392	1	2	1	1	5	ADEQUATE	benign	0	1	2	0	3	ADEQUATE	benign	
10	722	anandhi	23	f	2380	1	2	1	1	5	ADEQUATE	benign	0	1	1	1	3	ADEQUATE	benign	
11	739	munisamy	48	m	2479	1	2	2	1	6	ADEQUATE	malignant	1	2	2	2	7	SUPERIOR	malignant	scc
12	954	kaja moideen	65	m	3077	0	0	1	0	1	UNSUITABLE	Nondiagnostic	0	1	1	0	2	UNSUITABLE	Nondiagnostic	
13	974	seliyan selvaraj	40	m	2912	0	1	2	0	3	ADEQUATE	benign	1	1	2	1	5	ADEQUATE	benign	
14	508	bakyanathan	65	m	5548	1	2	2	1	6	ADEQUATE	benign	1	2	2	2	7	SUPERIOR	malignant	scc
15	2589	kanagasabai	70	m	13097	1	1	2	1	5	ADEQUATE	benign	1	2	2	1	6	ADEQUATE	malignant	scc

C-Cellularity B-Background M-Morphology A-Architecture S-Score adc-Adenocarcinoma lcc-Large cell carcinoma Scc-Squamous cell carcinoma Smcc-Small cell carcinoma

KEY TO MASTER CHART

C – CELLULARITY

0 - Minimal or absent: Diagnosis not possible

1 - Sufficient for cytodiagnosis

2 - Abundant : Diagnosis simple

B – BACK GROUND OBSCURED BY BLOOD AND PROTEINACEOUS MATERIAL

0 - Large amount: Diagnosis greatly compromised

1 - Moderate amount: Diagnosis possible

2 - Minimal amount: Diagnosis easy

M – MORPHOLOGY cellulardegeneration and cellular trauma.

0 - Marked: Diagnosis impossible

1- Moderate: Diagnosis possible

2 - Minimal: good preservation

A – ARCHITECTURE appropriatearchitecture and cellular arrangement

0 - Minimal to absent: nondiagnostic

1 - Moderate: some preservation eg: follicles, papillae,acini, synctia or single cell pattern

2 - Excellent architectural display, closely reflectinghistology: diagnosis obvious

QUALITY

Diagnostically unsuitable (score 0-2)

Diagnostically adequate (score 3-6)

Diagnostically superior (score 7-8)