"DIAGNOSTIC UTILITY OF THE CELL BLOCK METHOD VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY"

By

REG NO: BN0119003

Dissertation

Submitted to the

KLE Academy of Higher Education and Research
Belagavi, Karnataka
In partial fulfilment of the requirements for the degree of

DOCTOR OF MEDICINE
IN
PATHOLOGY

DEPARTMENT OF PATHOLOGY
J. N. MEDICAL COLLEGE,
BELAGAVI, KARNATAKA

APRIL – 2022

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

BELAGAVI, KARNATAKA

Endorsement by Head of Department and Principal / Head of the Institution

This is to certify that the dissertation entitled **"DIAGNOSTIC UTILITY OF THE CELL BLOCK METHOD VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY"** is a bonafide research work done by **REG NO: BN0119003.**

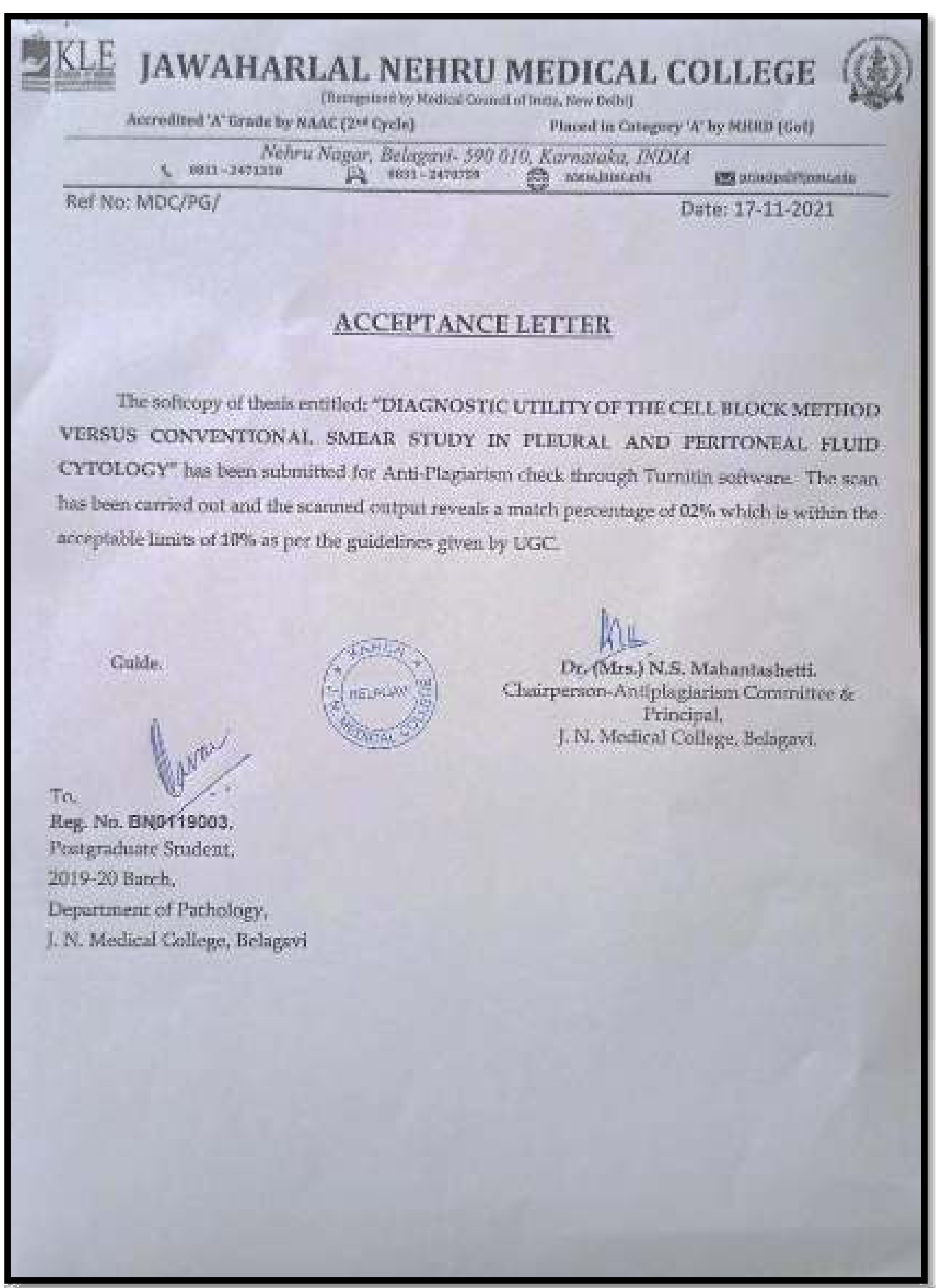
DR. Ranjit Kangle MD,
Professor and HOD
Department of Pathology,
J. N. Medical College,
Belagavi, Karnataka

Date:
Place: Belagavi.

DR.(Mrs) N. S. Mahantashetti MD (Paed).
Principal
J. N. Medical College,
Belagavi, Karnataka.

Date:
Place: Belagavi.

PLAGIARISM ACCEPTANCE LETTER



KLE **JAWAHARLAL NEHRU MEDICAL COLLEGE**

(Recognised by Medical Council of India, New Delhi)

Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)

Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

Ref No: MDC/PG/ Date: 17-11-2021

ACCEPTANCE LETTER

The softcopy of thesis entitled: "DIAGNOSTIC UTILITY OF THE CELL BLOCK METHOD VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY" has been submitted for Anti-Plagiarism check through Turnitin software. The scan has been carried out and the scanned output reveals a match percentage of 02% which is within the acceptable limits of 10% as per the guidelines given by UGC.

Guide.

Dr. (Mrs.) N.S. Mahantashetti.
Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BN0119003,
Postgraduate Student,
2019-20 Batch,
Department of Pathology,
J. N. Medical College, Belagavi

LIST OF ABBREVIATIONS USED

CS	-	Conventional smear
CB	-	Cell block
H&E	-	Haematoxylin and eosin
PAP	-	Papanicolaou
MGG	-	May-Grunwald Giemsa
IHC	-	Immunohistochemistry

ABSTRACT

DIAGNOSTIC UTILITY OF THE CELL BLOCK VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY

BACKGROUND : The cytological examination of body fluids is one of the commonly performed investigations. The aim of this study is to compare the cytological features of pleural and peritoneal fluids by cell block (CB) and conventional smear (CS) method to assess the cellular features and diagnostic efficiency of both the methods

METHODS :. 100 pleural and peritoneal fluid samples were evaluated by both CB and CS method. CB was preparad by fixing in l0% formalin and adding plasma thrombin. The morphological details like cellularity, architectural pattern, nuclear and cytoplasmic details of cells were studied by both the methods.

RESULTS : CS gives better results for nuclear and cytoplasmic details whereas CB is more superior in terms of cellularity, architectural pattern and equal in bringing out the cytoplasmic and nuclear details. The diagnostic efficiency of cell block method was greater compared to CS method.

CONCLUSIONS : CB preparation is simple, rapid and inexpensive method for serous fluids in which better morphololological features gives definitive diagnosis and should be performed routinely.

KEYWORDS : Cell block, Conventional smear, Pleural fluid, Peritoneal fluid

TABLE OF CONTENTS

SL. NO.	TOPIC	PAGE NO.
1	INTRODUCTION	1-3
2	AIM AND OBJECTIVES	4
3	REVIEW OF LITERATURE	5-28
4	METHODOLOGY	29-35
5	OBSERVATION AND RESULTS	36-46
6	DISCUSSION	47-57
7	SUMMARY	58-60
8	CONCLUSION	61
9	BIBLIOGRAPHY	62-70
10	ANNEXURES	71-88
	ANNEXURE I – ETHICAL CLEARANCE	71
	ANNEXURE II – CONSENT FORM	72-74
	ANNEXURE III – PROFORMA	75-76
	ANNEXURE IV- PHOTOMICROGRAPHS	77-80
	ANNEXURE V – KEY TO MASTER CHART	81-83
	ANNEXURE VI- MASTER CHART	84-88

LIST OF TABLES

Table No	TABLES	Page. No
1	Cytomorphological differences between Reactive mesothelial hyperplasia (RMH), Mesothelioma and Adenocarcinoma	**10**
2	Criteria for assessment of conventional smear and cell block	**31**
3	Distribution of fluids according to age and gender	**37**
4	Mean distribution of Age	**38**
5	Background obscuration in CS and CB	**39**
6	Amount of diagnostic material present in CS and CB	**39**
7	Retention of cellular architecture in CS and CB	**39**
8	Interpretation of Kappa score	**40**
9	Mean scores for all criteria's in CS and CB	**40**
10	Interpretation of Quality of slide in CS	**41**
11	Interpretation of Quality of slide in CB	**41**
12	Comparison between diagnostic category in CS and CB	**43**
13	Descriptive statistics for total score obtained in CS and CB	**43**
14	Primary sites in metastatic effusion in male and female	**45**
15	Distribution of Age and Gender in various studies	**48**

16	Comparison of p values for each category in various studies	**49**
17	Assessment of quality of slides in various studies	**50**
18	Clinical diagnosis in negative for malignancy category in various studies	**51**
19	Comparison of cytodiagnosis in present study with other studies	**52**
20	Pleural fluid analysis for primary lesions in various studies	**54**
21	Peritoneal fluid analysis for primary lesions in various studies	**55**
22	Additional yield of malignancy in various studies by cell block	**56**

LIST OF FIGURES

Fig. No	Figures	Page. No
1	Showing major serous cavities	6
2	Fluid sample	33
3	Test tubes, slides, pipette, coplin jar, xylene, mounting media, coverslips	33
4	Centrifuge machine	33
5	Slide warmer	33
6	PAP staining station	34
7	MGG stains	34
8	PAP, MGG and CB slides	34
9	Scalpel, Tissue cassettes , Filter paper	35
10	Tissue Processor	35
11	Rotary Microtome	35
12	Pie chart showing distribution of fluids	36
13	Showing genderwise distribution of cases	37
14	Diagnostic categories on CS	42
15	Diagnostic categories on CB	42
16	Clinical diagnosis of Negative for malignancy in Pleural fluids	44
17	Clinical diagnosis of Negative for malignancy in Peritoneal fluids	44
18	Distribution of diagnosis in CS and CB	45

LIST OF PHOTOMICROGRAPHS

Sl. No	Photomicrographs	Page. No
A	Mesothelial cell cluster on CS (PAP stain, 40X)	77
B	Mesothelial cell cluster on CB (H &E stain, 20X)	77
C	Mesothelial cell cluster on CB (H &E stain, 40X)	77
D	Malignant cells on CS (PAP stain, 20X)	78
E	Malignant cells on CS (PAP stain, 40X)	78
F	Malignant cells on CB (H &E stain, 40X)	78
G	Malignant serous Papillae on CS (PAP stain, 40 X)	79
H	Malignant serous Papillae on CB (H &E stain, 40X)	79
I	Malignant cells on CS (PAP stain, 40X)	80
J	Malignant cells on CB (H &E stain, 40 X)	80

INTRODUCTION

Accumulation of fluid other than blood in pleural and peritoneal cavities is called an effusion, which in the abdomen is called ascites. Serous effusions are accumulation of fluid in excess of the normal small amount in serous cavities. All effusions are pathological regardless of their cellular constituents [1,2].

These effusions are classified into two types- transudates and exudates. The transudates are clear, straw-colored fluids characterized by a low specific gravity often below 1.010 and low protein content (usually below 3g/dL). It is seen in conditions with increased venous pressure such as congestive heart failure or liver cirrhosis and decreased oncotic intravascular pressure as in hypoproteinemia or nephrotic syndrome.

The exudates are characterized by high protein content (usually above 3g/dL) and have a high specific gravity of more than 1.015. There are several causes of exudates which include Inflammatory conditions that are usually caused by infectious processes in the organ enclosed by the membrane, Tumors that may be primary or metastatic and other miscellaneous causes [3].

Cytologic examination of pleural and peritoneal effusions reveals information about inflammatory conditions of the serous membranes, parasitic infestations, infections with bacteria, fungi or viruses. It also supplies evidence of the presence of a fistulous connection with a serous cavity [4].

Cytological examination of serous fluids is one of the commonly performed investigations as it is simple, reliable, rapid and cost effective. It reveals information about inflammatory and malignant lesions of serous membranes. Cancers like

pulmonary, breast and gastric carcinoma commonly present with malignant pleural effusion. Cytological examination assists the clinician in formulating the etiology of effusion and list of differential diagnosis. It also helps to follow the results of therapy and prognosis [5].

Processing of Fluids :

The fluids are divided into two equal parts. One part is kept for conventional cytology (centrifuged smear – CS) and the other part for cellblock (CB).

Conventional Smear:

The fluid is centrifuged at 2000 rpm for 10 minutes in glass test tubes and supernatant is discarded. Minimum of two thin smears are prepared from the sediment according to the method of Papanicolaou.

Cell Block :

Plasma thrombin method :

1. Add 2 or 3 drops of plasma to the sediment. Mix the sediment and the plasma together.
2. Add 3 or 4 drops of thrombin solution to the mixture. Mix again.
3. Allow the mixture to clot.
4. Add 10% buffered formalin to the clot.
5. With a pair of small scissors, cut the clot(s) into small pieces and allow them to fix for 30 min.
6. Process the fragments (spontaneous and induced clot) as tissue [3].

In conventional cytology of smears, the accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem. There is lower sensitivity due to overcrowding of cells, cell loss and its processing methods.

To overcome these limitations, cell block method was developed as it provides better tissue architecture; multiple sections can be obtained from same material[5]. Cell block method can very well differentiate between malignant and non malignant cells.

It also increases the sensitivity of detecting malignancies and has the ability to reduce false-positive interpretations. This method is simple and inexpensive and bridges the gap between cytology and histology. The slides can be further processed for special stains and Immunohistochemistry. Hence the present study will be undertaken to evaluate the utility of cell block preparation as it increases the sensitivity in diagnosis of fluids in effusions received in laboratory

AIMS AND OBJECTIVES

1. To compare cytological features of pleural and peritoneal fluids by cell block method and conventional smear study
2. To assess utility of a combined approach for cytodiagnosis of pleural and peritoneal effusions.

REVIEW OF LITERATURE

The History of Thoracic Drainage:

The thought of draining substances from the thoracic cavity has been documented since ages. The oldest acknowledged reference to thoracic drainage dates back to the fifth century B.C.E. according to Hippocrates firstly, cut the skin between the ribs with a scalpel; then wrap a lancet with a piece of cloth, leaving the point of the blade exposed a length equal to the nail of your thumb, and insert it [6]. Hippocrates successfully treated inflammation with this procedure.

The first description of a water-seal chest drainage system was attributed to Playfair in 1873 in the treatment of pectoral inflammatory disease. In 1875, Gotthard Bülau also used the closed water-seal chest drainage to treat an empyema. He used a trocar to puncture the pleural space and then introduced a rubber tubing with a distal clamp. Monaldi, in 1950 suggested draining the thoracic cavity with a more superior approach at the second or third intercostal space [7].

The modern three chamber thoracic drainage system was initially described by Howe in 1952. In 1968, Heimlich designed a flutter valve to attach to catheters and replace the cumbersome underwater drainage bottles. The advantages included simplicity, safety ,sterility, disposability, in the event of disconnection, and allowance for patient ambulation [8].

Currently the plastic three chamber single unit system is used which allows allows efficient collection of intrapleural fluid and evacuation of air. This system is better in patient comfort and safety compared to the original Heimlich valve. Recently, Pleural drainage systems with digital quantification of air leaks and pleural pressure have been introduced as well.

SEROUS EFFUSIONS

Serous cavity effusions are relatively easy to drain and are collected for therapeutic and diagnostic purposes. Proper handling of specimens from the initial stage of collection to final stage of interpretation is important. The personnel involved includes clinicians, cytotechnologists and pathologists who should be familiar with the intricacies of specimen collecting, processing and interpreting for optimal results.

Anatomy, Histology and Cytology

The major serous cavities include the two pleural cavities and the peritoneal cavity. These cavities are lined by parietal and visceral mesothelial layer. The serous cavity histology and serous fluid cytology are significantly identical without any site specific differences [9] Mesothelial cells are derived from mesoderm. The mesothelial cells are supported by a thin layer of fibrous connective tissue with a varying amount of adipose tissue, small blood vessels and lymphatics.

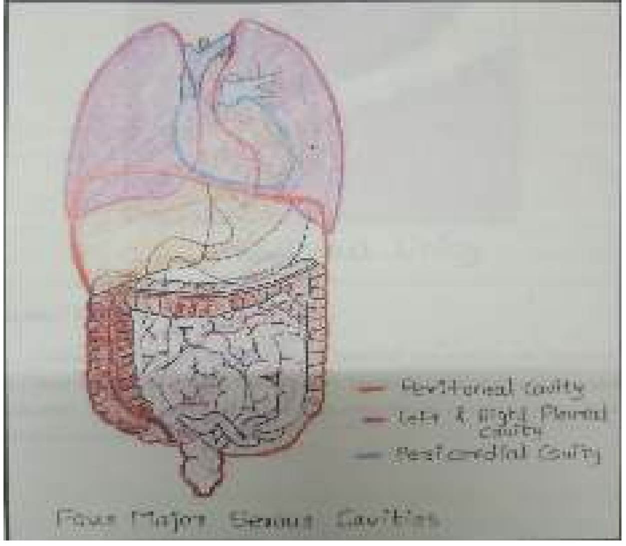


Fig 1. Showing major serous cavities

Cells of Serous Fluid

The normal mesothelial lining is composed of a single layer of flattened cells. Parietal and visceral layers of mesothelium are separated by a layer of a lubricating fluid. This fluid facilitates the movement of two membranes against each other. Accumulation of excess fluid other than blood is known as effusion. Effusion in abdomen is called as ascitis [3]. The normal count of cells in pleural and peritoneal fluid is less than 1000 WBC/cc

Mesothelial cells : mesothelial cells are round about 25um in diameter and have a single central or eccentric nucleus. The cells are flat and in sheets in their natural state, however when they are shed off naturally or by force , the cells are scattered and each individual cell tends to ball up. In PAP stained smears the cells have dense grey-green cytoplasm showing two zone phenomena , a narrow zone of pale ectoplasm and dense endoplasm.

Mesothelial window : The microvilli of mesothelial cells tend to prevent adjacent cells from completely opposing each other, thereby creating a gap between two cells. The space between adjacent mesothelial cells is referred as mesothelial window. These spaces are not limited to mesothelial cells and may be seen in other type of cell groups including metastatic cancers.

Reactive mesothelial cells : Various pathological processes such as inflammation, trauma, infection lead to reactive changes in mesothelial cells. Mesothelial cell hypertrophy and proliferation leads to remarkably wide morphological spectrum which may even overlap with malignant cells. Such exfoliated mesothelial cells are referred to as reactive mesothelial cells. They have wide range of appearances. The cell size ranges from 15 to 30 um but may be as large as 50um in diameter with

variable amount of cytoplasm. The nuclei are enlarged and show variation in size and shape with conspicuous nucleoli. Binucleation and multinucleation is seen frequently. Few cells may show high nuclear cytoplasmic ratio with scant cytoplasm and slightly hyperchromatic nuclei with prominent nucleoli.

Reactive cells with cell enlargement, nuclear enlargement, multinucleation, vacuolation, hyperchromasia, irregular nuclear membrane may mimic malignancy

1. **Macrophages :** The size varies from 15- 100 um. They have an eccentric, round to bean shaped nuclei with lightly staining lacy cytoplasm . The cytoplasm usually contains leucocytes, nuclear particles, RBCs, lipid droplets etc.

2. **Red blood cells (RBCs)** : RBCs found in small numbers are not significant and are probably due to trauma of needle insertion. Large number of RBCs indicate traumatic hemorrhage, neoplastic effusion or hematoma due to massive infarction of organs.

3. **Neutrophils** : neutrophils are seen in wide variety of neoplastic and non neoplastic conditions like tuberculosis, arthritis and various malignancies.

4. **Eosinophils** : eosinophilic effusion can be seen in cases of allergy, autoimmune disorders, pneumonia, pulmonary infarction, tuberculosis, pneumothorax and malignant neoplasms.

The narrow gap of 5- 10 micron that separates the parietal and visceral layers of the serosa is widened in the presence of effusion of fluid into the serous cavity. All effusions are pathological irrespective of their cellular composition. Effusions may be of two type either transudates or exudates. Effusions can also be classified according

to the etiological process, which may be due to reactive benign (infective/inflammatory) conditions or due to malignant deposits into the cavity. Peritoneal cavity can accumulate up to 15 – 20L while the pleural cavities can hold up to 3 L of fluid in each cavity.

Reactive effusions: [10]

In adults most effusions are due to benign conditions such as congestive heart failure, cirrhosis of liver or pericarditis. Cytomorphological features of reactive mesothelial cells overlap significantly with malignant cells and may be misinterpreted as malignant.

Malignant effusions: [10]

Effusions secondary to carcinoma are mostly recurrent and hemorrhagic. Non traumatic massive hemorrhagic effusions almost always have neoplastic etiology. Malignant neoplasms from almost any site (except CNS) can metastasize to serous cavities and present as an effusion. They are usually secondary to adenocarcinoma from breast, lungs, gastrointestinal and genito-urinary tract or due to lymphoma/leukemia spillover. Among malignant pleural effusions, primary carcinoma of lung is the most common cause, followed by GIT and pancreatic carcinomas in men while in women; carcinoma of breast is followed by lung and ovarian malignancy. In the malignant peritoneal effusions, cancers of gastro intestinal tract, and pancreas predominate in men while in women; carcinoma of ovary is followed by GIT.

Table 1: Cytomorphological differences between Reactive mesothelial hyperplasia (RMH), Mesothelioma and Adenocarcinoma [3, 20-23]

FEATURE	RMH	MESOTHELIOMA	ADENOCARCINOMA
Cellularity	Variable	Hypercellular	Hypercellular
Morphology of cells	Monolayered with knobby outlines	2-3 dimensional cell groups with knobby outline	2-3 dimensional groups with smooth borders
Papillary structures	Rare	May be seen	May be seen
Cellular variation	Mild variation	Great variation	Great variation
Multinucleated cells	Sparse	Frequent	Frequent
Nuclei	Round with smooth contours	Enlarged , round , generally smooth contour	variable size often irregular in contour
N: C ratio	Low	Usually low	High
Intercellular windows	Present	Present ; Large windows may resemble acini	Absent; narrow acini may resemble window
Cytoplasm	usually abundant and dense with peripheral fuzziness	usually abundant and dense with peripheral fuzziness	Variable, not dense, sharp cell border
Cytoplasmic vacuolation	may be seen	may be seen	often seen
Cytoplasmic mucin	not seen	not seen	Often seen

Cytological examination of serous effusions has been done for nearly a century in the diagnosis of malignancy and eventually the detection of primary site. It helped in the staging, diagnosis and prognosis of malignant tumors and also gave information regarding various inflammatory lesions of the serous membrane. The effusion positive for malignancy was often considered as a definitive diagnosis [11].

Presently the examination of body fluids for the presence of malignant cells is accepted as a routine laboratory procedure for the detection of unsuspected malignancies and also for detection of metastasis of unknown primary.

In 1875, Quincke demonstrated that the milkiness of cancerous ascitic fluids was not due to fat but due to an albuminous substance, probably from the neoplasm (? carcinoma cells). In 1882, he also noticed the predominance of leukocytes in inflammatory exudates, mesothelial cells in tuberculosis and malignant effusions. He described clusters of large irregular cells containing vacuoles in ascitic fluid. Clinically this case was considered at first to be one of liver cirrhosis, but on microscopic examination of ascitic fluid due to the presence of large cells, the diagnosis of peritoneal carcinosis was made and was proven to be correct at autopsy[12].

In 1895, Rieder recorded many large vacuolated cells showing mitotic figures in the ascitic fluid of a patient with carcinoma ovary. This was for the first time that cells free in a fluid medium were seen undergoing mitotic division. Further, Geigel and Voit suggested that abnormal cells can be found in fluids and mitoses were of great significance[12].

Rieder suggested that presence of numerous polymorphous, large, unequal cells specially with vacuoles lying in clusters, favored neoplastic process rather than

benign reactive mesothelial cells. however, morphological changes were not specific for neoplastic process; such changes were also seen in inflammatory etiology[13].

In 1897, Dock described atypical cells from pleural and ascitic fluid exhibiting striking variations in size, shape and nuclear arrangement and many atypical mitotic configurations. Warthin also described spindle cells floating free in the pleural fluid in a case of fibro sarcoma. Quensel emphasized the presence of dark staining nucleoli as evidence of the neoplastic nature of the cells in an effusion. In 1899, Benecke reported pigment-containing cells in the peritoneal fluid in a case of melanocarcinoma[12].

In study conducted by Takagi F on 183 effusion samples, they concluded that the most reliable and useful criteria for diagnosis of malignancy in fluids were:

1. Clusters of malignant cells with an organoid pattern
2. Individually lying malignant cells
3. Cells staining positive for mucin [14]

Fluids in which the small round cells were found representing undifferentiated carcinoma arising in glands (ovary, stomach, breast,) were extremely difficult to diagnose. They also suggested that if the number of tumor cells were small, it was impossible to differentiate the reactive mesothelial cells from the tumor cells [14]

In 1954, Foot NC et al studied 219 effusions of proven malignancy to identify the primary sites and types of metastatic tumor from exfoliated cells in serous fluids. They found that cells from malignant tumors could be readily recognized in smear preparations of serous fluids and also proposed that it was possible to diagnose the

source and type of malignant tumor cells in serous effusions with an overall accuracy of 48% [15].

In 1956, Foot NC analyzed smears from 2029 persons, found that examination of smears were of distinct value in confirming or ruling out suspicion of a tumor. He proposed smear examination might not assist in early detection of malignant growth, since these are already far advanced when cells are exfoliated into these effusions [16].

Malignant cells in ascitic or pleural fluids were almost always indicative of metastatic tumors as primary malignancy arising from mesothelial cell lining these spaces were very rare. When mesothelial primary was present, tumor cells were usually numerous and seen in clumps [17].

In 1972 Murphy WM et al, studied 117 malignant effusions retrospectively from various sites in to determine the primary site of malignancy. Their study was comprised of well-documented cases of pleural and ascitic serous effusions containing malignant tumor cells derived from the carcinoma of the ovary, breast, lung, endometrium and stomach.

According to the study malignant tumor cells could be identified in approximately 90% of the effusions related to malignant neoplasm. They found that cytoplasmic vacuoles are not characteristic. In fact, the occurrence of vacuoles in more than 5% of the cells was a major factor in differentiating ovarian from mammary neoplasm. Uniformity of the cell size and shape was found to be a hallmark of breast cancer in fluids. Major characteristics of lung tumors in effusions were irregularity of cells with vacuoles and macro nucleoli, large percentage of isolated cells and the prominence of multinucleated cells [18].

Most of the authors [18,19] were in agreement to the fact that the examination of cell patterns (for example, Indian file pattern, proliferation spheres in cases of mammary cancers, prominence of vacuolation in ovarian cancers, etc.) assisted in identification of the primary site. In a study done by Sears and Hajdu, the most common primary sites causing malignant effusions were carcinoma of breast, lung, lymphoreticular neoplasms, ovary and gastrointestinal tract.

Riego et al concluded that megakaryocytes in serous fluids signified an advanced hematopoietic malignancy and most of the patients showed marrow replacement by a metastatic process. Their study also suggested that searching for primary lung carcinoma should be of high priority in such cases [24]

In a study done by Hallman et all in 1994, they observed that majority of the pediatric neoplasms were benign and malignant effusions were usually of the small cell type; it was difficult to distinguish these small cell types from the mononuclear inflammatory cells [25].

In pediatric neoplasms, cytological examination showed a higher proportion of positive effusion cytology with lymphoma/leukemia, neuroblastoma and Wilms tumor. Pleural fluids were the most common type of effusions in pediatric patients and showed the highest proportion of positivity [26]

In 1958, Foot NC et all found that mesothelial cells and histiocytes undergo metaplastic change in certain circumstances and can be readily mistaken for cancerous elements [27]

In 1996, Sujathan K et al. studied 100 samples of serous effusions and concluded that AgNOR count is significant in differentiating malignant cells from reactive mesothelial cells in ascitic and pleural fluid samples[28].

In 1979, Singh G et al found that the antimesothelial cell serum could be used as a diagnostic reagent in confirmation of the diagnosis of mesothelioma [29]

Booth S.N. et al in 1977 studied 56 cases of serous effusions (ascites and pleural effusions) and demonstrated that CEA levels in cancerous effusions were frequently several times higher than corresponding serum levels in contrast to the fluid levels of pregnancy associated $\alpha 2$ glycoprotein (PAG). Thus, the measurement of carcinoembryonic antigen (CEA) and pregnancy associated $\alpha 2$ glycoprotein (PAG) in serum and effusion fluids would increase the diagnostic accuracy in patients with effusions [30].

In 1985, Churg A et al studied immunohistochemical staining of keratin and vimentin in malignant mesothelioma. Mesotheliomas produce a variety of keratins and significant levels of vimentin, the intermediate filament considered characteristic of mesenchymal cells. Rapidly dividing mesothelial cells in culture as well as cultured cells derived from mesotheliomas produced large amounts of vimentin, but resting cells produced much less of this substance[31]

In 1992, Mezger et al used 4 pan epithelial antigens on cytospin preparations (EMA, Egp-34, BW-495 and TAG-72) along with CEA in their study involving 170 patients in identification of carcinoma cells in ascitic and pleural fluid. They concluded that BW-495 appeared to be a suitable marker for demonstration of carcinoma cells in 13 samples of pleural and ascitic fluid and it has a higher degree of sensitivity than does either TAG-7 or CEA [32]

In a study done by Mullick SS et all on 75 pleural effusion samples proved that p53 immunostaining is highly specific and moderately specific marker for malignancy in serous fluids [33].

The accurate identification of cells as either malignant or benign reactive mesothelial cells is a diagnostic problem in conventional smears. The reactive mesothelial may show reactive changes such as nucleomegaly, cytomegaly, multinucleation, mitotic figures and high nuclear cytoplasmic ratio. These changes are commonly seen in cirrhosis, allergic pleurisy, polyarteritis and cardiovascular diseases [14]

Morphological study of serous effusions may lead to diagnostic problems whenever there is little or no morphological distinction (for example mesothelial cells and poorly differentiated malignant cells).

The need to use a panel of markers and recurrent usage of immunocytochemical stains on was not possible on centrifuged smears (CS) due to scanty material, hence cell block (CB) plays a significant role in these situations.

CELL BLOCK (CB):

The cell block technique is among one of the oldest method which includes paraffin embedding of sediments of fluids with further processing by histologic techniques.

In 1896, Bahrenberg allowed a large amount of fluid to clot spontaneously. The supernatant fluid was poured off and the clot was shrunken and hardened by successive addition of alcohol. The process was repeated until a small stringy mass was obtained. Finally, this was embedded in celloidin and cut like a tissue. With the

help of this procedure, Bahrenberg was able to find epithelial cells in two ascitic fluids. The autopsy in these cases later revealed carcinoma [12,34,35,36]

In 1917, Mandelbaum devised a technique for the cell block preparation. Zemansky, Schlesinger and Honigman showed that the method was not only technically practical but also diagnostically useful if properly carried out. He concluded that the examination of the body fluids for evidence of malignant neoplasm by the cellblock technique is of great significance and dependable procedure [37]

Most cytologists used this technique of cellblock introduced by Bahrenberg and Mandlebaum till 1945. Later the smear technique became a routine method especially after the introduction of Papanicoloau stain, which highlighted better nuclear features.

In tumors that are difficult to diagnose on smears, cellblocks prepared from residual tissue or fluids can be useful. This technique is easy, safe and reproducible. Further the effectiveness of cellblock depends on the availability of diagnostic material for further histological examination, special stains and IHC studies for better classification of the tumor. Infectious causes can be identified with microbiologic stains.

Khan et al suggested that cellblocks gave better architectural and morphological details with respect to the presence of nucleoli, pseudoacinar or acinar structures. They also found that cell block was a significant technique in the identification of the acinar structures in majority of adenocarcinomas [38]

The diagnosis of carcinoma is more reliable when based upon cell clusters rather than individual cells [39]

The advantages of the CB are summarized as:

1. Recognition of histological patterns of diseases that can be difficult to identify in conventional smears [40]
2. It is Possible to study multiple sections by routine staining, special staining and immunocytological procedures [41]
3. Less cellular dispersal provides easier microscopic observation.
4. Less difficulty in spite of hemorrhagic background.
5. Slides can be stored for retrospective studies. Storage of the CS is a practically not possible.

The disadvantages with cellblock technique are:

1. Delay in the diagnosis as compared to conventional smears [3]
2. At times , there is a risk of losing material during processing [3]
3. Sometimes mesothelial cells may form rosettes or pseudoacini because of centrifugation that can lead to misdiagnosis [42]
4. Longer histological preparation time and increased cost. Jonasson JG et al concluded that routine use of cellblock is not a cost effective method of detecting malignancy in body cavity fluids [43]

Cellblock from serous effusions can be prepared by various methods.

A) FIXED SEDIMENT METHOD :

This technique was originally. described by Mandlebaum [3]. The main disadvantage with this technique is the risk of losing material.

The steps are as follows

1. Sediment or tissue fragments are mixed in a fixative. Fibrin clots are removed
2. The mixture is centrifuged for 15 minutes at 2000 r.p.m and allowed to stand overnight without disturbing the sediment [41]
3. Supernatant is poured off.
4. The sediment is carefully removed and wrapped in a filter paper and placed in a labeled tissue cassette.
5. Tissue is processed by routine tissue processing

B) BACTERIAL AGAR METHOD

1. Step 1 to 3 is same as fixed sediment method.
2. Sediment is gently removed from the tube and placed on a paper towel.
3. The sediment is sliced into half and cut side is placed on a pool of melted agar on a glass slide or petri dish. It is then allowed to harden for few minutes.
4. The excess agar is trimmed and rest of the agar button is placed in tissue cassette and processed. (3 % agar is prepared by dissolving 3.0 gm of bacterial agar in 100 ml of boiling water)

C) PLASMA THROMBIN CLOT METHOD

In this method, outdated plasma obtained from blood bank was mixed with unfixed sediment. Alcohol inhibits the clotting action of plasma and thrombin hence, If the sample is previously fixed with alcohol, the sediment is thoroughly washed several times with a salt solution. Equal amount of thrombin solution and plasma are

mixed together and added to sediment. The clot is formed in 1 to 2 minutes. The clot is then placed into lens paper and added to tissue cassette.

The following dehydration and embedding procedure is used –

1.	70 % ethyl alcohol	1 hour
2.	95% ethyl alcohol	1 hour
3.	95% ethyl alcohol	30 min
4.	95% ethyl alcohol	30 min
5.	Absolute alcohol	1 hour
6.	Absolute alcohol	1 hour
7.	Absolute alcohol	1 hour
8.	Xylol	1 hour
9.	Xylol	1 hour
10.	Paraffin	2 hours
11.	Paraffin	2 hours

In 1993, Leung et all introduced a simple miniblock technique in cytology by using setting and gelling reagent [44]. In this method the material obtained for cell block preparation was fixed in Omnifix for 1 hour. It was later centrifuged at 3000 r.p.m for 15minutes. The supernatant was discarded. The sediment was mixed with a setting reagent in a ratio of 1:2 . forming a gel like cell button. The button was then placed into the cytofunnel and spun at 1500 r.p.m for 5 minutes. The gel button was collected in the well of the cassette. For hardening one drop of setting reagent was added. It was processed further as routine surgical specimen.

In 1997, Bibbo [45] described a cell block preparation by using plasma thromboplastin. Later they added 10 % tinted buffer formalin. The buffer was cut into small pieces and fixed for 30 minutes. These small pieces were processed as tissue.

In 1997 Belloti et al [46] described the use of microwave in cell block preparation. Microwave reduced the dehydrating, clearing and impregnating time. They processed material collected by FNA samples.

D) COMPACT CELL BLOCK TECHNIQUE

In 1998 Yang et all studied 250 body fluids . in their technique, cyto rich red solution is added to fresh cellular sediment in a centrifuge tube at a ratio of 1:1. After 2 minutes, two to three drops of plasma and topical thrombin is added. The tube is then gently agitated for two to three minutes, until a gelatinous clot is obtained. The clot is then slid onto a lens tissue on top of paper towels. The lens tissue is folded once over the clot. By gently squeezing the excess fluid from it through the lens tissue into the paper towels, the clot is transformed into a compact, flat ,densely cellular aggregate, which is painted with mercurochrome prior to fixation in formaldehyde. They concluded that the compact cell block is about 10-20% the size of a conventional cell block, yet more number of cells are on display, thus decreasing the need for deep cuts and screening time while increasing the efficiency of diagnosis. The compact cell block technique is particularly useful for endometrial brush biopsies [47]

In 2000 Nathan et all described a new technique of cell block preparation. He used improvised ethanol formalin fixative (9 parts of 100% ethanol and 1 part of 40% formaldehyde) followed by paraffin processing. This preparation imparts detailed

cytomorphological features which correspond closely to cells in pap smear. It also ensures preservation of immunocytochemical properties [48]

E) AGAR CYTO CELL BLOCK

Kersten et al developed a new method in 2000 [49], Agar cyto cell block procedure for multiple molecular diagnostic analysis on a single scrapping from the uterine cervix. They observed that over more than 20 specimens were prepared from a single agar cyto cell block. Immunohistochemistry for Ki-67 and p53 and in situ hybridization for the centromere of human chromosome 1 and HPV type 16. They concluded that agar cyto cell block protocol was an excellent tool for diagnostic and prognostic markers of cervical cancer.

F) HISTOGEL TECHNIQUE OF CELLBLOCK:

By Varsegi and Shidham [50]- an improved technique to increase the chance of capturing individually scattered cells with the inclusion of AV marker. This helps to visualize the level at which the cells are concentrated, thus preventing the histotechnologist from cutting too deeply into the block.

Protocol for Histogel Method:

- Histogel may be used if the pellet formed during centrifugation is too scanty or not solid enough
- Add 1-2 drops of molten histogel to the pellet in a flat bottomed centrifuge tube.
- Mix by wooden applicator stick
- Place the centrifuge tube into refrigerator for 5-10 minutes or until solid

- Carefully transfer the pellet in the gel form from the tube onto a paper inside the tissue cassette
- Drop the cassette into 10% formalin and process for paraffin embedding.

These techniques received not much attention, probably due to lack of standardized cost effective methods that achieve better diagnostic results. The routine use of cell block by agar or Histogel methods are not cost effective as it requires additional materials and consumption of extra time compared to earlier conventional methods.

If the tissues were fixed and processed according to standard histopathology laboratory schedule, the cells appeared shrunken, the nuclei were small and dark rendering assessment of chromatin pattern difficult and the nucleoli were less conspicuous. For maximum usefulness of cellblock, fixation and processing of the samples have to be modified. Specimens fixed in 2.5% buffered formalin gave an appearance of reminiscent of autolysis. The cells were widely separated from each other and nuclear membranes were crenated or wrinkled. On the other hand, by using more than 10% formalin gave rather pyknotic nuclei and obscured chromatin pattern [51]

Dekker and Bupp also reported the advantages of cellblock in cytology, which included valuable diagnostic evidences that cannot be observed in smears. However some authors opined that carefully prepared smears were a better diagnostic medium than cellblock. According to their study, there was not a single case in which the cellblock was negative for malignant lesions and the smear was positive for the same [41]

In a study done by Sujathan et al [52] , in cellblocks the diagnostic yield of malignancy increased from 17 to 21 and atypical cases reduced from 10 to 1. Study done by Bodele et al [53] found that additional 7% positivity for malignant lesions in cellblock when compared to standard smears.

Richardson et al [54] opined that the very best degree of accuracy would be obtained by utilizing smears as principal diagnostic weapon and supplementing with cellblocks of material remaining after preparation of the smears. consistent with various studies, more than 55% of original smear diagnosis was improved after the cellblock was examined. The overall accuracy rate with cellblock technique was 97% with a sensitivity of 95% and specificity of 100% for malignant lesions.

37 cases of serous effusions were studied by Bista P he concluded that better architectural display and good yield of diagnostic material with minimal degenerative changes with CB. He suggested that findings of both CS and CB will complement one another to arrive at a diagnosis [54]

It was possible to diagnose primary site in 81% of cases in cell block preparation compared to conventional smears. Special stains can be easily performed on the sections prepared from cell blocks.

Price et al studied 286 cell blocks and found that pericellular lacunae were seen in majority of the neoplasms. These neoplasms originated in the breast, ovary, lung and GIT which helped in distinguishing adenocarcinoma from reactive mesothelial cells [55]

In a study done by Meenu Thapar et al on 190 cases, concluded that combination technique yielded 13% more malignant cases than that were detected

using smears by themselves. Primary site was determined in 83.3 % cases in malignant effusion [40]

Kumar SH et al studied 117 body fluids and concluded that CB has a better diagnostic yield of malignancy and helps in providing a definitive diagnosis for cases that are suspicious for malignancy on CS. In their case CB increased yield of malignancy by 8.3 % [56]

PLEURAL EFFUSIONS

Santiago Romero et al evaluated different criteria's for separation of transudates and exudates. They showed that the criteria of Light et al is the gold standard for initial categorization of pleural effusions and also offers the highest accuracy for segregating the transudates from exudates [57]

In a study done by Rashmi Kushwaha on 100 pleural fluids showed that the most useful test in establishing the diagnosis of pleural effusion is pleural fluid cytology and pleural fluid cell count. They also concluded that cytologic study of pleural fluid is a complete diagnostic modality, which aims at pointing out the cause of effusion and also a means of prognostication of disease process [58]

Kumar et al reported a case of eosinophilic pleural effusion and concluded that a well-recognized cause of eosinophilic pleural effusion is chest trauma. Other causes include pneumonia, heart failure, asthma, pulmonary infarction, malignant disorder and tuberculosis [59]

Frist B et al in 1979, compared diagnostic values of biopsies of the pleura and cytological evaluation of pleural fluids. They showed that the cell population present in the sediment was representative of a much larger area of pleural surface than that

obtained by needle biopsy. Cytology therefore has a greater opportunity compared to needle biopsy technique to retrieve malignant cells in the presence of scanty malignant deposits on the pleura [60]

Udasimath S et al studied 60 pleural fluid samples with combined conventional smears and cell block method. They reported 15% increased yield for malignancy by cellblock method and also concluded the CB method provided high cellularity, better architectural patterns, morphological features and additional yield of malignant cells hence thereby increasing the sensitivity of cytodiagnosis [61]

In a study done by Koksal D et al. on 40 patients with lung cancer found increased ratio of diagnosis of malignancy by 10%. They could also subtype lung cancer as adenocarcinoma in 35% of cases and concluded that CB combined with CS increased the diagnostic yield in exudative pleural effusions accompanying lung cancer [62]

Renshaw AA studied malignant pleural effusions and found that most of the patients with pulmonary adenocarcinoma had unresectable lesions. Patients with malignant effusions due to breast and ovary had significantly longer survival compared to lung carcinoma. lymph node metastasis or direct local extension in lung carcinoma had worst prognosis. He also concluded that malignant effusion developing in patients more than two years following resection of a pulmonary adenocarcinoma are likely to be secondary. Lymph node and pleural involvement at the time of resection are important risk factors for patients in developing malignant pleural effusions after surgical resections of pulmonary adenocarcinoma [63]

In a study done by Khan N on 58 cases of malignant effusion from different serous cavities, found that lung carcinoma was the most common malignancy in pleural effusions of males and breast carcinoma in females [38]

Johnston WW studied 584 specimens of pleural effusion from 474 patients, and concluded that in both males and females, the order of frequency was lungs, lymphoma/leukemia, breast, female genital tract, and GIT. In 90.5% of patients a cytopathological diagnosis conclusive of malignancy was obtained on the first specimen of fluid [64]

PERITONEAL EFFUSIONS

The peritoneal cavity is normally a fluid free space covered with a layer of mesothelial cells resting on thin basement membrane. In humans, it has a surface area of about 1700 sq cm. because it is extremely sensitive, the peritoneum frequently reacts to a wide array of stimuli with increased permeability and effusion formation with either inflammatory or neoplastic causes. The positive peritoneal effusion is an important prognostic indicator in oncologic patients and is useful in proper identification of the primary tumor [65]

DiBonito et al studied 215 cases with cyto-histologic correlation and found that in males – cancers of GIT, pancreas and liver were most frequent, whereas in females, gynecological sites were most common. Cells from extra abdominal tumors were pleural mesothelioma in males and breast carcinoma in females [66]

In 1987, Bergmann J et al studied elevation of CA-125 in patients with benign and malignant ascites using the biotin–avidin immunoperoxidase technique, CA-125 was detected in peritoneum, pleura, pericardium, the mullerian duct and the amnion.

Concentrations in the ascitic fluid were found to be higher than in the serum and it is suggested that CA125 synthesized by the peritoneum could be considered as a marker of peritoneal inflammation. In 1989, Bollinger DJ et al. studied peritoneal malignant mesothelioma versus serous papillary adenocarcinoma. They found CA 125 as a sensitive marker for serous carcinoma [67]

In 2006, Sudha A et al reported a case of 34-year-old male whose cytological smear of ascitic fluid showed whorls of signet ring cells in a background of abundant mucin. A diagnosis of pseudomyxoma peritonei was followed by peritoneal biopsy, which confirmed a signet ring cell carcinoma. Pseudomyxoma peritonei indicates peritoneal involvement by a mucin – producing neoplasm of GIT or ovaries containing variable cellularity. They opined that cytomorphologic features of epithelial cells in peritoneal washing material can accurately categorize cases of pseudomyxoma peritonei and that this categorization appeared to have important prognostic significance [68]

MATERIAL AND METHODS

The present study titled as "DIAGNOSTIC UTILITY OF THE CELL BLOCK METHOD VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY" was carried out in the Department Of Pathology, Jawaharlal Nehru Medical College KAHER, Belagavi after obtaining permission from Ethics Committee from January 2020 to December 2020.

Study Design : Prospective Study

Time period : 1 year from January 2020 to December 2020

Selection of cases : The study included fresh body fluids (Pleural and Peritoneal) from 100 patients with relevant clinical details of both sexes and all ages.

A proforma was formulated and the data collection of each case was done by filling proforma (Annexure II)

Inclusion criteria:

1. Pleural fluid
2. Peritoneal fluid

Exclusion criteria :

1. All other type of body fluids

History : A detailed history regarding age, sex , general examination, relevant investigations and clinical diagnosis was taken.

Reagent and Equipments:

For preparation of conventional smear: Glass slide of 75x 25x 1.35 mm, coplin jar with 95% ethanol fixative, cover slips, mounting media, test tubes, pipettes, laboratory centrifuge machine

For preparation of cell block: Glass slide of 75x 25x 1.35 mm, cover slips, mounting media, outdated plasma, thromboplastin, test tubes, pipettes, forceps, scalpel, tissue cassettes, filter paper,jars, centrifuge machine, automated tissue processor, paraffin embedding bath, microtome.

Fluids were divided into 2 parts. One part was processed for conventional smear and other part for cell block preparation.

Processing of fluid :

CONVENTIONAL SMEAR PREPARATION

1. Fluid from the container was transferred to centrifuge tube labeled with specimen identifier and centrifuged for 5 min at 2000 rpm
2. Supernatant fluids were discarded and sediments were spread on the slide with the help of glass rod
3. Slides were fixed in 95% methanol and stained with papanicolau stain
4. Dry smears were stained with MGG

CELL BLOCK PREPARATION

Plasma –Thromboplastin method

1. Plasma and thromboplastin reagent were used to prepare cell block

2. Fluid from the container was transferred to centrifuge tube labeled with specimen identifier
3. Spin for 10 min at 2500 rpm
4. Decant the supernatant fluid
5. Mix 2-3 drops of plasma with sediment
6. Add 2-3 drops of thromboplastin reagent and mix
7. Add 10% buffered formalin
8. Let it fix in formalin for 30 minutes
9. The sediment then wrapped in filter paper and processed in histokinette as a routine paraffin section
10. Hematoxylin and eosin staining was done.

TABLE NO 2: criteria for assessment of conventional smear and cell block

Criteria	0	1	2
1. Volume /blood obscuring background	Large (diagnosis greatly compromised)	Moderate (diagnosis possible)	Minimal (diagnosis easy)
2. Architecture	Minimal (diagnosis not possible)	Moderate (some preservation)	Excellent architectural display
3. Cellularity (amount of diagnostic material present)	Minimal (diagnosis not possible)	Moderate (sufficient for diagnosis)	Abundant (diagnosis easy)

Each individual slide was analyzed for background, architecture, cellularity, cytoplasmic and nuclear details using the scoring system in table no. 2

According to the criteria mentioned above, the quality of the slides was determined by qualitatively grouping them into 3 categories

Quality of slide :

1. Diagnostically unsuitable (0)
2. Diagnostically adequate (1-4)
3. Diagnostically superior (4-8)

Diagnostic categories:

1. **Negative for malignancy**
   a) Scant cellularity
   b) Acute inflammatory infiltrate
   c) Lymphocyte rich
   d) Reactive mesothelial cell reaction

2. **Suspicious for malignancy**

3. **Positive for malignancy**

Statistical Methods : Kappa statistics was used for each criteria to determine the agreement between the two methods. In comparing mean values of CS and CB Students Unpaired t Test is used.

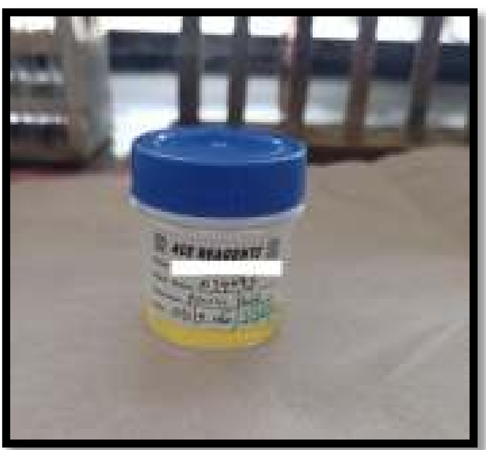


Fig 2. Fluid Sample

Fig 3 .Test tubes, slides, pipette, coplin jar, xylene, mounting media, coverslips

Fig 4. Centrifuge Machine

Fig 5. Slide warmer

Fig 6.   PAP Staining station

Fig 7. MGG STAINS

Fig 8. PAP, MGG and CB Slides

Fig 9. Scalpel, Tissue cassettes , Filter paper

Fig 10. Tissue Processor

Fig 11. Rotary Microtome

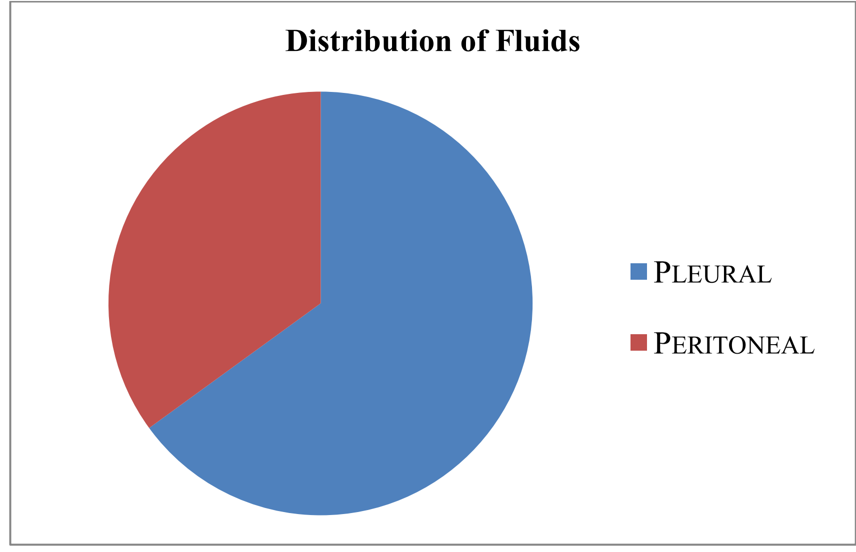
OBSERVATION AND RESULTS

This study was carried out in Department Of Pathology, Jawaharlal Nehru Medical College KAHER, Belagavi from January 2020 to December 2020.

Study material consisted of pleural and peritoneal fluid samples which were processed by conventional smear method and cell block method. Slides were evaluated individually.

A total of 100 fluid samples were evaluated with both the techniques.

FIGURE 12 : Pie chart showing distribution of fluids



As seen in the above figure out of 100 fluids, 65 % were pleural fluids and 35% were peritoneal fluids.

FIGURE 13 : Showing Genderwise distribution of cases

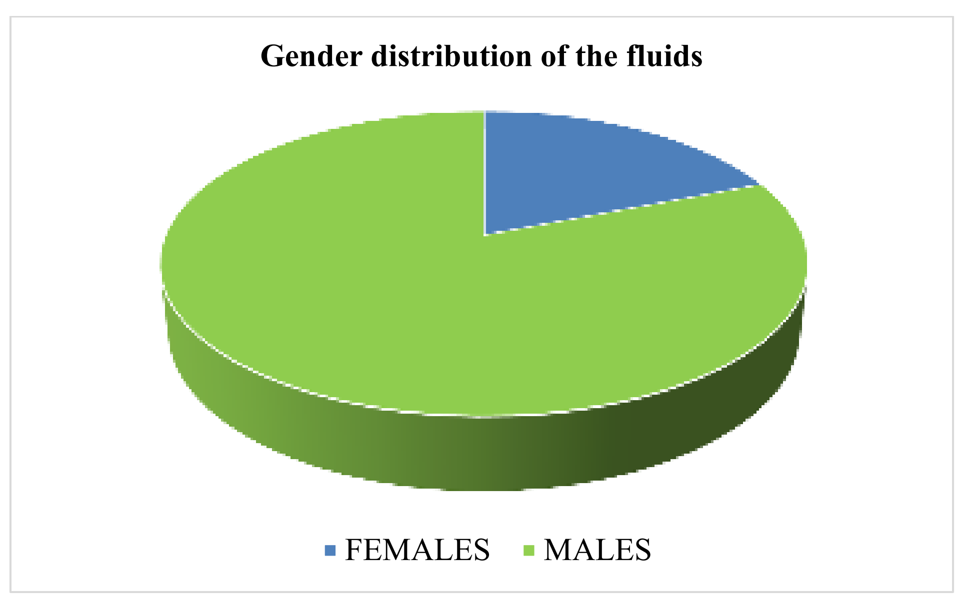


TABLE 3: Distribution of fluids according to Age and Gender

AGE	FEMALE	MALE	TOTAL
20 - 29	2	3	5
30 - 39	0	8	8
40 - 49	0	11	11
50 - 59	2	20	22
60 - 69	7	25	32
70 - 79	6	13	19
80 - 89	2	1	3
TOTAL	19	81	100

TABLE 4 : Mean distribution of Age

	MEAN	S.D.	MINIMUM	MAXIMUM
OVERALL	58.72	13.47881	28	80
FEMALE	64.68	14.59	28	80
MALE	57.32	12.9	28	80

As evident from the figure 2, table 3 and table 4 out of 100 cases 81 % were males and 19% were females. 32 % patients belonged to the age group of 60-69 years while only 3 % belonged to age group 80-89 years. Overall mean age was 58 years.

Conventional smear (CS) and cell block (CB) method were used for scoring the samples based on 3 categories like background, cellular yield and morphology and architecture.

The Kappa test was used for statistical analysis of each category.

In comparing mean values of CS and CB Students Unpaired t Test is used

NS	Not significant
VS	Very significant
HS	Highly significant

TABLE 5: Background obscuration in CS and CB

score	CS1 (category 1)	CB1 (category 1)
0	15%	1%
1	45%	18%
2	40%	81%

KAPPA STATISIC FOR THE ABOVE TABLE IS 0.0512 ($p = 0.2240$, NS)

TABLE 6: Amount of diagnostic material present in CS and CB

Score	CS2 (category 2)	CB2 (category 2)
0	18%	1%
1	51%	17%
2	31%	82%

KAPPA STATISIC FOR THE ABOVE TABLE IS 0.2601 ($p = <0.0001$, HS)

TABLE 7: Retention of cellular architecture in CS and CB

Score	CS3 (category 3)	CB3 (category 3)
0	5%	3%
1	41%	18%
2	54%	79%

KAPPA STATISIC FOR THE ABOVE TABLE IS 0.3332 ($p <0.0001$, HS)

TABLE 8 : Interpretation of Kappa score

Kappa	Agreement
<0	No agreement
0.00-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-1.00	Almost perfect agreement

As observed from the above tables, the first criterion that is background had kappa score 0.0512 indicating slight agreement between two methods CS and CB. The second and third criterion that is cellularity and cellular architecture had kappa score of 0.2612 and 0.3332 indicating difference in agreement between CS and CB

TABLE 9: Mean scores for all criteria's in CS and CB

Criteria	Mean ± SD		P value	Inference
	CS	CB		
Blood obscuring the background	1.28± 0.75	2.02±0.47	<0.0001	HS
Diagnostic material present	1.18±0.78	1.93±0.57	<0.0001	HS
Architecture	1.53±0.66	1.88±0.88	0.0002	HS

The mean score of CB is slightly higher than CS. To determine if there is significant difference between particular criterion between CS and CB Students Unpaired t test was used with the p value result shown in table no 9 which reveals there is significant difference between two methods for all the three criteria's ($p < 0.05$)

TABLE 10 : Interpretation of Quality of slide in CS

	Score in CS		
Category	**1**	**2**	**3**
1	0	1	0
2	1	17	0
3	1	0	40
TOTAL	2	18	40

TABLE 11 : Interpretation of Quality of slide in CB

	Score in CB		
Category	**1**	**2**	**3**
1	0	1	1
2	1	15	40
3	0	0	42
TOTAL	1	16	83

KAPPA STATISIC FOR THE ABOVE TABLES (11 AND 12) IS 0.2346 ($p = 0.0003$, HS)

The quality of the slide was determined on the basis of total score obtained on the 3 criteria's for each method. Slides with score 0, 1-4 and more than 5 were categorized as 1,2 and 3 respectively as diagnostically inadequate, diagnostically adequate and diagnostically superior.

The interpretation of slide quality was done using kappa statistics which yielded value of 0.2346 indicating fair agreement between two methods.

FIGURE 14 : Diagnostic categories on CS

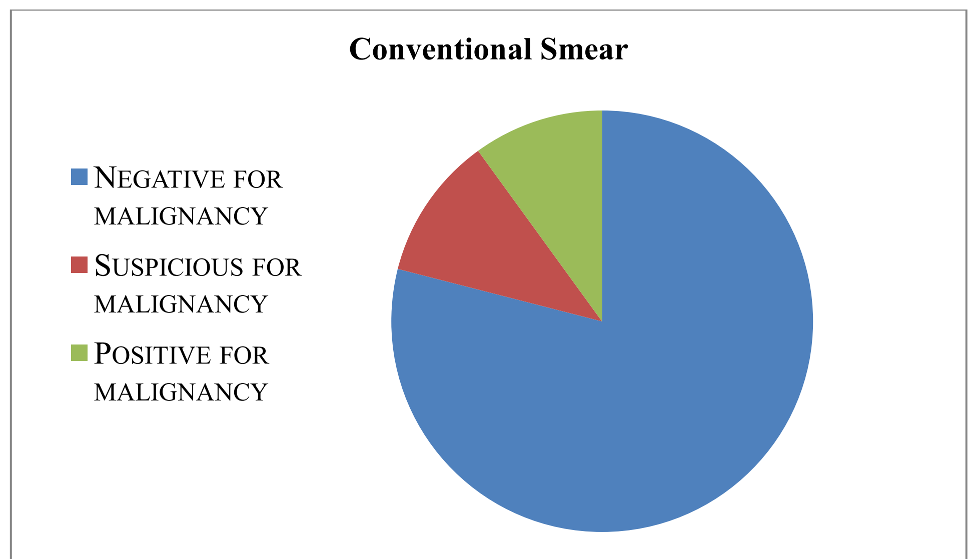


FIGURE 15: Diagnostic categories on CB

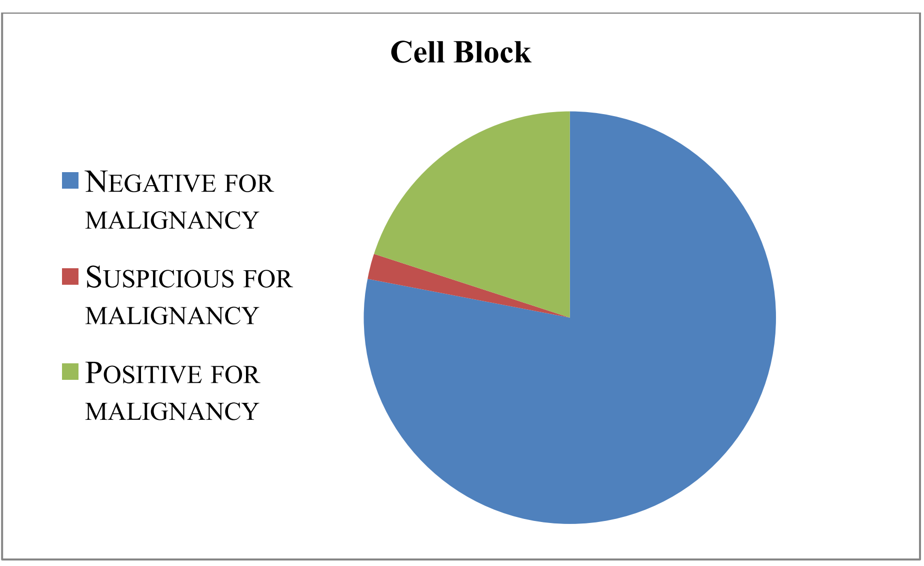


TABLE 12 :Comparison between diagnostic category in CS and CB

DIAGNOSTIC CATEGORY	CS	CB
NEGATIVE FOR MALIGNANCY	79	78
SUSPICIOUS FOR MALIGNANCY	11	2
POSITIVE FOR MALIGNANCY	10	20

Out of 100 samples 10 cases were diagnosed as positive for malignancy on CS while 20 cases were positive on CB. 10 suspicious for malignancy cases were confirmed as positive for malignancy on CB hence CB yielded additional 10% malignant cases compared to CS.

TABLE 13 : Descriptive statistics for total score obtained in CS and CB

Descriptive statistics	Negative for malignancy		Suspicious for malignancy		Positive for malignancy	
	CS	CB	CS	CB	CS	CB
Mean ± SD	2.46±0.99	2.26±1.45	4±1.74	3±0.68	4.09±0.8	6.12±1.98
Median	3	3	3	3	4	3
Range	(0-4)	(0-5)	(2-4)	(3-7)	(3-5)	(4-8)

Above table shows the descriptive statistics for total score for CS and CB according to diagnosis. In Positive for malignancy group, CS had mean score of 4.09±0.8 while CB had mean score of 6.12±1.98. The difference between the two scores was statistically significant with p value< 0.0001. In suspicious group mean score by CS was 4±1.74 while for CB it was 3±0.68, The difference between the two scores was statistically significant with p value< 0.0006. For negative for malignancy, the difference between mean scores was statistically insignificant with p value of 0.14

FIGURE 16 : Clinical diagnosis of Negative for malignancy in Pleural fluids

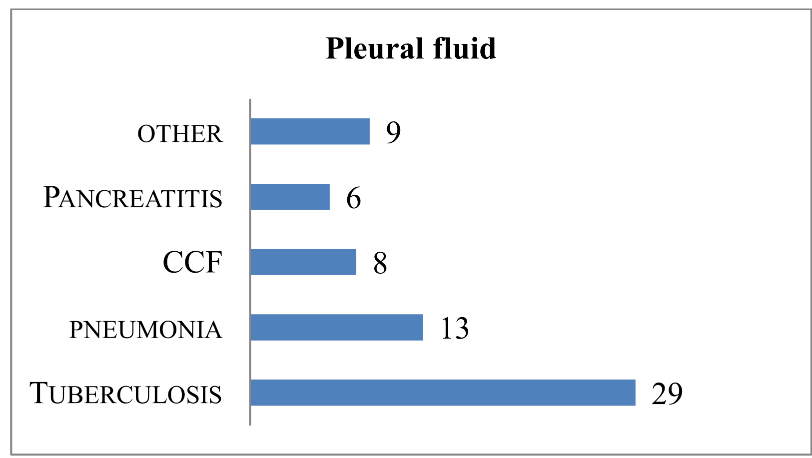
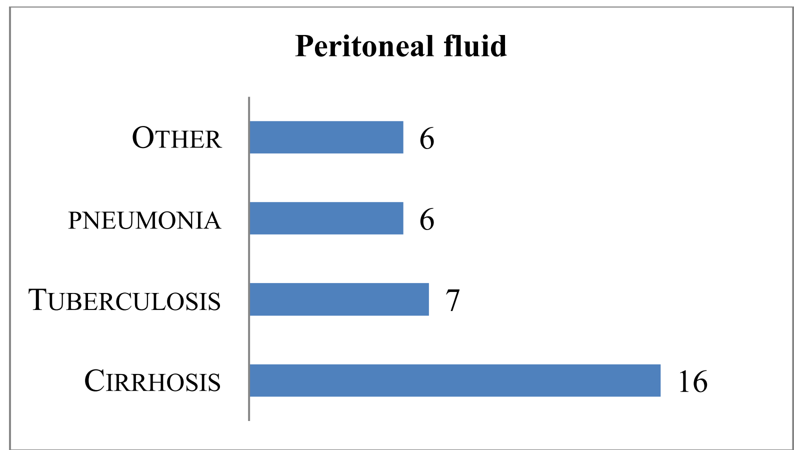


FIGURE 17 : Clinical diagnosis of Negative for malignancy in Peritoneal fluids

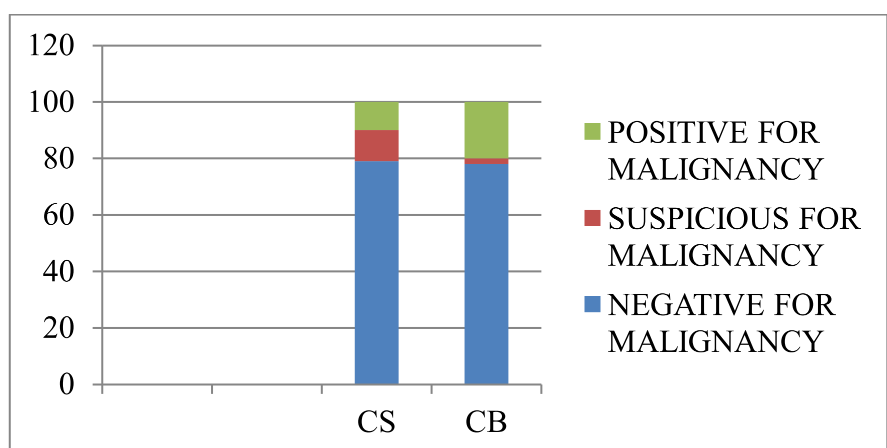


In the category negative for malignancy out of 79 cases maximum cases were diagnosed as tuberculosis (36) followed by pneumonia (20), cirrhosis (16), congestive cardiac failure (8) and pancreatitis (6)

In pleural fluid samples (65) maximum cases were diagnosed as tuberculosis (29) followed by pneumonia (13), congestive cardiac failure (8) and pancreatitis (6)

In peritoneal fluid samples (35) maximum cases were diagnosed as cirrhosis (16) followed by tuberculosis (7) and pneumonia (6)

FIGURE 18 : Distribution of diagnosis in CS and CB



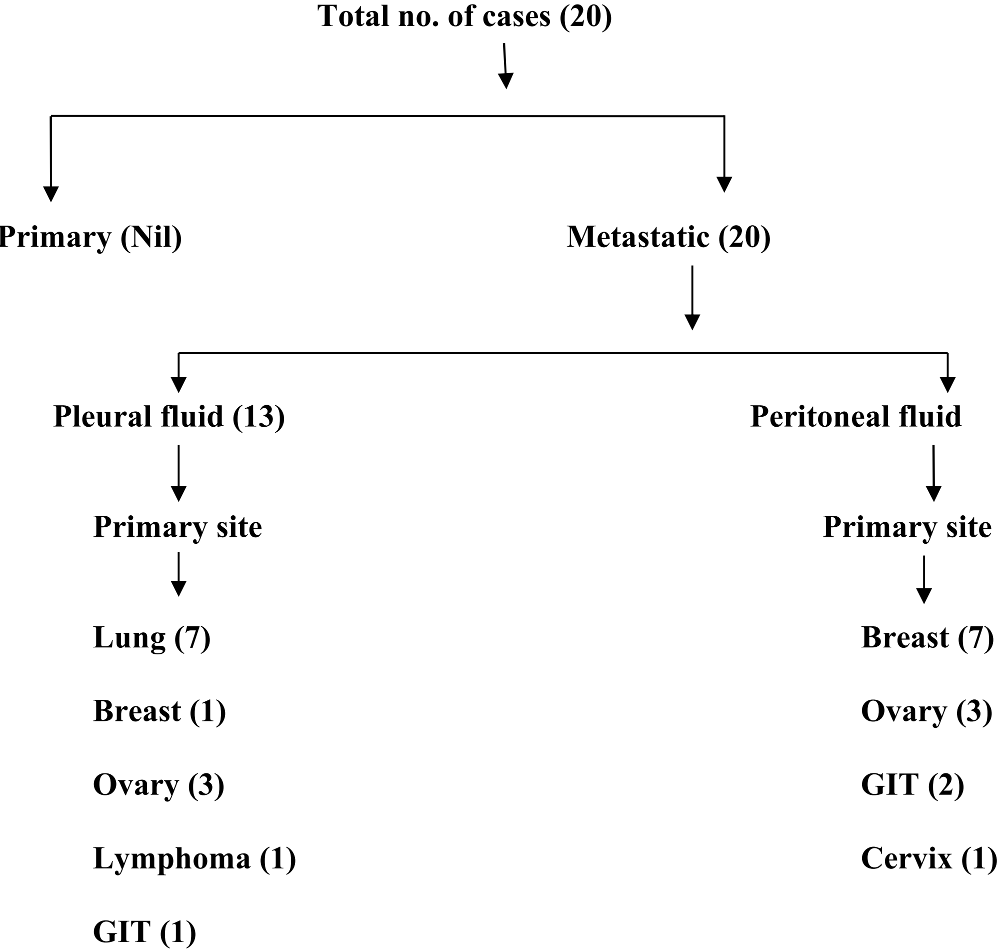
Out of 100 cases total 20 were diagnosed as positive for malignancy , 13 pleural fluid and 7 peritoneal fluid cases.

TABLE 14: Primary sites in metastatic effusion in male and female

Primary site	Male	Female	No. of cases	%
Lung	6	1	7	35
Ovary	0	6	6	30
Breast	0	2	2	10
Cervix	0	1	1	5
GIT	2	1	3	15
Lymphoma	0	1	1	5
Total	8	12	20	100

Above table shows the gender distribution of metastatic effusions. Out of 12 females, maximum (6) were ovarian malignancies. Out of 8 males, maximum (6) were lung carcinoma.

FLOWCHART SHOWING MALIGNANT CASES DISTRIBUTION



The above flowchart shows primary site in malignant effusions, most common site was lung in pleural fluids while it was ovary in peritoneal fluids.

DISCUSSION

Diagnostic cytology is the art of interpretation of cells from the human body that exfoliate or are removed from their physiologic milieu. The cytologic study of fluids represents the cell population from a much larger surface area than that obtained by needle biopsy [60.] Cytology has a higher probablity than needle biopsy to retrieve malignant cells in the presence of malignant deposits.

Cytological examination of serous effusions is of significant importance in the diagnostics and has therapeutic as well as prognostic implications. Reactive mesothelial cells, plenty of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional centrifuged smears[28].

In the present study due consideration was given to age, sex, site of effusion, quality of slides in both conventional smear and cell block method. The final diagnosis was correlated with radiological and clinical findings. Cell blocks provided additional or complimentary information to that obtained from conventional smears.

In our study cell block was prepared by plasma thromboplastin method. For this method outdated plasma from hematology laboratory was used. This method was cost effective, reproducible and it was possible to obtain more cellular material from the solid cell button. Cell block gave better cellularity and architectural preservation. Kulkarni MB [69] et al used similar method for cell block preparation. Thapar [40] et al, Udasimath [61] et al used 10% formalin fixative method while Sujathan [28] et al used sediment method with ethanol acetic acid and formalin as fixative. Both these methods gave better cellularity and architecture compared to CS, the only

disadvantage of these methods was it could be only applied to exudative fluids with significant amount of sediment.

In India, Pleural effusion is more common than peritoneal effusion affecting significant bulk of population. In the present study total 100 fluid samples were analyzed by both conventional smear and cell block method. Out of which 65 were pleural and 35 were peritoneal fluids. In a study done by Sujathan [28] et al out of 86 fluid samples 32 were pleural and 54 were peritoneal effusions while in a study done by Bansode S [70] et al 80 % were pleural fluids and 20 % were peritoneal fluids while in a study done by Matreja SS[73] et al pleural fluids were 58.2 % and peritoneal fluids were 41.8 %. Such difference was because of random selection of cases.

TABLE 15: Distribution of Age and Gender in various studies

STUDY	MEAN AGE	MALE	FEMALE	TOTAL
Goswami[80] et al	46	45	23	68
Kushwaha R[58] et al	62	56	44	100
Aswini G[71] et al	57	64	50	114
Present Study	58	81	19	100

The age ranged from 20 years to more than 80 years of age. Maximum cases belonged to 5th decade in both female and male patients. Mean age was 58 years. Regarding gender distribution , maximum (81 %) were male patients and 19% were female with a ratio of almost 4:1 favoring male population. As depicted in Table 15 male preponderance was also seen in studies done by Goswami[80] et al, Kushwaha R[58] et al and Aswini G[71] et al.

In the present study each slide was assessed on the basis of Miar's point scoring system which was also followed by Sumitha P [82]et al, Matreja S[73] et al and Bansode[70] et al. each slide was observed and scored on the basis of background (category 1), cellularity (category 2) and architecture (category 3). The p value for each criteria was calculated.Cytoplasmic and nuclear details were also studied.

TABLE 16 : Comparison of p values for each category in various studies

STUDY	p value for category 1	p value for category 2	p value for category 3
Sumitha P[82] et al	0.001	0.001	<0.001
Matreja S[73] et al	< 0.005	< 0.005	< 0.005
Bansode[70] et al	0.026	0.0010	0.0098
Present Study	<0.001	<0.001	0.0002

The p value for each criteria was found to be statistically significant favoring cell block. Similar results were seen in a study done by Sumitha P [82]et al, Matreja S[73] et al and Bansode[70] et al.

Kappa statistics was used for each criteria to determine the agreement between the two methods [72] it indicated slight agreement between the two methods for background criterion while for cellularity and cellular architecture there was difference in agreement for two methods. Similar findings were found in study done by Bista P[54] et al and Bansode[70] et al

The quality of the slide was determined on the basis of total score obtained on the 3 criteria's for each method. Slides with score 0, 1-4 and more than 5 were categorized as 1,2 and 3 respectively as diagnostically inadequate, diagnostically adequate and diagnostically superior.

TABLE NO 17: Assessment of quality of slides in various studies

Study	diagnostically inadequate		diagnostically adequate		diagnostically superior	
	CS	CB	CS	CB	CS	CB
Matreja S[73]	0%	0%	40%	44%	53%	56%
Thapar[40] et al	26%	0%	20%	21%	54%	67%
Bista P[54]	21%	8%	35%	28%	43%	68%
Present Study	2%	1%	58%	16%	40%	83%

As depicted in above table 40 % cytological smears were diagnostically superior while 58% were diagnostically adequate. On the other hand, 83% CBs studied were diagnostically superior and 16% were diagnostically adequate. 2 % cases were found to be diagnostically unsuitable on CS and 1 % on CB study. Our findings were similar to Matreja S[73] et al . Study done by Thapar[40] et al and Bista P[54] et al showed slightly higher percentage in diagnostically inadequate criteria.

TABLE 18 : Clinical diagnosis in negative for malignancy category in various studies

Study	Most common	2nd most common	3rd most common
Thapar[40] et al	Tuberculosis	CHF	Liver cirrhosis
Matreja S[73] et al	Tuberculosis	Trauma	Pneumonia
Kushwaha R[58] et al	Tuberculosis	Pneumonia	Empyema
Bista P[54] et al	Tuberculosis	Pneumonia	CHF
Present Study	Tuberculosis	Liver cirrhosis	Pneumonia

In negative for malignancy category ,the most common diagnosis was Tuberculosis (36) followed by liver cirrhosis (16) , pneumonia (13), congestive cardiac failure (8) and pancreatitis (6). Tuberculosis was the most common cause in various studies as depicted in table no. 18. Scant cellularity was seen in cases of congestive cardiac failure. Most of the effusions in negative for malignancy criteria were transudative having low cellular contents which was later correlated with cell block.

Among transudates majority of the cases did not yield material on cell block. Meenu Thapar[40] et al and Udasimath S [61] suggested that CBs helped in non-neoplastic effusions with identical morphology as in CS.

Clinical , radiological, microbiological and biochemical investigations has helped in the diagnosis of non neoplastic cases. Cases of pneumonia were having pleural effusions associated with acute febrile illness, cough and chest radiographs showed pulmonary infiltrates. High level of serum amylase was found in pancreatitis

cases. Cases with lymphocyte rich exudates were having exudative effusions with ADA level more than 50 IU and also mycobacterium tuberculosis positivity was confirmed on culture.

Diagnostic categories were divided into negative for malignancy, suspicious for malignancy and positive for malignancy. P value for each diagnostic category was calculated and it was found to be statistically significant for suspicious of malignancy and positive for malignancy criteria while it was statistically insignificant for negative for malignancy category.

TABLE 19: COMPARISON OF CYTODIAGNOSIS IN PRESENT STUDY WITH OTHER STUDIES

Sr. No	Study and Year	No of cases	Negative for malignancy		Suspicious		Positive for malignancy	
			CS	CB	CS	CB	CS	CB
1.	Khan[38] et al (2010)	75	23 (31%)	14(19%)	10(13%)	7(9%)	42(56%)	54(72%)
2.	Bansode[70] et al(2015)	142	102 (72%)	106(74%)	19(13%)	11(8%)	21(15%)	25(18%)
3.	Thapar[40] et al (2016)	190	142 (74%)	138(72%)	10 (8%)	6(5%)	38(54%)	46(66%)
4.	Theerada[74] et al (2017)	150	118 (79%)	111(74%)	3(2%)	0 (0%)	29(19%)	39(26%)
5.	Matreja[73] et al (2019)	150	140 (91%)	139(90%)	11 (7%)	2 (3%)	9(69%)	12(92%)
6.	Gude[71] et al(2020)	114	90 (63%)	86(75%)	18(15%)	0 (0%)	6 (5%)	38 (32%)
7.	Present Study	100	79(79%)	78(78%)	11(11%)	2(2%)	10(10%)	20(20%)

In the present study maximum cases were in negative for malignancy category with 79% on CS and 78% on CB. Similar findings were seen in a study done by Theerada[74] et al, Gude[71] et al and Matreja[73] et al.

In CS distinguishing between reactive mesothelial cells and malignant mesothelial cells is a diagnostic problem due to overlapping of microscopic features, these cases were confirmed as negative for malignancy on cell block. Shivkumarswami U[79] et al stated that the difficulty is either secondary to marked atypia of mesothelial cells caused by the, chemical, physical, immunological, microbiological or metabolic insults to the serous membranes or to the subtle cytomorphological features of some malignant neoplasms, particularly in cases of well-differentiated adenocarcinomas. The problem may become compounded by artefacts from poor fixation, preparation, or staining techniques.

One case of negative for malignancy on CS was diagnosed as positive for malignancy on CB. On further Histopathological examination the case was diagnosed as adenocarcinoma of lung. CB has a advantage that it concentrates more amount of cellular material, forms a cell button and increases cellular material also architecture is well maintained on CB compared to CS.

In suspicious category the number of cases was higher on CS (11%) compared to CB (2%). Similar findings were noted by Bansode [70]et al, Thapar [40] et al, Matreja[73] et al and Gude[71] et al as shown in the table 19. Out of 11 cases(CS) malignancy was confirmed in 9 cases on CB. Acinar architecture was seen in cases diagnosed as adenocarcinoma.

Out of 100 samples 10 cases were diagnosed as positive for malignancy on CS while 20 cases were positive on CB. Similar findings were also seen in a study done by Bansode [70]et al and Matreja [73] et al. Thapar [40] et al , Theerada[74] et al and Gude [71]et al also had additional yield of malignancy on cell block. The identification of primary site was not possible on CS due to loss of architectural pattern and inability to evaluate immunoreactivity. CB on the other hand revealed histologic aspect of primary neoplasms with architectural patterns like papillae, acini , rosettes etc. it helped to identify possible type of primary site. The commonest tumours were of adenocarcinoma type from lung, ovary and breast. Papillae were seen in cases of papillary serous cystadenocarcinoma of ovary. In the cases where there was dilemma clinicoradiological correlation helped to identify the primary site of malignancy.

In terms of cytomorphology, the cytoplasmic and nuclear details were more clear on CS compared to CB.

TABLE 20 : Pleural fluid analysis for primary lesions in various studies

Sr. no	Study	Lung %	Breast %	Ovary %	GIT %	Others %	Unknown %
1.	Sears and Hajdu[75] et al	19	24	5	4	29	19
2.	Lopez[76] et al	21	16	4	3	7	49
3.	Johnstonet[64] al	36	15	8	6	16	19
4.	Khan[38] et al	69	12	0	0	0	19
5.	Bansode[70] et al	61	28	0	0	11	0
6.	Present Study	56	7	23	7	7	0

In the present study among the positive for malignancy cases diagnosed on cell block in the analysis of pleural fluids, most common primary sites was lung (56%) , followed by ovary (23%) breast (7%) and GIT (7%). Similar results were observed by Khan[38] et al , Bansode[70] et al and Lopez[76] et al .It was possible to know primary site in all cases with clinical, radiological, biochemical and histopathological correlation.

In a study done by Khan[38] et al carcinoma of the lung was the most common site followed by carcinoma of ovary and carcinoma of GIT. Similarly study done by Murphy[77] et al showed that the most common primary malignant lesions were in lung followed by breast and ovary. Takagi[14] et al noted similar results. Spieler[78] et al described that common primary lesions identified in serous effusions were breast followed by ovary, lung and GIT.

TABLE 21 : Peritoneal fluid analysis for primary lesions in various studies

Sr. no	Study	Ovary %	GIT %	Lung %	Breast %	Others %	Unknown %
1.	Sears and Hajdu[75] et al	32	11	4	15	23	15
2.	Lopez et[76] al	32	32	0	9	13	14
3.	Khan[38] et al	28	9	0	0	30	33
4.	Bansode [70]et al	61	23	0	0	15	0
5.	Present Study	42	28	0	14	14	0

In present study for peritoneal fluid, the most common primary site was ovary (42%) followed by GIT (28%) ,breast(14%) and cervix (14%). Similar results were observed by Khan[38] et al , Bansode[70] et al and Sears and Hajdu[75] et al. Lopez[76] et al observed equal number of cases (32%) from ovary and GIT.

TABLE 22 : Additional yield of malignancy in various studies by cell block

Sr. No	Study	(%)
1	Dekkar and Bupp et al[41]	38
2	Khan et al[38]	20
3	Shivakumarswami et al[79]	15
4	Bodele et al[53]	7
5	Richardson et al[81]	5
6	Bansode et al[70]	8
7	Present Study	10

In our study diagnostic yield of malignancy was significantly increased by CB method. Present study identified 10% (10 cases) malignant lesions by CB compared to CS.

Various other studies revealed additional diagnostic yeild with CB. In a study done by Bodele et al[53], additional 7% (10 cases) was identified by cell block[53]. Dekkar et al[41] reported that the diagnostic yield in samples obtained by combined CB and CS method was double compared to CS and CB technique alone. By using CB method they demonstrated tumor in 38% cases which were reported as negative or atypical on cytology.

In a study done by Khan et al[38] additional diagnostic yield was 16 % for malignant cases. Takagi F [14] identified additional 18 cases positive for malignancy. In other study done by Khan et al titled as usefulness of cell block versus smears in malignant effusion cases reported that recovery rate for malignant lesions by cell block was 20 % more compared to conventional smear alone.

According to various studies additional diagnostic yield for malignancy was noted if conventional smear technique is supplemented by cell block method. In present study we diagnosed malignant lesions in 20 % cases by CB method while only 10 % by CS method.

In present study identification of primary site was possible in 100 % of the cases. In a study done by Khan et al[38] primary site could be identified in 81 % of cases while Thaper et al [40] identified the same in 83 % of cases. Clinico-radiological and Histopathological correlation helped in confirmation of primary site.

SUMMARY

- This was an observational study comparing the efficacy of Centrifuged smear vs Cell block in pleural and peritoneal fluids received in the section of cytology, in department of Pathology, Jawaharlal Nehru Medical College, Belagavi
- In the present study 100 fluid samples were evaluated by both conventional smear and cell block method.
- Fluids were divided into 2 parts. One part was processed for conventional smear and other part for cell block preparation by plasma thromboplastin method
- Out of 100 samples 65 % were pleural fluids and 35% were peritoneal fluids
- Male preponderance was noted with male to female ratio of 4:1
- Maximum patients belonged to the age group of 60-69 years.
- CS and CB smears were scored on the basis of background, cellular yield, cellular morphology
- p value was calculated for each category and it was found to be significant for cellular yield and cellular architecture favoring cell block
- Cytoplasmic and nuclear features were well made out on conventional smear
- Kappa statistics was used for each category and quality of slide which showed slight agreement between two methods

- For diagnosis three categories were used negative for malignancy, suspicious for malignancy and positive for malignancy
- p value was calculated for each category and it was found to be significant for suspicious and positive for malignancy category while it was insignificant for negative for malignancy.
- In the category negative for malignancy out of 79 cases maximum cases were diagnosed as Tuberculosis followed by Pneumonia, Cirrhosis, Congestive cardiac failure and Pancreatitis
- In pleural fluid samples (65) maximum cases were diagnosed as Tuberculosis (29) followed by pneumonia (13), congestive cardiac failure (8) and Pancreatitis (6)
- In peritoneal fluid samples (35) maximum cases were diagnosed as cirrhosis (16) followed by tuberculosis (7) and pneumonia (6)
- One case of negative for malignancy on CS was diagnosed as positive for malignancy on CB. On further Histopathological examination the case was diagnosed as adenocarcinoma of lung.
- In suspicious for malignancy criteria in CS (11 cases) malignancy was confirmed in 9 cases. 2 cases were suspicious on CB too.
- Out of 100 samples 10 cases were diagnosed as positive for malignancy on CS while 20 cases were positive on CB.

- 10 suspicious for malignancy cases were confirmed as positive for malignancy on CB .
- Out of 20 positive for malignancy cases, 13 were pleural fluids and 7 peritoneal fluid cases.
- In metastatic effusions, Out of 12 females, maximum (6) were ovarian malignancies. Out of 8 males , maximum (6) were lung carcinomas.
- The identification of primary site was not possible on CS due to loss of architectural pattern and inability to evaluate immunoreactivity.
- It was possible to know primary site in all cases with clinical, radiological, biochemical and histopathological correlation.
- In pleural fluids, most common primary sites was lung (56%) , followed by ovary (23%) breast (7%) and GIT (7%).
- In present study for peritoneal fluid, the most common primary site was ovary (42%) followed by GIT (28%) ,breast(14%) and cervix (14%).
- CB yielded additional 10% malignant cases compared to CS.

CONCLUSION

The conclusions derived from the present study are:

- Cell block technique is easy, simple, reproducible and reliable method for cytodiagnosis
- Cell block preparation is cost effective and uses routine laboratory reagents and processing
- The advantages of cell block are concentration of all cellular material, increased cellular yield and preservation of architectural pattern
- The use of cell block technique almost eliminates the suspicious for malignancy category and gives more definitive diagnosis
- Cell blocks are useful in establishing the primary site of malignancy
- In cell block technique multiple sections can be obtained from same material for ancillary techniques like special stains and immunohistochemistry

BIBILIOGRAPHY

1. Kumar, Vinay, Abul K. Abbas, Nelson Fausto, Stanley L. Robbins, and Ramzi S. Cotran. Robbins and Cotran Pathologic Basis of Disease (8^{th}ed). Philadelphia: Elsevier Saunders, 2005.
2. Pleural, peritoneal and pericardial effusions - a biochemical approach. Kopcinovic LM, Culej J. *Biochem Med (Zagreb)* 2014;24:123–37
3. Koss LG et al: Effusions in the absence of cancer. In Diagnostic Cytology and its Histopathologic Bases, 5th edition. Edited by Koss LG, Melamed MR, Vol 2, Philadelphia, J. B. Lippincott, 2006, p 919-48.
4. Bernard Naylor: Pleural, peritoneal and pericardial effusions. In Comprehensive Cytopathology, 3rd edition. Edited by Marluce Bibbo, Wilbur DC: Saunders Elsevier, 2008, p515 -78.
5. Bhanvadia VM, Santwani PM, Vachhani JH. Analysis of diagnostic value of cytological smear method versus cell-block method in body fluid cytology: Study of 150 cases. *Ethiop J Health Sci.* 2014;24:125–31.
6. Hippocrates, (Potter P, trans.). Hippocrates Volume VI. Cambridge (MA): Harvard University Press, 1988: 39-43.
7. Desimonas N, Tsiamis C, Sgantzos M. The Innovated "Closed Chest Drainage System" of William Smoult Playfair (1871). Surgical Innovation. 2019;26(6):760-62.
8. Gogakos A, Barbetakis N, Lazaridis G, et al. Heimlich valve and pneumothorax. *Ann Transl Med*. 2015;3(4):54. doi:10.3978/j.issn.2305-5839.
9. Shidham VB and Falzon M: Serous effusions. In Diagnostic Cytopathology. Edited by Winifred Gray, Churchill Livingstone, Elsevier, 3rd edition, 2010:115-74.

10. Hjerpe, A, Abd Own, S, Dobra, K. Integrative approach to cytologic and molecular diagnosis of malignant pleural mesothelioma. Transl Lung Cancer Res. 2020;9(3):934-43.
11. Nathan NA, Narayan E, Mary M et al. Cell block cytology: Improved preparation and its efficacy in diagnostic cytology. Am J Clin Pathol 2000; 114: 599- 606.
12. Zemansky AP, Jr. Examination of fluids for tumor cells: Analysis of 113 cases checked against subsequent examination of tissue. Am J M Sc 1928; 175: 489-504.
13. Rieder MJ, Taylor SL, Clark AG, Nickerson DA. Sequence variation in the human angiotensin converting enzyme. Nat Genet. 1999 May;22(1):59-62
14. Takagi F. Studies on tumor cells in serous effusion. Am J Clin Pathology 1954; 24:663-75.
15. Foot NC. Identification of types and primary sites of metastatic tumors from exfoliated cells in serous fluids. Am J Pathol 1954; 30: 661-77.
16. Foot NC. The identification of neoplastic cells in serous effusions. Am J Pathol 1956; 32: 961-77.
17. Velios F, Griffin J. Examination of body fluids for tumor cells. Am J Clin Pathol 1954; 24: 676-81.
18. Murphy WM, Alan BP. Determination of primary site by examination of cancer cells in body fluids. Am J Clin Pathol 1972; 18: 479-88.
19. Luse SA, Reagen JW. A histological study of effusions. 1. Effusions not associated with malignant tumors. Cancer 1954; 7: 1155-66.
20. Tao LC: The cytopathology of mesothelioma. Acta Cytol 23:209-13.

21. Kimura N, Dota K, Araya Y, Ishidate T, Ishizaka M. Scoring system for differential diagnosis of malignant mesothelioma and reactive mesothelial cells on cytology specimens. Diagn Cytopathol. 2009 Dec;37(12) 885-90.
22. Effusion cytology, Dr. Alpha Tsui, Royal Melbourne hospital 2008, 137.189.150.85
23. Sehgal S, Agarwal R, Goyal P, Singh S, Kumar V, Gupta R. Primary serous carcinoma of peritoneum: A case report. International Journal of Case Reports and Images 2012;3(10):16–20.
24. Riego AG, Cuinas C, Vilanova JJ, Ibarrola, R. Extramedullary hematopoietic effusions. Acta Cytol 1998; 42: 1116-20.
25. Hallman JR, Geisinger KR. Cytology of fluids from pleural, peritoneal and pericardial cavities in children. A comprehensive survey. Acta Cytol 1994; 38:209-17.
26. Wong JW, Pitlik D, Abdul-Karim FW. Cytology of pleural, peritoneal and pericardial fluids in children. A 40 years summary. Acta Cytol 1997; 467-73.
27. Foot NC, Identification of mesothelial cells in sediments of serous effusions. Cancer 1959; 12: 429-437.
28. Sujathan K, Kannan S, Pillai KR, et al. Significance of AgNor count in differentiating malignant cells from reactive mesothelium cells in serous effusions. Acta Cytol 1996; 40: 724-28.
29. Singh G, Whiteside TL, Dekker A. Immunodiagnosis of mesotheslioma. Use of antimesothelial cell serum in an indirect immunofluoresence assay. Cancer 1979; 43:2288-96.
30. Booth SN, Lakin G, Dykes PW, Burnett D and Bradwell AR. Cancer associated proteins in effusion fluids. J. Clin Path 1977; 30: 537-40.

31. Churg A. Immunohistochemical staining for vimentin and keratin in malignant mesothelioma. Am J Surg Pathol 1985; 9: 360-65.
32. Mezger J, Stotzer O, Schilli G, Bauer S, Wilmanns W . Identification of carcinoma cells in Ascitic and pleural fluid. Comparison of four panepithelial antigens with carcinoembryonic antigen. Acta Cytol 1992; 36(1): 75-81.
33. Mayall F, Heryet A, Manga D, et al. p53 immunostaining is a highly specific and moderately sensitive marker of malignancy in serous fluid cytology. Cytopathology 1997;8: 9-12.
34. Bahrenburg LPH. On the diagnostic results of the microscopical examination of the ascitic fluid in two cases of carcinoma involving the peritoneum. Cleveland Med Gaz 1986; 11:274-78.
35. Zito FA, Gadaleta CD, Salvatore C, Filatico R, Labriola A, Marzullo A et al. A modified cellblock technique for fine needle aspiration cytology. Acta Cytol 1995;39: 93-99.
36. Wojcik EM, Selvaggi SM. Comparison of smears and cell blocks in the fine needle aspiration diagnosis of recurrent gynecologic malignancies. Acta Cytol 1991;35(6): 773-76.
37. Chapman CB and Whalen EJ. The examination of serous fluids by cell block technique. New Engl J Med 1947; 237(7): 215-20.
38. Khan N, Sherwani KR, Afroz N, Kapoor S. Usefulness of cellblocks versus smears in malignant effusion cases. Journal of Cytology 2006;23(3): 129-32.
39. Leung SW, Bedard YC. Methods in Pathology. Simple mini block technique for cytology. Mod pathol 1993; 6(5): 630-32.
40. Thapar M. Mishra RK. Sharma A et al. Critical analysis of cell block versus smear examination in effusions, J Cytol 2009 Apr; 26(2):60-4.

41. Dekker A, Bupp PA. Cytology of serous effusion: An investigation into the usefulness of cell blocks versus smears. Am J Clin Pathology 1978; 70: 855-60.
42. Bernard Naylor: Pleural, peritoneal and pericardial effusions. In Comprehensive Cytopathology, 3rd edition. Edited by Marluce Bibbo, Wilbur DC: Saunders Elsevier, 2008, p 51-78.
43. Jonasson JG, Ducatman BS, Wang HH. The cell block for body cavity fluids: do the results justify the cost? Mod Pathol 1990,3(6):667-70.
44. Leung SW, Bedard YC. Methods in Pathology. Simple mini block technique for cytology. Mod pathol 1993; 6(5): 630-32
45. Bibbo M. pleural,peritoneal and pericardial effusions. in comprehensive cytopathology edn 3rd Philadelphia: Saunders Elsevier, 1997;1 515-78.
46. Bellotti MS, Elsner B, Pisilli L. Use of the microwave oven for cell block preparation. Acta Cytol. 1997 ;41(2):610-11.
47. Yang GC, Wan LS, Papellas J, Waisman J. Compact cell blocks. Use for body fluids, fine needle aspirations and endometrial brush biopsies. Acta Cytol 1998; 39: 93-99
48. Nathan NA, Narayan E. Smith MM, Horn MJ cell block cytology : improved predation and its efficacy in diagnostic cytology Am. J Clin pathol 2000; 114; 599- 606.
49. Kersten HM, Robben JC , Poddighe PJ,Melchers WJ. Agar Cyto : A novel processing method for multiple molecular diagnostic analysis of the uterine cervix. J Histochem Cytochem 200: 48; 709-18

50. Varsegi GM, Shidham V. Cell block preparation from cytology specimen with predominance of individually scattered cells. Journal of Visualized Experiments 2009;29: e1316, 1-7
51. Kung IT, Yuen RW, Chan JK. Technical notes. Optimal formalin fixation and processing schedule of cell blocks from the fine needle aspirates. Pathology 1989; 21:143-45.
52. Sujathan K, Pillai KR, Chandralekha B, Kannan S, Mathew A, Nair MK. Cytodiagnosis of serous effusions: A combined approach to morphological features in Papanicolaou and May-Grunwald Giemsa stained smears and modified cell block technique. Journal of Cytology 2000; 17(2): 89-95.
53. Bodele AK, Parate SN, Wadadekar AA, Bobhate SK, Munshi MM. Diagnostic utility of cell block preparation in reporting of fluid cytology. Journal of Cytology 2003;20(3):133-35.
54. Bista P. Camparison of the diagnostic accuracy of cell block with cytology smear in serous effusions. Journal of pathology of Nepal 2013; 3: 482-86.
55. Price BA, Ehya H, Lee JH. Significance of pericellular lacunae in cellblocks of effusions. Acta Cytol 1992; 36(3): 333-7.
56. Kumar SH, S S, Shetty D, Rao R. Clinicopathological Study of 117 Body Fluids: Comparison of Conventional Smear and Cell Block Technique. *Curr Health Sci J*. 2020;46(4):336-43.
57. Romero S, Candela A, Martin C. et al. Evaluation of different criteria for the separation of pleural transudate from exudates. Chest 1993;104(2): 399-404
58. Kushwaha R, Shashikala P, Hiremath S, et al. Cells in pleural fluid and their value in differential diagnosis. Journal of Cytology 2008; 25(4): 138-43

59. Nanda kumar U, Varkey B, Mathai G. Post traumatic pleural fluid and blood eosinophilia. JAMA 1975: 234(6): 625-26.
60. Frist B, Khan AV and Koss KG. Comparison of the diagnostic values of biopsies of the pleura and cytologic evaluation of pleural fluids. Am J Clin Pathol 1979; 72:48-51.
61. Udasimath S, Arakeri SU, Karigowdar MH, et al. Diagnostic utility of the cell block method versus the conventional smear study in pleural fluid cytology. Journal of cytology 2012;29(1): 11-15.
62. Koksal H, Demirag F, Bayiz H, et al. The cellblock method increases the diagnostic yield in exudative pleural effusions accompanying lung cancer. Turk Patoloji Derg 2013; 29: 165-70
63. Renshaw AA, Madge R, Sugarbaker DJ, et al. Malignant Pleural effusions after resection of pulmonary adenocarcinoma. Acta Cytol 1998; 42(5): 1111-15.
64. Johnston WW. The malignant pleural effusion. A review of cytopathologic diagnosis of 584 specimens from 472 consecutive patients. Cancer 1985; 56:905- 09
65. Van de Molengraft FJJM, Vooifs GP: The interval between the diagnosis of malignancy and the development of effusion with reference to the role of cytologic diagnosis. Acta Cytol 1988; 32: 183-87
66. Bonito LD, Falconieri G, Colautti I, Bonifacio D, Dudine S. The positive peritoneal effusion. A retrospective study of cytopathologic diagnosis with autopsy confirmation Acta Cytol 1993; 379(4): 483-88.

67. Bollinger DJ, Wick MR, Dehner LP, et al. Peritoneal malignant mesothelioma versus serous papillary adenocarcinoma. Am J Surg Pathol 1989; 13(8): 659-70.
68. Sudha A, Fatima A. Pseudomyxoma Peritonei- signet ring cell type diagnosed by ascitic fluid cytology. Journal of Cytology 205; 22(3): 137-39.
69. Manisha B Kulkarni,Sangeeta B Desai,Dulhan Ajit,R.F.Chinoy.Utility of the thromboplastin-plasma cell block technique for fine needle aspiration and serous effusions.Diagnostic Cytopathology 2009 Feb;37(2):86-90.
70. Bansode, Shubhada & Nayak, S. & Kumbhalkar, D.. (2013). Evaluation of Cell Block Technique in the Cytodiagnosis of Body Fluids. ISSN 2319-7064
71. Gude, Dr & Mala, Dr & Kamidi, Dr. (2020). Cell Block as a Tool of Diagnostic Refinement in Cytological Examination of Pleural and Peritoneal Effusions. Saudi Journal of Pathology and Microbiology. 5. 431-36
72. Viera AJ et al.(2005) Understanding Interobserver Agreement: The Kappa Statistic. Family Medicine 37(5): 360-63
73. Matreja SS, Malukani K, Nandedkar SS, Varma AV,Saxena A, Ajmera A. Comparison of efficacy of cell block versus conventional smear study in exudative fluids. Niger Postgrad Med J 2017;24:245-9.
74. Assawasaksakul T, Boonsarngsuk V, Incharoen P. A comparative study of conventional cytology and cell block method in the diagnosis of pleural effusion. J Thorac Dis 2017;9(9):3161 -67
75. Sears D, Hajdu SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. Acta Cytol 1987; 31(2): 85-97
76. Lopez and Cardoz PL. A critical evaluation of 3000 cytologic analysis of pleural fluid and peritoneal fluid. Acta Cytol 1966;10: 455-60

77. Murphy WM, Alan BP. Determination of primary site by examination of cancer cells in body fluids. Am J Clin Pathol 1972;18:479-88
78. Spieler P, Gloor F. Identification of types and primary sites of malignant tumours by examination of exfoliated tumor cells in serous fluids. Comparison with diagnostic accuracy on small histologic biopsies
79. Shivkumarswami U. Surekha U.Arakeril, Mahesh H. Karigowdar, B.R. Yelikar: The role of cell block method in the diagnosis of malignant ascitic fluid effusion Journal of clinical and diagnostic research, 2012; September, vol-6(7): 1280-83
80. Falguni J Goswami, H P Srilakshmi, Roopam K Gidwani Diagnostic Utility of Cell Block Preparation with Conventional Cytological Smears. A Cross Sectional Study Pacific group of eJournals , 2019; January, vol 1 : 19-23
81. Richardson HL, Koss LG, Simon TR. An evaluation of the concomitant use of cytological and histological techniques in the recognition of cancer in exfoliated material from various sources. Cancer 1995;8: 948-50
82. Sumitha MP, Thejasvi K, Niveditha SR. Role of cell block technique by fixed sediment method in fluid cytology. *Ann Clin Cytol Pathol.* 2017;3(1):1051-52.

ANNEXURE-I-ETHICAL CLEARANCE LETTER

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 258 **Date: 24/12/2019**

To,

PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"DIAGNOSTIC UTILITY OF CELL BLOCK METHOD VS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE II – CONSENT FORM

INFORMED CONSENT

DIAGNOSTIC UTILITY OF CELL BLOCK METHOD VERSUS CONVENTIONAL SMEAR STUDY IN PLEURAL AND PERITONEAL FLUID CYTOLOGY

The principal investigator of the study is Dr. ______________ under the guidance of Dr. _________ (guide).

Purpose of the study: The purpose of this study is to compare cell block method with conventional smear study. This study will help in determining a better diagnostic tool for serous effusions. You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo Thoracocentesis or paracentesis you will be included in this study.

Procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge . If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know a better method for diagnosis of body fluid cytology which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and

withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. If you have any queries about your rights as a study subject, you may call Dr. Roopa Bellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

Name of the participant:

(signature/thumbprint)

Name of the witness :

(signature/thumbprint)

Name of the investigator:

(signature)

Date :

ANNEXURE III - PROFORMA

Case No: **Cytology No:**

Name: **Age:** **Sex:** **Ward:** **Department:**

Clinical Diagnosis:

Presenting Complaints:

Breathlessness/ Fever / Pain In Abdomen/ Jaundice / Cough /

Vomiting / Weight Loss/ Malaena / Haematemesis/

Past History:

TB/ HTN/ DM/ Surgery / Accident / Malignancy/ Autoimmune Disease

Personal History:

Sleep/ Appetite / Bladder/ Bowel Habits

Smoking/ Alcohol / Tobacco

General Physical Examination :

Built / Pulse Rate / Pallor / Icterus Clubbing / Lymphadenopathy/ Edema

Systemic Examination:

CVS RS P/A CNS

Investigation:

Details of Body Fluid:

Nature of fluid Colour

Volume Appearance

1. **Conventional Smear :**

   Total count Cell Type

   PAP, MGG , H & E

2. **Cell Block :**

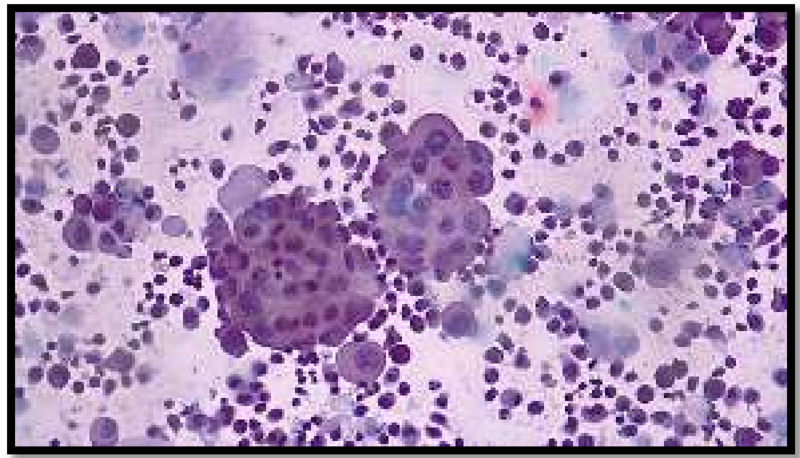
   H & E

   Special Stain if any

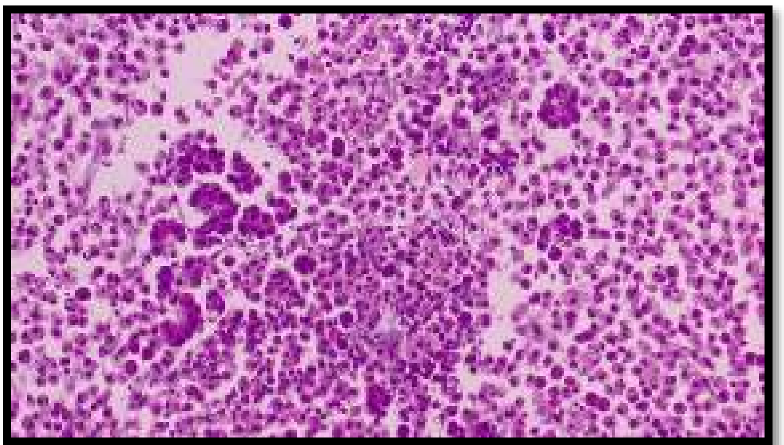
	CS	**CB**
BACKGROUND (volume of blood obscuring background)		
ARCHITECTURE		
CELLULARITY (amount of diagnostic material present)		
NUCLEAR FEATURES	**size** **pleomorphism** **mitotic activity**	**size** **pleomorphism** **mitotic acivity**
CYTOPLASMIC FEATURES		
DIAGNOSIS		

ANNEXURE IV-PHOTOMICROGRAPHS

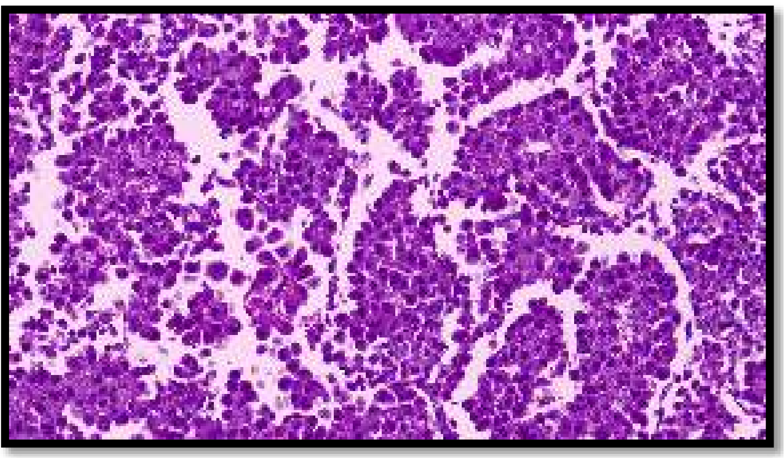
1. Case of Alcoholic Liver Disease



A-Mesothelial cell cluster on CS (PAP stain, 40X)

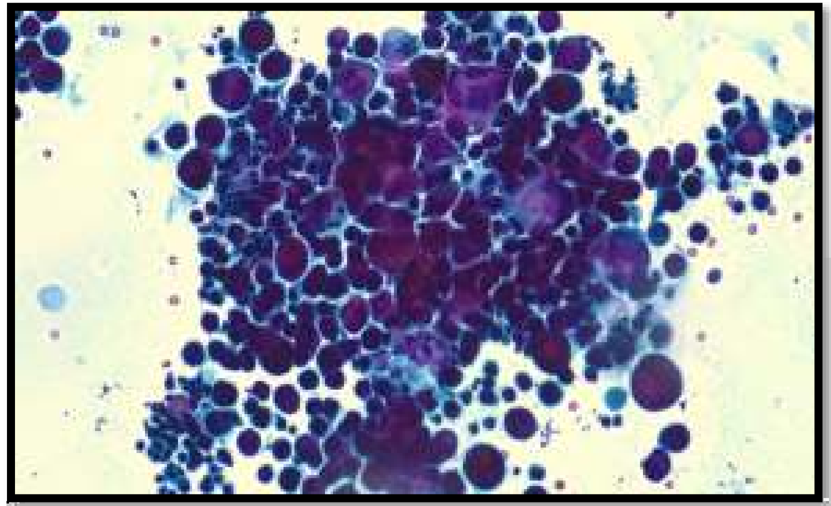


B- Mesothelial cell cluster on CB (H &E stain,20X)

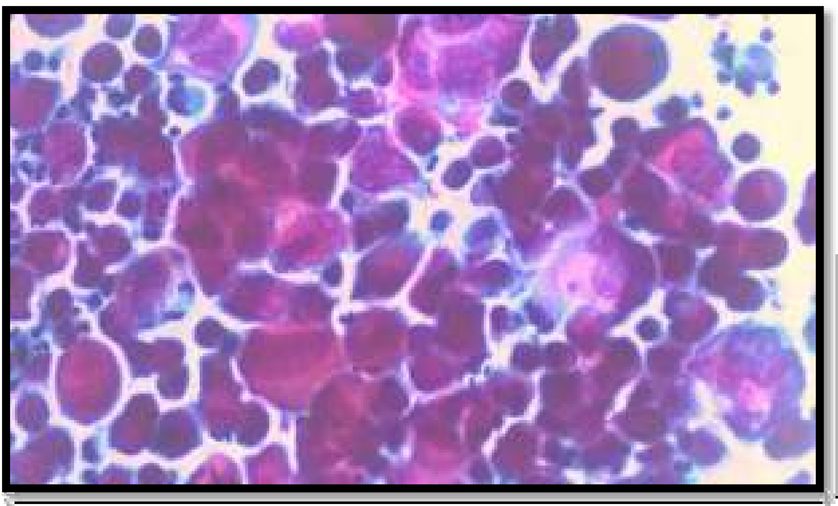


C-Mesothelial cell cluster on CB (H &E stain, 40X)

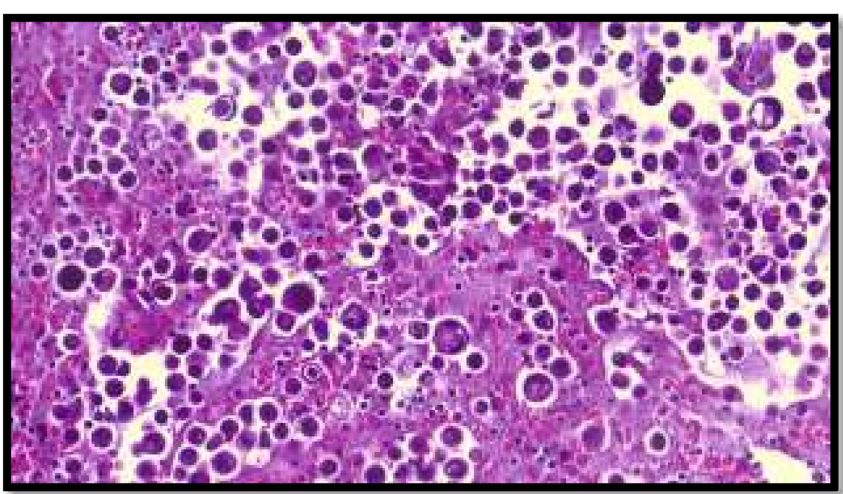
2. Case of Metastatic Pleural effusion



D- Malignant cells on CS (PAP stain,20X)

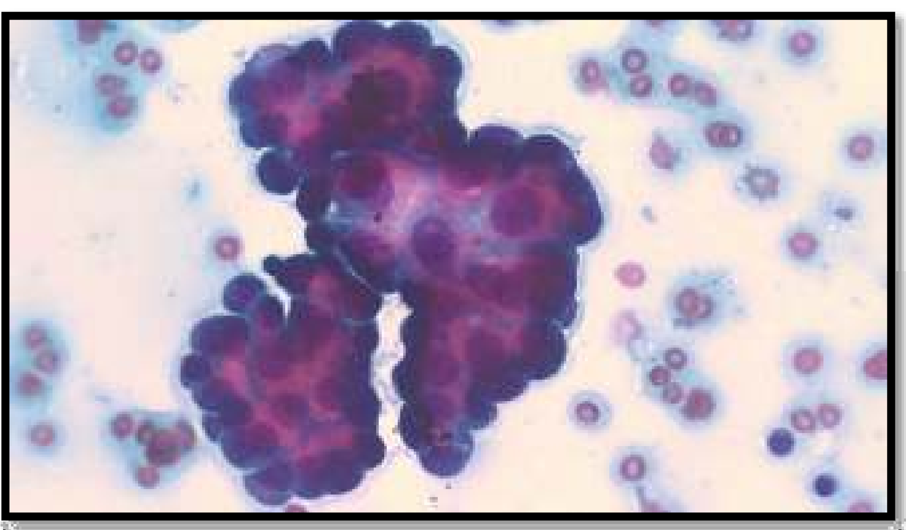


E- Malignant cells on CS (PAP stain,40X)

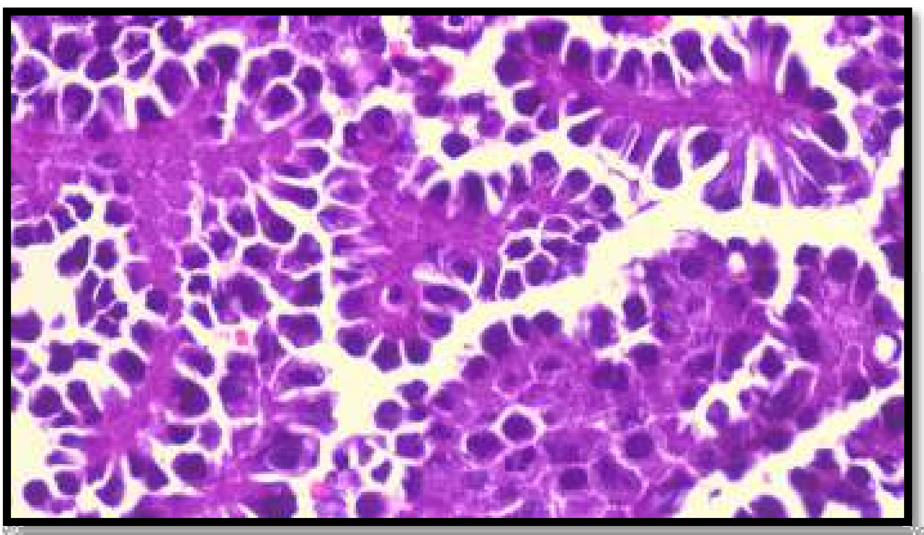


F-Malignant cells on CB (H &E stain, 40X)

3. Case of Papillary serous cystadenocarcinoma of ovary

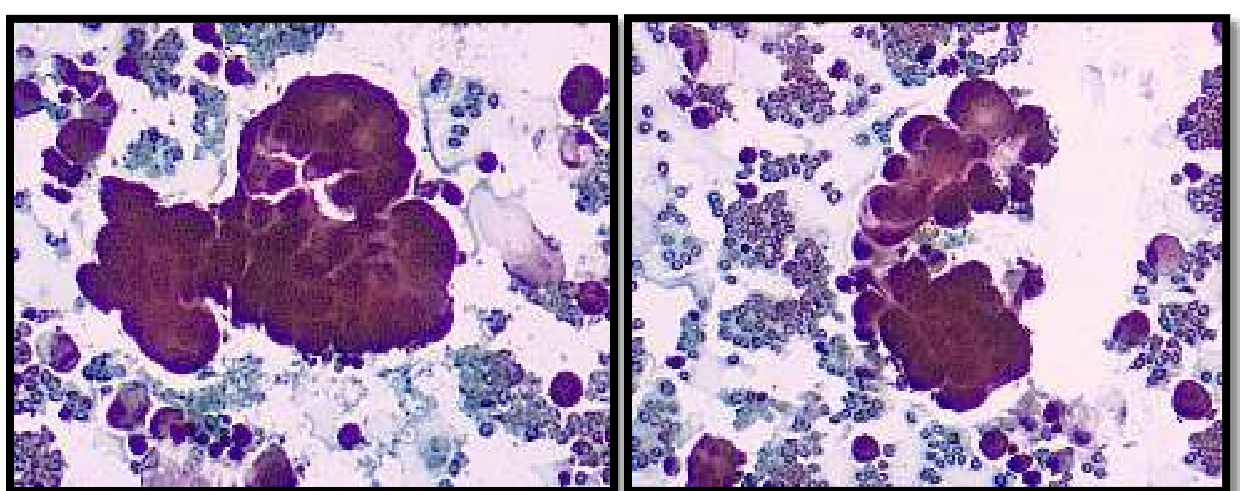


G- Malignant serous papillae on CS (PAP stain, 40 X)

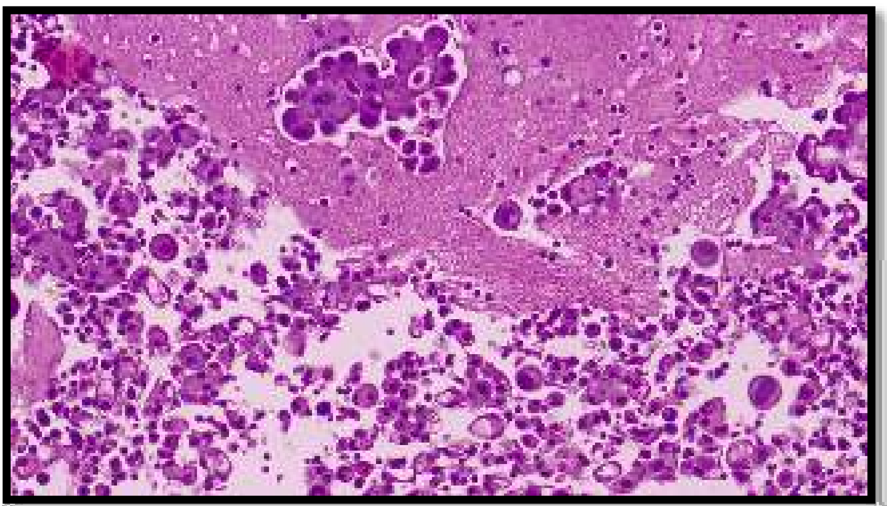


H-Malignant serous papillae on CB (H &E stain, 40X)

4. Case of Adenocarcinoma of Lung



I- Malignant cells on CS (PAP stain, 40X)



J-Malignant cells on CB (H &E stain, 40 X)

ANNEXURE V- KEY TO MASTER CHART

GENDER : M – Male F- Female

TYPE OF FLUID : A – PLEURAL FLUID B- PERITONEAL FLUID

QUANTITATIVE SCORE FOR CONVENTIONAL SMEAR (CS) AND CELL BLOCK(CB)

Criteria for assessment of smear and cell block

Criteria	0	1	2
1. Volume /blood obscuring background	large (diagnosis greatly compromised)	Moderate (diagnosis possible)	minimal (diagnosis easy)
2. Architecture	minimal (diagnosis not possible)	moderate (some preservation)	excellent architectural display
3. Cellularity (amount of diagnostic material present)	minimal (diagnosis not possible)	moderate (sufficient for diagnosis)	adundant (diagnosis easy)

Diagnostic categories:

1. **Negative for malignancy**
    a) Scant cellularity
    b) Acute inflammatory infiltrate
    c) Lymphocyte rich
    d) Reactive mesothelial cell reaction

2. **Suspicious for malignancy**

3. **Positive for malignancy**

Quality of slide

1: diagnostically unsuitable

2: diagnostically adequate

3: diagnostically suitable

Final Diagnosis:

1: Tuberculosis

2: Carcinoma Lung

3: mucionous cystadenocarcinoma ovary

4: serous cystadenocarcinoma ovary

5: alcoholic liver disease

6: chronic liver disease

7: Pancreatitis

8: Cholangiocarcinoma

9: Hydropneumothorax

10: Inflammatory Bowel disease

11: Pneumonia

12: carcinoma breast

13: Periampullary carcinoma

14: congestive cardiac failure

15: adenocarcinoma stomach

16: carcinoma cervix

17: adenocarcinoma pancreas

18: Non alcoholic fatty liver disease

19: Lymphoma

PRIMARY SITE OF MALIGNANCY:

1: lung

2: ovary

3: Breast

4: Cervix

5: GIT

6: lymphoreticular malignancy

ANNEXURE VI -MASTER CHART

SR. NO	CYTO NO	AGE	SEX	TYPE	QUANTITATIVE SCORE						QUALITY OF SLIDE		DIAGNOSIS		CLINICAL DIAGNOSIS	PRIMARY SITE
					CONVENTIONAL SMEAR			CELL BLOCK								
					CS1	CS2	CS3	CB1	CB2	CB3	CS	CB	CS	CB		
1	48/20	28	M	A	2	1	2	2	2	2	3	3	1c	1c	11	
2	152/21	65	M	A	2	1	1	2	2	2	2	3	1b,d	1b,d	10	
3	18/20	48	M	A	0	0	1	2	1	1	2	2	1b	1b	9	
4	584/20	52	M	A	2	0	2	2	1	2	2	3	1b,d	1b,d	17	
5	117/20	63	M	A	2	1	1	2	1	1	2	2	1c	1c	11	
6	132/20	35	M	A	0	0	1	2	1	2	2	3	1b	1b	9	
7	639/20	59	M	A	0	1	2	2	2	2	2	3	2	3	2	1
8	39/20	38	M	B	1	1	1	2	2	2	2	3	1c	1c	18	
9	41/20	28	F	A	2	0	0	2	2	0	2	2	1a	1a	11	
10	61/20	80	F	A	1	1	2	2	2	2	2	3	3	3	4	1
11	58/20	61	M	B	2	0	2	2	2	2	2	3	1b,d	1b,d	5	
12	88/20	68	M	A	2	1	2	2	2	2	3	3	3	3	2	1
13	270/20	64	M	B	2	1	2	2	2	2	3	3	1c,d	1c,d	6	
14	173/20	72	M	B	1	0	1	2	1	1	2	2	1c	1c	5	
15	559/20	64	F	B	1	0	2	2	2	2	2	3	2	3	3	2
16	563/20	58	M	A	1	1	1	2	2	2	2	3	1b	1b	5	
17	571/20	67	M	A	1	1	1	2	2	2	2	3	1c,d	2	17	

18	135/20	62	M	B	1	1	1	2	2	2	2	3	1b	1b	2	
19	565/20	58	M	B	0	1	0	2	2	2	2	3	1c	1c	5	
20	273/20	70	M	B	1	1	1	2	1	1	2	2	1c	1c	5	
21	148/20	29	M	A	2	1	2	2	2	2	3	3	1c	1c	11	
22	631/20	72	M	A	2	1	2	2	2	2	3	3	3	3	2	1
23	101/20	78	F	A	0	1	2	2	2	2	2	3	3	3	4	2
24	128/20	65	M	B	1	1	1	2	2	2	2	3	1c	1c	5	
25	421/20	68	M	A	1	1	2	2	2	2	2	3	1c,d	1c,d	1	
26	671/20	74	M	A	1	1	2	2	2	2	2	3	3	3	2	1
27	235/20	58	M	B	0	1	1	2	2	2	2	3	1b	1b	2	
28	193/21	60	F	A	0	0	1	2	1	1	2	2	1b	1b	12	
29	49/20	59	M	A	0	0	1	2	1	2	2	2	1b	1b	7	
30	565/20	67	M	B	2	1	1	2	2	1	2	2	1d	1d	5	
31	136/21	71	M	B	2	1	1	2	2	2	2	3	1c,d	1c,d	6	
32	84/20	53	M	A	0	0	1	2	2	2	1	3	1b	1b	9	
33	632/21	76	M	A	2	2	2	2	2	2	3	3	3	3	15	5
34	297/21	68	F	A	0	1	1	1	1	2	2	2	3	3	4	2
35	175/20	48	M	A	1	2	2	2	2	2	3	3	1b	1b	1	
36	139/21	40	M	B	0	0	1	2	2	1	2	3	1c	1c	5	
37	163/21	62	M	A	1	1	1	2	2	2	2	3	2	3	13	5
38	176/21	58	M	A	1	2	2	2	2	2	3	3	1b	1b	5	
39	270/21	63	M	A	1	2	2	2	2	2	3	3	1c,d	1c,d	6	
40	38/20	42	M	B	0	0	0	2	1	0	1	2	1a	1a	5	
41	477/21	78	F	A	0	0	2	2	2	2	2	3	2	3	4	2
42	59/21	80	M	A	1	1	2	2	2	2	2	3	1c,d	1c, d	14	
43	13/21	56	M	B	1	1	1	2	2	2	2	3	1c	1c	5	

44	37/21	70	F	B	1	1	1	2	2	2	2	3	1c	1c	10	
45	95/21	60	M	A	1	1	2	2	2	1	2	3	1b	1b	5	
46	129/21	56	M	A	1	1	2	2	1	1	2	2	1c	1c	11	
47	134/21	34	M	A	2	0	2	2	2	2	2	3	1b	1b	9	
48	239/21	28	F	B	0	0	2	2	2	1	2	3	1b	1b	11	
49	177/21	35	M	A	1	1	1	2	2	2	2	3	1b	1b	1	
50	191/21	67	F	B	1	0	1	2	2	1	2	3	2	3	3	2
51	556/21	68	M	B	2	2	2	2	2	2	3	3	1d	1d	2	
52	40/21	47	M	B	2	2	0	0	0	0	2	0	1c	1a	7	
53	23/21	71	M	A	1	1	1	2	1	2	2	3	1c	1c	1	
54	81/21	46	M	A	2	1	2	2	2	2	3	3	1c,d	1c,d	2	
55	186/21	68	F	A	1	1	2	2	2	2	2	3	1c,d	1c,d	2	
56	62/21	80	F	A	2	1	2	2	2	2	3	3	2	3	12	3
57	75/21	59	M	B	1	1	2	2	2	2	2	3	1c	1c	5	
58	49/21	28	M	A	0	0	1	1	2	1	2	2	1a	1a	11	
59	41/21	37	M	B	1	1	2	1	1	1	2	2	1c,d	1c,d	18	
60	56/21	52	M	A	1	1	2	2	2	2	2	3	1c	1c	6	
61	62/21	38	M	B	1	1	0	2	2	1	2	3	1b	1b	5	
62	425/20	42	M	B	2	2	1	2	2	2	3	3	1b	1b	5	
63	123/20	59	F	A	1	0	1	1	1	1	2	2	1a	1a	7	
64	53/20	62	M	B	1	2	2	2	2	2	3	3	1c	1c	1	
65	236/20	78	M	A	1	1	1	1	2	2	2	3	3	3	13	5
66	198/20	56	F	A	1	1	1	2	1	2	2	3	1c,d	1c,d	6	
67	48/21	48	M	A	2	1	1	2	2	2	2	3	1b	1b	11	
68	45/20	59	M	B	1	1	2	2	2	2	2	3	1c	1c	6	
69	252/20	64	M	A	1	2	2	2	2	2	3	3	1b	1b	5	

70	479/21	72	M	A	2	2	1	2	2	3	3	3	1c,d	1c,d	1	
71	177/21	72	F	A	1	1	1	3	3	3	2	3	2	3	12	3
72	419/21	78	M	A	1	1	1	2	3	3	2	3	1a	1c	2	
73	59/21	68	M	A	1	2	2	2	2	2	3	3	2	1b,d	2	
74	558/21	70	F	B	2	2	2	3	3	3	3	3	3	3	16	4
75	178/21	48	M	A	2	2	2	3	3	3	3	3	1 c,d	1c,d	1	
76	129/21	50	M	B	2	1	2	2	2	2	3	3	1b	1b	5	
77	776/20	58	M	A	2	2	2	2	2	2	3	3	1b	1b	1	
78	270/20	62	M	B	3	3	2	3	3	3	3	3	1b,d	1b,d	5	
79	487/20	59	M	A	3	2	3	3	3	3	3	3	1b	1b	7	
80	631/20	72	M	A	2	2	2	3	3	3	3	3	2	3	2	1
81	421/20	69	F	B	2	1	1	2	2	2	3	3	2	3	19	6
82	163/21	37	M	A	2	2	2	3	3	2	3	3	1b,d	1b,d	9	
83	42/21	68	M	A	1	2	2	2	2	2	3	3	1a	1a	1	
84	36/21	70	F	B	3	3	3	3	3	3	3	3	1c	1c	10	
85	44/20	52	M	A	2	2	2	2	2	2	3	3	1b,d	1b,d	5	
86	94/21	60	M	B	2	3	3	3	3	3	3	3	1b,d	1b,d	5	
87	203/20	47	M	A	2	2	2	3	3	3	3	3	1 c,d	1 c,d	6	
88	48/21	52	M	A	2	2	2	2	2	2	3	3	1b,d	1b,d	1	
89	55/20	66	M	A	1	1	1	1	2	1	2	2	1a	1a	1	
90	64/20	62	M	A	1	1	1	1	1	1	2	2	1c,d	1c	2	
91	223/20	59	M	A	1	2	2	2	2	2	3	3	1b	1b	5	
92	157/20	39	M	B	2	3	3	3	3	3	3	3	1c	1c	14	
93	99/21	67	M	B	2	3	2	2	2	2	3	3	1b,d	1b,d	5	
94	411/20	63	M	A	1	2	2	2	2	2	3	3	1c	1c	6	
95	88/20	42	M	A	2	1	1	2	2	2	3	3	1b,d	1b,d	1	

96	75/21	77	M	A	2	2	2	2	2	2	3	3	3	3	2	1
97	335/20	54	M	B	1	1	1	2	1	1	3	3	1c	1c	7	
98	323/20	64	F	A	1	2	1	2	2	2	3	3	2	3	4	2
99	33/21	66	M	A	2	1	1	2	2	2	3	3	1b	1b	10	
100	75/21	72	M	A	1	2	2	2	2	2	3	3	1a	1a	18	