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Diagnostic Significance of Cytomorphological Analysis of Body Fluids

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I. INTRODUCTION

The study of cells within the fluids of serous cavities ,mainly the pleural,peritoneal and pericardial is known as effusion cytology.Cytomorphologiclal analysis of these cells helps to understand the pathophysiological process of the disease and helps in reaching a final diagnosis.(1,2)

Effusion cytology is the first line of investigation in a suspected case of malignancy.The cell population in the sediment of an effusion sample presents a much higher diagnostic yield as compared to needle biopsies as they are representative of a much larger surface area.(3-5)

The pathologist often reports such samples as Positive/negative for malignancy or atypical cells/clusters noted.Cytological report of atypical cells/clusters warrants further ancillary investigations such as cell count,biochemical,microbiological evaluation,cell block,histopathological evaluation and Immuno histochemistry (IHC)study for final confirmation of diagnosis.The study was carried out to know the diagnostic significance of cytomorphological analysis in body fluids.

II. MATERIAL AND METHODS

A retrospective hospital based study was carried out on 2267 samples submitted in the Department of Pathology at a Tertiary care hospital in North India from September 2018 to September 2019.All cases of neoplastic and non neoplastic diseases with effusion of pleural,peritoneal and pericardial cavity along with CSF were included which comprised 419 samples.Urine cytology ,Pap smears,Brush cytology,nipple discharge,BAL & peritoneal wash samples were excluded.Positive report was included in the study once in case of multiple samples from the same site of one patient.Cytospin was used.Smears were made from the sediments.In case of haemorrhagic fluids,glacial acetic acid was used as the hemolysing agent and then the sample was processed routinely. Two smears were available from each effusion sample,one of which was air dried and stained by Giemsa while the other was wet fixed and stained by standard Papanicolaou's method.Relevant clinical data and radiological investigations were obtained for each case.Gross analysis was done,cell counts were noted on improved Neubaur chamber,predominant cell type was evaluated and cytomorphologcal analysis of the body fluid was done.In cases were more than 10ml of fluid was sent,cell blocks were made,the sediment was fixed in formalin and processed like a routine histopathology specimen.Markers like CA-125,CK-7,CK-20,TTF etc were used for IHC studies whenever required.

III. RESULTS

Our study comprised of 155 pleural,143 peritoneal and 01 pericardial effusion samples.The study also included 120 CSF samples.The age of the patient ranged from 2 to 91 years(mean age 46.5years).Male preponderance was observed in our study with a M:F ratio of 1.22:1.Maximum number of cases were seen in the age group of 41-60years(42%).(Table 1)

Table 1: Age wise distribution of samples

AGE(In years)	NUMBER OF CASES(in %)
0-20	1.43
21-40	17.89
41-60	42
61-80	34.12
81-100	4.53

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Out of the 419 cases,300(71.59%)effusion samples were reported as negative for malignancy,38(9.06%) as atypical cell clusters noted and 81(19.33%) as positive for malignancy(Table 2).Microscopic examination revealing single scattered bizarre cells or occasional clusters of atypical cells showing increased nucleocytoplasmic ratio,irregular cell contours and occasional prominent nucleoli were classified under the category of atypical cell clusters.These samples were subjected to further analysis by other ancillary techniques and relevant clinical data.

Table 2:Cytological diagnosis in effusion sample

EFFUSION SITE	POSITIVE FOR MALIGNANCY	ATYPICAL CELL CLUSTER	NEGATIVE FOR MALIGNANCY
PLEURAL(n=155)	32	21	102
PERITONEAL(n=143)	47	17	79
PERICARDIAL(n=01)	01	00	00
CSF(n=120)	01	00	119
TOTAL(419)	81	38	300

The most common cause for malignant pleural and peritoneal effusion was attributed to adenocarcinomas in our study.The definitive diagnosis of a classifiable malignancy included 76.54% Adenocarcinoma cases,20.98% Squamous cell carcinoma and 2.46% uncommon malignancies.On further analysis of 32 malignant pleural effusions,the most common primary site was found to be lung(62.5%) followed by breast(18.75%),gastrointestinal tract (9.37%),ovary(6.25%) and bladder(3.12%). The most frequent site for adenocarcinomas presenting with peritoneal effusion was ovary(70.21%) followed by Gastrointestinal tract(25.53%) and kidney(4.25%)(Table 3).

CSF analysis of 120 samples revealed blast cells(0.83%) in a known case of Acute lymphoblastic leukemia.01 case of poorly differentiated adenocarcinoma was found in a single pericardial effusion sample in a case of carcinoma breast.

Table 3: Distribution of Adenocarcinomas in effusions correlating with primary malignancy.

EFFUSON SITE	LUNG	BREAST	OVARY	GIT	KIDNEY	BLADDER
PLEURAL(n=32)	20	06	02	03	00	01
PERITONEAL(n=47)	00	00	33	12	02	00
PERICARDIAL(n=1)	00	01	00	00	00	00
CSF(n=120)	00	00	00	00	00	00
TOTAL	20	07	35	15	02	01

Analysis of 120 samples of CSF revealed abnormal findings in 63 samples(52.5%) as shown in Table 4.

Table 4: Abnormal CSF findings in various diseases(n=63)

ETIOLOGY	ABNORMAL CSF FINDINGS(in %)
Meningitis	92.06%
Malignancy	3.17%
Demyelinating disease	3.17%
IVH	1.58%

Gross appearance of CSF varied from clear to hazy to opaque.Marked elevation in protein levels(≥ 100 mg/dl)were seen in bacterial and tubercular meningitis and moderate increase in protein (≥50mg/dl)was observed in viral meningitis.Low sugar level(≤45mg/dl)was seen in bacterial meningitis cases .CSF Adenosine Deaminase (ADA) levels were determined in 51 cases.19 cases of tubercular meningitis showed increased ADA levels with a mean value of 26.2IU/L.

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Cell counts were markedly elevated in bacterial and tubercular meningitis.Lymphocytic pleocytosis was observed in tubercular and viral meningitis.No organism was isolated in majority of the cases except tubercular bacilli in two and Cryptococcus in one case.All three cases with an identifiable organism were HIV positive.

Cytospin examination done in 56 cases of suspected malignancy showed leukemic blast cells in only one known case of ALL indicating metastasis to CNS.The blast cells showed increased nuclear-cytoplasmic ratio and prominent nucleoli.

Two cases of Intraventricular haemorrhage(IVH) and two cases of demyelinating disease was found in our study.

01 case of poorly differentiated adenocarcinoma was found in a single pericardial effusion sample in a case of carcinoma breast.

The distribution of effusion samples on predominant cell type and cytological evaluation is shown in Table 5.

Table 5: Predominant cell type and cytological examination of effusion samples.

EFFUSION SITE	TRANSUDATE	NEUTROPHIL RICH EXUDATE	LYMPHOCYTE RICH EXUDATE	MALIGNANT CELLS	ATYPICAL CELLS	TOTAL
PLEURAL	18	26	58	32	21	155
PERITONEAL	24	14	41	47	17	143
PERICARDIAL	00	00	00	01	00	01
CSF	102	04	13	01	00	120

IV. DISCUSSION

Etiological diagnosis of effusion samples is challenging and cytomorphological analysis provides valuable information in evaluation of body fluids.It also helps in staging of malignancies and assessing the prognosis of the disease.(1-3)

In our study,pleural fluid was the most common effusion sample(36.99%) followed by peritoneal(34.12%) which was in accordance with the study done by Sudha et al(6) but in contrast with the study of Chakrabarti et al(5) who found peritoneal samples more frequent than pleural effusion samples.

Male preponderance with a M:F ratio of 1.22:1 as seen in our study was also observed by Chakrabarti et al(5).This was in contrast with the study of Sudha et al(6) which had female preponderance.

Primary Adenocarcinoma of the lung (Fig 1)was the most common cause of malignant pleural effusion followed by Carcinoma Breast(Fig 2) and Primary squamous cell carcinoma(Fig 3) which correlated well with the studies of Chakrabarti et al(5),Sudha et al(6),Kushwaha et al(7) ,Tetikkurt et al(8) and Gupta et al(9).We had 21 cases showing atypical cell clusters.Amongst the 21 cases,15 cases on histopathological revealed Adenocarcinoma of the lung(Fig 4),03 cases squamous cell carcinoma of the lung and 03 biopsies were inconclusive.Further work up on atypical cell clusters in such cases was not available in other studies.One rare case of metastatic Transitional Carcinoma in a known case of Carcinoma Bladder was observed in our study(Fig 5).

Total protein ≥3gm was taken as inclusion criteria for exudates.Pleural fluid analysis revealed lymphocyte rich exudates(58,37.41%) as the most common finding followed by malignant effusion(32,20.64%).This was in accordance with the study of Sudha et al(6) and Kushwaha et al(7) but in contrast with the study of Chakrabarti et al(5) and Shulbha et al (10) who observed transudates as the most common effusion in their studies.Tubercular etiology was considered as the clinical diagnosis of all the lymphocyte rich exudates.AFB staining was done for all these samples and only one sample was found to be positive.The remaining cases were further evaluated using ancillary technique such as microbiological evaluation and radiological investigations for final diagnosis.

Cell counts revealed more than 300 cells/cumm in all pleural effusion samples with more than 50% mature lymphocytes in all lymphocyte rich effusion samples.This compared well with studies done by Kushwaha et al(7) and Tetikkurt et al(8).

Evaluation of 143 samples of peritoneal fluid revealed that malignant effusion was the most common cause (32.86%).Primary adenocarcinoma of the ovary (70.31%)was the commonest etiology(Fig 6 & 7).Similar findings were reported in Chakrabarti et al (5),Sudha et al(6) and other studies(9,11,12).Gastrointestinal malignancies

attributed to 25.53% malignant effusion in the peritoneum.Adenocarcinoma of the stomach is the commonest gastrointestinal malignancy causing peritoneal effusions which was similar to findings observed by Jha et al(13) in their study and in contrast to findings by Sudha et al who observed only two cases of carcinoma stomach affecting the peritoneal fluid.

06 cases of peritoneal effusion showed atypical cell clusters.Further work up showed 10 cases of carcinoma stomach,04 cases Carcinoma endometrium,02 cases of hepatocellular carcinoma,02 cases of carcinoma gall bladder,02 cases of renal cell carcinoma and 01 case of squamous cell carcinoma cervix.Similar work up with other ancillary technique was not available in other studies.

Amongst the 143 samples,lymphocyte rich exudates was the next frequent case of effusion after malignancy which was in contrast to many studies.(5,6,10-12).In these cases cell count was ≥300 cells/cumm and the commonest cause attributed was liver cirrhosis.

Higher number of normal CSF samples were observed in neonates(54.16%) to rule out meningitis in suspected cases of febrile or hypocalcaemic seizures.These cases didnot present with any signs of meningeal irritation.These findings were similar to studies conducted by Laving et al(14),Grages et al(15) and Brouwer et al(16).Lymphocytic pleocytosis was observed in Tubercular meningitis with considerable variation in polymorphs from (2-46%).This was in correspondence to studies done by Quan C et al(17),Pinto VL et al(18),Kulkarni et al(19),Khanna A et al(20) and Tan CB et al(21).This could be attributed to release of tubercular protein during treatment leading to Hypersensitivity reaction.

Zn stain for AFB was positive in only two cases(1.66%).Similar findings of low yield was also observed by Quan C et al (17) and Pinto VL et al (18).Newer modalities like Gene expert and CSF ADA are being used increasingly to aid in diagnosis(22-23).A cut off of CSF ADA ≥ 10 IU/L has been considered as diagnostically significant in our study.The sensitivity of the test varies from 44-100% and specificity varies from 71-100%(22,24).However,culture remains the gold standard and also helps in drug sensitivity of the case.(24)

Subarachnoid haemorrhage and intraventricular haemorrhage was diagnosed by imaging modalities(25,26).Albuminocytological dissociation was observed in demyelinating diseases which was similar to findings observed by Winer JB et al(27),Akbayram S et al(28) and Drulovic J et al(29).

In Acute lymphoblastic leukemia,CNS involvement is afflicted in 3-5% of children(30).Only one case(0.83%)revealed leukemic blast cells in CSF in a known case of ALL(Fig 8).These findings question the utility of CSF analysis in screening for CNS involvement which was in accordance with the findings of Asha et al(31).

Only 1% prevalence of pericardial effusion samples is found in patients of a tertiary care hospital(32).Common causes of pericardial effusion are malignancy,infective,idiopathic,radiation,drug induced and in some autoimmune disorders(33).The accuracy for cytological diagnosis of malignancy in pericardial fluid varies from 57-100%.(34-36).We evaluated only one pericardial fluid in our study which revealed poorly differentiated adenocarcinoma in a known case of carcinoma breast(Fig 9).

V. CONCLUSION

Cytomorphological analysis of body fluids is essential in diagnosis and staging of metastatic disease. However,uncommon malignancies clinically presenting as effusion pose diagnostic challenge to the pathologist and require detailed clinical history and other ancillary investigation for final confirmation.

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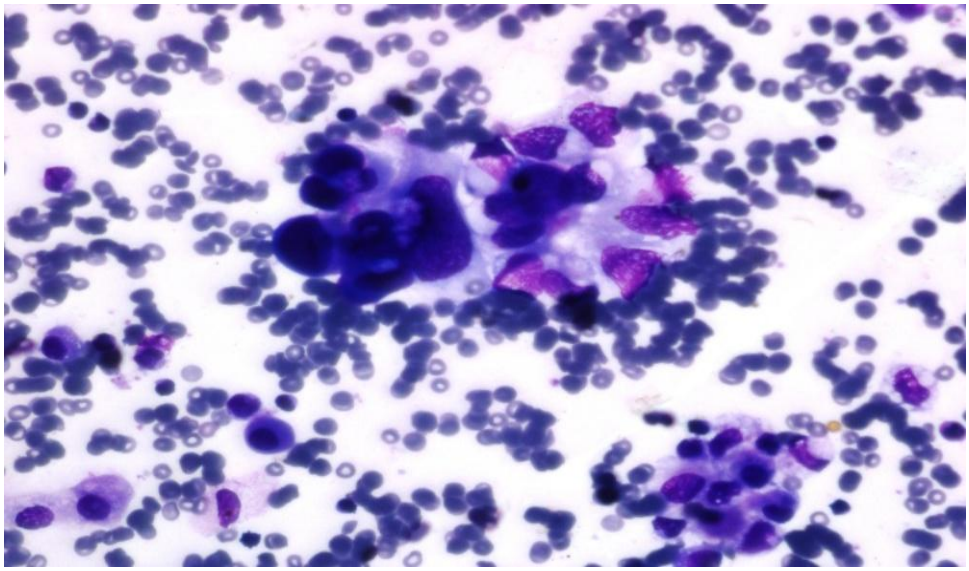

Fig 1:Pleural Fluid cytology showing malignant cells from Adenocarcinoma Lung

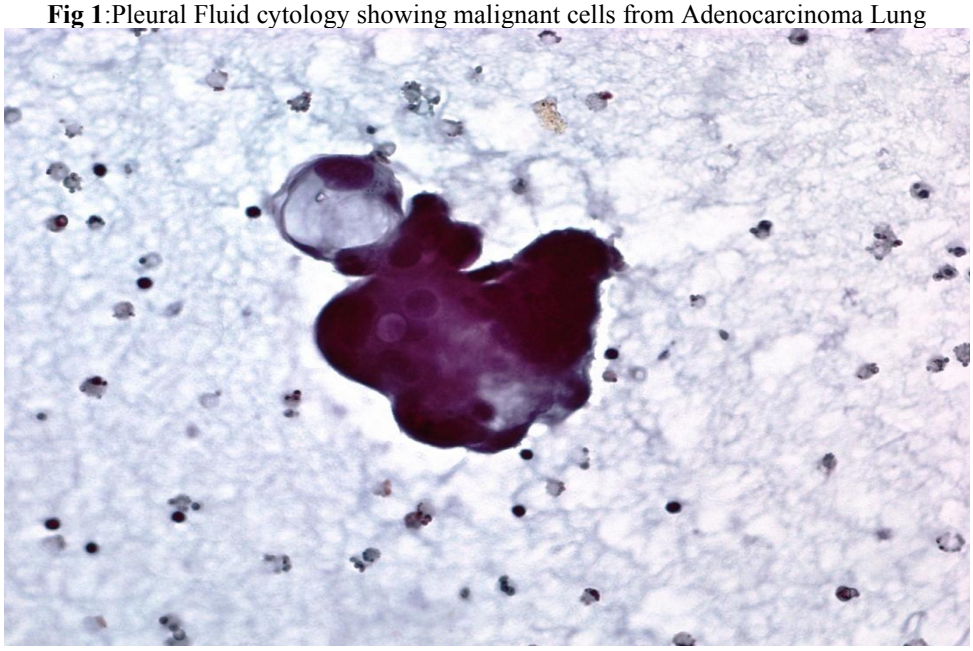

Fig 2: Pleural Fluid cytology showing Metastasis from Carcinoma Breast

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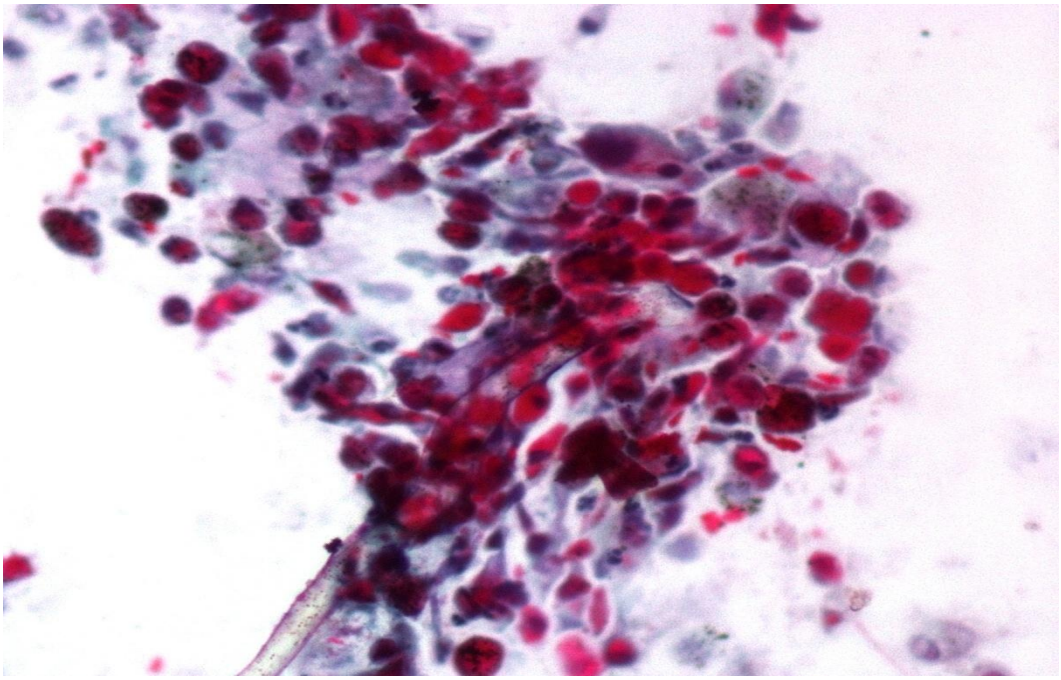
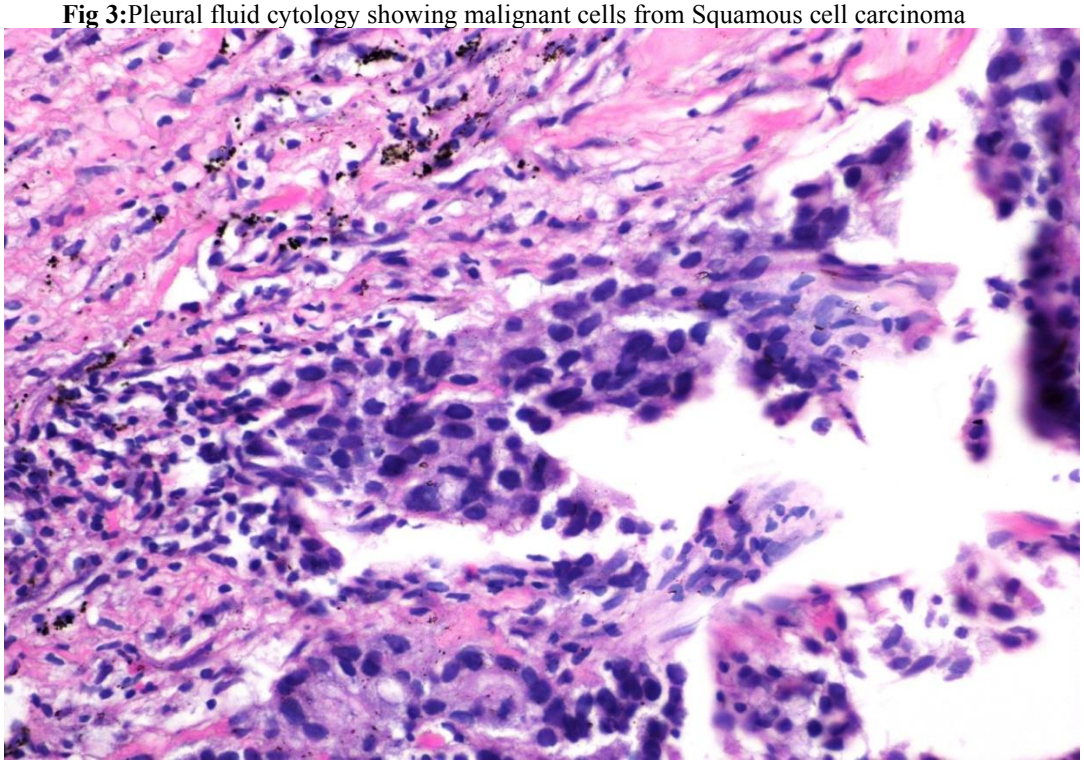


Fig 3: Pleural fluid cytology showing malignant cells from Squamous cell carcinoma



Lung

Fig 4:Histopathological Image of Adenocarcinoma Lung

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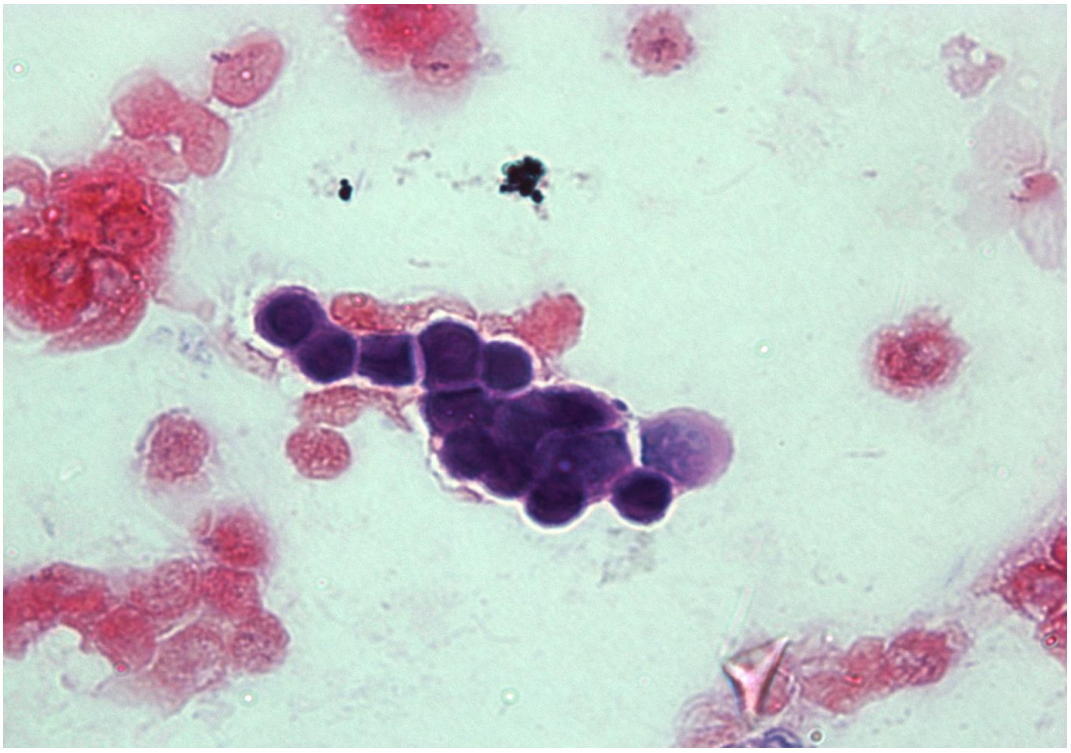

Fig 5:Pleural Fluid cytology showing Metastasis from Transitional Cell Carcinoma Bladder

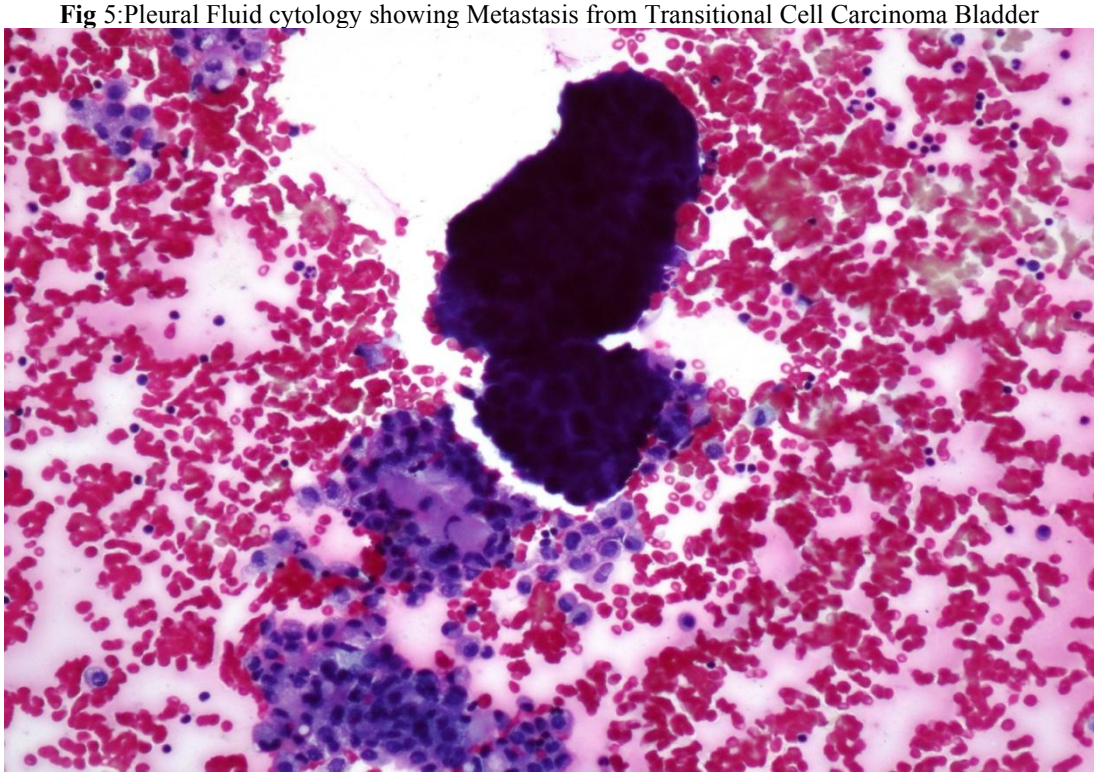

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Fig 6: Peritoneal Fluid cytology showing malignant cells from Adenocarcinoma Ovary

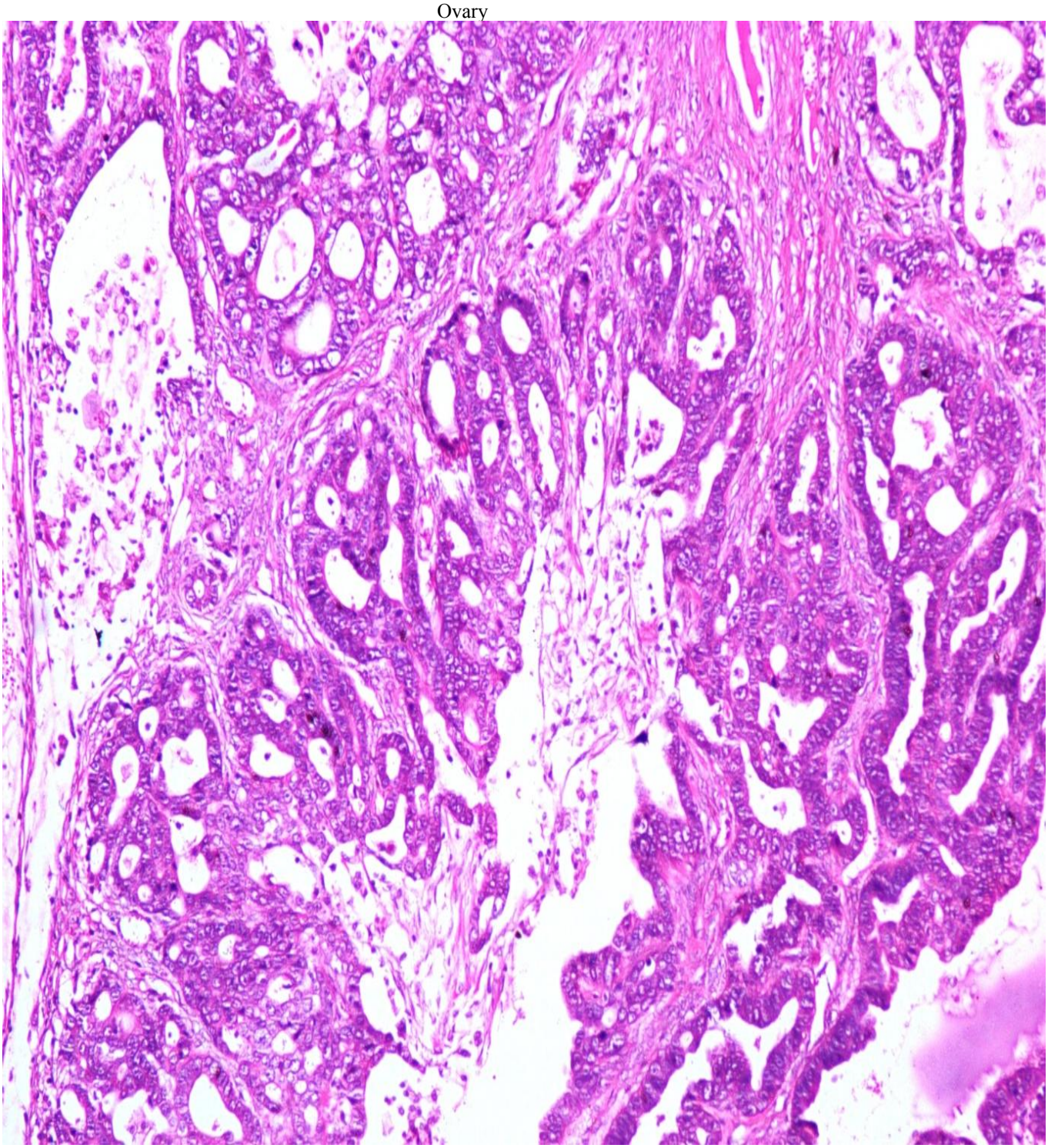

Fig 7: Histopathological image of Cystadenocarcinoma Ovary

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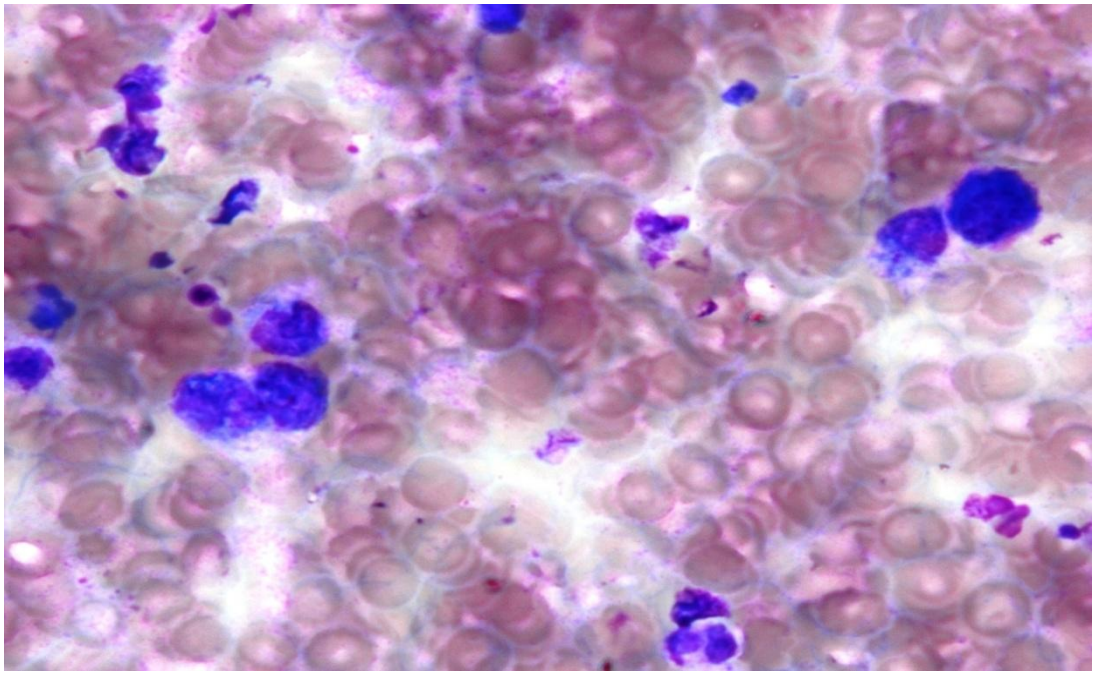

Fig 8:Blast Cell in CSF from a known case of ALL

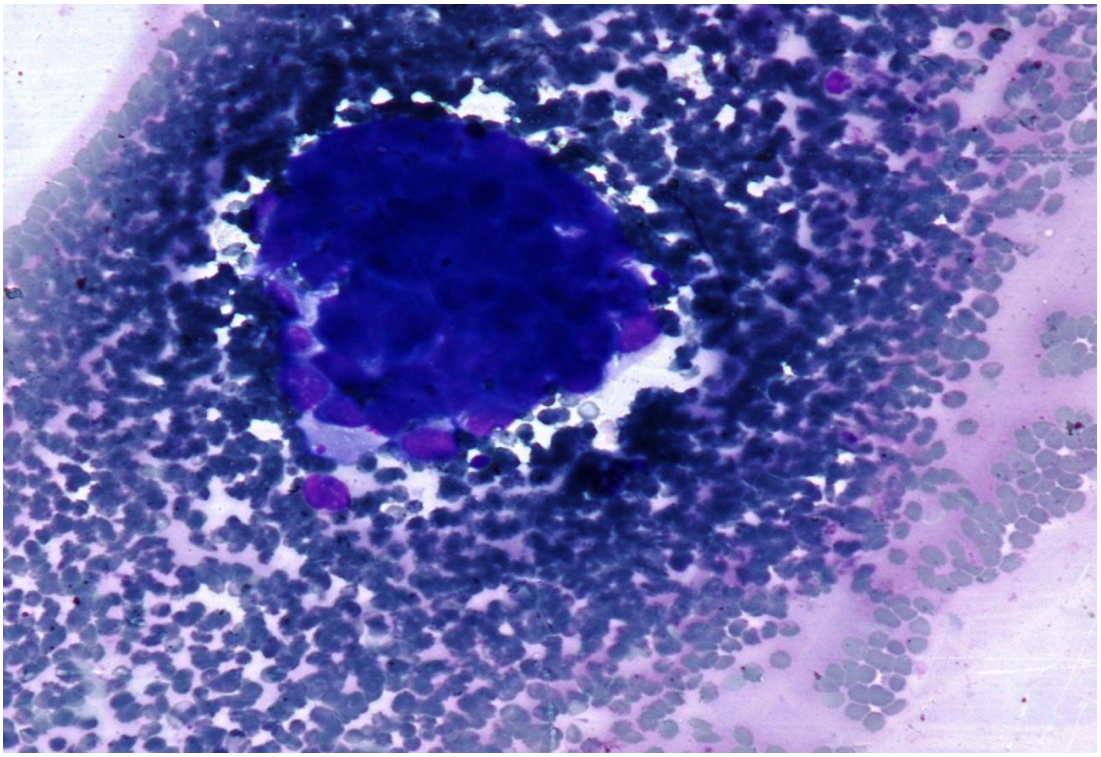

Fig 9: Pericardial Fluid showing Metastasis of poorly differentiated carcinoma from Carcinoma Breast