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ROLE OF SEROUS FLUID CYTOLOGY IN BOTH NON-MALIGNANT AND MALIGNANT LESIONS WITH EMPHASIS ON CYTOMORPHOLOGY OF RARE METASTATIC MALIGNANCIES TO SEROUS CAVITY

Pathology

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ABSTRACT

Context: Serous effusion cytology is an important investigation for diagnosis of neoplastic and non-neoplastic diseases. Malignant cells in serous fluid cytology indicates advanced stage. **Aims:** To evaluate the utility of serous fluid cytology in differential diagnosis of malignant and non-malignant serous effusions and to emphasize the cytomorphology of rare metastatic malignancies. The importance of cell block with immunocytochemistry for diagnostically challenging cases was also highlighted. **Settings and Design:** This was a one year retrospective study in which cases were classified into malignant and non-malignant. Immunohistochemistry was performed on cell blocks of difficult to diagnose cases. **Methods and Material:** Total 356 serous fluids (pleural, peritoneal and pericardial fluids) were centrifuged, cytocentrifuged and stained with Papanicolaou and Giemsa. Clinical history, radiological findings and other relevant parameters were noted. **Results:** 184 cases were of pleural fluid, 168 of peritoneal fluid and four were of pericardial fluid. Lung malignancies and ovarian malignancies were the most common primary sites in pleural and peritoneal fluids respectively. Out of 40 positive for malignancy cases, 22 were first confirmed on cytology. Clinical diagnosis of Boerhaave's syndrome was confirmed on cytology. Rare presentations of metastasis of squamous cell carcinoma, pleomorphic lobular carcinoma and clear cell type renal cell carcinoma were diagnosed on cytology. **Conclusions:** Rare metastatic malignancies to serous cavity pose diagnostic challenges on cytology. Both, cytopathologists and clinicians should be aware of these rare malignancies. A vigilant cytopathologist can identify malignant cells based on cytomorphological features and give accurate diagnosis with the help of ancillary tests and lead clinicians for further work up, evaluation and treatment of the patients.

KEYWORDS

Adenocarcinoma, Cannibalism, Boerhaave's Syndrome, Pleomorphic lobular carcinoma, Squamous Cell Carcinoma

INTRODUCTION:

Serous effusion is defined as pathological accumulation of fluids within serous cavities like pleural, peritoneal, pericardium and effusion cytology is study of cells within these cavities. Accumulation of fluids occur when there is imbalance of factors that favour the flow of fluids from vascular space and/or when there is exudation of fluid through infection or malignant implantation.[1]

Aim of our study is to evaluate utility of serous fluid cytology in differential diagnosis. We have discussed how cytology of effusions is important for guiding clinicians to diagnose and manage both non-malignant and malignant cases. Rare cases of malignancies metastasizing to pleural and peritoneal fluids are also discussed.

METHODS:

This study was carried out in the cytology section of department of Pathology of a tertiary care hospital for one year period. 371 serous fluids (pleural, peritoneal and pericardial) received during this one year were studied. The gross findings were noted. These fluids were centrifuged at 2000 rpm for five minutes and cyto-centrifuged at 2000 rpm for three minutes.

Both air-dried and alcohol fixed smears were made for both types of samples. Air-dried smears were stained with Giemsa stain while alcohol fixed were stained with Papanicolaou (Pap) stain. Cell blocks were made and stained with Hematoxylene and Eosin stain. Immunocytochemistry (ICC) was performed for diagnostically difficult cases using Cytokeratin7 (CK7), Cytokeratin20 (CK20), Cytokeratin19 (CK19), Calretinin, Wilms Tumour1 (WT1), Thyroid Transforming Factor1 (TTF1), P63, GATA3, GCDFP etc. Cytological findings were noted and correlated with clinical, radiological and histopathological findings.

RESULTS:

From the 371 samples received, 15 samples had no cellularity or had degenerated cells and were excluded from the study. In the remaining 356 samples, 184 were pleural, 168 were peritoneal and 4 were pericardial fluids.

Age range of the cases was three years to 90 years with peak age range at 41-50 years (26.7%) for peritoneal fluids, 31-40 years (17.39%) and 51-60 years (17.39%) for pleural fluids. Female preponderance with 85 numbers (50.6%) for peritoneal fluids and male predominance with 110 numbers of males (59.78%) for pleural fluids was seen.

Out of 184 cases of pleural fluids, 162 cases (88.04%) were non-malignant and 21 (11.41%) cases were malignant and one case was suspicious for malignancy. Lymphocyte rich exudates were most common (91, 49%) of all non-malignant cases, followed by mixed inflammatory effusions (35, 19%). Mesothelial rich fluids were only 9 or 5% of the total pleural effusions.

Out of 168 cases of peritoneal fluids, 145 cases were non-malignant, while 20 (11.90%) cases were malignant and three were suspicious for malignancy. Maximum cases were of mixed inflammatory effusions (57, 34%), followed by mesothelial rich effusions 45 (27%).

Out of four cases of pericardial effusions, two cases were lymphocyte rich effusion, one each were polymorph rich effusion and mesothelial rich effusion.

Majority cases of pleural fluids (75, 82.41%), three peritoneal fluid cases and one pericardial fluid case with lymphocyte predominance were associated with tuberculosis. 45 pleural fluid cases, peritoneal and pericardial fluid cases, were not suspected for tuberculosis clinically. Only six cases had adenosine deaminase more than 50. However, based on cytological findings, followed by clinical, radiological and microbiological investigations, they were started on anti-koch's treatment and responded to treatment.

The cases with polymorphs predominant effusion were associated with Pneumonia, liver abscess, pyosalphynx and empyema. There was a 27 years old male, who was chronic alcoholic with history of forceful

vomiting. He was suspected of having esophageal rupture. This case was confirmed of having Boerhaave's syndrome by presence of fungal colonies and squamous epithelial cells on cytology.

Mixed inflammatory effusions in peritoneum were associated with liver disease, acute pancreatitis, cases operated for ovarian torsion and simple ovarian cyst. While in pleural fluid, they were associated with tuberculosis and pneumonia.

Nearly 80% of decompensated liver cirrhosis cases showed mesothelial predominant effusions. There was a case of pericardial effusion with mesothelial predominance, who was started on Anti-Koch's treatment and responded to treatment.

We had three cases of eosinophil rich effusions, all in pleural fluid. Two of them had hydropneumothorax on radiology. Both cases were started on anti-kochs treatment and they responded to treatment. Unfortunately we lost the follow up of one case.

Macrophages predominant effusions were associated with many nonspecific inflammatory conditions like chronic liver disease, renal disease, tuberculosis, pneumothorax, post chemotherapy cases.

Two cases had necrotizing granulomatous inflammation in pleural fluid. Both were started on Anti-Koch's treatment.

There was a case with peritoneal fluid, which was detected to be having many fungal colonies. Initially it was suspected of contamination. However second fresh sample also showed similar findings. Gastric contents of the patient were similar on gross. It was greenish, granular and dirty looking. Since the appearance and findings of both peritoneal fluid and gastric contents were similar, diagnosis of gastric perforation was made. Microbiological follow up could have helped clinicians to treat the patient. Unfortunately this patient died.

We had total 41 confirmed malignant cases and four were suspicious for malignancy.

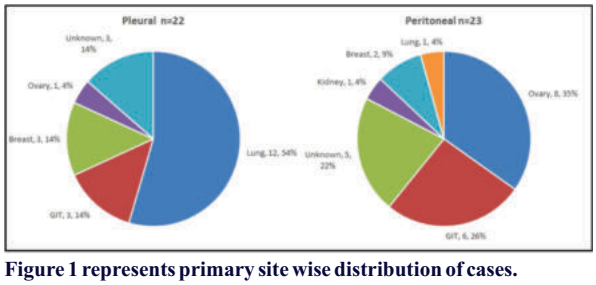

Figure 1 represents primary site wise distribution of cases.

There were total eight cases where primary site of malignancy could not be assessed due to inadequate sample for making cell block. Out of these, three had disseminated malignancy, were not operable and died. We lost to follow up remaining five cases.

Cytological findings of adenocarcinoma were correlated with histopathology and radiological findings. 22 cases (including two suspicious cases) were not suspected clinically or on initial radiological finding and were diagnosed solely on cytology [Table 1].

Table 1. Correlation of malignancies on Cytology with Radiological finding.

Cytology Diagnosis		Initial Radiological Finding	
		Suspected	Not Suspected
Pleural Fluids	21	9	12
Peritoneal Fluids	20	12	8
Suspicious	4	2	2
Total	45	23	22

Adenocarcinoma and mesothelial cells were easily differentiated on Giemsa stain than Pap stain indicating importance of Giemsa stain (shown in Fig. 2 a, b).

Four cases were suspicious for malignancy. Clinical correlation was done for all cases and histopathology follow up was done for two cases. Out of these four cases, one case was a known case of breast carcinoma with past history of modified radical mastectomy surgery done eight years back; on follow up patient had multiple metastatic deposits and was inoperable. One case had malignant germ cell tumor on histopathology and one had mucinous cystadenocarcinoma on histopathology. We lost to follow up one case.

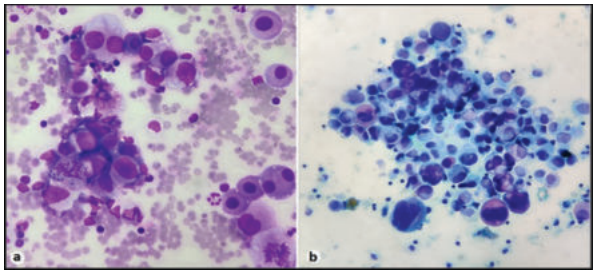

Figure 2:

There was a 65 year old male, presented with ascites. He had history of nephrectomy done in past for conventional Renal Cell Carcinoma (RCC). Peritoneal fluid was received. On cytology there were singly scattered clusters of cells with lipid vacuoles and granules in cytoplasm which were easily seen on Giemsa. On Pap stain we could see enlarged nuclei with coarse chromatin pattern and irregular nuclear membrane. The diagnosis of metastasis of conventional RCC was given based on the above findings. We followed up the CT scan findings which were suggestive of metastatic deposits in peritoneum, liver, spleen and pelvis confirming the diagnosis of metastasis of RCC. There was a 54 year old male presented with both pleural and peritoneal effusion. On cytology many 3D ball cluster of malignant cells were seen having eccentric, round to oval hyperchromatic nuclei and vacuolated cytoplasm. It was given as positive for malignancy, adenocarcinoma. ICC performed on cell block. TTF1 positivity confirmed the primary from lung.

There was a 54 year old female, known operated case of MRM for metaplastic Squamous Cell Carcinoma (SCC) breast. She was on chemotherapy and radiotherapy. After 8 months of diagnosis of breast cancer, she presented with ascites. On cytology, it was a cellular smear showing many papillaroid fragments having eccentric, hyper chromatic nuclei with vacuolated cytoplasm. It was given as positive for malignancy, adenocarcinoma. Clinical suspicion of breast metastasis and GIT malignancy were ruled out by GATA3 and P63 negativity and CK 20 negativity respectively. CT scan showed pelvic mass and right ovary was not separately visualized from mass along with omental metastasis, peritoneal metastasis and pelvic metastasis. WT1 and CK7 positivity and Calretinin negativity confirmed primary site being ovary (shown in Fig. 3 a, b).

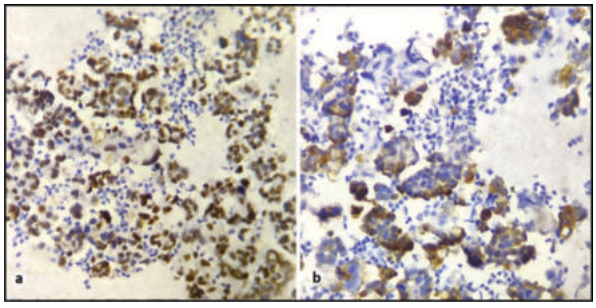

Figure 3: Adenocarcinoma showing a) 40x-WT1 positivity b) 40x – CK7 positivity

This patient was started on chemotherapy for ovarian adenocarcinoma and her subsequent PET CT scan showed reduction in the sizes of lesions indicating response to therapy.

There was a 55 year old female with known case of carcinoma esophagus, presented with pleural effusion. On cytology there were discrete and few clusters of malignant cells which were polygonal in shape with large hyperchromatic round nuclei with occasional cells showing vacuoles in cytoplasm. However morphology was of high grade carcinoma with difficult to categorize in particular malignancy.

Hence we performed ICC on cell block which showed strong positivity to P63 and Calretinin was negative in malignant cells leading towards diagnosis of squamous cell carcinoma metastasis with primary being esophagus.

There was a 65 year old female with past history of MRM done for breast malignancy with ongoing chemotherapy, presented with pleural effusion. On cytology, there were many discrete, singly scattered cells which were difficult to differentiate from mesothelial cells. However, there were many small necrotic cells having pyknotic nuclei which were easily appreciated on Giemsa stain (shown in Fig. 4 a, b).

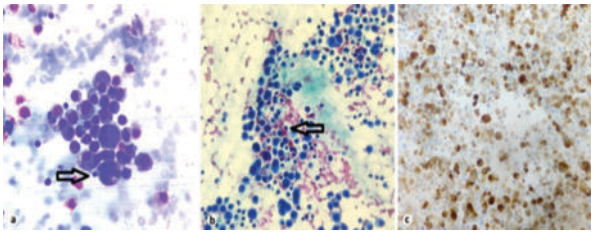

Figure 4:

Occasional cells had irregular, thick nuclear membrane which were easily seen on Pap smear. The diagnosis of high grade carcinoma was given. This was a case of pleomorphic lobular carcinoma breast on histology. Immunocytochemistry showed strong positivity to GCDFP in all large malignant cells (shown in Fig. 4 c). Since these cells were difficult to differentiate from mesothelial cells, Calretinin negativity in malignant cells could rule out possibility of mesothelioma.

Table 2 represents the comparison of our study with other studies. [2],[3],[4]

Studies	Present Study	Rasik Hatila et al (2004)[2]	Swali et al (2017)[3]	Archana et al (2014)[4]
Number of cases	356	355	150	150
Age Range (Decade)	4th – 6th	3rd – 5th	4th – 6th	4th – 5th
Male of Female Ratio	1.2:1	1.4:1	1.4:1	1.05:1
Most common primary site of malignancy in Pleural Fluid	Lung	Lung	Lung	-
Most common primary site of malignancy in Peritoneal Fluid	Ovary	-	Ovary	-

DISCUSSION:

Maximum number of malignant effusions, including suspicious for malignancy cases, were pleural (22, 49%) followed by peritoneal effusions (23, 51%). All malignancies were of metastatic origin. Maximum number of metastasis to pleural fluids were from lung adenocarcinoma (12, 54%) and ovarian adenocarcinoma to peritoneal fluid (8, 34%). Other malignancies which metastasized to pleural fluid were from breast, GIT, ovary and unknown. These findings were similar to the study done by Cakir et al.[5] While in peritoneal fluid, metastasis were from GIT, unknown, kidney, breast and lung.

Though the most significant role of cytology of serous effusion is to detect malignant cells, it is also very important in diagnosis of certain non-malignant cases on cytology, like in our study cytology confirmed the suspected case of Boerhaave's syndrome (shown in Fig. 5 a, b).

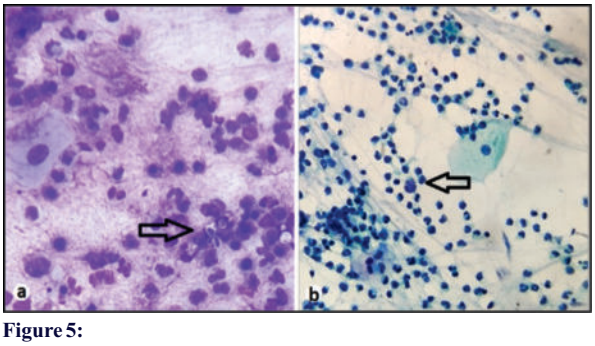

Figure 5:

It is a rare disease in which there is esophageal rupture in mediastinum due to forceful vomiting. Since the unusual and late clinical presentation and grievous prognosis, detection of it is utmost important for further management. We also detected disseminated fungal infection in one case. But this patient did not survive. We did not have microbiology follow up, otherwise this could have guided clinicians for typing and further management.

All air dried smears were stained with Giemsa and wet fixed smears were stained with Pap stain. Since the information provided by Giemsa stain and Pap stain is unique and complimentary, both types of stains are essential for accurate cytological diagnosis.[6] Pap, Hematoxylene and Eosin stains give better nuclear details and generally perform better on thick or extensively necrotic smears. Romanowsky type stains allow better estimation of relative cell and nuclear size and superior visualization of cytoplasmic details, smear background elements and intercellular matrix components.[6] In our study, cytoplasmic details of all malignant samples were better appreciated on Giemsa. Cytoplasmic granules, cytoplasmic vacuoles (mucin or lipid) were better appreciated on Giemsa than on Pap. Also the morphology of mesothelial cells was also better appreciated on Giemsa stain such as three colored zones of cytoplasm – perinuclear clearing surrounded by dark zone of concentrated organelles and peripheral zone of microvilli.

Although histological type most commonly seen is adenocarcinoma, variety of other malignancies can cause effusions.[6] In our study unusual malignancies presenting as serous effusions were Squamous cell carcinoma in pleural fluid (one case), Conventional RCC in peritoneal fluid (one case), pleomorphic lobular carcinoma in pleural fluid (one case) and ovarian carcinoma in peritoneal fluid (one case) in a known case of metaplastic squamous cell carcinoma of breast.

Occurrence of pleural effusion in SCC is rare and ranges from (0.5 - 2.7%).[7] Although it is very common neoplasm, SCC can be difficult to differentiate from adenocarcinoma and malignant mesothelioma on cytology and also immunocytochemistry overlaps.[7] Since SCC is amenable to chemotherapy and radiotherapy, it is important to recognize SCC in these patients to avoid unnecessary investigations and surgery.[5]

Metastasis of RCC in peritoneal fluids has only 3% incidence. Many studies have found papillary RCC to be the most common type presenting with serous effusion. Detection is very difficult since cells are bland and nondescript, with few distinguishing features.[8],[9] To our knowledge only two cases of conventional RCC have been studied in the literature in last 10 years. In our case, subtle cytomorphological features like clustering of cells, lipid vacuoles and granules in cytoplasm and slightly enlarged round to oval nuclei with irregular nuclear membrane and coarse chromatin could differentiate these cells from mesothelial cells and macrophages (shown in Fig. 6 a, b).

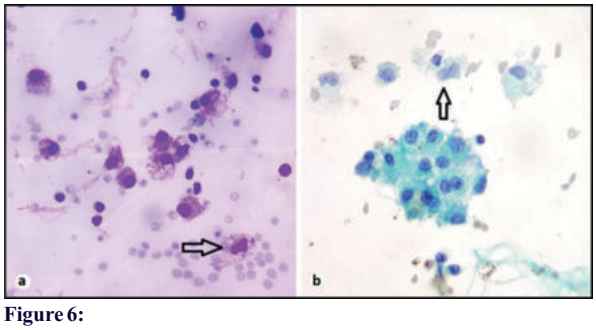

Figure 6:

In this case, lipid vacuoles and granules were well seen on Giemsa stained smears, indicating the importance of Giemsa stain. Diagnosis of these malignant cells and their differentiation from mesothelial cells and macrophages is utmost important, since its occurrence indicates unfavorable prognosis.

In body cavities, lobular carcinoma metastasis is difficult to diagnose because of benign looking cells and discohesive clusters.[10] Hence as compared to duct carcinoma, lobular carcinoma is a more frequent cause of false negative diagnosis.[11],[12] Pleomorphic lobular carcinoma is a subtype of lobular carcinoma with high grade cytological features and aggressive clinical behavior. It has increased risk of recurrence and decreased overall survival.[13], [14], [15] Pleomorphic lobular carcinoma is difficult to differentiate from mesothelial cells.[10], [16] Like in our case, the cells were polygonal with central round nuclei with prominent

nucleoli. Clinical history of know breast carcinoma, occasional cells showing thick nuclear membrane and necrotic cells on cytology could led us to the diagnosis of high grade carcinoma. In this case ICC was helpful to confirm breast origin.

Calretinin is a very good marker for mesothelial cells. [17],[18] In our study negative staining of Calretinin helped differentiate mesothelial cells from malignant cells along with other panel of markers.

In our study we saw cannibalism in three cases, two were of breast primary metastasizing to peritoneal fluids and one lung adenocarcinoma metastasis to peritoneal fluid (shown in Fig. 7).

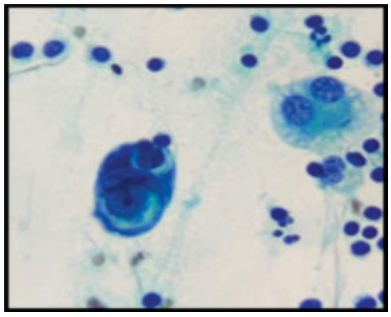

Figure 7:

Cell cannibalism was first described by Leyden in 1904. He called it as bird's eye cell, which is a feature of survival advantage in adverse condition like low nutrient supply. [19] Detection of these cells in fluids indicate disseminated malignancy.[20]

Cytological examination of pleural, peritoneal and pericardial effusions, is helpful to detect both, malignant and non-malignant cases. This leads the clinicians for further work up, evaluation and treatment of the patients.

Though adenocarcinoma is most common malignancy found in serous fluids, both cytopathologists and clinicians must keep in mind the rarely occurring malignancies in routine practice for better approach to diagnosis.

The cytopathologist should be more vigilant to differentiate malignant cells of RCC and pleomorphic lobular carcinoma from mesothelial cells and macrophages. Background necrotic cells with pyknotic nuclei can also be the clue for malignancy.

Presence of cell cannibalism in fluids is an indicator of disseminated malignancy.

Although the Pap stain may be the best known stain in practice of cytopathology, Giemsa stain remains an indispensable.

Combined with ICC on cell block, fluid cytology is the best aid to diagnose morphologically challenging cases.

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