Cytomorphology of metastatic squamous cell carcinoma in effusions: A retrospective study

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Abstract: Introduction: Metastatic squamous cell carcinomas (SCC), though common, are rarely seen in effusions. According to recent literature, the incidence of metastatic squamous cell carcinoma in body cavity fluids is 0.5 – 2.7%. Hence a misdiagnosis of metastatic SCC is very common. However some cytomorphological features can aid us in the accurate diagnosis. A study of the morphological features was done and the morphological features are described in detail for the diagnosis of metastatic SCC. We aim to study the cytomorphological features of metastatic squamous cell carcinoma in effusion fluids. Material and methods: All effusion fluid specimens reported as positive for malignancy with a squamous component from January 2014 to January 2019 were included in the study. Adequate clinical details were collected from the database. Results: In the study period, 21 effusion fluid specimens, which included 10 peritoneal and 11 pleural fluids from 16 patients, were reported as squamous cell carcinoma (SCC). 14 of these cases had histopathological correlation and diagnosis. Diagnosis of metastasis was made either based on established morphological features or when a discrepancy was encountered, the aid of immunocytochemistry or immunohistochemistry with p63 facilitated in arriving at the diagnosis. Conclusion: Although rare, SCC can be identified in serous effusions. To prevent misdiagnosis, correlation with clinical history and type of original tumor is necessary, along with increased awareness of the cytomorphological types and IHC overlap between carcinomas. Furthermore, the cytological diagnosis of metastatic SCC will give the patient a dismal prognosis

Index Terms: cytology, squamous cell carcinoma, peritoneal fluid, pleural fluid, effusions, cytomorphology, metastatic carcinoma. (key words)

I. INTRODUCTION (HEADING 1)

Metastatic squamous cell carcinomas (SCC), though common, are rare in effusions. According to recent literature, the incidence of metastatic squamous cell carcinoma in body cavity fluids is 0.5 – 2.7%. The most common malignant neoplasm in serous effusion is adenocarcinoma (72%) with other entities, such as small cell carcinoma (14%), malignant mesothelioma (8%), lymphoproliferative disorders (3.5%) and squamous cell carcinoma (SCC) (0.5- 2.7%) being less prevalent.[3] This incidence in pleural effusions of metastatic SCC from the lung is mainly because of the central location of lung SCC and peripheral location of adenocarcinomas, which we quite often see in malignant cytologic smears of pleural fluid analysis.

Cytological examination of effusion fluids is a quick and accurate method to diagnose metastatic effusions. Hence, the first line of investigation of a suspected neoplastic effusion is often the cytological examination.[4] After the advent of personalized medicine, the cytopathologist carries the responsibility to identify the cancer cells accurately and further categorize the tumor type, also giving the primary if possible.

The largest series of SCC in serous effusions to date was published in 1989, in which a total of 46 patients with metastatic SCC to pleural, peritoneal, or pericardial cavities were identified in 9297 serous effusions during a period of 33 years.[5]

The discovery of squamous cells in serous effusions is an uncommon occurrence whether benign or malignant. SCC can be difficult to discern in effusions from adenocarcinomas or malignant mesotheliomas due to significant morphological and immunohistochemical (IHC) overlap. If the morphology of SCC is well differentiated, the diagnosis is quite straightforward; however, with a poorly differentiated morphology, we take the aid of immunohistochemistry to arrive at a diagnosis.

There is limited information of morphologic features and differential diagnosis of the metastases in effusion fluids. The present study was undertaken to analyze the cytomorphological features that aid in diagnosis of SCC.

II. MATERIALS AND METHODS

Specimens were retrieved from the Pathology database at the Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry from January 2014 and January 2019 (5 years), while a total of 18,730 serous fluids were accessioned. All fluid specimens reported as positive for malignancy with a squamous cell component were included in the study, 3 cases were excluded as 2 of them were collected from pelvic fluid and 1 from psoas abscess fluid collection. All patients had a confirmatory tissue diagnosis of SCC, with 2 cases having an upfront diagnosis in fluid specimens.

A total of 21 fluid specimens from 16 cases (Table 1) comprising eleven pleural fluids and ten peritoneal fluids had a diagnosis of SCC. Of these, 3 patients had multiple fluid specimens with a gap of one week. Demographic data and relevant clinical information were obtained from the patient's medical records and database.

Table 1- Basic demographic data and number of each effusion fluid specimens

Case#	Age & sex	Body fluid	Number of specimens
1.	53/F	Pleural	1
2.	51/M	Pleural	3
3.	58/F	Pleural	1
4.	48/M	Pleural	1
5.	49/F	Pleural	2
6.	25/M	Pleural	2
7.	50/M	Pleural	1
8.	42/F	Peritoneal	1
9.	60/F	Peritoneal	1
10.	67/F	Peritoneal	1
11.	37/F	Peritoneal	1
12.	30/F	Peritoneal	1
13.	42/F	Peritoneal	1
14.	50/F	Peritoneal	1
15.	54/F	Peritoneal	2
16.	60/F	Peritoneal	1

For each case, two smears were prepared, one air-dried May-Grünwald-Giemsa-stained smear and one alcohol-fixed Papanicolaou-stained smear. A cell block was processed whenever necessary. All cases had a documented histopathological diagnosis with squamous cell component.

Each case was microscopically re-evaluated by 2 pathologists. Any disagreement regarding morphologic interpretation or cell type was resolved by consensus at the time of simultaneous double screening. The poorly differentiated cases were confirmed by immunocytochemical (ICC) and immunohistochemical (IHC) staining performed on either smears or cell block sections respectively.

III. RESULTS

Clinical information

Twenty one specimens were collected from 16 patients. The mean age of the study population was 47.2 years. 12 of the patients were females and 4 were males with a male: female ratio of 1:3. Effusion fluid specimens comprised pleural fluid (n=11, 45%) and peritoneal fluid (n= 10, 55%). Three of the cases had multiple fluid specimens, one of which had 3 specimens and two of them had 2 specimens.

Fourteen of the cases had known prior diagnosis of SCC and 2 cases were diagnosed upfront in effusion fluid. Of these, primary sites were cervix (n= 10), lung (n= 3), oesophagus (n= 1), vault (n=1) and squamous cell thymic carcinoma (n= 1). None had a known second malignancy.

Of the 16 cases, primary cervical SCC (10 cases) accounted for 64% of total metastasis and 90% of all peritoneal fluid metastasis followed by primary lung carcinoma (3 cases) accounting to 22% of total metastasis and 43% of all pleural fluids.

Table 2- Summarized results comprising information about primary diagnosis and Immunocytochemistry and immunohistochemistry

Case #	Age & sex	Body fluid (number)	Original cytological Diagnosis	Site and time of primary carcinoma tissue diagnosis	ICC(smear)/IHC (cell block)
1.	**53/F**	**Pleural (1)**	**Adeno-squamous Carcinoma***	**Cervix - synchronous**	**IHC p63+**
2.	51/M	Pleural (3)	SCC	Esophagus - prior diagnosis	ICC p63+
3.	58/F	Pleural (1)	SCC	Cervix - prior diagnosis	N/A
4.	48/M	Pleural (1)	SCC	Lung – prior diagnosis	N/A
5.	49/F	Pleural (2)	SCC	Lung - prior diagnosis	ICC p63+
6.	**25/M**	**Pleural (2)**	**Squamous cell thymic carcinoma***	**Thymic cell carcinoma–upfront diagnosis**	**IHC p63+, CD5+, CD117-, PLAP-**
7.	50/M	Pleural (1)	SCC	Lung – prior diagnosis	ICC p63+
8.	42/F	Peritoneal (1)	SCC	Cervix – prior diagnosis	ICC p63+
9.	60/F	Peritoneal (1)	SCC	Vault– prior diagnosis	IHC p63+
10.	67/F	Peritoneal (1)	SCC	Cervix – prior diagnosis	ICC p63+
11.	37/F	Peritoneal (1)	SCC	Cervix – prior diagnosis	N/A
12.	30/F	Peritoneal (1)	SCC	Cervix – prior diagnosis	N/A
13.	42/F	Peritoneal (1)	SCC	Cervix – prior diagnosis	ICC – p63+
14.	50/F	Peritoneal (1)	SCC	Cervix - prior diagnosis	ICC – p63+
15.	54/F	Peritoneal (2)	SCC	Cervix - prior diagnosis	ICC – p63+
16.	60/F	Peritoneal (1)	SCC	Cervix - prior diagnosis	N/A

*The cases diagnosed upfront in pleural fluid cytology
SCC- Squamous cell carcinoma, ICC- Immunocytochemistry, IHC- Immunohistochemistry,
N/A- No ancillary methods performed

Morphological features:

The morphological features of SCC metastasis in fluids that were encountered after evaluation of the smears were:

- Refractile rings (Figure 1A) -When glassy, hyalinizing cytoplasm acquires successive concentric layers (or shells), and their interfaces are best seen as "rings" when viewed perpendicularly to the plane of focus. Cells with refractile rings can have a spectrum of colors with the Papanicolaou stain. They are often, but not obligatorily orangeophilic.
- Malignant polygonal cell (Figure 2B)- Cells are flat and angulated with sharp cytoplasmic borders and a nucleus that is larger and darker with coarser, dispersed chromatin.
- "Cell in cell" appearance (Figure 1B)
- Intercellular bridging (Figure 1C)
- Keratin pearl- This is created by concentric ringing or layering of at least 2 cells in a whorl-like fashion (Figure 1D)

- Fiber cell - A cell with elongated bipolar cytoplasmic processes and a centrally placed nucleus that can be flattened almost to the verge of being pointed.

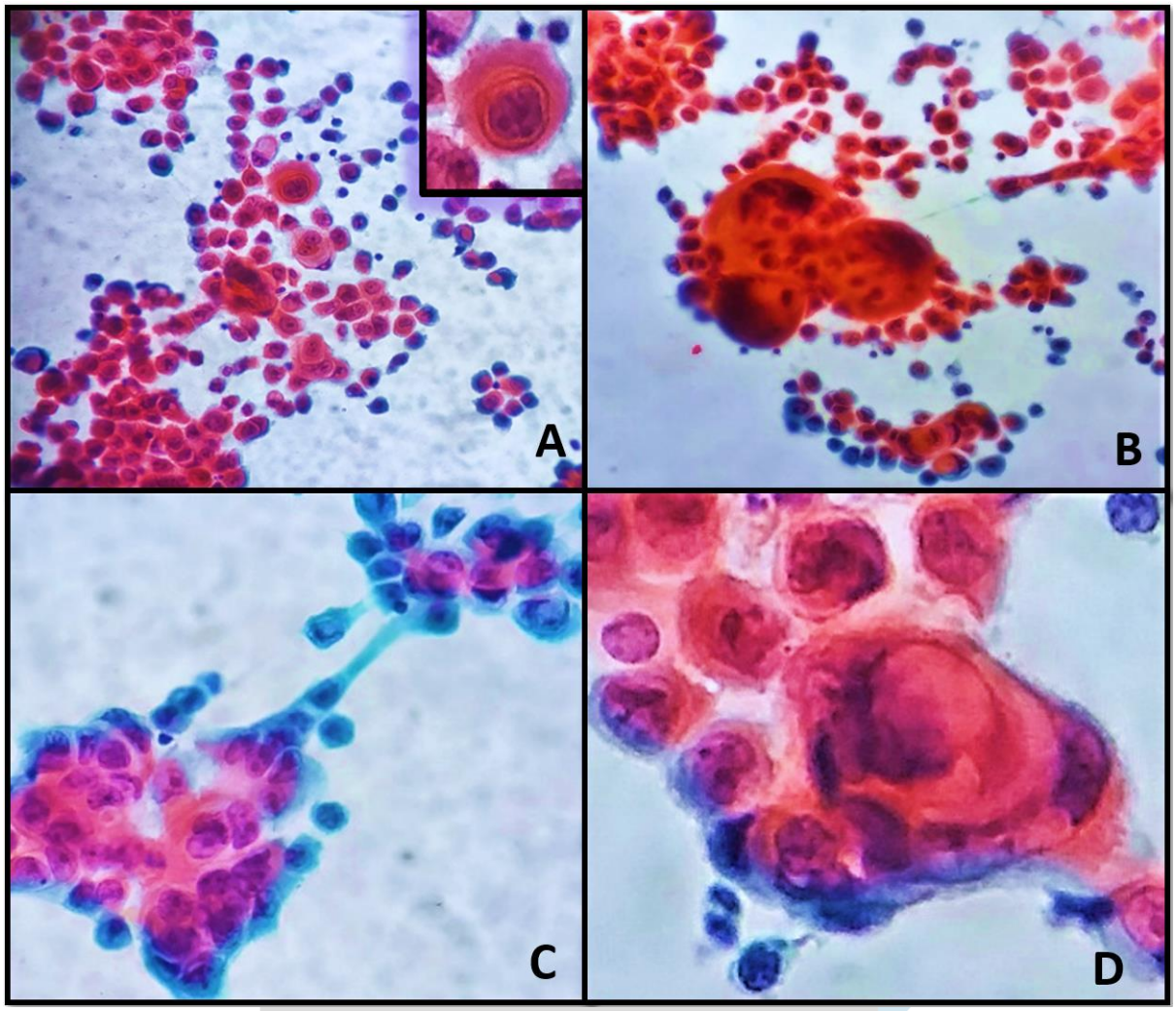

Figure 1: Papanicolaou stained smears with A- dense orageophilic cytoplasm and refractile rings (inset), B- cell in cell appearance, C- intercellular bridging, D- fiber cell encasing a keratin pearl

- Poorly differentiated rounded cell, so-called "Third-type cell" (Figure 2D)- The cell as defined by Graham is a malignant round-oval cell having a centrally located hyperchromatic nucleus, inconspicuous nucleolus, and an irregular chromatin pattern that ranges from coarsely clumped to completely dispersed.[7] Homogenous, dense cytoplasm is typically light blue (Romanowsky stain) or acidophilic, basophilic, or orangeophilic when keratinized (Papanicolaou stain) and completely envelops the nucleus. Refractile rings often appear with advanced keratinization. Variations include cytoplasmic vacuoles, eccentric rather than central nuclear placement, nuclear pyknosis, and multinucleation; the latter producing a multinucleated "giant" tumor cell.
- Tadpole cell - An elongated cell with an ovoid nucleus at the rounded bulbous end and a thin elongated unipolar extension of cytoplasm at the other end.
- Undifferentiated cell - A rounded cell having a variable nuclear shape (occasionally pleomorphic with pronounced anisonucleosis), clumped irregular chromatin with perhaps discrete nucleoli, and only a small amount of nondescript cytoplasm. The meager amount of cytoplasm typically does not envelop the entire nucleus.

The predominant morphological clue was high cellularity (Figure 2A) with atypical keratinization of cytoplasm (cytoplasmic orangophilia) in polygonal or tadpole-like cells exhibiting hyperchromatism and pyknosis. The predominant cell type was a poorly differentiated rounded cell "third cell type". 8 of 21 specimens showed background inflammation comprising neutrophilic infiltrate. Most of the cases showed cytoplasmic vacuolations of variable degrees along with intercellular bridging.

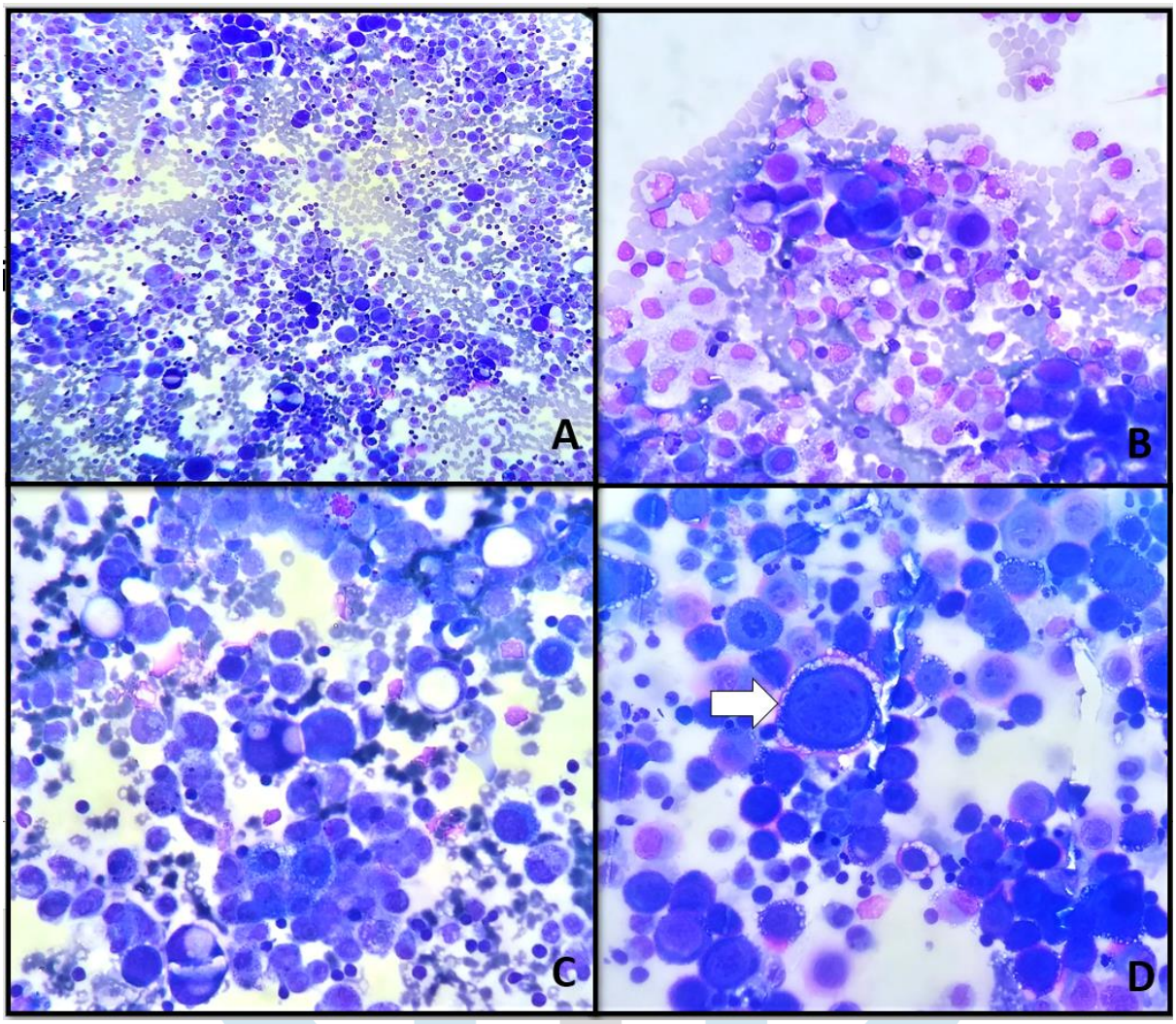

Figure 2: May-Grünwald-Giemsa-stained smears with A- exhibiting high celularity, B- a typical polygonal cell with dense cytoplasm, C- cytoplasmic vacoules ,both fine and coarse vacuolations, D- poorly differentiated rounded cell- "third cell type"

In discrepant cases or smears with poorly differentiated morphology, immunocytochemistry with p63 (Figure 3B) was performed which was helpful. Further, cell block preparation and immunohistochemistry with p63 facilitated in arriving at the diagnosis and confirming metastases. (Figure 3D)

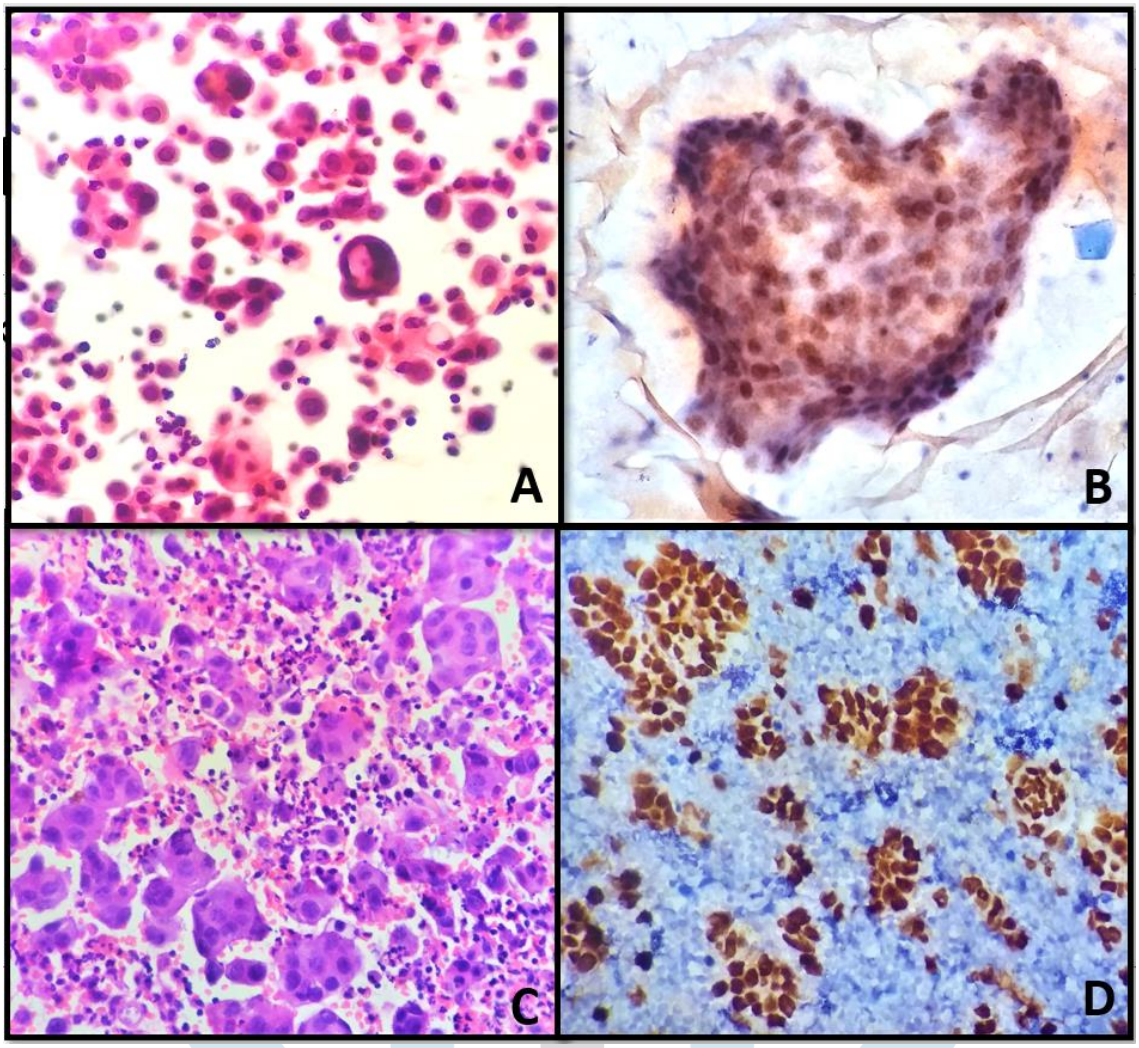

Figure 3: A- Papanicolaou stained smear with dyskeratotic cells with orangiophilia of the cytoplasm, B- Immunocytochemistry (ICC) with p63 in plueral fluid cytology smear, C- Hematoxylin and Eosin stained cell block preparation in pleural fluid cytology showing tumor cells in clusters and scattered cells exhibiting moderate nuclear pleomorphism, dense cytoplasm and hyperchromatism. The background shows neutrophilic infiltration along with nuclear debris, D- Immunohistochemistry (IHC) with p63 in plueral fluid cytology cell block

2 cases diagnosed upfront in cytology:

One case of adenosquamous carcinoma of cervix which turned out to have both adeno and squamous components for which the cell block performed confirmed the expression of p63 by IHC in only a group of tumor cells. Further, we received a cervical biopsy which confirmed 2 components of tumor cells by IHC.

Another case of thymic SCC was also diagnosed upfront in pleural fluid cytology, which had a poorly differentiated morphology. The patient presented with mediastinal mass along with pleural effusion. The smears were highly cellular with tumor cells which are predominantly dyscohesive along with loose clusters exhibiting high N:C ratio, scant cytoplasm, coarse chromatin, and prominent nucleoli and mixed with mature lymphocytes and neutrophils in slight blood mixed background. ICC showed positivity for p63 and CD5 and negative for CD177, PLAP, CD3, CD20 which were done to rule out germ cell tumor and lymphoma.

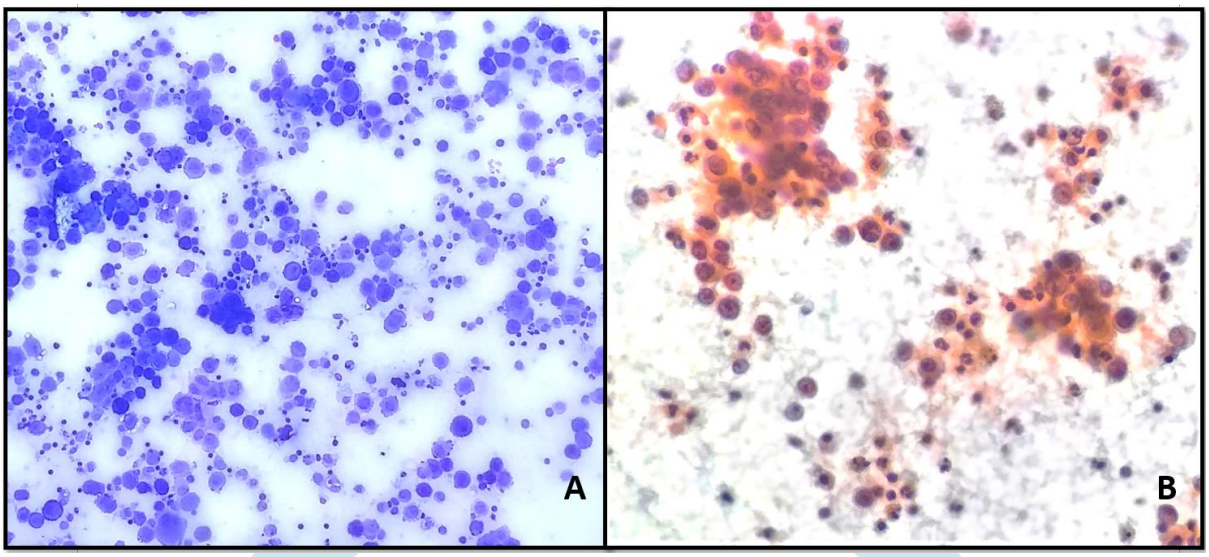

Figure 4: Thymic carcinoma- squamous cell carcinoma (A- May-Grünwald-Giemsa-stained smear, B- Papanicolaou stained smear

IV. Discussion

A variety of neoplasms can metastasize to serous cavities. The lining of the serous cavity is rich in lymphatics, and lymphatic lacunae open directly via the lining's tiny openings, or stomas. Carcinomas mostly spread to the serosa through lymphatic capillaries, which can get obstructed and result in effusion. Malignant effusions can arise from direct extension of primary carcinomas of serosal membrane-lining organs, such as the lung, intestines, liver, ovary, etc. By default, cytopathologic analysis of serous effusions presumes the presence of malignant cells unless otherwise noted. [6-8] Cytologic evaluation gives a prompt and reliable diagnosis, especially in this era of personalized medicine, where cytopathologists play an eminent role in fast forwarding the diagnosis.

Pleural effusions are a frequently encountered manifestation of metastatic disease and can occur in 15% of the patients with cancer.[14] However, the most frequent metastatic malignancy encountered are adenocarcinomas mainly having this propensity because of the peripheral location of pulmonary adenocarcinomas. SCC metastasis to effusion fluids is uncommon. Hence differentiating metastatic SCC from other common malignant effusions is a challenge for cytopathologists. Morphology may be misguiding, and a wrong diagnosis of adenocarcinoma, which is commonly seen, may be made. Especially the confusion is when the primary diagnosis is unknown. Thorough examination of the morphological features described above will be helpful in accurate diagnosis along with proper clinical details. Molecular tests done on cell blocks of pleural fluids are done quite often, mainly for non-small cell lung carcinoma, in specific adenocarcinomas, followed by SCC. The tests usually performed are EGFR, ALK, ROS as a first line molecular investigation with surrogate IHCs available for them. These gene abnormalities when detected, can be given targeted therapy.

Immunohistochemical stains are commonly used to distinguish differential diagnoses in some difficult case[6]. Previous studies show that a panel of K903, CK5/6, p63, calretinin, WT-1 and Ber-EP4 has a very high sensitivity and specificity in the differentiation of SCC from mesothelial cells or adenocarcinoma[7]. It is important to recognize that some of these markers may be positive in Malignant mesothelioma, SCC or adenocarcinoma. Therefore, a focused immunohistochemical panel should always be used and interpreted judiciously[8].

Reactive mesothelial cells may have significant morphologic overlap with cancer cells. Such reactive mesothelial cells may be a major or a minor component of the malignant effusion. Some of these factors are significant diagnostic pitfalls, which may lead to false-positive interpretations. This is particularly applicable to cases with a previous history of carcinoma. Although a false-positive diagnosis may be difficult to disprove, it may subject the patient to improper management decisions and emotional distress. [16] We would like to emphasize on the morphological features of SCC in fluids and without hesitation take the aid of IHC for an optimal diagnosis.

V. Conclusion

Although rare, SCC can metastasize to serous effusions. To prevent misdiagnosis, correlation with clinical history and type of original tumor is necessary. The cytomorphological features and IHC markers discussed will aid in the accurate diagnosis of SCC in effusion fluids.

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