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Original Research Article

# Sarcomas in fluid cytology: Experience from a tertiary care centre in India

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ABSTRACT

**Background:** Cytological evaluation of fluids for sarcomatous exfoliation is extremely rare. However, recently studies have come up describing characteristics of sarcomatous malignancies exfoliating into fluids but study from an Indian experience is still lacking.
**Objective:** Description of clinico-pathological landscape in known cases of sarcomas, involving body fluid with emphasis on cytomorphologic details.
**Material and Methods:** Study includes cases reported as positive for involvement by sarcoma on fluid cytology including pleural, ascitic and cerebrospinal fluid (CSF) specimens between Jan 2016 to June 2022. Cases were retrieved and reviewed for cytomorphologic features which were subsequently correlated with its parent histology and IHC. IHC was applied on cell blocks for two cases where involvement was doubtful.
**Results:** In total, 21 fluid samples/cases including 4 CSF, 6 ascitic and 11 pleural fluid specimens were incorporated. Case spectrum comprised of 6 cases of Ewing Sarcoma (ES), 3 cases of rhabdomyosarcoma (RMS), 3 cases of osteosarcoma (OS), 2 cases of malignant peripheral nerve sheath tumor (MPNST), 2 cases of synovial sarcoma (SS), a case each of chondrosarcoma (CS), leiomyosarcoma (LMS) angiosarcoma (AS) and two cases of other malignancies with exfoliation of their sarcomatous component. Two cases presented as isolated latent metastasis so an IHC panel was applied to exclude involvement by secondary malignancy and prove involvement by primary diagnosed sarcoma.
**Conclusion:** Majority of exfoliated sarcomas presented with epithelioid to pleomorphic morphology where at times it becomes obligatory to rule out occurrence of a secondary malignancy. It's the first study from an Indian institute's perspective that reflects upon such diversity of sarcomas with variability in morphology on exfoliation which can be overwhelming for a cytopathologist at times. IHC panel might be used when clinical background is unknown or when involvement is uncertain. More studies are needed that can help come up with recommendations that address such problems.



## 1. Introduction

Adenocarcinomas are among the tumors that exfoliate into body cavities most commonly, while sarcomas are among the rare malignancies that do so. Treatment must be modified if malignant cells are discovered in body fluids which indicates advanced disease and poor prognosis. Studies have shown that fine needle aspiration cytology (FNAC) has good sensitivity and specificity in identification of bone and soft tissue tumors,[1] but the approach in exfoliative fluid cytology is still not well understood. This is not only due to its rarity but also due to the morphologic alterations that tumor cells may undergo in fluids.[2] In addition, paucity of pertinent literature describing

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clinicopathologic characteristics of sarcomas in cytology is rare. Hence, our effort aims to expand the present knowledge of sarcomas in exfoliative fluid cytology. It is the first study from India that describes this particular variety of cases. Our main objective was to thoroughly examine the cytomorphological characteristics of the exfoliated tumour cells while taking into account potential differential diagnoses, which is crucial in cases of latent metastasis in particular. The results of IHC and histomorphology were also compared.

## 2. Materials and Methods

Patients reported as positive for sarcomatous tumour exfoliation into body fluids between January 2016 and June 2022 at Gujarat Cancer and Research Institute in Ahmedabad were subjects of the current retrospective descriptive analysis. CSF, pleural, and ascitic fluid samples were included. Clinical and demographic details were obtained from patient's files. Cytosmears, cell block sections and their respective tissue sections on which initial diagnosis was made were retrieved from departmental archives and reviewed. Specifics of cytomorphology were correlated with its parent histomorphology in each case. Immunohistochemistry was the principal tool for initial tissue diagnosis. Since the diagnosis was done in a setting with limited resources, no molecular techniques were used.

### *2.1. Effusion fluid processing*

For effusion fluids, including pleural and ascitic fluid specimens, they were centrifuged at 600 g for 10 minutes, and from the sediment, two slides were prepared, which were fixed in alcohol subsequently and stained with the Papanicolaou stain. The remaining sample was used for cell block preparation by the fixed sediment method. 3 ml of 100% methanol was added to the sediment and centrifuged again as stated before. The supernatant was discarded, and thereafter the sediment was kept in 3 ml of 10% NBF (neutral buffered formalin) overnight. The next day, the supernatant was discarded and the sediment was collected to be processed as a small biopsy in the tissue processor.

### *2.2. CSF processing*

The sample was centrifuged at 600 g for 10 minutes. Subsequently, the supernatant was decanted into another tube. 4-6 drops (200-300 ul) of polyethylglycol (PEG) were added to the pilot tube and mixed thoroughly. All the cell suspension was poured into the chamber of the cytospin holder. After charging the sample, the cytospin was spun at 800 rpm for 3 minutes. Following this, the slide and cytofunnel were unclipped. The smear prepared was immediately transferred to 100% methanol. After 20 minutes, the smear was taken out to be stained with Papanicolaou stain.

### *2.3. Reviewed cytomorphologic features*

The description of individual cell morphology was given as either pleomorphic, spindle cell, small round cell, or epithelioid cells. Other features, including cell arrangement, cell size, nuclear, and cytoplasmic details, were also studied and compared to tissue histomorphology.

### *2.4. Immunocytochemistry (ICC)*

ICC was employed to reach a conclusion in cases where involvement of fluid by a sarcoma was unsure at the time of reporting. CB sections (3 $\mu$m thick) were cut and tested for antigen presence with the Ventana Benchmark XT auto-immunostainer and the Ultra View DAB detection kit. Sections were deparaffinized using the EZ prep buffer, and antigen was retrieved by the CC1 buffer at pH 9. All slides are incubated with various antibodies. Different antibodies had their own incubation times. After that, an HRP enzyme-conjugated secondary antibody was added and incubated for 8 minutes before being exposed to 3% H2O2 and DAB chromogen for 8 minutes. Counterstaining with hematoxylin was done, and decolorization with blueing reagent was done for 4 min. Then finally, mounting was done with DPX.

For two patients, both of which showed isolated metastases after a period of remission, an ICC panel was applied on cell blocks. The examination of both cases shared a panel of two epithelial markers, BerEP4 and MOC-31, as well as two mesothelial markers, calretinin and mesothelin. This was done to rule out the development of a second cancer following treatment. Desmin and actin were used to prove LMS involvement, while CD31 and CD34 were utilised to prove involvement by angiosarcoma.

## 3. Results

A total of 21 cases of body fluids involved by sarcoma were identified. Clinical and treatment details of patients are enlisted in Table 1.

Age range of cases in our study varied from 2 to 66 years. 3 out of 21 cases were pediatric, while the rest were either young adults, middle-aged adults, or elderly. There were 19 cases that showed fluid metastasis subsequent to a histologic diagnosis and were under follow-up. Time interval between initial diagnosis and fluid presentation varied from one month to seven years. In two instances, there was a contemporaneous presentation, where biopsies and fluids that were received both tested positive for cancer. In these situations, application of IHC on biopsy was preferred over a cell block to make the initial diagnosis.

The most common fluid to be involved was pleural in 50% (10/21) cases, followed by ascitic 30% (6/21) and CSF 20% (4/21) cases. There were 7 cases of Ewing sarcoma (ES), 4 cases of rhabdomyosarcoma (RMS), 3 cases of high-grade osteoblastic osteosarcoma (OS), 2 cases of malignant

**Table 1:** Patient particulars, histological diagnosis, time to develop effusion and survival details

| Histologic diagnosis (n cases) | Age/ Sex | Primary site | Treatment | Fluid involved | Time interval to develop exfoliation | Survival after fluid reported positive |
|---|---|---|---|---|---|---|
| Ewing sarcoma (6 cases) | 42/M | Rt femur | CT | pleural | 84 months | 3 months |
| | 25/M | Rt Scapula | CT | pleural | 8 months | 1 month |
| | 17/F | Lt scapula | CT | CSF | 3 months | 2 months |
| | 19/F | Lt tibia | CT | CSF | 30 months | Lost to follow up |
| | 13/M | Lt femur | CT | CSF | 9 months | 24 months |
| ES in immature teratoma | 27/F | Retropritoneal mass | CT | ascitic | 6 months | 1 month |
| Osteosarcoma (2 cases) +osteoblastic (osteosarcoma) | 30/F | Rt ovary | CT+ op | ascitic | 6 months | 18 months |
| | 58/M | Rt Femur | CT+ op | pleural | 24 months | 20 days |
| | 16/m | sphenoid | CT | CSF | 16 months | 1 month |
| | 42/M | Lt humerus | CT+ op | ascitic | 9 months | 1 month |
| Rhabdomyosarcoma (3 cases) (1 embryonal +2 alveolar) | 26/M | Urinary bladder | CT+ op | ascitic | 12 months | 3 months |
| | 2/M | Mass on neck | CT | pleural | 5 months | 2 months |
| | 5/M | Mass on shoulder | CT | pleural | 9 months | 8 months |
| Carsinosarcoma with RMS (1 case) | 63/M | uterus | CP | ascitic | CP | 7 days |
| MPNST (2 cases) | 34/M | Rt Shoulder | CT+ RT | pleural | 12 months | 4 months |
| | 54/m | Gluteal region | CT | pleural | 1 month | 1 month |
| SS (2 cases) | 35/M | Lt shoulder | CT | pleural | 15 months | 11 months |
| | 32/m | Rt lung | CP | pleural | CP | Alive last follow up of after a month |
| Chondrosarcoma WD (1 case) | 66/M | Rt femur | Post op | ascitic | 1 month | Lost to follow up |
| LMS (1 case) | 56/F | uterus | CT+ op | pleural | 24 months | 1 month |
| Epithelioid angiosarcoma (1 case) | 33/F | Buccal mucosa | CT+ op | pleural | 22 months | Alive last follow up of after 2 months |

peripheral nerve sheath tumour (MPNST), 2 cases of synovial sarcoma (SS), and 1 case each of chondrosarcoma (CS), leiomyosarcoma (LMS), and angiosarcoma (AS).

Following the identification of fluid involvement, majority (17/21) of the patients died. Survival time varied from seven days to two years. Two cases were lost to follow-up and two cases were alive at the last follow-up. A 13-year-old boy who developed pleural fluid metastases and had been diagnosed with Ewing sarcoma a year back was the longest survivor. He lived on while receiving therapy for a further two years.

Detailed histological, IHC and cytomorphologic analysis for each diagnosis is given in Table 2.

Although certain deviations were noted in morphology of exfoliated cells, prior clinical and histomorphologic information for most of the cases was available in our study which saved time and expense. But it is important to keep in mind that history is never the only factor to take into account; rather, it serves as a tool to facilitate cytomorphologic examination. With the exception of two instances, which presented with isolated metastases after a period of remission, their cytomorphologic and clinical presentation coincided sufficiently to favour involvement by initially diagnosed sarcoma. To evaluate these two cases ICC was applied on cell blocks. In one case tumour cells were positive for CD31 and CD34 (Figure 5c), proving involvement by angiosarcoma. While in other case tumour cells were positive for actin and desmin (Figure 4c) proving involvement by LMS. In both the cases, tumour cells were negative for BerEP4 and MOC-31, with a few background mesothelial cells showing calretinin and mesothelin positivity.

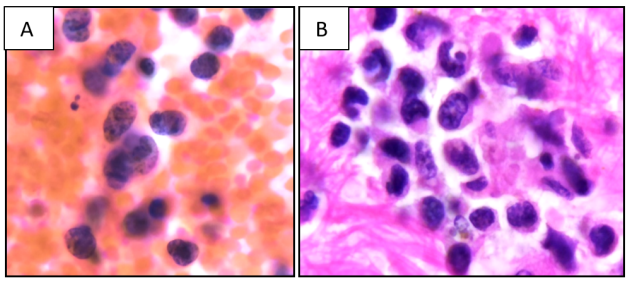


**Fig. 1:** MPNST–**A& B**: epitheliod cells in cytosmears (PAP stain 400X)

## 4. Discussion

Fluid examination for both neoplastic and non-neoplastic reasons may be performed on a cancer patient. Cancerous effusion may result from either direct involvement of bodily cavities, which is known to be extremely uncommon for sarcomas, or via lymphatic involvement. A few researches focused on sarcomatous tumours that exfoliate into bodily fluids and have found that the incidence is less than 1%.

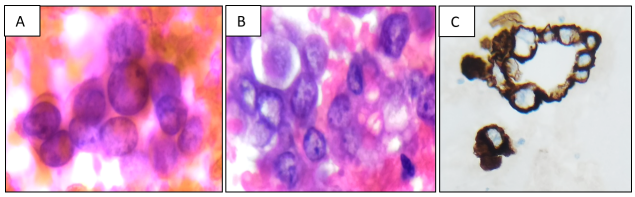


**Fig. 2:** Angiosarcoma **A)**: Epithelioid cells on cytosmear (PAP stain 400X); **B)**: Cell block section showing lumina formation (H&E 400X); **C)**: CD31 positive on cell block (IHC 200x)

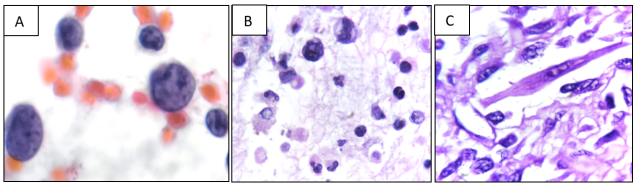


**Fig. 3:** RMS- **A)**: Small to medium sized round cells oncytosmear (PAP stain 400X); **B)**: Pleomorphic and multinucleated cells on cellblock; **C)**: Histology (H&E 400X)

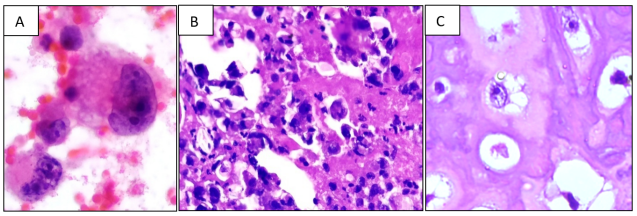


**Fig. 4:** Osteosarcoma–**A**): Bizzare cells on cytosmear (PAP stain 400X); **B)**: Cell block (H&E 200X); **C)**: Histology section (H&E 400X)

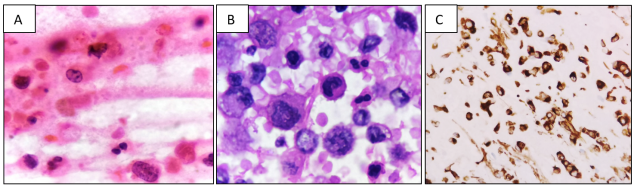


**Fig. 5:** LMS **A)**: Occasional viable nuclei on a necrotic background (PAP stain 400X); **B)**: Bizzare cells with irregular nuclear membrane on cell block (H&E 400X); **C)**: Desmin positive (IHC 200X)

The first series in this area was published by Abadi et al.,[3] who described 24 cases. However, their spectrum was very constrained and included some cases of malignant fibrous histiocytoma, a term that has recently changed in line with our constantly evolving understanding of other soft tissue tumours. The largest study examined 20 years' worth of data from their institute and involved 36 cases of bone and soft tissue sarcomas in CSF and effusion fluids.[4] They had a broader range of cases than we did, such as adipocytic and undifferentiated sarcomas, which we did not see in

**Table 2:** Comparison of histomorphologic, IHC and cytomorphologic details

| Primary histomorphology | Final Diagnosis after IHC | Cytologic features | | | |
|---|---|---|---|---|---|
| | | Cell arrangement | Morphologic category | Nucleus | Cytoplasm |
| Undifferentiated round cell tumor. | CD99: + FLI-1: + LCA-, Synaptophysin-, Desmin – ES | Usually appreciable dual population. Small cells and larger cells (seen on cell block) | Round cell tumor | Smaller cells appear hyperchromatic and larger cells have a comparatively lighter chromatin with inconspicous nucleoli. | Scant, sometimes with vacuoles |
| Small round to spindle and stellate cells | Desmin+, myogenin+, MyoD1+ Embryonal RMS/Alveolar RMS | Predominantly singly scattered with variability in individual cell morphology. | Small round to epithelioid to pleomorphic | Fine to coarse with variable presence of nucleoli. Occasional multinucleation can be seen | Occasional rhabdomyoblast with eccentric nucleus, plenty of eosinophilic cytoplasm sometimes with inclusions. (Figure 1) |
| Spindly to pleomorphic cells | SOX10+, S100 patchy+, Actin –, TLE-caldesmon-, MPNST | Singly scattered or in small groups | Epithelioid to pleomorphic cells | Irregular nuclear membrane Hyperchromatic, coarse chromatin with variably conspicuous nucleoli | Moderate to abundant amount of cytoplasm with ill-defined cell borders (Figure 2) |
| Spindle cell tumor | TLE+, BCL2+, S100-, CD34-, D2-40-, Desmin-SS | Singly scattered cells mainly seen as naked nuclei. Hypo and hyper cellular areas seen on cell block. | Monotonous oval to fusiform elongated cells | Irregular nuclear membrane with nuclear folding. Fine granular chromatin with variably conspicuous nucleoli. | Scant and delicate |
| Pleomorphic cells with evident osteoid | IHC none. High grade OS | Singly scattered (No stroma no osteoid) | Pleomorphic with very bizarre cells | Irregular nuclear membrane with fine chromatin, single to multiple prominent nucleoli | Delicate with poorly defined cell borders (Figure 3) |
| Lobules of neoplastic cartilage with minimal atypia | IHC none. CS- WD | Singly scattered cells (no matrix) | Pleomorphic | Fine chromatin with conspicuous nucleoli | Scant and pale |
| Spindly pleomorphic cells | Desmin+, h-caldesmon+, SOX10-, CD117- LMS | Singly scattered (in necrotic background) (Figure 4) | Pleomorphic with bizzare cells | Irregular thickened nuclear membrane. Coarse chromatin variably prominent nucleoli | Dense moderate to abundant |
| Epithelioid cells forming ill-defined vascular channels | CD31+, CD34+, SOX-10 -, H-caldesmon -, desmin- AS | Singly scattered cells (Figure 5) | Epithelioid to pleomorphic cells | Irregular nuclear membrane. Coarse chromatin prominent nucleoli | Moderate to abundant sometimes with intracytoplasmic lumina. |

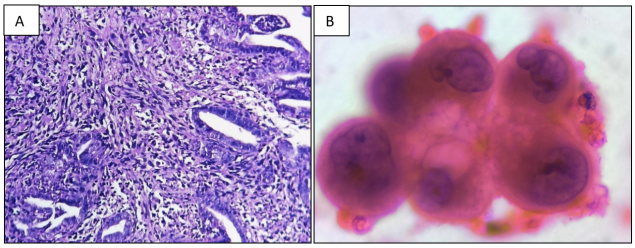


**Fig. 6:** Carcinosarcoma with round cell component; **A)**: Malignant glandular epithelium along with malignant round cells in stroma. Section from uterus (H&E 100X); **B)**: Malignant round to epithelioid cells seen on cytosmears (PAP stain 400X)

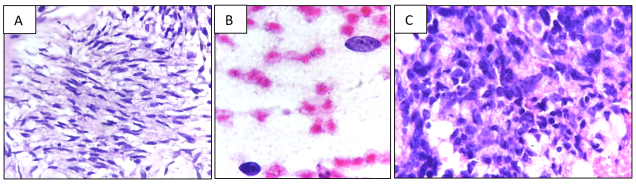


**Fig. 7:** Synovial sarcoma **A)**: Section from lung biopsy (H&E 400X); **B)**: Cytosmear showing occasional scattered naked plump oval nuclei (PAP stain 400X); **C)**: Cell block showing spindle plump cells (H&E 400X)

our study. However, we observed two cases of MPNST exfoliation in pleural fluid, which has only been documented in one other study.[5] Additionally, studies have combined the description of a few rare metastases with sarcomas in fluid cytology, which is only mentioned in one other paper.[5] Several unexpected metastases have also been described in fluid cytology alongside sarcomas.[6]

The childhood group is thought to be the most frequently impacted, according to prior studies, however in our analysis, majority of patients were middle-aged, followed by elderly, young people, and followed by the paediatric population. There were no appreciable age differences in any category in our analysis since there were a relatively small number of cases. According to reports, osteosarcomas are the most prevalent sarcoma in India, although more cases of soft tissue sarcomas were observed peeling into body fluids.[7] This may be because soft tissue sarcomas are a more diversified group than osseous tumours, and as a result, IHC findings are particularly helpful in determining the diagnosis. Therefore, for the two cases with concurrent biopsy and fluid presentation an IHC workup was done on biopsy material. In the first case, a sample of pleural effusion fluid was submitted along with a biopsy from a lung mass. Both showed distinct hypo- and hypercellular areas in addition to monotonous spindle cells (Figure 6). A finding of spindle cell neoplasm was extracted from both specimens, awaiting immunohistochemical analysis of the biopsy material. Diagnosis favouring synovial sarcoma was made when an applied IHC panel revealed positivity for TLE and negative for S100, SOX-10, CD34, STAT-6, D2-40, and AE1. Second was a consultation-based case of uterine carcinosarcoma (Figure 7), identified as having a round cell component. In addition, cytologic analysis of the patient's ascitic fluid revealed pleomorphic round to oval cells with coarse chromatin, noticeable nucleoli, and an abundance of cytoplasm. Actin, Desmin, and MYOD1 were all positively detected by IHC on the histology section for the heterologous component. As a result, carcinosarcoma containing a component of rhabdomyosarcoma was identified. In a related investigation three cases of non-epithelial gynecologic cancer were discovered by Sarah et al.[8] Some tumours may exhibit sarcomatous differentiation or component, and their preferential exfoliation in fluids was noted. Another example of this was a second case of immature teratoma with undifferentiated round cell component. IHC panel applied on tissue sections revealed CD99 and FLI-1 positivity, so a diagnosis of Ewing sarcoma was favoured. The patient was appropriately managed as a result, and six months later she came with an isolated ascitic fluid effusion that was malignant round cell positive. The inference was fluid involvement by Ewing sarcoma, which corresponds to the prior history.

Also, we discovered that Ewing sarcoma exfoliates into body cavities most frequently. This might be due to the fact that, after osteosarcoma, it is the second most prevalent sarcoma in India. Although pleural fluid was the most frequently involved fluid in our analysis, metastatic illness isn't always indicated by fluid involvement. It is viewed as locally progressed disease if the fluid comes from a body cavity in proximity to the tumour, and it is a metastasis if it comes from a different body cavity. This has management repercussions because the goal of treatment for metastasis is palliative care, whereas the goal of treatment for locally advanced disease is comprehensive care.

According to our observations, majority of the sarcomas we saw had cytomorphologies that were epitheliod and/or pleomorphic, with hyperchromatic to coarse chromatin and ill-defined cell boundaries. Given that these tumours are resistant to treatment, the frequent pleomorphism may be a side effect of the treatment or a change into higher grade. In our investigation, fluid cytology revealed plump oval to fusiform shaped cells in tumours with a predominance of spindle cell on histology. This agrees with the information published in the literature.[14] While reviewing the slides, the primary impression for most of the cases was a poorly differentiated carcinoma rather than a sarcoma, except for cases where there was a small round cell morphology. There are no discernible variations between the exfoliative cytomorphology of small round cells and histomorphology. Therefore, fluid cytology does not always lend itself to the broad classification of epithelioid, pleomorphic, small cell, and spindle cell employed in FNAC. Because variable cell populations and patterns may be visible on cell blocks, such

**Table 3:** Differentials for a sarcomatous exfoliation in body fluids

| Differential diagnosis | Similarities to sarcomatous effusion | Morphological differences |
|---|---|---|
| Melanomas [9] | Epithelioid, plasmacytoid to spindly to round cells. | Mostly central nuclei, prominent nucleoli with defined cytoplasmic borders. Melanin pigment can be demonstrated. |
| Lymphomas | Small round cell with scant cytoplasm. Occasional pseudo cohesive clustering may be seen. | Mainly discohesive singly scattered cells with hyperchromatic to clumped nuclei and inconspicuous nucleoli |
| Small cell neuroendocrine carcinoma [10] | Small round cell morphology | Coarsely clumped "salt and pepper chromatin" with inconspicuous nucleoli |
| Desmoplastic round cell tumor | Small clusters of small round cells. | Fine granular chromatin with variably conspicuous nucleoli with delicate cytoplasm sometimes with vacuoles |
| Neuroblastoma [11] | Clusters of small round cells. Sometimes with rosetting. | Coarsely clumped "salt and pepper chromatin". Inconspicuous nucleoli. Occasional neuropil like material |
| Wilms tumor [12] | Small clusters of round cells representing the blastema. | 3-4 times the size of a normal lymphocyte with pale chromatin and conspicuous nucleoli. Sometimes with epithelial element. |
| *Mesothelioma [13] | Epithelioid look with variation in cell size and frequent bi/multinucleation | Cellular smears with large fragments composed of spheres, papillae, berry like morules. Large nuclei with subtle atypia and prominent nucleoli |
| Poorly differentiated carcinoma | Singly scattered or small clusters of epithelioid to pleomorphic cells | Cells with defined cytoplasmic membranes, peripheral orientation of nuclei in clusters vesicular or coarse chromatin, single to multiple prominent nucleoli. Strap cells, tadpole cells or signet ring cells. |

as dual staining of cells in cases of Ewing sarcoma or the formation of an abortive lumina in a vascular tumour, it appears that the common misconception that patterns and stroma are not common features of exfoliative cytology is only partially accurate.

Anytime there is a questionable sarcomatous exfoliation into fluids, a line of potential differentials that can involve fluid should be considered. A large number of non-sarcomatous round cell tumours, such as neuroendocrine tumours, lymphomas, melanomas, and mesenchymal tumours, can make diagnosis challenging especially in the paediatric population (Table 3). Although it is extremely uncommon for sarcomas and their mimics to show together in fluids, it is well recognised that they can coexist with carcinomas and that either one could arise during or after therapy for another cancer. Therefore, in both cases of latent solitary metastasis in our study, IHC was used to rule out the emergence of other cancers such poorly differentiated carcinoma or mesothelioma that might also exhibit epithelioid morphology in addition to supporting the inference of sarcoma recurrence.

When Ewing sarcoma recurred years later, tumour cells had a small round cell shape with focal clustering, favouring involvement by the same with a contemporaneous biopsy was available to demonstrate recurrence.

Aside from the research described above, there are solitary case reports and very few case series in the literature that contribute to our current understanding. Studies from India are also much harder to find. [15–18] Our web research indicates that this is the first Indian paper to talk about encounters with sarcomas in fluid cytology. We have found that sarcomas are fatal if fluid cytology results are positive. Patients with locally advanced illness or metastases did not have significantly different survival rates.

It is essential to make greater use of the chance presented by sarcomas peeling into body cavities to assess their biological and molecular behaviour and use this knowledge to enhance therapeutic care. Increasing the quantity and sample size of such studies would probably be necessary for this.

## 5. Conclusion

Sarcomatous infiltration in fluids is rarely diagnosed. It is believed that changes in cell morphology brought on by exfoliation in fluids can make diagnosis challenging, however our research shows that, with the aid of cell block sections, this morphological gap can be narrowed down which brings the perception closer to parent

histomorphology. Although there were no cases in which a first-hand diagnosis was given on fluid cytology, a thorough evaluation of the morphology and clinical information can help to broadly classify exfoliated neoplastic cells as sarcomatous and plan the necessary ancillary testing should one come across a situation. In times of uncertainty or in the absence of tumour-specific markers, eliminating epithelial malignancy and figuring out the type of background cells can also aid in establishing whether fluid is being affected by a sarcoma. In addition to describing a clinoco-pathologic spectrum, this study also shows how sarcomatous exfoliation is assessed in a country with limited resources, such as India.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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