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Original Research Article

Analysing diagnostic utility and malignancy risk stratification in pleural fluid cytology specimen by application of international system of reporting serous fluid cytopathology

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Abstract

Introduction: Pleural fluid accumulate in the serous cavity as a result of various etiologies and fluid cytology can provide a diagnostic information. In order to standardise the reporting among different laboratories, the International System for Reporting Serous Fluid Cytopathology (ISRSFC) was proposed by the International Academy of Cytology and the American Society of Cytopathology.
Aim of the study: To apply the ISRSFC for reporting pleural fluid cytology and to assess the risk of malignancy of each category
Materials and Methods: All the pleural fluid specimens from January 2019 to August 2021 were retrieved from the data received in the cytopathology lab in the department of pathology JNMC, Aligarh. The cases were reviewed and re-categorised according to ISRSFC into 5 categories: Non-diagnostic (ND), Negative for malignancy (NFM), Aypia of uncertain significance (AUS), Suspicious for malignancy (SFM), and Malignant (MAL). The risk of malignancy (ROM) for each category was then evaluated after correlating cytohistological reports.
Results: Among the total 704 cases we studied, there were 194 ND, 297 NFM, 11 AUS, 59 SFM and 143 MAL cases. The ROM for the categories were found to be 19.5%, 24.9%, 63.63%, 88.13%, and 100% respectively.
Conclusions: Our study highlights the significance of categorising fluids according to ISRSFC, thereby standardising the management protocol as well as better prognostication. It helps in estimating the risk of malignancy in each category. The study also shows significantly higher risk of Malignancy in SFM as compared to AUS, hence supports the separate diagnostic importance of these 2 categories.

Keywords: Aypia, Malignancy, Cytopathology, Malignancy risk.

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1. Introduction

Both the lungs are covered by two membranes, namely parietal and visceral pleural. Pleural cavity is the cavity between these two membranes and accumulation of pleural fluid in this cavity indicates a certain ongoing pathology.[1] Pleural effusion can be related to causes, such as para-malignant effusions, renal or heart failure, medications, pulmonary embolism, malnutrition, hypoalbuminemia, or reduced lymphatic drainage in the setting of thoracic duct obstruction. Therefore, fluid cytology examination is of prime significance in cases of undiagnosed pleural effusion, independent of pleural fluid chemistry.[2] Pleural fluid sensitivity varies according to cancer site and histologic type and varies between 40 to 90%.[3,4]

We already have reporting systems in cytology such as the Bethesda System for Reporting Cervical Cytology, the Bethesda System for Reporting Thyroid Cytopathology, the Paris System for Reporting Urinary Cytology, and the Milan System for Reporting Salivary Gland cytopathology.[5-8] The International Academy of Cytology (IAC) and the American Society of Cytopathology (ASC) proposed the new reporting system named The International System for Reporting Serous Fluid Cytopathology (ISRSFC).[9-11]

The 5 proposed diagnostic categories are nondiagnostic, negative for malignancy (NFM), atypia of undetermined

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significance (AUS), suspicious for malignancy (SFM), and malignant (MAL). The 5 proposed diagnostic categories are nondiagnostic, negative for malignancy (NFM), atypia of undetermined significance (AUS), suspicious for malignancy (SFM), and malignant (MAL). This tiered system helps to improve communication between the clinicians and cytopathologists, which directs them to better patient management and therapeutic strategies.[1,12]

In our study we recategorised pleural fluid samples into the categories recommended by ISRSFC classification system. The risk of malignancy for each of the proposed categories was also calculated.

2. Materials and Methods

All the pleural fluid specimens from January 2019 to August 2021 were retrieved from the data received in the cytopathology lab in the department of pathology JNMC, Aligarh. Other respiratory specimens like sputum, and those obtained by bronchoalveolar lavage and bronchial washings were excluded from this study. Patient's age, sex, clinical details and imaging are also noted. The cytology smears are reviewed for the cytosmear findings.

The samples were centrifuged and the supernatant discarded. Sediment obtained was resuspended in 2ml of CytoRich Red fluid. The vials were then vortexed for 1-2 mins at 3000rpm. Vortexing randomizes the sample and disintegrates any cell clusters. 6 ml of Tris buffer was then added. We then vortexed and transferred the entire content into a 12 ml tube. The complete assembly was put into a centrifuge bucket. Centrifugation was done at 1050 rpm for 2 mins.

Smear fixed in 95 % alcohol are rehydrated by dipping them sequentially in descending order of ethyl alcohol (80%, 70% and 50%) and then in distilled water. (10 dips in each). Stained with Harris's Haemotoxylin for approximately 6 minutes. Rinsed in tap water -2 rinses of 10 dips each. Differentiated in 0.25% aqueous hydrochloric acid (6 dips each) until the cytoplasm is decolourised and only the nuclei retain the stain. Blueing is done in tap water or lithium carbonate until the desired staining intensity was reached. Next, slides are passed sequentially through ascending grades of ethyl alcohol (50%,70% 80% and 2 changes of 95%) -10 dips in each, to ensure complete dehydration.

Stained with OG-6 for approximately 2 minutes. Dipped in 95 ethyl alcohol-3 changes of 10 dips each.

All the cytosmears are examined are examined. Recategorisation was performed using ISRFSFC classification, and cases were allocated to one of the five proposed categories. Different criteria are used for allocating into each categories.

2.1. Criteria used for recategorisation

2.1.1. Non diagnostic (ND)

This category includes all smears of hemorrhagic, scant cellularity (benign cells usually <10cells). These cells included macrophages, mesothelial cells, lymphocytes and RBCs.

2.1.2. Negative for malignancy (NFM)

This category includes different cell types of benign macrophages, lymphocytes and mesothelial cells. Cells shown no evidence of malignancies.

2.1.3. Atypia of undetermined significance (AUS)

This category included few atypical cells or atypical lymphocytes or atypical mesothelial cells or atypical cells of unknown origin. However, cytology features are not enough to keep in SFM and low cellularity to keep in NFM.

2.1.4. Suspicious for malignancy (SFM)

This includes atypical cells, atypical lymphocytes, atypical mesothelial cells. These are markedly atypical cells or specimen collected from highly suspicious patient or suspicious of malignancy on radiology. But we're quantitatively insufficient to put in the malignant category.

2.1.5. Malignancy (MAL)

This category includes highly cellular or large tissue fragments in the smears having either primary and secondary malignant cells. Cells show nuclear enlargement, irregular nuclear membrane, macro nucleoli, frequent binucleation, cellular pleomorphism, atypical mitosis.

2.2. Risk of malignancy

Presence or absence of malignancy in each case is confirmed by available histopathological tissue diagnosis. The risk of malignancy (ROM) for each category was calculated. The ROM was calculated using the following formula:

The number of patients with a tissue diagnosis of malignancy * 100/total number of cases in a given category with available follow up tissue diagnosis.

3. Results

Total 704 cases are studied over a period of 3 years (January 2019 to August 2021). Age of the patients ranged from 4years to 90years. Most of the patients were males (n=492). Female accounts for n=212. The volume of fluid ranged between 1-100ml.

3.1. Recategorisation

The fluid cytology diagnosis varied from one pathologist to another in our hospital. Out of the total cases, 189 (26.8%) cases were inadequate for opinion, which included mainly hemorrhagic cases and scant cells. No strict criteria were used

for classification. Benign cases were 303 (42.8%), suspicious for malignancy were 69 (9.9%). After recategorisation few cases from benign group were shifted to Non diagnostic. The total 69 cases of suspicious of malignancy were redistributed to atypia of undetermined significance (11 cases 1.6%) and suspicious for malignancy (59 cases 8.3%). Malignant cases were 143 (20.3%), both before and after recategorization (**Table 1**).

Table 1:

ISRFSC categories	n (%)	Total no of follow up	Risk of Malignancy
Non-diagnostic (ND)	194 (27.5%)	38 (malignant)	19.5%
Negative for malignancy (NFM)	297 (42.1%)	74 (malignant)	24.9%
Atypia of undetermined significance (AUS)	11 (1.6%)	7 (malignant)	63.63%
Suspicious for malignancy (SFM)	59 (8.3%)	52 (malignant)	88.13%
Malignant (MAL)	143 (20.3%)	143 (malignant)	100%

3.2. Categories

3.2.1. Non diagnostic

After recategorisation 194 cases were reported as non-diagnostic. Most the cytosmear of this category showed only haemorrhage. Few cases shown scattered lymphocytes or degenerated mesothelial cells. **Figure 1** shows two cases diagnosed as non-diagnostic.

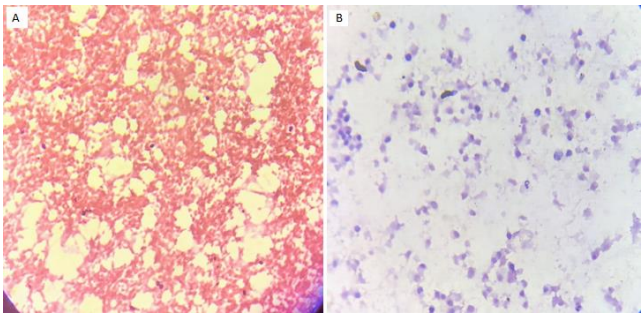

Figure 1: Two cases categorized as "non-diagnostic". **A:** Cytospin smear shows blood only; **B:** Smear shows very few scattered lymphocytes only.

3.2.2. Negative for malignancy

297 cases were reported as negative for malignancy. Smears shown mesothelial cells, neutrophils and lymphocytes. No atypical cells were seen. Main underlying cause of pleural effusion in this category were infective origin. **Figure 2** shows 2 cases categorised as NFM.

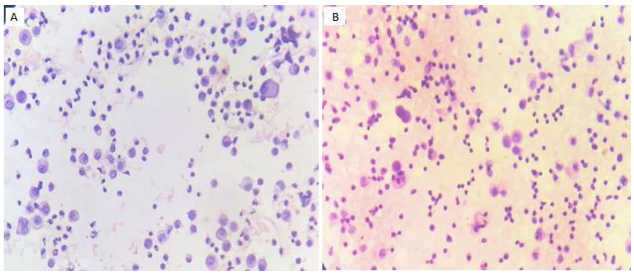

Figure 2: Two cases categorized as Negative for malignancy; **A:** Cytospin smear shows adequate cellularity with presence of reactive mesothelial cell, macrophages and mixed inflammatory infiltrates; **B:** Cytospin smear shows adequate cellularity with presence of mesothelial cells, macrophages and dense lymphocytic infiltrates

3.2.3. Atypia of undetermined significance

11 cases out of 704 cases are categorised as AUS. Smears shown reactive mesothelial cells with lymphocytes, and occasional large atypical cells. These typical cells are of epithelial in origin. Atypical cells are having increased N:C ratio, hyperchromasia, irregular nuclear membrane. **Figure 3** and **Figure 4** shows 2 cases categorised as AUS.

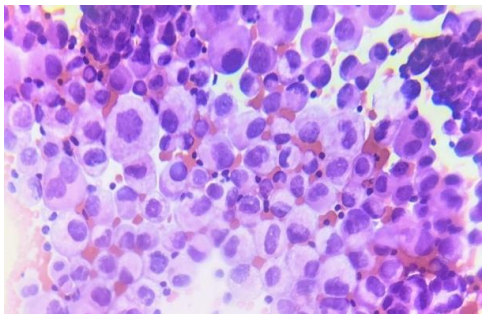

Figure 3: A case categorized as atypia of uncertain significance. Cytospin smear shows few reactive mesothelial cells, lymphocytes and occasional larger atypical cell.

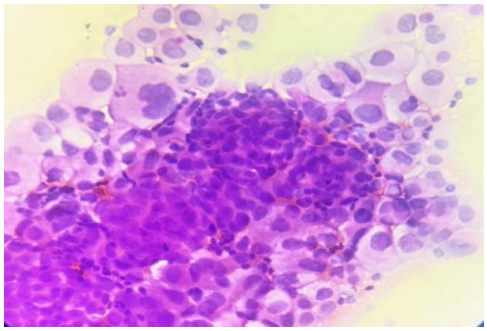

Figure 4: Cytospin smear shows few reactive mesothelial cells, lymphocytes and occasional larger atypical cell

3.2.4. Suspicious for malignancy

59 cases were diagnosed as SFM. These smears shown mostly reactive mesothelial cells, lymphocytes along with few atypical cells trying to form a cluster also seen (**Figure 5**a and b).

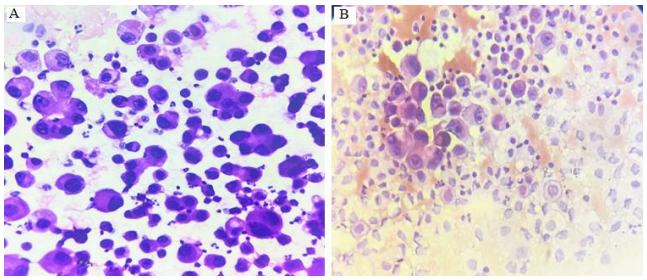

Figure 5:

2 cases categorized as Suspicious for malignancy. Cytospin smear show low cellularity, with mostly reactive mesothelial cells, macrophages and lymphocytes. Few scattered larger cells were seen having enlarged hyperchromatic nuclei

3.2.5. Malignancy

143 cases were diagnosed as malignancy. These cases were mostly known case of lung carcinoma followed by, breast carcinoma and ovarian carcinoma (**Figure 6**a and b).

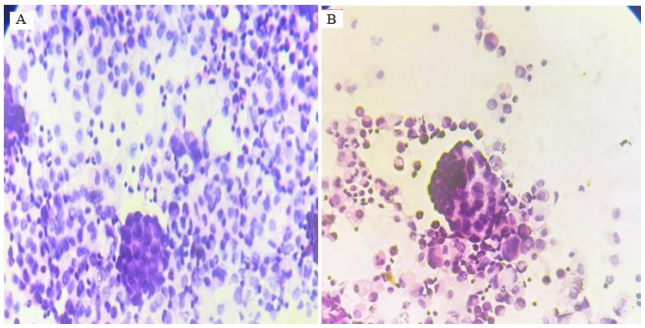

Figure 6:

2 cases categorized as malignant. Cytosmears were highly cellular and showed cohesive ball and papillae like clusters of cells with enlarged hyperchromatic nuclei

3.3. Follow up and risk of malignancy

Risk of malignancy is calculated after follow up with the available histopathological tissue diagnosis (**Figure 7** and **Figure 8**). Among the 194 Non diagnostic cases 38 cases came for follow up and diagnosed as malignant. In NFM 74 cases shown malignancy on HPE. AUS shown 63.3% ROM. SFM and MAL shown 88.13% and 100% ROM respectively (**Table 2**).

Table 2:

ISRFSC categories	n (%)	Total no of follow up	Risk of Malignancy
Non-diagnostic (ND)	194(27.5%)	38(malignant)	19.5%
Negative for malignancy (NFM)	297(42.1%)	74(malignant)	24.9%
Atypia of undetermined significance (AUS)	11(1.6%)	7(malignant)	63.63%
Suspicious for malignancy (SFM)	59(8.3%)	52(malignant)	88.13%
Malignant (MAL)	143(20.3%)	143(malignant)	100%

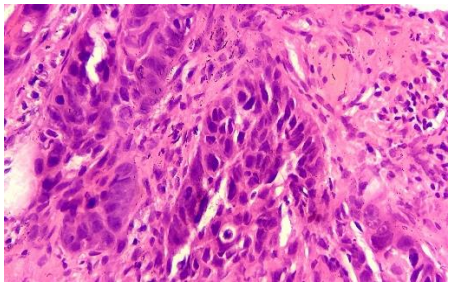

Figure 7:

A case diagnosed as SFM on fluid cytology, on HPE showing Moderately differentiated squamous cell carcinoma. H and E stained section shows atypical cells showing pleomorphism, increased N:C ratio, hyperchromatic nuclei and moderate amount of eosinophilic cytoplasm. Focal keratinization is seen.

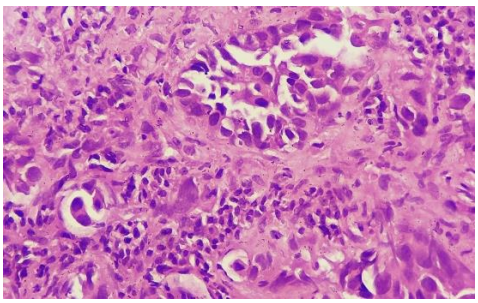

Figure 8:

A case diagnosed as MAL on fluid cytology, on HPE showing Adenocarcinoma. H and E stained section shows irregular glands lined by atypical cells showing pleomorphism, increased N:C ratio, hyperchromatic nuclei and scant cytoplasm.

4. Discussion

In this study we applied the new ISRSFC reporting for recategorisation of pleural fluid cytosmear. Our study consisted of 194 (27.5%) non diagnostic cases, 294 (42.1%) negative for malignancy, 11 cases (1.6%) AUS, 59 cases (8.3%) SFM, and 143 cases (20.3%) malignant cases. The number of cases increased in the non-diagnostic category from 180 to 194. The number of benign cases reduced after the reclassification from 303 to 297. This can be due to the adequacy criteria, i.e hemorrhagic smears, scant cellularity with <10cells, scattered, mesothelial cells, macrophages, lymphocytes or polymorphs are categorised as Non diagnostic.

The majority of the samples in this study, which is similar to Rodriguez et al., 2020, were classified as NFM.[13]

This may be caused a centre receives majority of the cases as reactive and infective aetiologies.

In light of very low cellularity that is insufficient for producing an absolute negative result, certain patients that were earlier classified as negative for malignancy were reclassified into the Non diagnostic category. Atypical cells in instances labeled as benign were previously missed during slide review, which resulted in their reclassification as AUS and SFM.

The risk of malignancy ROM for the categories ND, NFM, AUS, SFM and MAL were found to be 19.5%, 24.9%, 63.63%, 88.13% and 100% respectively. Our study is similar to Farhani et al.2019, the risk of malignancy for ND, NFM, AUS, SFM and MAL was 17.4%, 20.7%, 65.9%, 81.8% and 98.9% respectively.[1]

The ROM in our study is similar to the ROM given by ISRSFC, i.e % ROM (SE) for ND, NFM, AUS, SFM and MAL are 17% (± 8.9%), 21% (± 0.3%), 66% (± 10.6%), 82% (± 4.8%) and 99% (± 0.1%) respectively.[14]

In our study the ROM of AUS and SFM were 63.63% and 88.13% respectively, which was similar to the studies done by Farhani et al., 2019 and Jha et al.,2021.[1,15] But the difference between the ROM of two categories in our study was not statistically significant (p value 0.175). This is similar to jha et al.,2021 and contrary to the studies done by Hou et al.,2021.[12,15]

Malignant cases in our study were 20.3% and ROM was 100%, because all the cases were able to follow up on HPE. ROM in our study in the malignant category is according to ISRSFC and the study done by Jha et al.,2021.[14,15]

5. Conclusion

The current study highlights the significance of categorising fluids according to ISRSFC. Thereby standardising the management protocol as well as better prognostication. It helps in estimating the risk of malignancy in each category. The study also shows significantly higher risk of malignancy in SFM as compared to AUS, hence supports the separate diagnostic importance of these two categories.

6. Conflict of Interest

None.

7. Source of Funding

None.

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