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CATEGORIZATION OF SEROUS EFFUSIONS USING THE INTERNATIONAL SYSTEM FOR REPORTING SEROUS FLUID CYTOPATHOLOGY AND ASSESSMENT OF RISK OF MALIGNANCY WITH DIAGNOSTIC ACCURACY

Pathology

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ABSTRACT

Serous effusion cytology is a key diagnostic tool for evaluating pleural, peritoneal, and pericardial effusions. It is rapid, cost-effective, and minimally invasive, yet presents challenges, particularly in distinguishing malignant cells from reactive mesothelial cells due to overlapping features. The International System for Reporting Serous Fluid Cytopathology (ISRSFC) was recently introduced to address these challenges by standardizing reporting and enhancing risk stratification.

KEYWORDS

Serous Effusion Cytology, International System for Reporting Serous Fluid Cytopathology (ISRSFC), Risk of Malignancy (ROM), Diagnostic Accuracy.

INTRODUCTION

Serous cavity fluid is one of the most frequently received specimen types in cytopathology laboratories for high-sensitivity preliminary assessments of effusion aetiology. Serous effusion is a common symptom of various illnesses, including inflammatory, benign, and malignant neoplasms. Between 10% and 60% of all effusions are due to malignancy. The majority of malignant effusions arise from metastases of adenocarcinomas from the lungs, breasts, gastrointestinal tract, and genital tract, including the ovaries. Additionally, between 10% and 53% of malignant mesothelioma cases present with serous effusion alongside metastases. In many instances, serous effusions may be the first or only indication of cancer.[1,2]

Most cytology samples commonly encountered in practice come from serous effusions located in the peritoneal, pleural, and pericardial cavities. These samples offer an easy-to-understand, relatively non-invasive, and economical approach to aiding patient management. Serous effusions reflect a broad spectrum of clinical conditions, each having its own distinct cellular composition and originating from either non-neoplastic or neoplastic sources. Causes of serous effusions can vary widely, including cancer, autoimmune disorders, and metabolic conditions. Malignancy accounts for 10% to 25% of serous effusions. Cytological analysis of these effusions is a simple, cost-effective, and minimally invasive method for determining malignancy and guiding further patient care. In cases of atypical cells, additional methods such as immunocytochemistry, flow cytometric immunophenotyping, and molecular analysis may be employed for further typing and confirmation.[3]

In 2020, the International System for Reporting Serous Fluid Cytology (ISRSFC) was introduced to standardize the reporting of cytological findings in serous effusions. The initiative, developed through the collaboration of experts in cytopathology and related fields, aimed to ensure clarity, consistency, and uniformity in diagnostic practices. The ISRSFC promotes better communication among healthcare providers and enhances patient care by providing a standardized framework for interpreting serous fluid cytology results.

ISRSFC proposes five diagnostic categories: Nondiagnostic (ND), Negative for Malignancy (NFM), Atypia of Undetermined Significance (AUS), Suspicious for Malignancy (SFM), and Malignant (MAL). Specimens classified as AUS exhibit enough morphological features to rule out being nondiagnostic but lack the qualitative or quantitative cytological traits needed to be clearly identified as benign or malignant.[4,5]

The SFM category includes specimens showing cytological features commonly associated with malignancy, but that are not sufficient in either quantity or quality to confirm a malignancy diagnosis. Studies by Hou et al. show a significant difference in the risk of malignancy (ROM) between SFM and AUS ($P < 0.01$), underscoring the importance of classifying these two groups separately.[6]

The ISRSFC categories are clearly defined as follows:[7]

- **I. Nondiagnostic (ND):** Insufficient cellular elements for a cytologic interpretation.
- **II. Negative for Malignancy (NFM):** No evidence of mesothelial or non-mesothelial malignancy.
- **III. Atypia of Undetermined Significance (AUS):** Slight cellular atypia (nuclear/architectural), e.g., papillary clusters.
- **IV. Suspicious for Malignancy (SFM):** Features are suspicious but not definitively diagnostic for malignancy.
- **V. Malignant (MAL):** Definitive findings and/or supportive studies indicating mesothelial or non-mesothelial malignancy.

MATERIAL AND METHODS

All fluids, regardless of amount, that were obtained and drawn into the laboratory were handled promptly. If the fluids could not be processed right away for technical reasons, they were kept in a refrigerator at 4oC and processed at a later time. The fluids were observed visually for their volume, color, and appearance, and the results were recorded.

Processing Of Fluids:

All fluids (irrespective of volume), received and aspirated in the laboratory were processed at the earliest. If the fluids were not processed on time, they were stored in a refrigerator at 4°C and processed later. The fluids were examined grossly for volume, color and appearance and findings were noted. After that a conventional smear was made.

Conventional Smear:

The regular smear involved spinning the fluids at 2500 rpm for 10 minutes in plastic test tubes and pouring off the supernatant. At least two thin smears were made from the sediment and both H&E staining and Giemsa staining were performed on the centrifuged smears.

The **Haematoxylin and Eosin (H&E) staining** procedure requires specific reagents, including Harris Haematoxylin, 1% Eosin, and 1% Acid alcohol. The technique begins with fixing the smear in 95% alcohol for 15 minutes, followed by washing with water. The smear is then stained with Harris Haematoxylin for 5 minutes and rinsed with

water. Afterward, the slide is dipped in 1% Acid alcohol, rinsed again with running tap water until bluing occurs, and counterstained with 1% Eosin. For dehydration, the slide is rinsed sequentially in 70% alcohol for 30 seconds, 90% alcohol for 90 seconds, and absolute alcohol for 30 seconds. The final steps involve rinsing the slide in two changes of xylene, clearing, and mounting it with D.P.X.

The **Leishman-Giemsa cocktail stain** involves preparing a Giemsa working solution by mixing an equal volume of Giemsa stain with water (1:1 dilution). Then, an equal volume of filtered Leishman's stain is mixed with the Giemsa working solution. After air-drying, the smears are flooded with the Leishman-Giemsa cocktail for one minute. An equal volume of buffer or distilled water is then added and left on the slides for six minutes. Finally, the slides are washed with tap water, dried, cleared, and mounted for examination.

RESULTS

In the present study, a total of 273 serous effusion samples were analyzed, with 202 samples (74%) being pleural fluid and 71 samples (26%) being peritoneal fluid**.** The analysis focused on age, gender, area of residence, histological confirmation, and volume of effusions to assess the prevalence and characteristics of malignant versus non-malignant cases.

Age Distribution:

A distinct age-related pattern in the occurrence of malignancy was observed. Among younger age groups (≤20 and 21-30 years), all cases were found to be non-malignant, both in clinical follow-up and cytological findings, where "Negative for Malignancy" (NFM) diagnoses predominated. In the middle age groups (31-50 years), a gradual increase in malignancy was noted, with 2.2% malignancy in the 31-40 years group and 5.1% in the 41-50 years group. Additionally, cytological categories such as Atypia of Undetermined Significance (AUS) and Suspicious for Malignancy (SFM) started to appear more frequently. Among older age groups (51-70 years and >70 years), the prevalence of malignant findings rose significantly. Malignancy was identified in 16.3% of cases in the 51-60 years group, 22.2% in the 61-70 years group, and 20.0% in those over 70 years. Cytological findings in these groups mirrored this trend, with more frequent diagnoses of suspicious and malignant cases.

Gender Distribution:

The study included more male participants (162 cases or 59.3%) compared to females (111 cases or 40.7%). However, females exhibited a higher percentage of malignancy (13.5%) than males (2.5%). Among females, genital tract malignancies, particularly ovarian cancers, were the most common cause of malignant effusions. The higher malignancy rate in females likely reflects the prevalence of ovarian and other genital tract cancers that often present with malignant effusions.

Area of Residence:

A comparison between rural and urban populations revealed a slightly higher prevalence of malignancy in urban areas (7.4%) compared to rural areas (6.6%). This difference may be attributed to better access to healthcare services, earlier detection, and more advanced diagnostic tools available in urban areas, which could lead to earlier identification of malignancies.

Histological Correlation:

Histological confirmation of malignancy was available in 40 cases (14.7%). Of these, 11 cases (4.1%) were confirmed as malignant, while 29 cases (10.6%) were confirmed as non-malignant. The remaining 233 cases (85.3%) did not undergo histological examination. Among the confirmed malignant cases, 7 were peritoneal fluids from females, with 5 originating from ovarian malignancies, including mucinous cystadenocarcinoma, serous carcinoma, and papillary adenocarcinoma.

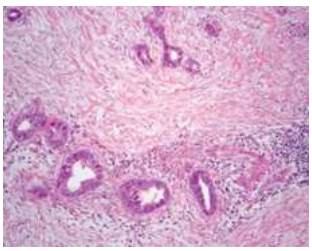

Figure 1: Photomicrograph showing adenocarcinoma of the gall bladder on histopathology from the case diagnosed as suspicious for malignancy on cytology. (H&E X100)

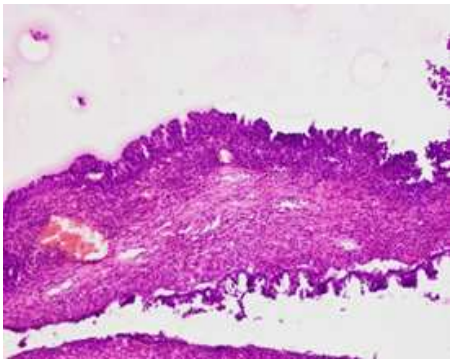

Figure 2: Photomicrograph showing papillary adenocarcinoma ovary on histopathology from a case diagnosed as malignant on cytology.(H&E x100)

The other peritoneal malignancies included adenocarcinomas of the colon and gall bladder. Four pleural fluid cases were confirmed as adenocarcinomas of the lung. These findings further emphasized the higher malignancy rate among females due to the predominance of genital tract cancers.

Effusion Volume:

The volume of serous effusions showed a noticeable correlation with malignancy. Malignant cases were more frequent in larger volume samples, particularly those greater than 20ml, where 40% of cases were malignant. The lowest percentage of malignancy (3.27%) was observed in the 11-15ml volume range.

DISCUSSION

In the present study, 16.11% of cases were classified as non-diagnostic which is notably higher than most studies, except for the study done by Mandava et al.,[13] in the same year that is 2024 which reported an exceptionally high (82.2%) than the present study.

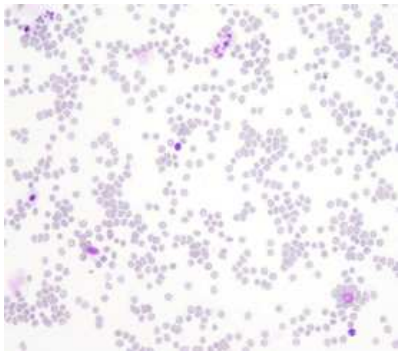

Figure 3 : Photomicrograph shows a non diagnostic (ND) pleural effusion cytology because of haemorrhage and absence of mesothelial cells.(Giemsa stain x 100)

For cases negative for malignancy, the present study reported 73.62%, a figure that aligns closely with study done by Kolte s et al[9] in 2022, Rakheja G et al[10] in 2023, and Kala et al[20] in 2023, who in their study stated the number of cases belonged to negative for malignancy as 71.6%, 72.7% and 75.75% respectively.

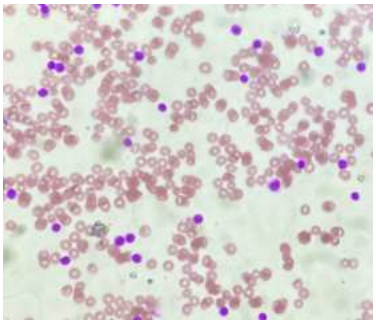

Figure 4 : Photomicrograph shows negative for malignancy (NFM) peritoneal effusion showing predominantly lymphocytic population and haemorrhage. (Giemsa x400)

The category of atypia of undetermined significance accounted for 4.39% of cases in the present study. This finding of our study is slightly higher than the other studies done in past like Kolte s et al[9](2.4%) in 2022, Rakheja G et al[10](3.2%) in 2023, and Kala et al[20](0.51%) in 2023. This could be due to difference in expertise of different pathologists in reporting according to ISRSFC.

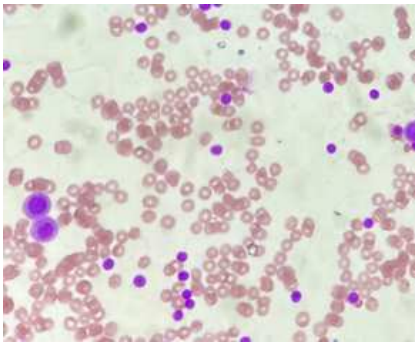

Figure 5 : Photomicrograph shows atypia of undetermined significance (AUS) pleural effusion showing occasional atypical mesothelial cell with binucleation along with lymphocytes.(Giemsa x400)

In terms of cases suspicious for malignancy, the present study reported 2.19% of the cases, which is similar to the studies done by Sachan R et al[11](2.3%), Rakheja G et al (1.5%) and Kala et al[12](2.46%).

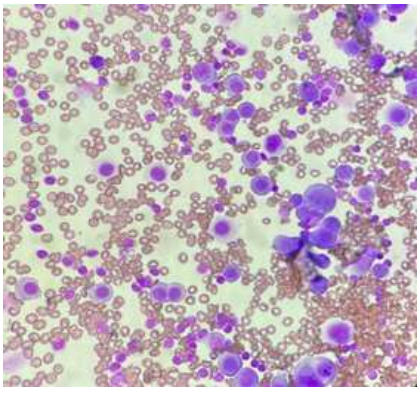

Figure 6: Photomicrograph of suspicious for malignancy (SFM) peritoneal effusion sample showing atypical mesothelial cells present in clusters showing pleomorphism, high nuclear to cytoplasmic ratio from a patient of adenocarcinoma gall bladder confirmed on histopathology. (Giemsa x400)

Finally, the percentage of malignant cases in the present study was 3.66%, which is almost similar to the study done by Amita K et al[14] in 2024mandava, reported 3.82% of the total cases. Zhu YL et al.[8] reported the highest of the total cases at 47.8%.

Overall, the present study shows higher rates of non-diagnostic cases and lower rates of confirmed malignant cases compared to similar studies, while the distribution of negative for malignancy and atypia of undetermined significance cases falls within the range observed across different studies.

Table 1: The Comparision Of The Distribution Of Our Samples According To Isrsfc.

Categories	Present study(%)	Zhu YL et al	Kolte S et al	Rakheja G et al	Sachan R et al	Kala et al	Mandava et al	Amit K et al
Non Diagnostic	16.11	0.5	2	15.2	2.5	0.17	82.2	4.6
Negative for malignancy	73.62	30.3	71.6	72.7	71	75.75	8.6	88.51
Atypia of undetermined significance	2.19	18.6	4.7	1.5	2.3	2.46	4.8	0.85
Suspicious of malignancy	3.66	47.8	19.3	7.3	22	21.11	8.6	3.82
Malignant								

Overall, the present study demonstrates lower ROM for non-diagnostic and negative for malignancy categories compared to most other studies, while maintaining consistent high ROM in the suspicious of malignancy and malignant categories.

Table 2: The Comparison Of Overall Risk Of Malignancy Of Our Samples And Comparison With Other Studies.

cc	Present study(%)	Zhu YL et al(%)	Kolte S et al(%)	Rakheja G et al(%)	Sachan R et al(%)	Kala et al(%)	Mandava et al(%)	Amit a K et al(%)
Non Diagnostic ROM	2.3	38.5	23	16.9	57.1	25	25	0
Negative for malignancy ROM	0.5	28.6	25	12.1	9.9	17.9	8.5	0
Atypia of undetermined significance ROM	8.3	52.1	56	50	66.7	66.7	50	40
Suspicious of malignancy ROM	100	99.4	80.6	80	66.7	75.4	77	100
Malignant ROM	100	100	90	100	97.2	96.5	100	100

DISCUSSION

In the present study, 16.11% of cases were classified as non-diagnostic, which is notably higher than most studies, except for the study done by Mandava et al.,[13] in the same year that is 2024 which reported an exceptionally high (82.2%) than the present study. For cases negative for malignancy, the present study reported 73.62%, a figure that aligns closely with study done by Kolte s et al[9] in 2022, Rakheja G et al[10] in 2023, and Kala et al[12] in 2023, who in their study stated the number of cases belonged to negative for malignancy as 71.6%, 72.7% and 75.75% respectively. The category of atypia of undetermined significance accounted for 4.39% of cases in the present study. This finding of our study is slightly higher than the other studies done in past like Kolte s et al[9](2.4%) in 2022, Rakheja G et al[10](3.2%) in 2023, and Kala et al[12](0.51%) in 2023. This could be due to difference in expertise of different pathologists in reporting according to ISRSFC.

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Overall, the present study shows higher rates of non-diagnostic cases and lower rates of confirmed malignant cases compared to similar studies, while the distribution of negative for malignancy and atypia of undetermined significance cases falls within the range observed across different studies.

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REFERENCES

1. DeBiasi EM, Pisani MA, Murphy TE, Araujo K, Kookoolis A, Argento AC, et al. Mortality among patients with pleural effusion undergoing thoracentesis. Eur Respir J. 2015;46:495-502
2. Shidham VB, Layfield LJ: Approach to diagnostic cytopathology of serous effusions. Cytojournal. 2021, 18:32.
3. Thakur N, Alam MR, Abdul-Ghafar J, Chong Y. Recent application of artificial intelligence in non-gynecological cancer cytopathology: A systematic review. Cancers. 2022;14:3529.
4. Chandra A, Crothers B, Kurtycz D, Schmitt F. Announcement: The International System

for Reporting Serous Fluid Cytopathology. Acta Cytol. 2019;63:1–3.
5. Hou T, Landon G, Stewart J, Roy-Chowdhuri S. The value of a tiered cytology diagnostic reporting system in assessing the risk of malignancy in indeterminate serous effusions. Cancer Cytopathol. 2021;129(1):75–82.
6. Chandra A, Crothers B, Kurtycz D, Schmitt F. Announcement: The International System for Reporting Serous Fluid Cytopathology. Acta Cytol 2019;63(5):349–351.
7. Pinto D, Chandra A, Schmitt F. The International System for Reporting Serous Fluid Cytopathology: How to Incorporate Molecular Data in Cytopathology Reports. Journal of Molecular Pathology. 2021;2(2):66-76.
8. Zhu YL, Ren WH, Wang Q, Jin HZ, Guo YY, Lin DM. A retrospective analysis of serous effusions based on the newly proposed international system for reporting serous fluid cytopathology: a report of 3633 cases in an oncological center. Diagnostic Pathology. 2022;2;17(1):56.
9. Kolte S, Zaheer S, Aden D, Ranga S. Application of the international system for reporting serous fluid cytopathology on reporting various body fluids; experience of a tertiary care hospital. Cytojournal. 2022; 19:52.
10. Rakheja G, Singh M, Priyadarshnee B, Marimuthu B, Dhar L, Jain S, Khurana N, Rathore A. Categorisation of peritoneal serous effusions using the International System for Reporting Serous Fluid Cytopathology—a study on gynaecological samples. Cytopathology. 2023;34(2):138-45.
11. Sachan R, Gupta A, Awasthi PN, Singh P, Anand N, Chandra S, Gaur G, Husain N, Sachan KD. Application of international system for reporting serous fluid cytology (ISRSFC) in effusion samples-a prospective study in an oncology setting. Journal of the American Society of Cytopathology. 2023;12(5):351-61.
12. Kala C, Kala S, Singh A, Jauhari RK, Bajpai A, Khan L. The International System for Reporting Serous Fluid Cytopathology: An Institutional Experience on its Implication and Assessment of Risk of Malignancy in Effusion Cytology. Journal of Cytology. 2023;40(4):159-164.
13. Mandava H, Renuka IV, Potti R, Mounica B, Kalla I. Deciphering Serous Effusions Using the New International System for Reporting Serous Fluid Cytopathology. Cureus. 2024;16(5).
14. Amita K, Suresh R, Kalappa P, Sanjay M. Effectiveness of International System for Reporting Serous Fluid Cytology in Routine Practice – A Cross Sectional Study at a Tertiary Care Centre. Online J Health Allied Scs. 2024;23(1):7.