DOI: 10.7860/NJLM/2025/73145.2889

Original Article

Pathology Section

Application of International System for Reporting Serous Fluid Cytology and Risk of Malignancy Assessment in Serous Effusion: A Cross Sectional Study

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ABSTRACT

Introduction: Serous fluid cytology is a safe, effective and minimally invasive procedure helping in the early diagnosis and prognosis of malignant effusions. However, due to the lack of a uniform reporting system and the morphological overlap between certain reactive and malignant conditions, there are limitations. The International System for Reporting Serous Fluid Cytology (ISRSFC) divides the serous effusions into five categories to assist in diagnosing and managing cases of serous effusion according to the Risk of Malignancy (ROM) in different categories.

Aim: To reclassify serous fluids according to the ISRSFC and evaluate the ROM in each category.

Materials and Methods: The present retrospective cross-sectional study was conducted in the Department of Pathology, Chirayu Medical College and Hospital, Bhopal, Madhya Pradesh, India, from January 2023 to December 2023. In the present study, serous effusion cases were cytologically evaluated and categorised according to the ISRSFC, and the ROM for each category was calculated. A total of 570 cases of serous fluids were evaluated cytologically and classified according to the ISRSFC. The ROM in different categories was calculated. Data was presented as number and percentage.

Results: The age of the patients ranged from 12-81 years, with a mean age of 43 years. The volume of the samples ranged from 1-1000 mL. The ROM in different categories for pleural, peritoneal, and pericardial fluid, respectively, was as follows: Category I {Non Diagnostic (ND)} constituted 1/15 (6.6%) and 1/19 (5.2%); Category II {Negative for Malignancy (NFM)} showed 3/186 (1.6%), 4/220 (1.8%), and 0/3 (0%); Category III {Atypia of Undetermined Significance (AUS)} comprised 1/4 (25%) and 0/3 (0%); Category IV {Suspicious for Malignancy (SFM)} showed 81.8% (9/11) and 75% (6/8); and in Category V {Malignant Lesions (MAL)}, the ROM was 100% (20/20, 22/22, 2/2) for all.

Conclusion: The ISRSFC is a standardised and effective system for serous fluid cytology. It provides consistent reporting terminology and better communication with clinicians and thus improving patient care. In the present study, the authors assessed the efficacy of this novel classification both as a means of communication between clinicians and pathologists and as a guideline for patient management.

Keywords: Atypical cells, Peritoneal fluid, Malignant, Non diagnostic

INTRODUCTION

Serous fluid is an ultrafiltrate of plasma present between serous membranes. An increase in fluid, termed effusion, can accumulate in serous cavities due to various neoplastic and non neoplastic conditions, such as hypoproteinemia, organ failure, infection, inflammation and tumours [1-3]. Serous fluid cytology is a simple, safe, cost-effective and minimally invasive procedure that aids in early diagnosis and helps determine the stage, prognosis and recurrence in cases of Malignant Lesions (MAL) [4-8]. It also provides material for molecular testing. However, there is wide variability in the reporting of fluid cytology because of different collection and processing techniques employed in different laboratories, and due to different levels of experience of different cytopathologists [4,9].

To overcome this issue, the International Academy of Cytopathology and the American Society of Cytopathology (IACASC) developed a standardised reporting system called the ISRSFC to provide uniform guidelines for diagnosis and reporting [2,9-11]. The aim of the ISRSFC is to provide better communication between clinicians and pathologists, thereby improved patient care. It provides standardised terminology and ROM for each category, therefore minimises the interobserver variation observed in the reporting of fluid cytology. The ISRSFC gives five categories: 1) ND (Negative Diagnosis); 2) NFM (Negative for Malignancy); 3) AUS (Atypical Cells of Undetermined Significance); 4) SFM (Suspicious for Malignancy); and 5) MAL, which encompasses both primary and secondary malignancies [2].

The main objectives of the present study were to reclassify serous fluids according to the ISRSFC and evaluate the ROM in each category, thereby contributing in setting guidelines for clinical management.

MATERIALS AND METHODS

The present retrospective cross-sectional study was conducted in the Department of Pathology, Chirayu Medical College and Hospital (tertiary care hospital), Bhopal, Madhya Pradesh, India, from January 2023 to December 2023.

Inclusion criteria: All cases of peritoneal, pleural, and pericardial fluids submitted to the Department of Pathology between 1st January 2023 to 31st December 2023 were included in the study.

Exclusion criteria: Peritoneal washings, Bronchoalveolar Lavage (BAL) fluid and other body fluids were excluded from the study.

Study Procedure

Cytology smears and relevant clinical details, including medical records, cytology and histology reports, and ancillary studies along with patient management, were procured from the Institution's

archives. The original slides were reviewed by two experienced cytopathologists and reclassified into suitable categories. Fresh specimens received were either submitted in their entirety or as a representative 100 mL sample for processing. During the process, the samples were equally divided into two tubes after proper mixing and centrifuged for 10 minutes at 2500 rpm. Conventional smears were prepared from one of the tubes, with a minimum of two smears being air-dried and alcohol-fixed, then stained with May-Grünwald Giemsa (MGG) stain and Papanicolaou or Haemotoxylin and Eosin (H&E) stain, respectively. The cell button obtained from the other tube was processed as a cell block after fixation in formalin and stained with H&E stain. The parameters recorded were patient's age, gender, medical history, specimen volume, cytological features and cell block studies, wherever possible. A total of 570 cases were distributed into five main categories. Histological and clinical follow-up was done. The gold standard for the final diagnosis was based on histological or clinical diagnosis ROM was calculated using the following formula ROM. Number of cases with a final diagnosis of malignancy, based on histopathological and/or clinical diagnosis)×100 / Total number of cases in that category.

STATISTICAL ANALYSIS

For statistical analysis, the cases were distributed according to age, gender and site. Data was presented as number and percentages.

RESULTS

A total of 570 serous effusion samples were received, which included 265 pleural fluids, 300 peritoneal fluids, and five pericardial fluids, as per the inclusion criteria over a period of one year. There was a slight female preponderance, with 270 males and 300 females. The age of the patients ranged from 12 years to 81 years, with a mean age of 43 years. The volume of the samples ranged from 1 mL to 1500 mL, with a mean age 100 mL. The demographic and specimen features are shown in [Table/Fig-1], and the ROM calculated for each group is also shown in [Table/Fig-1]. Fifty-five cases were excluded from the ROM analysis due to the failure to achieve a clear final diagnosis by the corresponding gold standard.

Clinical data	Number of cases in each category		
Age distribution in years	**Pleural fluid**	**Peritoneal fluid**	**Pericardial fluid**
10-20	10	11	-
21-30	18	23	-
31-40	65	58	-
41-50	42	87	-
51-60	71	78	03
61-70	44	38	01
71-80	12	03	01
81-90	03	02	-
Gender distribution	**Pleural fluid**	**Peritoneal fluid**	**Pericardial fluid**
Total number of cases	265	300	05
Male (270)	136	134	00
Female (300)	129	166	05
Volume distribution	**Pleural fluid**	**Peritoneal fluid**	**Pericardial fluid**
1-10 mL	30	55	4
10-100 mL	137	140	1
100-500 mL	84	46	-
500-1000 mL	11	37	-
>1000 mL	3	22	-

[Table/Fig-1]: Demographic characteristics of study population and fluid distribution.

Pleural fluid: In total, 265 pleural effusion specimens from 260 patients were reclassified according to the International Serous Fluid Classification (ISRSFC) as mentioned: 15 (5.6%) were categorised as ND, 207 (78.1%) as NFM, 7 (2.6%) as AUS, 11 (4.1%) as SFM, and 23 (8.6%) as MAL [Table/Fig-2]. The ROM for each category came out to be 6.6%, 1.6%, 25%, 81.8%, and 100%, respectively [Table/Fig-2]. Among all the malignant cases in pleural fluid, lung adenocarcinoma was the most common malignancy, accounting for 10 (43.4%) cases, followed by breast cancer and haematological Malignancy with 4 cases of each [Table/Fig-3e,i] and 3 (13%) of gynaecological Malignancy {Carcinoma (Ca) ovary}. Two cases categorised as malignant pleural effusion could not be confirmed finally [Table/Fig-2].

Peritoneal fluid: A total of 300 specimens from 293 patients were studied and reclassified as follows: 19 (6.3%) as ND, 244 (81.3%) as NFM, 5 (1.6%) as AUS, 8 (2.6%) as SFM and 24 (8%) as MAL. Among the confirmed malignant cases of peritoneal fluid, ovarian carcinoma was the most common cause, followed by gastrointestinal tract cancers (including colon and stomach cancers), pancreatobiliary [Table/Fig-3h] and breast carcinoma [Table/Fig-3g,h].

DISCUSSION

The serous effusion cytological study is used to diagnose the aetiology of effusions and can guide clinical decision-making. The accuracy of effusion cytology depends on many factors, including specimen collection and processing, the experience and expertise of cytopathologists, and the overlapping features in benign and MAL. To achieve better interobserver agreement, a uniform reporting system and a set of diagnostic features for each category are proposed [12,13].

The present retrospective study was conducted based on the newly proposed international reporting system for serous fluid cytology for a period of one year. During the present study, the authors analysed a total of 570 serous fluids and categorised them into five diagnostic categories, along with calculation of the ROM for each category. The ratio of males to females was slightly lower in both peritoneal and pleural effusions. The volume of fluid received ranged from 1 mL to 1500 mL, and malignancy was detected even in volumes as low as 5 mL. Some studies suggest a minimum fluid volume of 50-75 mL as a cut-off for adequacy; however, there is no clear-cut evidence [12,14-17]. Volume as an adequacy criterion is not mentioned in the International System for Reporting Serous Fluid Cytology (ISRSFC) [4].

Category I: Samples with scant or no cellularity and haemorrhagic smears were placed in this category. In the current study, out of 265 pleural fluids and 300 peritoneal fluids, 15 and 19 were categorised as ND, respectively. Most of these cases were acellular or had scant cellularity [Table/Fig-3a]. Three cases showed haemorrhagic effusion with no visible cellular details. A single case from each of the pleural and peritoneal fluids came out to be positive. The ROM for pleural fluid was 6.6%, and for peritoneal fluid, it was 5.2%. This is in concordance with the study conducted by Farahani SJ and Baloch Z [13]. In other studies, the ROM was higher compared to the current study [18,19]. The difference may be attributed to the difference in sample size in their studies. Out of 5 cases of pericardial fluids, there were no cases in this category.

Category II: This category constitutes the bulk of cases, with 78.1%, 81.3% and 60% of pleural, peritoneal, and pericardial fluids, respectively. The majority of these effusions are inflammatory, comprising acute neutrophilic, lymphocytic [Table/Fig-3b], and mixed infiltrates. Around 5% of cases in both pleural and peritoneal effusions showed reactive mesothelial hyperplasia. All three of the total five cases of pericardial effusion in this category were inflammatory effusions.

These statistics correlate with other studies [9,12,13,20]. The underlying conditions included Chronic Obstructive Pulmonary Disease (COPD), end-stage renal and hepatic disease, tuberculosis, and patients undergoing chemotherapy. Even in the case of benign effusions, classifying them on the basis of type of inflammatory cells

S. no. Category	Pleural fluid			Peritoneal fluid			Pericardial fluid		
	n (%)	No. of malignant cases confirmed (n)	ROM (%)	n (%)	No. of malignant cases confirmed (n)	ROM (%)	n (%)	No. of malignant cases confirmed (n)	ROM (%)
Total (n)	265			300			5		
1. ND	15 (5.6%)	1/15	6.6%	19 (6.3%)	1/19	5.2%	-	-	-
2. NFM	207 (78.1%)	3/186	1.6%	244 (81.3%)	4/220	1.8%	3 (60%)	0/3	0%
3. AUS	7 (2.6%)	1/4	25%	5 (1.6%)	0/3	0%	-	-	-
4. SFM	11 (4.1%)	9/11	81.8%	8 (2.6%)	6/8	75%	-	-	-
5. MAL	23 (8.6%)	20/20	100%	24 (8%)	22/22	100%	2 (40%)	2/2	100%

[Table/Fig-2]: Different categories for pleural, peritoneal and pericardial fluid according to ISRSFC classification.

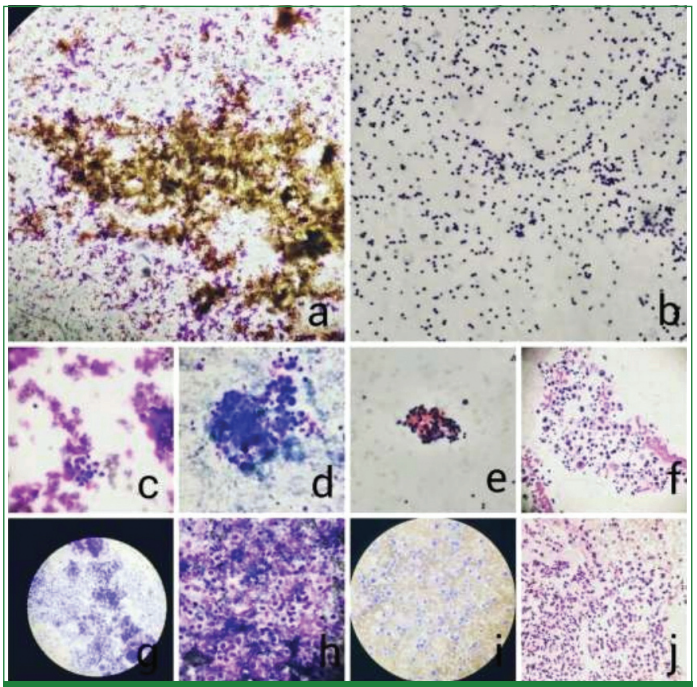
gives a clue to the aetiology of the effusion, thereby helps clinicians in making a decision. Florid mesothelial hyperplasia poses a diagnostic challenge [21]. Reactive hyperplasia of mesothelial cells with varied morphological features can occur due to a wide variety of stimuli. In the present study, all cases of reactive mesothelial hyperplasia came out to be negative.

The ROM in this category was 3/186 (1.6%) for pleural fluid, 4/220 (1.8%) for ascitic fluid and 0/3 (0%) for pericardial fluid. The present study results correlate with studies done by the previous studies [20,22]. The reason for the false negatives appears to be sampling error. Probably supernatant portion of the fluid, kept for a very long time was submitted for cytological examination.

Category III: Effusions classified as AUS are those that exhibit a cellular, nuclear and/or structural atypia but lack qualitative and quantitative features to be labeled as malignant [4,10,23]. In the present study, 7/265 (2.6%) of pleural fluid, 5/300 (1.5%) of ascitic fluid, and 0/5 (0%) of pericardial fluid comprised this category. These cases show a few scattered and/or clustered atypical cells [Table/Fig-3c]. Three cases of pleural fluid and two cases of peritoneal fluid were removed from the ROM calculation because they remained inconclusive. The ROM for pleural fluid was 1/4 (25%) and for ascitic fluid it was 0/3 (0%). No cases of pericardial fluid was kept in this category. In the present study, the ROM of category III was significantly lower compared to other studies [12,13,18,24]. The low rate of ROM in this category may be due to the small number of cases.

Category IV: This category includes cases that show cytological features worrisome of malignancy, having moderate to severe atypia [Table/Fig-3d]. A high level of clinical suspicion is also taken into consideration [2]. If the patient is a known case of malignancy, a few highly atypical cells can be diagnosed as category IV, SFM. While same few cells would be diagnosed as AUS in cases without a definite history of malignancy [2,25]. The ROM in this category was 9/11 (81.8%) for pleural fluid and 6/8 (75%) for peritoneal fluid. There were no cases of pericardial fluid in this category. These results are similar to the studies done by [13,19,21,22,26].

Category V: The malignant category accounted for 23/265 (8.6%) of pleural fluid, 24/300 (8%) of peritoneal fluid, and 2/5 (40%) of pericardial fluid. Lung carcinoma was the most common cause of malignant pleural effusion, followed by breast carcinoma and haematological malignancies [Table/Fig-3e,f,i,j]. The present study results are similar to the studies done by [2,12]. The most common cause of malignancy in peritoneal effusion was secondary to gynaecological malignancies, followed by gastrointestinal tract malignancies (colon, stomach) and pancreatobiliary malignancies [Table/Fig-3g,h]. These findings align with those in other studies. Out of five cases of pericardial fluid, two cases were malignant, specifically known cases of breast cancer and lung adenocarcinoma. The ROM in this category came out to be 100% for each fluid type. Three cases of pleural fluid and two cases of peritoneal fluid were not included in the calculation of ROM, as their final diagnosis could not be confirmed.



[Table/Fig-3]: a) Acellular ascitic fluid, (ND) (Geimsa; 10x); b) Lymphocytic pleural effusion (NFM), (Geimsa, 10x); c) very few atypical cells (AUS) (Geimsa, 40x); d) Atypical cells with high N/C ratio (SSFM) (Geimsa, 40×); e) Pleural fluid with atypical cells in a known case of Ca breast (Papanicolaou stain, 10x); f) Cell block of the same case; g) 3D clusters of malignant cells in peritoneal fluid in a case of Ca ovary (MAL) (Geimsa, 10x); h) Scattered malignant cells in peritoneal fluid in a case of Ca gall bladder (Geimsa, 40x); i) Atypical lymphoid cells/ pleural fluid involvement by NHL (Geimsa, 10x); j) Cell block of the case in i.

The diagnostic performance of the Institute laboratory is in accordance with that of other laboratories in earlier studies [Table/Fig-4] [2,20,27,28]. These results suggest that the ROMs in each category are comparable between the proposed ISRSFC and the reclassification studies done.

Type of fluid	Current study	Sun T et al., [20] 2022	Pergaris A et al., [27] 2021	Straccia P et al., [28] 2022	Yang H et al., [2] 2023
	ROM in percentage (%)				
Pleural fluid					
Category I	6.6	11.1	0	8.5	50
Category II	1.6	3.6	5.3	15	31
Category III	25	55	33.3	45.3	37
Category IV	81.8	83	93.3	93	91.8
Category V	100	100	100	100	100
Peritoneal fluid					
Category I	5.2	18.2	16.7	19.3	0
Category II	1.8	1.8	9	10.4	21
Category III	0	55	38.5	43.5	36.8
Category IV	75	100	83.3	100	85.2
Category V	100	100	100	100	100

[Table/Fig-4]: Risk of malignancy comparison with other studies [2,20,27,28].

This classification defines diagnostic criteria for each category, achieving a common platform with pathologists internationally,

better communication with clinicians, and setting guidelines for clinical management, thereby improving patient care.

Limitation(s)

There are a few limitations in the present study. The authors retrospectively analysed the 12-month cytology accessions of serous fluids, and there may be diagnostic bias due to the availability of clinical data, although follow-up was not available for all cases. Approximately 9% of cases were lost to follow-up for final diagnosis. Another limitation was the non availability of liquid-based cytology, which could have minimised the incidence of non diagnostic cases. Additionally, the number of pericardial effusion cases was very low, so no significant conclusions could be drawn from this data.

CONCLUSION(S)

The ISRSFC is a standardised reporting system for serous fluid cytology. It provides consistent terminology and is an effective, easy-to-understand, evidence-based reporting system. It enhances communication between the clinicians and cytopathologists and also gives risk stratification by providing the ROM in different categories, thereby contributing to appropriate management strategies. In the current study, the ROM was found to correlate with other studies, except in category III, which could be due to the small sample size in this category. Most of the serous effusions in the present study resulted in a therapeutically meaningful diagnosis. More studies are required, especially in indeterminate categories, for better performance analysis. Moreover, with sufficient sampling and ancillary methods like immunohistochemistry, more accurate results can be obtained.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 06, 2024
- Manual Googling: Aug 13, 2024
- iThenticate Software: Aug 21, 2024 (17%)

ETYMOLOGY: Author Origin

EMENDATIONS: 8

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? No
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **May 29, 2024**
Date of Peer Review: **Jul 04, 2024**
Date of Acceptance: **Aug 22, 2024**
Date of Publishing: **Jan 01, 2025**