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Reclassification of serous effusion samples according to the new International System for Reporting Serous Fluid Cytology with evaluation of the risk of malignancy in each new category

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Abstract

Background: Cytologic examination of serous effusions is an important diagnostic tool for the diagnosis of cancer, staging and prognosis of the patient. The newly proposed International System for Reporting Serous Fluid Cytopathology (ISRSFC) aims to standardize the cytologic reporting. **Aim:** The main objectives of our study were to reclassify serous effusion samples using the new International System for Reporting Serous Fluid Cytology (ISRSFC), compare cytological interpretation with histopathological diagnosis and/or clinico-radiological findings, to calculate risk of malignancy for each new category and to measure performance parameters. **Patients and methods:** This study included 510 cases of serous effusion that were sent for cytological assessment in Cytology unit, Pathology department, National Cancer Institute (NCI), Cairo University in the period between January 2018 and December 2020. Cases were reviewed and reclassified according to the ISRSFC. Risk of malignancy (ROM) and performance parameters were calculated. **Results:** On estimating ROM of the total 510 studied cases of both pleural and ascitic effusion, it was found that ROM was 27.2%, 20%, 37.5%, 94.3% and 99.6% for ND, NFM, AUS, SFM and MAL, respectively. For the total cases of pleural and ascitic effusion cases and after excluding ND cases and considering the SFM, and MAL categories as positive groups, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 83.5%, 98.3%, 99%, 77.5%, and 89%, respectively. By considering the AUS, SFM, and MAL categories as positive groups, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were determined to be 87.4%, 87.2%, 92.2 %, 80%, and 87.3 %, respectively. **Conclusion:** The newly proposed ISRSFC allows standardization of reporting through the development of diagnostic criteria, it also facilitates the communication with the clinicians, by defining the ROM for each category so improve clinical decision-making

Key words: ISRSFC, risk of malignancy, serous effusion

Introduction

Serous effusions result from an imbalance between the production and reabsorption of serous fluid **(1)**. Their presence is always considered a pathologic condition, and they reflect a wide range of etiologies, including infections, autoimmune and metabolic diseases, trauma and malignancy. It is estimated that around 10% to 25% of pleural, pericardial and peritoneal effusions are caused by malignancy **(2)**.

The diagnosis of serous fluid is based on clinical presentation, radiological findings and laboratory testing which including biochemical assays and cytological interpretation **(3)**.

Cytological assessment of serous effusion has been widely used in the initial evaluation of effusion. Its main role is to differentiate between benign and malignant effusions for proper decision making in choosing the appropriate therapeutic strategy and assessing patients' prognosis. It is very important to avoid the false positive cytological diagnosis, as this can lead to inappropriate expensive follow-up tests or even unnecessary treatment with psychological stress for the patient **(4)**.

Cytomorphological overlap between reactive mesothelial proliferations and malignant cells are the main challenge that faces cytopathologists while reporting serous effusion cytology **(5)**.

Previously, standardized guidelines for reporting serous effusions were lacking. International Academy of Cytology (IAC) and the American Society of Cytopathology (ASC) developed the International System for Reporting Serous Fluid Cytopathology (ISRSFC) that consists of five diagnostic categories to provide a uniform reporting format to improve interpretation of serous effusion cytology with good inter-observer agreement and provide well-defined risks of malignancy (ROM) for each category. It also aims at better communication between cytopathologists and clinicians by linking the reporting system with management options. In addition, it allows effective communication among different institutions worldwide **(6)**.

The aim of this work was to re-classify serous effusion samples according to the new International System for Reporting Serous Fluid Cytology (ISRSFC), to compare the cytological interpretation with the available corresponding histopathological diagnosis and/or clinico-radiological impression of serous membrane involvement in order to calculate the risk of malignancy (ROM) for each new category and to measure the sensitivity, specificity and diagnostic accuracy of effusion cytology.

Patients and methods

This retrospective study was conducted on 510 patients presented to NCI Outpatient Clinic with serous effusion, referred for aspiration and sent for cytological assessment in Cytology unit, Pathology department, National Cancer Institute (NCI), Cairo University in the period between January 2018 and December 2020.

Ethical consideration: Informed consents were initially obtained from all patients for performing cytological and surgical procedures and for using samples and tissues for research purposes. The study was approved by The Institutional Review Board (IRB) of National Cancer Institute (NCI), Cairo University (Approval Number 2309206002).

Inclusion criteria: Our study included cases with sample amount 50 ml or more. The included cases had available adequate clinical data, results of imaging studies & results of corresponding histopathological specimen (if present).

Exclusion criteria: Sample amount less than 50 ml, samples with no available clinical and radiological data and cases with missed or broken slides.

Methods:

Specimen collection and gross examination:

At medical or pediatric oncology departments, the fluid was tapped under sterile conditions in its most dependent location. It is collected in a clean dry container (such as a plastic bag, syringe, bottle, or other plastic container) that was labeled in patient's name and hospital number. Fluid was received unfixed in our laboratory as soon as possible after collection. If the specimen could not be sent immediately to our laboratory or could not be processed soon after submission, it was stored in a refrigerator at 4°C; the fluid was not allowed to freeze. The addition of fixatives or anticoagulants to effusions is not from our unit's recommendations. The fluid was examined for its gross features such as volume, color, clarity, and any unusual physical features such as bad odor or high viscosity.

Cytological preparations

The fluid was processed according to the technique described by **Keebler and Facik, (7)**. Any clots detected were removed and all fluid was extracted from them by pressing them against the side of the container with a spatula or tongue depressor leaving a firm, rubbery mass. The shrunken clot was then processed as a cell block. The remaining fluid was shacked up to disperse cells. An aliquot was poured off (up to 50 ml) into a centrifuge tube and the sample was centrifuged for 5 min. at 2000 rpm. The supernatant was then decanted by completely inverting the tube leaving the firm sediment in the bottom of the tube. The standard handling of effusion samples in our laboratory consisted of centrifugation and preparation of four conventional smears, three ethanol fixed for Papanicolaou staining and one air dried for Giemsa staining. Subsequently, residual material was used for liquid-based preparation (BD Sure path) following the manufacturer's protocol for material preservation and slide preparation. Cell blocks were made for all fluids when available using the plasma thromboplastin clot method. Ancillary testing in the form of immunocytochemistry on cell blocks was applied in selected cases attempting to reach a conclusive diagnosis following the standard immune-peroxidase methods using Ventana BenchMark. The antibodies used included Calretinin, CD3, CD10, CD15, CD20, CD68, CD79a, CD99, CD138, CDX-2, CEA, CK5/6, CK7, CK19, CK20, D2-40, Desmin, EMA, ER, GATA-3, HER-2, Ki67, MOC-31, Napsin-A, NKX-2, PAX-8, P53, PR, TDT, TTF-1, Vimentin, WT-1.

Cytology Reporting and Categorization

All cases were reclassified according to the International System for Reporting Serous Fluid cytology (ISRSFC) in five categories. The categories of ISRSFC as described by **Chandra et al., include:** Non-diagnostic (ND), Negative for malignancy (NFM), Atypia of undetermined significance (AUS), Suspicious for malignancy (SFM) and Malignant (MAL) **(6)**.

Validation of cytologic diagnosis

The cytological specimens were classified as either benign or malignant, based on the results of concomitant or follow-up biopsy and/or clinical-radiological impression, which determines the presence or absence of serous membrane involvement.

Statistical analysis

The ROM was calculated and presented as the proportion of cases in each category with serous membrane involvement in the confirmatory test (histopathological diagnosis and/or clinico-radiological impression). The cases in the ND category were excluded from further statistical

analysis as they could not be included as either negative or positive for malignancy. Performance analysis included the calculation of sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for pleural and peritoneal effusion cytology samples. Sensitivity = True positive/(True positive + False negative), Specificity = True negative/(True negative + False positive), PPV = True positive/(True positive + False positive), NPV = True negative/(True negative + False negative) and Diagnostic accuracy = (True positive + True negative)/All analyzed cases.

Results

Table (1): Distribution of studied cases according to International System for Reporting Serous Fluid Cytology (ISRSFC)

ISRSFC diagnostic category	Frequency	Percent
• **ND**	22	4.3%
• **NFM**	195	38.2%
• **AUS**	32	6.3%
• **SFM**	35	6.9%
• **MAL**	226	44.3%
Total	**510**	**100%**

The 510 studied cases were classified according to the criteria set by ISRSFC into: ND (22 cases, 4.3%), NFM (195 cases, 38.2%), AUS (32 cases, 6.3%), SFM (35 cases, 6.9%) and MAL (226 cases, 44.3%). Both NFM and MAL categories constituted the maximum number of the studied case (Table 1).

Table (2): Number and distribution of pleural and ascitic effusions per ISRSFC category

ISRSFC categories	ND N =22	NFM N =195	AUS N=32	SFM N =35	MAL N=226	Total N =510
Pleural N (%)	13 (7.1)	62 (33.9)	14 (7.7)	11 (6)	83 (45.4)	183 (100)
Ascitic N (%)	9 (2.8)	133 (40.7)	18 (5.5)	24 (7.3)	143 (43.7)	327 (100)

The total number of pleural and ascitic effusion specimens in our studied cases was 183 (36%) and 327 (64%) cases, respectively. No pericardial effusion cases were included in our study. Their distribution according to the ISRSFC categories is presented in (Table 2)

Table (3): Number of pleural effusion cases, age, gender, colour and volume for each ISRSFC category

ISRSFC categories	ND N =13	NFM N =62	AUS N =14	SFM N =11	MAL N =83	Total N =183
• **Age**						
Mean± SD	51 ±19	57 ±14	56 ±11	29 ±23	53 ±17	53 ±17
Median (range)	55 (18-80)	59 (6-81)	62 (38-69)	32 (3-61)	53 (3-87)	56 (3-87)
• **Sex**						
Male	4	25	7	3	25	64
Female	9	37	7	8	58	119
• **Colour**						
Yellow	1	36	6	6	50	99

Brown	2	4	0	1	1	8
Orange	1	10	0	0	8	19
White	0	1	0	0	0	1
Red (Hemorrhagic)	9	11	8	4	24	56
Volume range	**50-60**	**60-200**	**70-100**	**50-80**	**60-400**	**50-400**

Pleural effusion cases consisted of 64 males (35 %) and 119 females (65 %) patients (male to female ration = 1:1.8), with age ranging from 3 to 87 years old and median age equal to 56 years. Specimen volume ranged from 50 to 400 ml (Table 3).

Table (4): Number of ascitic effusion cases, age, gender, colour and volume for each ISRSFC category

ISRSFC categories	**ND**	**NFM**	**AUS**	**SFM**	**MAL**	**Total**
	N =9	**N =133**	**N =18**	**N =24**	**N =143**	**N =327**
• **Age**						
Mean± SD	54 ±8	47 ±17	62 ±12	49±14	55 ±13	52 ±15
Median (range)	57 (38-60)	48 (2-83)	61 (46-89)	52 (26-69)	57 (6-83)	55 (2-89)
• **Sex**						
Female	7	112	14	20	126	279
Male	2	21	4	4	17	48
• **Colour**						
Yellow	2	79	13	18	95	207
Brown	0	1	0	0	1	2
Clear	0	0	0	0	1	1
Green	0	1	0	0	0	1
Orange	1	11	1	0	9	22
White	0	1	0	0	0	1
Red(Hemorrhagic)	6	40	4	6	35	91
Gelatinous	0	0	0	0	2	2
Volume range	**50-80**	**80-1000**	**50-80**	**50-90**	**60-1000**	**50-400**

Ascitic effusion cases consisted of 48 males (14.7%) and 279 females (85.3%) patients (male to female ratio = 1:5.8), with age ranging from 2 to 89 years old and median age equal to 55 years. Specimen volume ranged from 50 to 1000 ML (Table 4).

Table (5): The risk of malignancy across ISRSFC categories in all studied cases.

ISRSFC categories	**ND N =22**	**NFM N =195**	**AUS N =32**	**SFM N =35**	**MAL N =226**
Final confirmed diagnosis					
Malignant	6	39	12	33	225
Negative for malignancy	16	156	20	2	1
ROM	**27.2%**	**20%**	**37.5%**	**94.3%**	**99.6%**

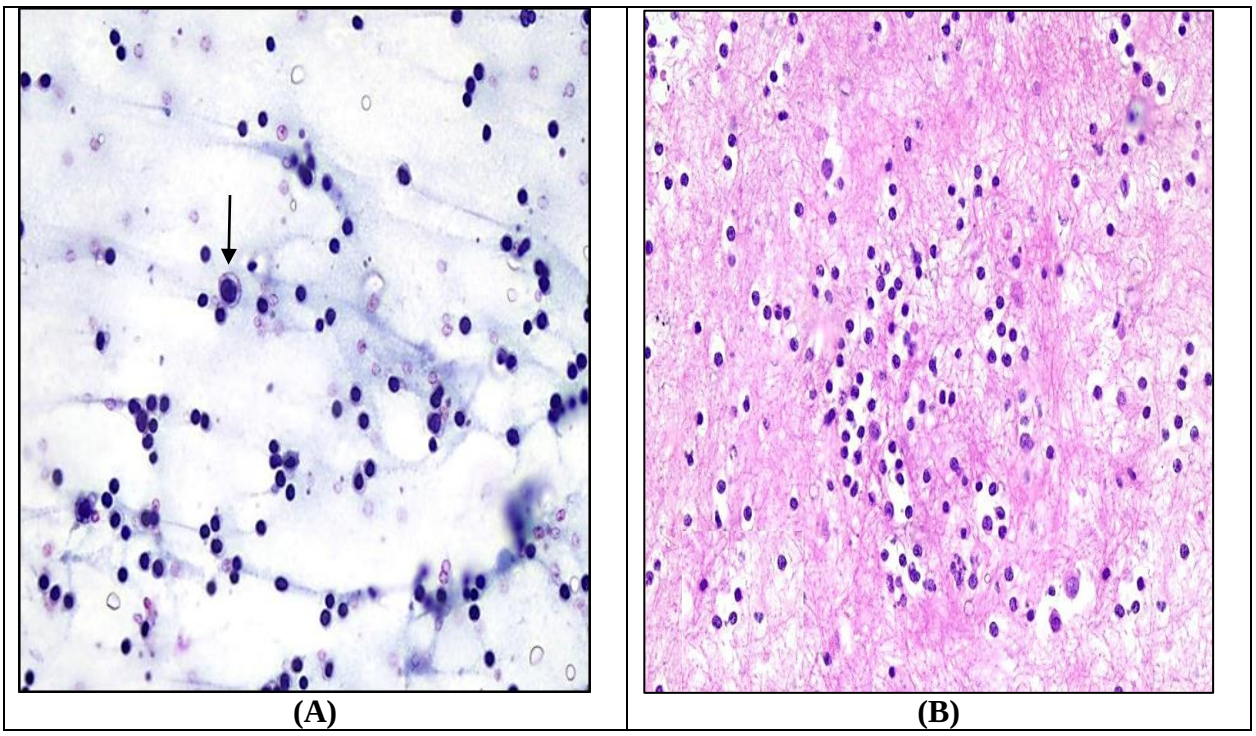
On estimating ROM of the total 510 studied cases of both pleural and ascitic effusion, it was found that ROM was 27.2%, 20%, 37.5%, 94.3% and 99.6% for ND, NFM, AUS, SFM and MAL, respectively **(Table 5)**

Table (6): Diagnostic performance in all studied cases:

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Overall accuracy (%)
Total cases					
Positive (MAL & SFM)	83.5	98.3	99	77.5	89
Positive (MAL, SFM & AUS)	87.4	87.2	92.2	80	87.3

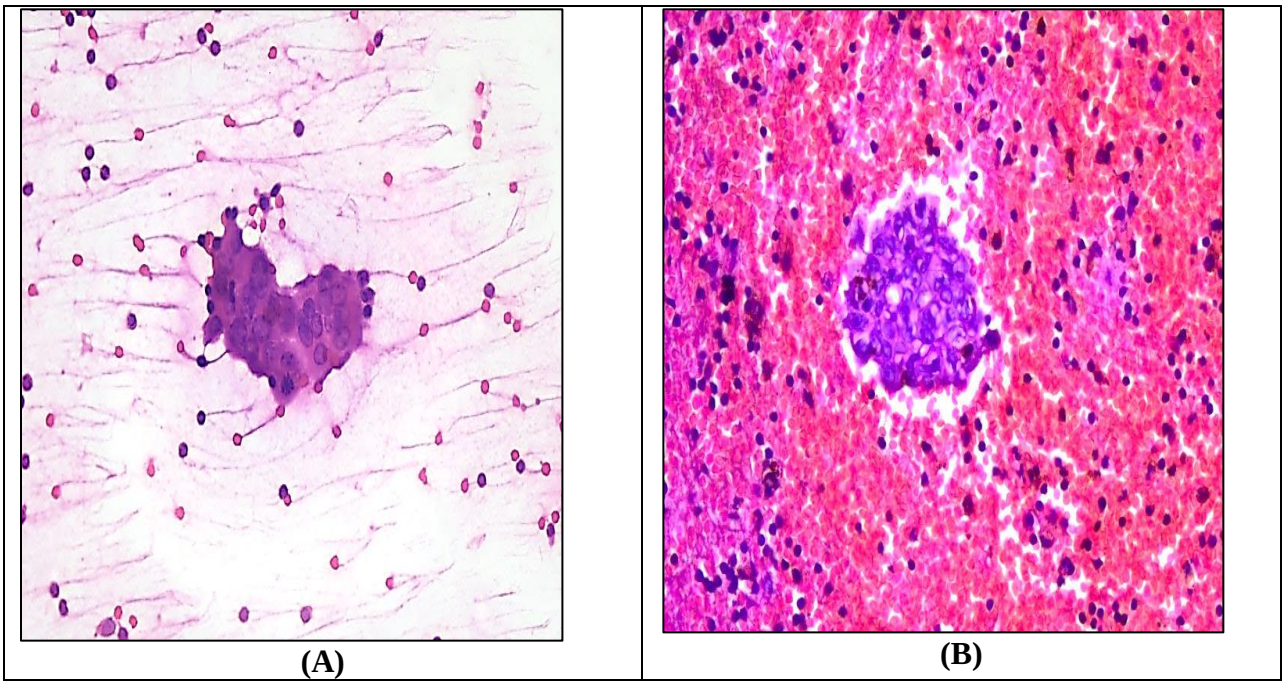
PPV: Positive predictive value, NPV: Negative predictive value

For the total cases of pleural and ascitic effusion cases and after excluding ND cases and considering the SFM, and MAL categories as positive groups, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 83.5%, 98.3%, 99%, 77.5%, and 89%, respectively. By considering the AUS, SFM, and MAL categories as positive groups, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were determined to be 87.4%, 87.2%, 92.2 %, 80%, and 87.3 %, respectively (Table 6).



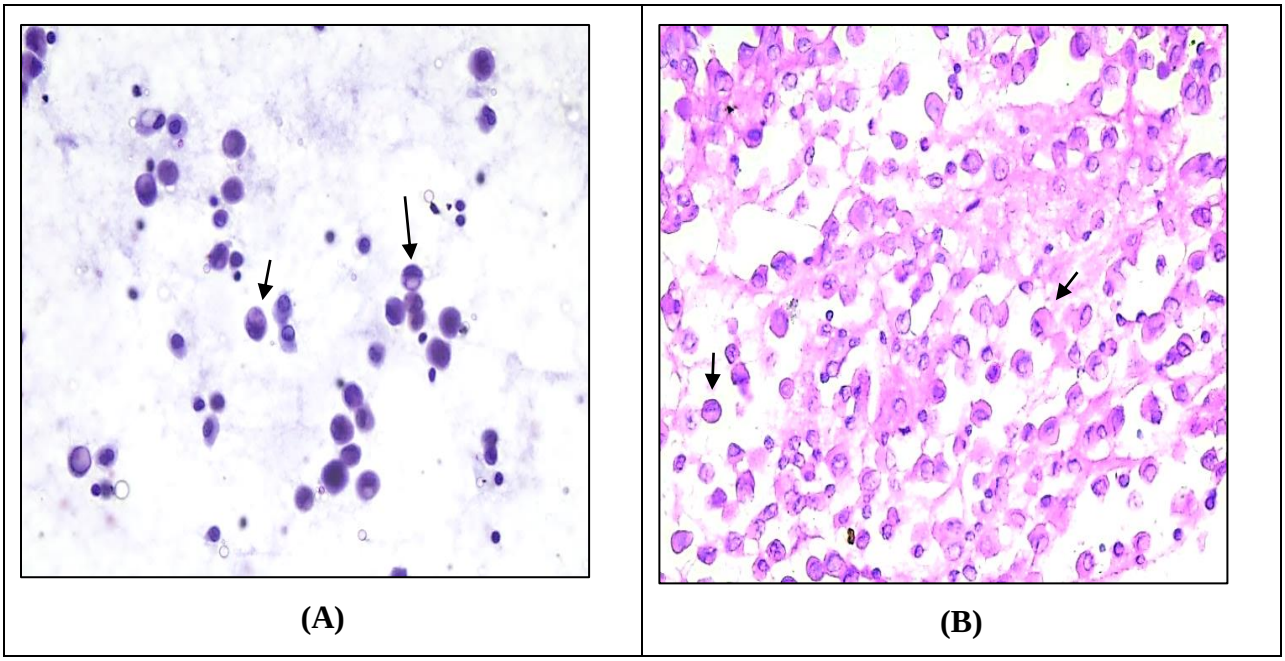
(A) **(B)**

Figure (1): Negative for malignancy (NFM), lymphocytic effusion. A male patient aged 51 years with right pleural effusion **(A)** Conventional smear (Papanicolaou stain, x400) and **(B)** cell block section (H&E stain x 400) show small mature lymphocytes with very few reactive mesothelial cells (arrow). The histopathological correlation of this case verified the benign nature, non-caseating granulomatous inflammation.

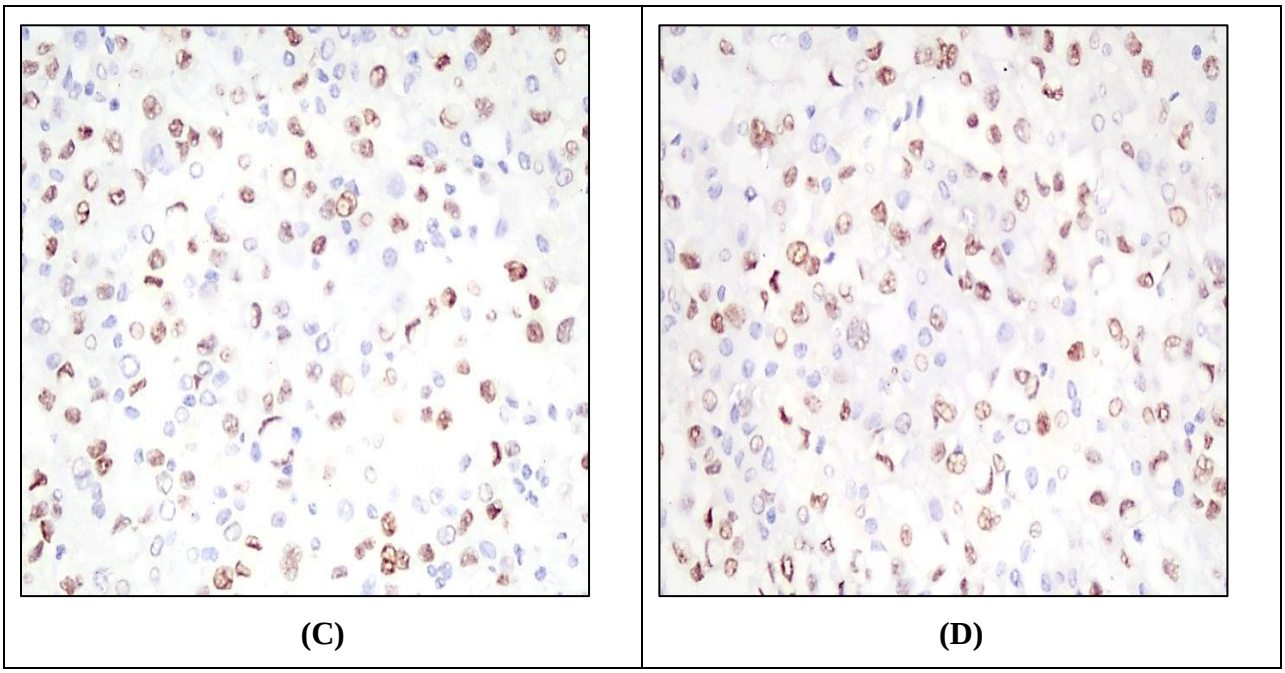


(A) **(B)**

Figure (2): Atypia of undetermined significance (AUS). **(A)** Conventional smear prepared from moderate amount of ascitic fluid show very few clusters of atypical mesothelial cells associated with chronic inflammation in a woman aged 39 years having an ovarian mass (Papanicolaou stain, x400) **(B)** cell block section showing group of cells with slightly increased N/C ratio (H&E, x400). Surgical follow up confirmed ovarian fibroma. The material was insufficient for additional testing.

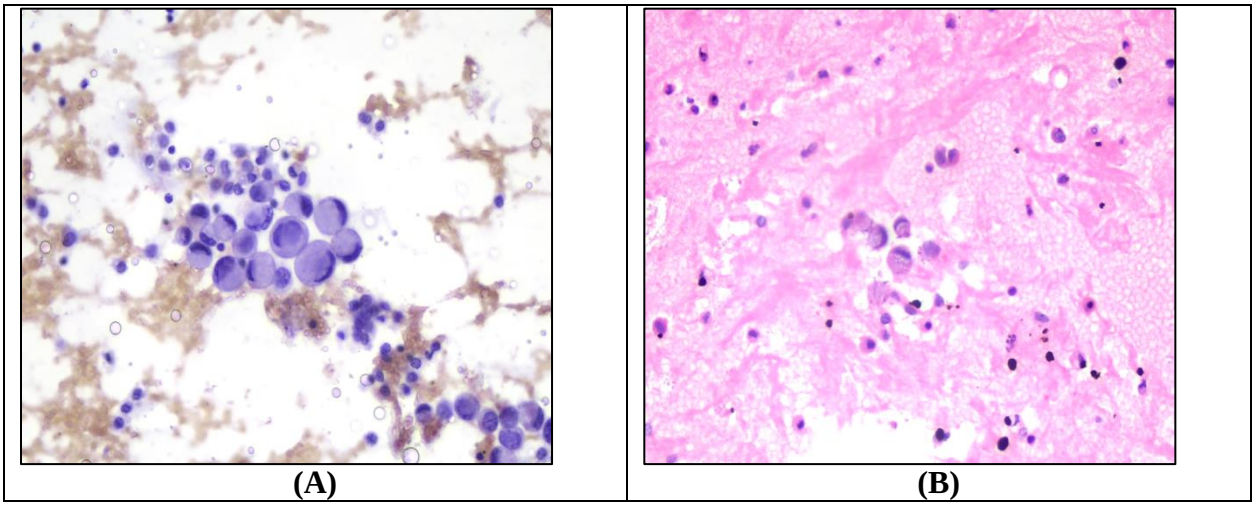


(A) **(B)**



(C) **(D)**

Figure (3): Suspicious for malignancy (SFM). Pleural fluid from a 60-year-old known case of breast lobular carcinoma. **(A)** Conventional Papanicolaou stained smear and (B) cell block H&E stained section shows numerous small isolated suspicious cells with large cytoplasmic vacuole and hyperchromatic nuclei (arrows) with no grouping or clustering in an inflammatory background showing reactive mesothelial cells (x400). The suspected cells are positive for GATA-3 **(C)** and ER **(D)** (Immunoperoxidase, x400), hence the case wasupgraded to metastatic adenocarcinoma of breast origin (MAL category) on the final report.



(A) **(B)**

Figure (4): Malignant (MAL). Ascitic fluid from a 55-year-old male having gastric signet ring cell adenocarcinoma. **(A)** Conventional Papanicolaou stained smear, x400 and **(B)** cell block H&E stained section x400 show numerous single neoplastic cells with enlarged nuclei, coarse chromatin, nuclear membrane irregularity, pleomorphism and intracytoplasmic vacuoles imparting a “signet ring” appearance.

Discussion

In the current study, cases were re-evaluated and distributed to ISRSFC categories as follows: 4.3% ND, 38.2% NFM, 6.3% AUS, 6.9% SFM and 44.3% MAL. Previous studies reported rates of ISRSFC categories ranging from 0.8 to 4.4% for ND, 63.1 to 81.4% for NFM, 0.6 to 6.6% for AUS, 2.3 to 4% for SFM and 10.5 to 56.2% for MAL (**8, 9, 10)**. Most of our studied cases belonged to the MAL category (226 cases, 44.3%). In addition, there is a wide discrepancy in the reported rate of NFM. These may be attributed to different patient populations and selection criteria. In addition, our study was carried out in cancer institute where most referral patients have malignant disease.

In our work, there were 7.1% of pleural and 2.8% of ascitic cases in non-diagnostic category. It varied from the study analyzed by **Lobo et al.,** where the number of cases in the ND category consisted of 0.8% for pleural and 0.7 % for ascitic effusions **(9)**.

Regarding the 195 cases of the NFM category, ascitic effusion constituted the majority of cases (133 cases) in comparison to pleural effusion which constituted 62 cases. This result was incomparable to a previous report by **Pergaris et al.,** where most cases of NFM category were pleural effusion **(10)**.

In this series, AUS constituted 7.7% cases of pleural fluid and 5.5% cases of ascitic fluid. **Lobo et al.,** reported 0.6% of pleural and 1.6% of ascitic cases in AUS category. In this regard, we had a slightly increased number of AUS cases. This could occur as a result of diagnostic bias and subjectivity in the interpretation of cytologically atypical cells in the setting of a patient with known malignancy and or distant metastasis **(9)**.

In our study, 6% of pleural and 7.3% of ascitic cases were kept in SFM category. **Ahuja and Malviya** observed 7.2% of pleural and 3.7% of ascitic cases in this category. They reported that SFM category should be viewed seriously by the clinician and managed as malignant until proven otherwise **(11)**.

The malignancy rate in this series was 45.4% for pleural and 43.7% for ascitic effusions. These data are almost like **Zhu et al.,** who reported malignancy rates of 47.7% for pleural and 49.9 % for ascitic effusions **(12**). However, **Straccia et al.,** reported malignancy rates of 10.5% for each pleural and ascitic effusions. Again, this difference may be explained by the source of our data from an oncological center **(13)**.

On estimating ROM of the total 510 studied cases of both pleural and ascitic effusion, it was found that ROM was 27.2%, 20%, 37.5%, 94.3% and 99.6% for ND, NFM, AUS, SFM and MAL, respectively.

Our results are nearly compatible with of **Kundu et al.,** who reported values for ND, NFM, AUS, SFM and MAL as 20%, 16.7%, 50%, 94.4%, and 100%, respectively **(14)**. On the other hand, **Jha et al.,** reported higher ROM for the aforementioned categories (87.5, 51.6, 88.2, 87.5 & 100% respectively). This difference can be explained by the gold standard used for establishment of the final diagnosis **(8)**.

For the total cases of pleural and ascitic effusion cases and after excluding ND cases and considering the SFM, and MAL categories as positive groups, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 83.5%, 98.3%, 99%, 77.5%, and 89%, respectively. By considering the AUS, SFM, and MAL categories as positive groups, the sensitivity, specificity, PPV, NPV, and diagnostic accuracy were determined to be 87.4%, 87.2%, 92.2 %, 80%, and 87.3 %, respectively.

These results of performance analysis were similar to the findings of **Lobo et al., (9).** On the other hand, **Ahuja and Malviya** reported higher sensitivity (97.3% & 92.5%) for pleural and

ascitic effusion, respectively when AUS, SFM, and MAL were considered as positive and a sensitivity of 89.3% and 87.5% for pleural and ascitic effusion, respectively when SFM, and MAL were considered as positive. This difference in sensitivity could be attributed to the different statistical methodology and reference tests. They included only histological correlation as a reference while histological, clinical, and radiological evidence of disease were used as a reference in the present study **(11)**.

In the present work, the highest diagnostic accuracy was seen when MAL and SFM considered as positive which is contrary to the results of **Lobo et al.,** where the highest diagnostic accuracy was observed when AUS, SFM, and MAL considered as positive **(9)**.

Conclusion

According to ISRSFC categories, most of our studied cases belonged to MAL category followed by NFM category. The ND category had the lowest cases. The indeterminate diagnostic categories (AUS and SFM) nearly fall within the reported and published ranges. The newly proposed ISRSFC allows standardization of reporting through the development of diagnostic criteria, it also facilitates the communication with the clinicians, by defining the ROM for each category so improve clinical decision-making.

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