Original Article

Cytological Diagnosis of Pleural Effusions Based on the International System for Reporting Serous Fluid Cytopathology and Its Histopathological Correlation

Mayursinh Sayaniya*, Hetal Joshi

Department of Pathology, Pramukhswami Medical College, Bhaikaka University, Karamsad, Anand, Gujarat, India

DOI: 10.21276/APALM.3554

***Corresponding Author**:
Dr Mayursinh Sayaniya
mayursayaniya995@gmail.com

Submitted: 23-Mar-2025
Final Revision: 18-Jun-2025
Acceptance: 19-Jun-2025
Publication: 30-Jun-2025

Abstract

Background: This study classifies pleural fluid cytology using the International System for Reporting Serous Fluid Cytopathology (TIS, 2021) and evaluates the Risk of Malignancy (ROM) through histopathological correlation. Conducted over two years at a tertiary care hospital, it aims to assess the diagnostic and prognostic value of TIS in managing serous effusions.

Materials and Methods: Pleural fluid samples were categorized using the International System for Reporting Serous Fluid Cytopathology, and histopathological slides were reviewed to assess the ROM. Statistical analysis was performed using descriptive statistics, and diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated to evaluate the effectiveness of the TIS classification in predicting malignancy.

Results: Among 260 cases, 4 (1.53%) were Non-Diagnostic (ND), 214 (82.31%) Negative for Malignancy (NFM), 9 (3.46%) Atypia of Undetermined Significance (AUS), 9 (3.46%) Suspicious for Malignancy (SFM), and 24 (9.23%) Malignancy (MAL). ROM was 33.33% in ND, 19.86% in NFM, 50% in AUS, 66.67% in SFM, and 50% in MAL. Sensitivity, specificity, and diagnostic accuracy varied across categories.

Conclusion: TIS is a reliable system for reporting pleural effusions. ROM is highest in the AUS, SFM, and MAL categories. Consistency in diagnostic criteria and further refinement in ND and NFM categories are necessary for improved clinical outcomes.

Keywords:

Serous effusion cytology, Pleural effusion, Cytopathology, Non-Diagnostic, Negative for Malignancy, Atypia of Undetermined Significance, Suspicious for Malignancy, Malignancy

Introduction

Serous fluids are critical specimens in cytopathology because they can be collected easily, cost-effectively, and with minimal invasiveness. Cytological analysis of these fluids plays a pivotal role in distinguishing between benign and malignant conditions, as well as determining whether a malignant tumor is primary or metastatic. This information is vital for prognosis, staging, and treatment planning [1–4]. Accurate pathological evaluation is thus integral to effective patient management.

Despite its clinical importance, the absence of a standardized approach for reporting effusion cytology has led to variability in diagnostic terminology and challenges in interpretation. Overlapping cellular features between benign and malignant conditions further complicate diagnosis. To address this issue, the International Academy of Cytology (IAC) and the American Society of Cytopathology (ASC) introduced the International System for Reporting Serous Fluid Cytopathology (TIS) in 2021. This system establishes uniform diagnostic guidelines and nomenclature for serous effusion cytology [5–9].

Our laboratory has recently adopted this standardized reporting system to improve diagnostic consistency and enhance clinical outcomes.

The aim and objectives for conducting this study were to implement this system, classify the pleural fluid according to these categories, and assess the ROM in each category.

Materials and Methods

Source of data: This retrospective and prospective observational study, conducted at the Surgical Pathology Section of a tertiary care teaching center from April 1, 2022, to March 31, 2024, focused on patients with clinical evidence of pleural effusion who were hospitalized during the study period. Data on pleural fluid cytology and histopathology were collected from the Laboratory Information System (LIS) and Hospital Information System (HIS).

Inclusion Criteria: All samples of pleural fluid received for cytological examination, along with all specimens of lung or pleural biopsy in which pleural fluid cytology was reported.

Exclusion Criteria: Lung biopsy or pleural biopsy in which pleural fluid was not sent for cytological examination.

Methodology: For cytology, the specimens were processed and smears stained with PAP and MMG stains. Each specimen was classified according to The International System for Reporting Serous Effusion Cytopathology (TIS) as follows: Non-Diagnostic (ND): Smears with few or no cells, showing scattered polymorphs, macrophages, lymphocytes, and mesothelial cells. Negative For Malignancy (NFM): Benign cells (polymorphs, lymphocytes, macrophages, mesothelial) without signs of malignancy. Atypia of Undetermined Significance (AUS): Specimens showing atypia in a few cells, not enough to be classified as neoplastic. Suspicious For Malignancy (SFM): Cells with atypia suggesting malignancy, but insufficient for a definitive diagnosis. Malignant (MAL): High cellularity with malignant cells in clusters and scattered singly.

Results

The study involved 260 patients with clinical signs of pleural effusion, admitted to a tertiary care hospital between April 2022 and March 2024. Pleural fluid from these patients was sent for cytological examination, and classification was done according to the International System for Reporting Pleural Fluid Cytopathology (TIS), followed by histopathological correlation to assess the Risk of Malignancy (ROM). Taking into account the number of patients and fluid samples received, a preliminary assessment of the cytological examination was made. Of the total patients, 167 (64.23%) were males and 93 (35.77%) were females, with a male-to-female ratio of 1.79:1.

Distribution of patients based on the International System for Reporting Pleural Fluid Cytopathology (TIS) is shown in [Table 1].

The age distribution showed the highest prevalence of SFM and MAL in the 4th to 8th decades of life, with 24.62% of patients aged 61–70, followed by 17.69% in both the 41–50 and 51–60 age groups.

Table 1: Distribution of Patients Based on The International System for Reporting Pleural Fluid Cytopathology (TIS) (N = 260)

Categories based on TIS	Number (%)
Non-Diagnostic (ND)	4 (1.53 %)
Negative for Malignancy (NFM)	214 (82.31 %)
Atypia of Undetermined Significance (AUS)	9 (3.46 %)
Suspicious for Malignancy (SFM)	9 (3.46 %)
Malignant (MAL)	24 (9.23 %)
Total	260

Of the 260 cases, 141 (54.23%) underwent pleural or lung biopsy. Among these, immunohistochemistry and molecular testing were performed in only 1.92% and 3.08% of cases, respectively. Adenocarcinoma was the most common morphology, followed by metastasis and granulomatous inflammation. However, 55.31% lacked a definitive diagnosis due to inadequate biopsy samples.

From all patients of pleural fluid cytology in this study, lung and/or pleural biopsies of 141 (54.23%) patients were available for cytohistological correlation. The distribution of cytological and histopathological examinations across TIS categories, with the Risk of Malignancy (ROM), is presented in [Table 2].

Table 2: Distribution of patients based on The International System (TIS) in different categories and Risk of Malignancy (ROM) of each category (N = 141)

Cytological categories based on TIS	ND	NFM	AUS	SFM	MAL	Total
No. of cases with cytological examination	4 (1.53 %)	214 (82.31 %)	9 (3.46 %)	9 (3.46 %)	24 (9.23 %)	260 (100 %)
Total no. of cases with histopathological examination	3 (1.15 %)	112 (43.08 %)	6 (2.31 %)	6 (2.31 %)	14 (5.38 %)	141 (100 %)
Non-Malignant cases	2 (66.67 %)	84 (59.57 %)	3 (50 %)	2 (33.33 %)	7 (50 %)	98 (69.50 %)
Total no. of cases with final positive diagnosis of malignancy in histopathology	1 (33.33 %)	28 (19.86 %)	3 (50 %)	4 (66.67 %)	7 (50 %)	43 (30.50 %)
Risk of malignancy (ROM)	33.33 %	19.86 %	50 %	66.67 %	50 %	30.50 %

Pleural fluid cytology diagnostic statistics showed that MAL alone had the highest sensitivity (87.5%) and PPV (53.85%), making it the strongest individual predictor. However, MAL + SFM + AUS showed the best balance of sensitivity (73.68%) and accuracy (66.67%). Specificity remained constant at 50%, while NPV was stable at 75%, ensuring reliable negative results. Notably, MAL + SFM achieved perfect diagnostic accuracy (100%), highlighting its strong complementary effect [Table 3].

In our study, considering only the MAL category as the positive group resulted in better sensitivity, NPV, and diagnostic accuracy compared to MAL + SFM + AUS or MAL + SFM. Overall, sensitivity and NPV were higher, while PPV and specificity were lower, possibly due to the absence of multiple fluid samples for cytology and biopsies not being taken from the targeted lesion.

Discussion

Serous cytology aids in malignancy diagnosis and treatment. Before TIS, inconsistent criteria caused variability, but TIS standardizes reporting, enhancing accuracy and clinical decisions [6]. Serous effusions constitute a major part of the cytopathology workload. Cytology helps diagnose cancer, analyze cell composition, determine etiology, and guide treatment [10].

Table 3: The performance analysis of Risk of malignancy (ROM) among pleural effusions based on The International System (TIS) for reporting pleural fluid cytopathology

	MAL + SFM + AUS	MAL + SFM	MAL
Sensitivity (%)	73.68	68.75	87.5
Specificity (%)	50	44.44	47.06
Positive Predictive Value (PPV) (%)	53.85	52.38	43.75
Negative Predictive Value (NPV) (%)	75	66.67	100
Diagnostic Accuracy (%)	60.47 5	55.88	60

The current study reports a high proportion of Negative for Malignancy (NFM) cases at 82.31%. Comparison of distribution of patients based on TIS with other studies (Table 4) emphasizes a strong focus on non-malignant conditions, highlighting the need to understand their contributing factors. Such insights can aid in developing preventive strategies and improving treatment interventions [6,11-13]. Compared to Zhu et al.'s larger dataset (2,366 vs. 260 cases), the current study shows a higher proportion of NFM cases, possibly due to demographic differences or diagnostic criteria. Low ND and AUS rates reflect improved reliability through strict TIS application and precise classification. Systematic handling of low-cellularity fluids further enhances diagnostic accuracy [16].

Table 4: Comparison of distribution of patients based on TIS with other studies.

Authors	Total number of cases	ND	NFM	AUS	SFM	MAL
Zhu et al[15]	2366	14 (0.59 %)	723 (30.56 %)	77 (3.25 %)	442 (18.68 %)	1110 (40.49 %)
Xu Y et al[11]	2454	30 (1.2 %)	1670 (68.1 %)	151 (6.2 %)	54 (2.2 %)	549 (22.4 %)
Lobo C et al[12]	1496	12 (0.80 %)	944 (63.10 %)	9 (0.60 %)	54 (3.61 %)	477 (31.89 %)
Kundu R et al[13]	1066	30 (2.81 %)	751 (70.45 %)	15 (1.41 %)	45 (4.22 %)	225 (21.11 %)
Pinto D et al[6]	350	5 (1.42 %)	253 (72.28 %)	7 (2.00 %)	4 (4.00 %)	71 (20.29 %)
Current study	260	4 (1.53 %)	214 (82.31 %)	9 (3.46 %)	9 (3.46 %)	24 (9.23 %)

Differences in SFM and MAL prevalence across studies reflect epidemiological complexity and the need for contextual analysis. Results from Pinto D., Xu Y., and Kundu R. show similarity with the present study [6,11,13].

Compared to Pinto D. et al., this study shows lower adenocarcinoma (14.89%) and metastasis (8.51%) rates, indicating a more balanced histological profile. Of 78 'No Definitive Opinion' cases, 21 had granulomatous inflammation and 12 lung metastases, mainly from breast cancer—highlighting the influence of contextual factors on interpretation and management [6,13,20,21]. Malignancy diagnosis was not based only on cell quantity. 91.54% of serous effusions were linked to NFM or MAL, with few ND, AUS, or SFM diagnoses, unlike Zhu YL et al., who reported fewer ND and AUS cases [15].

ROM was assessed by histopathological correlation with other studies (Table 5). The study's results indicate that the categories with SFM and MAL are significantly lower than the others. While ROM for ND, NFM, and AUS is similar to previous studies, SFM and MAL had lower ROM. False negatives may result from insufficient tissue or misinterpretation. ROM for ND and NFM is expected to improve with imaging and biopsies in high-suspicion cases [6,11-15,17,18].

Table 5: The Risk of Malignancy (ROM) in current study and comparison with a few other studies

Authors	Total number of cases	Risk of malignancy (ROM)				
		ND	NFM	AUS	SFM	MAL
Kundu et al[13]	1340	20 %	16.70 %	50 %	94.40 %	100 %
Farhani et al[14]	11799	17.40 %	20.70 %	65.90 %	81.80 %	100 %
Valerio E et al[18]	519	50 %	44 %	50 %	83.33 %	96.20 %
Lobo C et al[12]	1496	51.1 %	23.9 %	50 %	76.2 %	100 %
Xu Y et al[11]	2454	26.7 %	12 %	62.30 %	77.8 %	100 %
Pinto D et al[6]	350	40 %	20.16 %	42.86 %	78.57 %	100 %
Zhu YL et al[15]	2326	40 %	29.8 %	49.3 %	99.5 %	100 %
Current study	141	33.33 %	19.86 %	50 %	66.67 %	50 %

The performance analysis among pleural effusion with other studies (Tables 6, 7, and 8) shows that sensitivity and NPV align with other studies, supporting MAL, SFM, and AUS as positives, but our results differed in specificity, PPV, and diagnostic accuracy [6,12,18,19,22]. Sensitivity and NPV in studies by Zhu et al., Rossi ED et al., Lobo C et al., and Pinto D et al. were similar to the current study. Low specificity, NPV, and diagnostic accuracy are explored through root cause analysis [6,12,15,23].

The current study showed similar sensitivity, NPV, and diagnostic accuracy for MAL compared to other studies. However, specificity and PPV were slightly lower than those reported by Farhani et al., Pinto D et al., and Zhu et al. Possible reasons for the low specificity and NPV were explored through root cause analysis.

Table 6: The performance analysis (MAL + SFM + AUS) among pleural effusion with other studies

Authors	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Rodriguez EF et al[22]	84.9	86.4	94.8	66.0	85.3
Lobo C et al[12]	61.6	100	100	73.3	81.3
Zhu et al[15]	88.5	92.4	97.5	70.2	89.4
Pinto D et al[6]	62.50	96.65	92.39	79.84	83.2
Valerio E et al[18]	69.4	93.3	96.2	56	81.3
Current study	73.68	50	53.85	75	60.47

Table 7: The performance analysis (MAL + SFM) among different serous effusions with other studies

Authors	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Zhu et al[15]	86.5	99.4	99.8	68.3	89.4
Lobo C et al[12]	66.9	98.4	97.6	75.8	86.5
Pinto D et al[6]	60.29	98.56	96.47	79.23	82.3
Rossi ED et al[23]	87	100	98	100	98
Current study	68.75	44.44	52.38	66.75	55.88

Root cause analysis and recommendations for false negative cases: A false negative in pleural fluid analysis can occur when lymphatic obstruction prevents malignant cells from entering the fluid, highlighting the need for thorough diagnostics [24] (Figure 1). False negatives were mainly due to sampling or interpretation errors, with causes including non-exfoliating neoplastic cells, fibrinous coatings, and conditions like radiation or pneumonia. Cytology misinterpretation may result from changes in

cytomorphology [24]. Follow-up cytology is recommended for recurring effusions to improve malignancy detection. We identified error causes and proposed corrective actions for better sampling and interpretation accuracy.

Table 8: The performance analysis (MAL) among pleural effusions with other studies

Authors	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
Zhu et al[15]	62.0	100	100	43.5	70.6
Pinto D et al[6]	52.21	100	100	76.28	72.5
Farhani et al[14]	73.2 %	99.9	98.6	78.3	76.4
Current study	87.5	47.06	43.75	100	60

Root cause analysis and recommendations for false positive cases: False positives may arise from misinterpretation, fixation errors, or processing issues, highlighting the need for quality control and biopsy-cytology cross-referencing [24] (Figures 2 and 3).

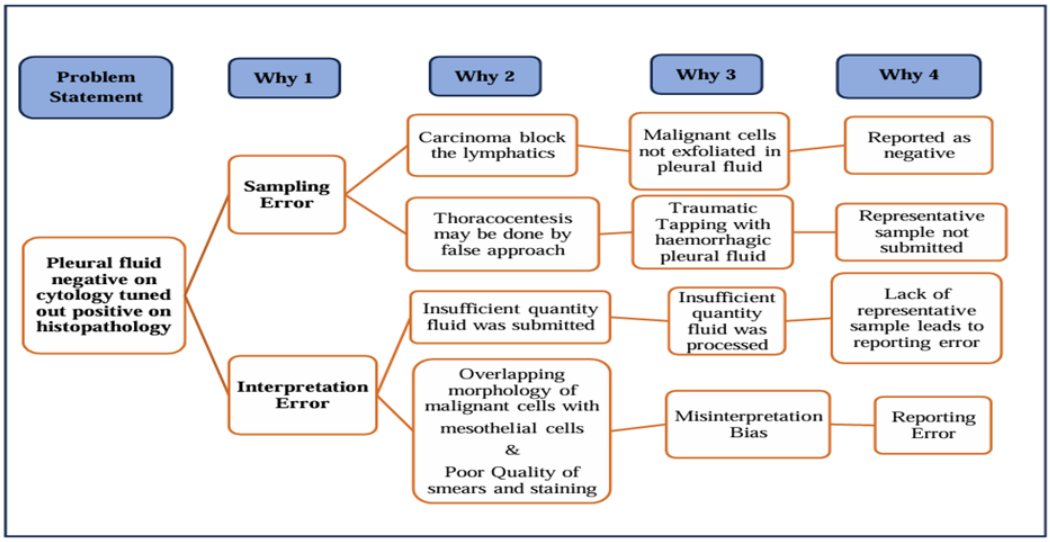

Figure 1: Root cause analysis of case in Negative for Malignancy (NFM) in cytology but Positive in histopathology

Proposed corrective actions for positive cytology and negative biopsy, as well as negative cytology and positive biopsy: Training and awareness of technical staff handling the specimen as well as the clinician performing the procedure. Updating the knowledge of consultants reporting the pleural fluid cytology and biopsy by attending seminars, CME, workshops, and conferences regularly. Improving the environment of the processing laboratory by trying to avoid interfering factors during processing. Performing only one task at a time; do not attend phone calls while performing tasks, etc. Regular maintenance of the equipment used for processing. Considering MAL alone or MAL, SFM, and AUS as positives improves sensitivity and NPV, but lowers specificity and PPV, likely due to false positives.

Limitations of the study: A limitation of our study is the small number of pleural fluid tests and cases without feasible biopsies. The high NPV is due to only one sample being examined, whereas at least three are recommended to rule out malignancy.

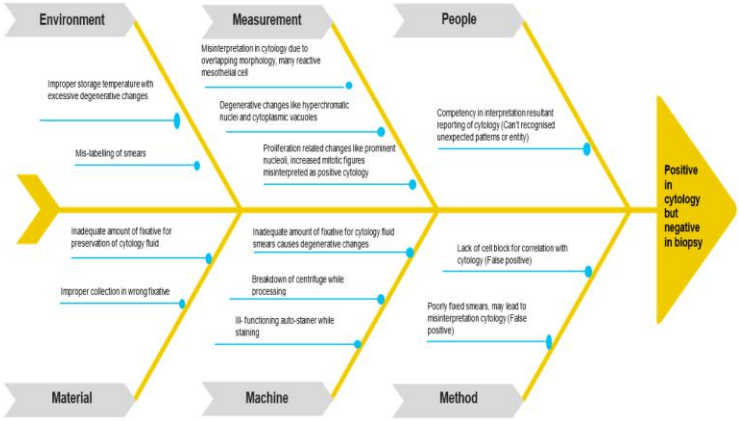

Figure 2: Root cause analysis – Cytological causes of false positivity

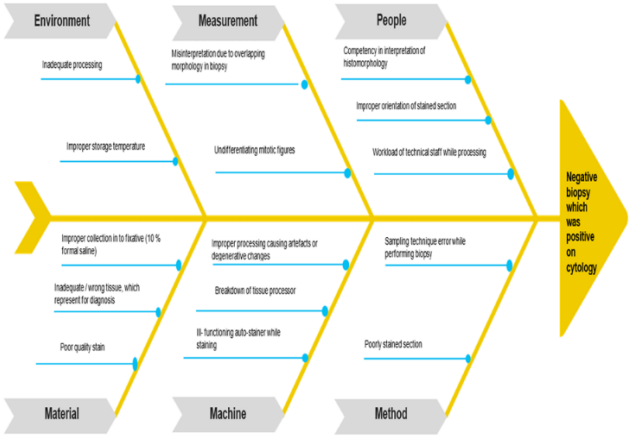

Figure 3: Root cause analysis – Histopathological causes of false positivity

Conclusion

Over two years, TIS was implemented at Shree Krishna Hospital's Central Diagnostic Laboratory, showing high sensitivity and NPV in detecting non-malignant diseases. While specificity and PPV were lower than in other studies, this was due to fewer samples analyzed. Root cause analysis highlighted areas for improvement, such as sampling and interpretation errors. The study supports TIS as a valuable tool for reducing diagnostic variability and improving patient management. To enhance accuracy, high standards in specimen processing, staff training, and equipment maintenance are essential for broader clinical use.

Acknowledgements: None

Funding: Nil

Competing Interests: None

References

1. Davidson B. Molecular testing on serous effusions. Diagn Cytopathol. 2021 May;49(5):640-6.
2. Yu GH, Glaser LJ, Gustafson KS. Role of ancillary techniques in fluid cytology. Acta Cytol. 2020 Jan 7;64(1-2):52-62.

3. Ebata T, Okuma Y, Nakahara Y, Yomota M, Takagi Y, Hosomi Y, et al. Retrospective analysis of unknown primary cancers with malignant pleural effusion at initial diagnosis. Thorac Cancer. 2016 Jan;7(1):39-43.
4. Zamboni MM, da Silva CT, Baretta R, Cunha ET, Cardoso GP. Important prognostic factors for survival in patients with malignant pleural effusion. BMC Pulm Med. 2015 Dec;15:1-7.
5. Ali SZ, Cibas ES. The Bethesda system for reporting thyroid cytopathology. New York: Springer; 2010 Feb 6.
6. Pinto D, Chandra A, Crothers BA, Kurtycz DF, Schmitt F. The international system for reporting serous fluid cytopathology—diagnostic categories and clinical management. J Am Soc Cytopathol. 2020 Nov 1;9(6):469-77.
7. Crothers BA, Chandra A. Proceedings of the American Society of Cytopathology companion session at the 2019 United States and Canadian Academy of Pathology meeting part 1: towards an international system for reporting serous fluid cytopathology. J Am Soc Cytopathol. 2019 Nov 1;8(6):362-8.
8. Wojcik EM, Kurtycz DF, Rosenthal DL, editors. The Paris system for reporting urinary cytology. New York: Springer; 2022 Jan 1.
9. Faquin WC, Rossi ED, Baloch Z, Barkan GA, Foschini MP, Kurtycz DF, et al., editors. The Milan system for reporting salivary gland cytopathology. Berlin: Springer International Publishing; 2018 Jan 31.
10. Pergaris A, Stefanou D, Keramari P, Sousouris S, Kavantzas N, Gogas H, et al. Application of the International System for Reporting Serous Fluid Cytopathology with cytohistological correlation and risk of malignancy assessment. Diagnostics (Basel). 2021 Nov 28;11(12):2223.
11. Xu Y, Hu AY, Wang SM, Wang Q, Pan YC, Zhang SH. A retrospective analysis of pleural effusion specimens based on the newly proposed International System for Reporting Serous Fluid Cytopathology. Diagn Cytopathol. 2021 Sep;49(9):997-1007.
12. Lobo C, Costa J, Petronilho S, Monteiro P, Leça L, Schmitt F. Cytohistological correlation in serous effusions using the newly proposed International System for Reporting Serous Fluid Cytopathology: experience of an oncological center. Diagn Cytopathol. 2021 May;49(5):596-605.
13. Kundu R, Srinivasan R, Dey P, Gupta N, Gupta P, Rohilla M, et al. Application of Indian academy of cytologists guidelines for reporting serous effusions: an institutional experience. J Cytol. 2021 Jan 1;38(1):1-7.
14. Farahani SJ, Baloch Z. Are we ready to develop a tiered scheme for the effusion cytology? A comprehensive review and analysis of the literature. Diagn Cytopathol. 2019 Nov;47(11):1145-59.
15. 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. Diagn Pathol. 2022 Jul 2;17(1):56.
16. Gonzalez M, Jhala N. Accuracy rate and risk of malignancy of liver cytology. J Am Soc Cytopathol. 2023 Sep 1;12(5):S52.
17. Jha S, Sethy M, Adhya AK. Application of the International System for Reporting Serous Fluid Cytopathology in routine reporting of pleural effusion and assessment of the risk of malignancy. Diagn Cytopathol. 2021 Oct;49(10):1089-98.
18. Valerio E, Nunes W, Cardoso J, Santos A, Bovolim G, Domingos T, et al. A 2-year retrospective study on pleural effusions: a cancer centre experience. Cytopathology. 2019 Nov;30(6):607-13.
19. Yang H, Zhu J, Wang P. Application of the International System for Reporting Serous Fluid Cytopathology (ISRSFC) in reporting serous effusion: a retrospective study. Medicine (Baltimore). 2023 Oct 27;102(43):e35707.
20. Sun T, Wang M, Wang H. Risk of malignancy assessment of the International System for Reporting Serous Fluid Cytopathology: experience in a community hospital setting and comparison with other studies. Cancer Cytopathol. 2022 Dec;130(12):964-73.
21. Goel S, Yeshvanth SK, Asnani R, Joshi D. Accuracy of bronchial cytological diagnosis in lung lesions in comparison with histopathology. J Cytol. 2022 Oct 1;39(4):163-8.
22. Rodriguez EF, Jones R, Gabrielson M, Santos D, Pastorello RG, Maleki Z. Application of the International System for Reporting Serous Fluid Cytopathology (ISRSFC) on reporting pericardial effusion cytology. Acta Cytol. 2020 Sep 2;64(5):477-85.
23. Rossi ED, Bizzarro T, Schmitt F, Longatto-Filho A. The role of liquid-based cytology and ancillary techniques in pleural and pericardic effusions: an institutional experience. Cancer Cytopathol. 2015 Apr;123(4):258-66.
24. Shidham VB. Diagnostic pitfalls in effusion fluid cytology. Cytojournal. 2021;18.