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Original Research Article

Cytological, cell block and immunohistochemical analysis of spectrum of malignant pleural effusion with special reference to non-small cell lung carcinoma: A hospital based study of North East India

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ABSTRACT

Introduction: Malignant pleural effusions (MPE) can result from primary malignancies of the pleura or from underlying intrathoracic or extrathoracic malignancies that reach the pleural space by hematogenous, lymphatic, or contiguous spread. Conventional smear and cell block (CB) technique is one of the oldest methods for the evaluation of body cavity fluids. The principal advantage of cell block over conventional smears are an additional yield of malignant cells and obtaining multiple sections for ancillary tests such as immunohistochemistry (IHC).

Materials and Methods: This study is a hospital-based cross-sectional study which was conducted for a period of 14 months from June 2021 to July 2022 in a tertiary care centre of North East India.

Results: Among 277 pleural effusion specimens 11.91% cases were positive for malignancy. Lung carcinoma was the most common cause of malignant pleural effusion. Non-small cell lung carcinoma comprised majority of lung carcinoma cases. Adenocarcinoma was the most common subtype of NSCLC with female preponderance. Cytological diagnosis had a Sensitivity of 76.19%, specificity of 100% and Accuracy of 96.5%. Immunohistochemistry of lung carcinoma in cell block preparation revealed 77.77% cases of adenocarcinoma of lung were positive for TTF-1 (P<0.018). Immuno-expression of P63 and P40 showed positive expression in both the cases of squamous cell carcinoma.

Conclusion: Conventional smear and cell block method with application of immunohistochemistry will help us in accurate diagnosis of primary site of the tumour aiding early treatment especially in resources restricted hospitals.

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1. Introduction

With an incidence of 150,000 new cases a year,¹ malignant pleural effusion has long been recognized as a cause of significant morbidity in cancer patients. Malignant pleural effusions (MPE) can result from primary malignancies of the pleura or from underlying intrathoracic or extrathoracic malignancies that reach the pleural space by hematogenous,

lymphatic, or contiguous spread. It is characterized by the presence of malignant cells in the pleural fluid.² The presence of MPE denotes systemic dissemination of cancer and has been staged as M1a disease, as per the American Joint committee on Cancer TNM staging system.³

Cytological examination of serous fluid is one of the commonest investigations executed worldwide. The accurate identification of cells as either malignant or reactive mesothelial cells is a diagnostic problem in conventional cytological smears. The cell block (CB)

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technique is one of the oldest methods for the evaluation of body cavity fluids.⁴ The principal advantage of cell block over conventional smears are morphological preservation of tissue architecture, an additional yield of malignant cells, thereby, increasing the sensitivity of the cytodagnosis and obtaining multiple sections for ancillary tests such as immunohistochemistry (IHC).

Immunohistochemistry (IHC) is the most common application of immunostaining. It selectively identifies the antigen in cells of a tissue by exploiting the principle of antigen antibody interaction in biological tissue which helps in accurate diagnosis of various neoplastic conditions. The aim of our study is to evaluate the common causes and frequency of malignant pleural effusions in North East India.

2. Materials and Methods

This study is a hospital-based cross-sectional study which was conducted for a period of 14 months from June 2021 to July 2022 in a tertiary care centre of North East India. Ethical clearance was taken from Institutional ethics Committee of Gauhati Medical College and hospital. 277 pleural effusion specimens were analysed both cytologically and using cell block (CB) method. Conventional smears were stained with May Grunwald Geimsa and Papanicolaou stain. Cell blocks were prepared using plasma thrombin method in our laboratory. CB sections were stained with Hematoxylin-Eosin. These slides were viewed under the microscope along with the assistance of proper history, examination and relevant investigation. They were categorized as non-diagnostic, benign, atypia of undetermined significance, suspicious for malignancy and positive for malignancy. Immunohistochemistry was performed in using cell blocks in selected cases based on the differential diagnosis generated by the cytomorphologic architecture, clinical history, examination along with relevant radiological and biochemical investigation. Photographs of the slides were taken at 400x magnification under Labomed vision 2000 microscope.

2.1. Ethical approval

Approved by Institutional ethical committee

2.2. Statistics

The results of conventional smear and cell block were statistically analysed using SPSS version 25 software. P value ≤ 0.05 was considered to be significant.

3. Results and Observation

Among lung carcinoma detected in MPE, 18 cases (75%) were that of adenocarcinoma, 2 cases (8.33%) were of

Table 1: Diagnostic Cytological categorization of pleural fluid reporting on conventional smear and cell block-

Category	Conventional smear	Cell block
Non-diagnostic	6	0
Benign	238	244
Atypical	4	0
Suspicious	7	1
Malignant	22	32
Total	277	277

Distribution of malignant and non-malignant pleural effusion cases

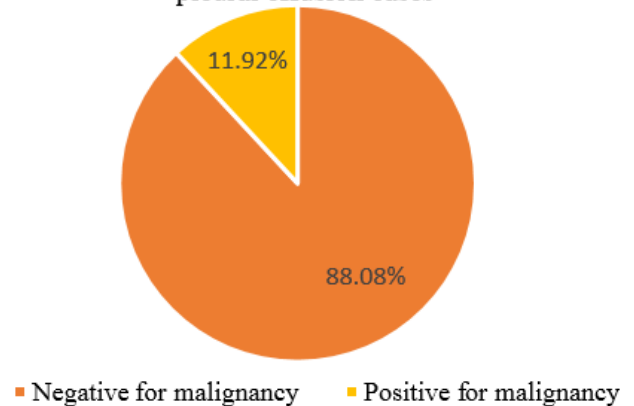


Fig. 1: Proportion of malignant and non-malignant pleural effusion cases

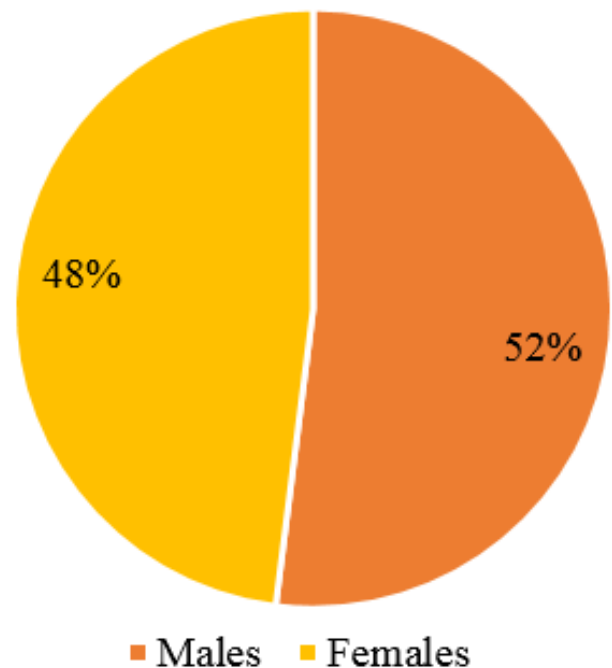


Fig. 2: Male: Female ratio of occurrence of malignant pleural effusion.

Table 2: Distribution of MPE cases in various age groups.

Age group (in years)	No. of cases	Percentage
11-20	3	9.09%
21-30	2	6.06%
31-40	0	00%
41-50	8	24.24%
51-60	8	24.24%
61-70	6	18.18%
71-80	4	12.12%
81-90	2	6.06%
Total	33	

Table 3: Distribution of Primary tumors with malignant pleural effusion-

Origin of metastasis (Primary tumor)	No. of cases	Percentage
1. Lung carcinoma	24	72.72%
2. Haemato-lymphoid neoplasm	4	12.12%
3. Breast carcinoma	2	6.06%
4. Ovary carcinoma	2	6.06%
5. Renal cell carcinoma	1	3.03%
Total	33	

Proportion of various malignancies detected in pleural effusion

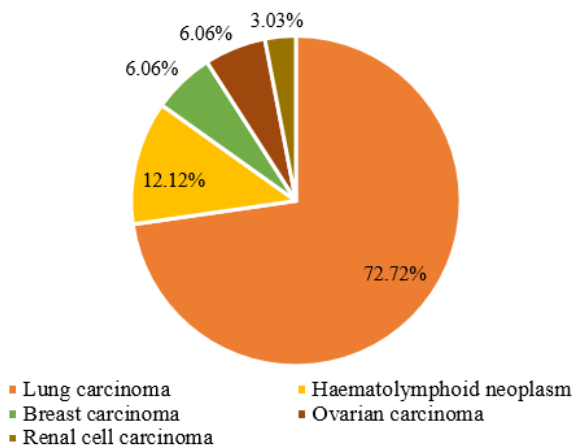


Fig. 3: Distribution of various primary malignant tumors with pleural effusion

squamous cell carcinoma and small cell lung carcinoma each. 2 cases (8.33%) were categorized as non-small cell lung carcinoma- NOS.

Adenocarcinoma was the most common type of lung carcinoma with a female predilection (58%).

Table 4: Statistical analysis of cytological diagnosis

32 True positive (TP)	0 False positive (FP)
10 False negative (FN)	244 True negative (TN)

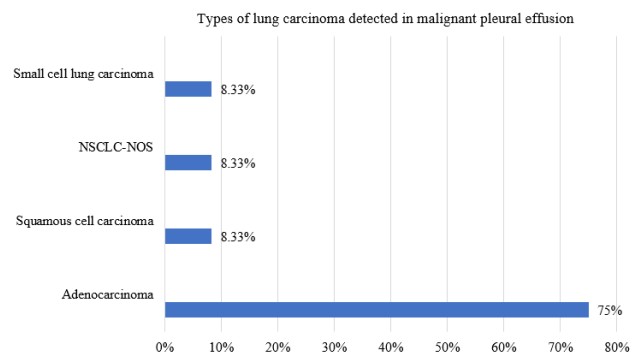


Fig. 4: Proportion of types of lung carcinoma detected in malignant pleural effusion

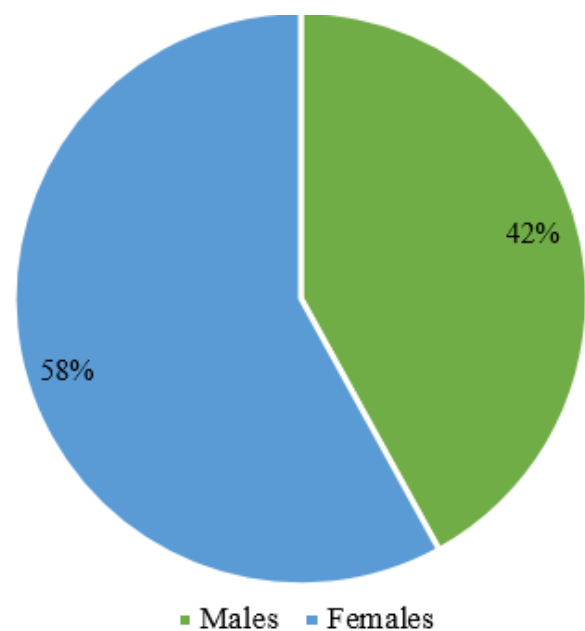


Fig. 5: Lung adenocarcinoma distribution in both the genders

Sensitivity is 76.19%, specificity is 100%, Accuracy-96.5%, Negative predictive value-96.06%, Positive predictive value- 100%

Chi square value is 6.188 and P value is 0.035 (P<0.05), statistically significant.

Immunohistochemistry of lung carcinoma in cell block preparation revealed 77.77% (n=14) cases of adenocarcinoma of lung were positive for TTF-1(P<0.018), whereas 22.22% (n=4) cases were negative. Immun-expression of P63 and P40 showed positive expression in both the cases of squamous cell carcinoma (n=2,100%)

Out of 2 cases categorized as NSCLC-NOS morphologically, 1 case showed TTF-1 positivity favouring adenocarcinoma. However, 1 case showed positivity for both P63 and TTF-1. Therefore, this case remained categorized as NSCLC-NOS.

Table 5: Comparison of conventional smear, cell block and immunohistochemistry findings in non-small cell lung carcinoma.

Type	Conv. smear CS	Cell block CB	Immunohistochemistry						
			TTF-1 positive	TTF-1 negative	P63 positive	P63 negative	p63 and TTF-1 positive	P40 positive	P40 negative
1. Adenocarcinoma	13	18	14 (77.77%)	4 (22.22%)	2 (11.11%)	16 (88.88%)	2 (11.11%)	0	18 (100%)
2. Squamous cell carcinoma	2	2	0	2 (100%)	2 (100%)	0	0	2 (100%)	0
3. NSCLC-NOS	2	2	1 (50%)	0	0	0	1 (50%)	0	0

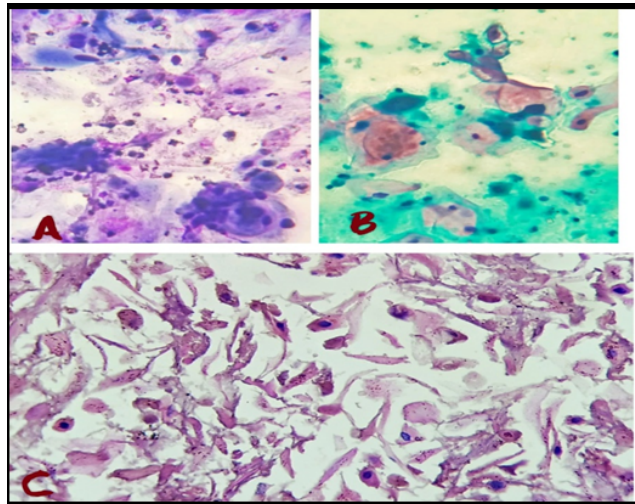


Fig. 6: A: MGG stain, B: PAP stain, C: Cell block architecture (spindle cell variant) of squamous cell carcinoma in pleural effusion

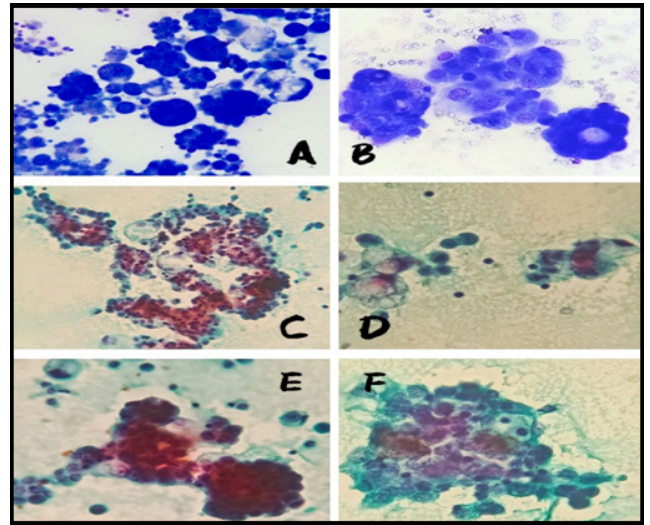


Fig. 8: A: 3D malignant cell cluster in Cytology Smear (CS), B: Gland formation in adenocarcinoma in CS (Both MGG stain), C-F: CS of lung adenocarcinoma (PAP stain).

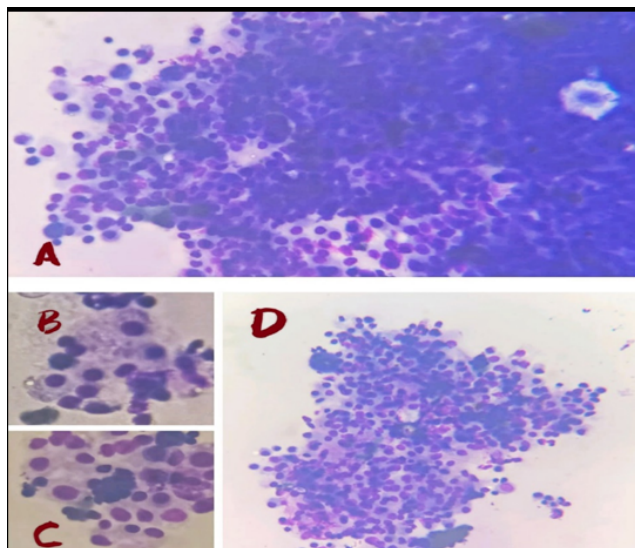


Fig. 7: Renal cell carcinoma in pleural fluid (MGG stain)

4. Discussion

The compelling role of fluid Cytology is the examination of serous effusion for the presence of malignant/cancer cells. Malignant effusions are often inceptive manifestation of cancer (especially in occurrence of lung, ovary, and mesothelial malignancies). An early and accurate diagnosis may warrant appropriate therapy and a better life expectancy for these patients.⁵⁻⁷

Table 6: Comparison of our study with other study on interpretation of conventional smears for pleural effusion:

	Negative for malignancy	Suspicious for malignancy	Positive for malignancy
Cakir et al ⁷	89%	3.2%	7.8%
Our study	88.08%	3.97%	7.94%

For diagnostic purpose both conventional smears and cell block preparation of serous effusions are recommended. Cytological diagnosis gives a fair sensitivity and very good specificity as can be perceived from our study. Cell block

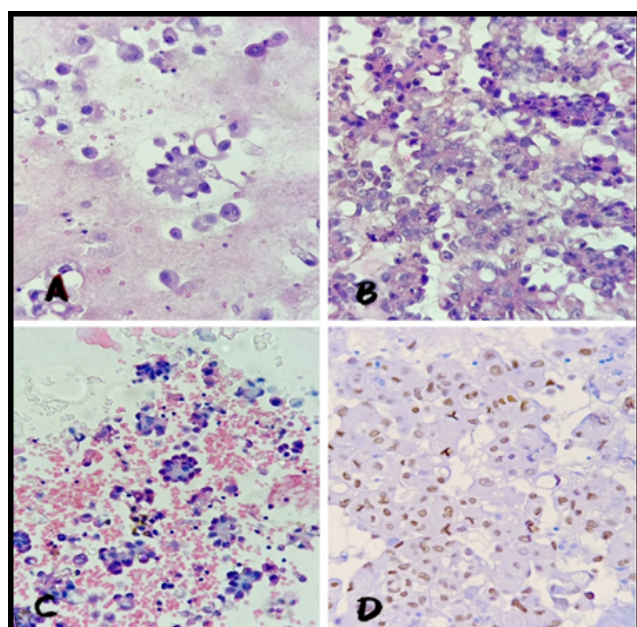


Fig. 9: A,B,C: cell block architecture showing papillary pattern in adenocarcinoma in malignant pleural effusion (H&E), D: TTF 1 positivity in adenocarcinoma lung in cell block section

sections provide good architecture and a better cellular yield which helps in confirming the diagnosis.

Table 7: Comparison of our study with other study for sensitivity and specificity of cytological diagnosis

	Sensitivity	Specificity
Dey et al ⁸	88.88%	86.98%
Ranieri S et al ⁹	92%	96%
Our study	76.19%	100%

The less sensitivity in our study might be due to technical errors or faulty interpretations. The amount of fluid submitted for examination, proper centrifugation, smearing and staining process plays an important role in diagnosing a case. Also, tumour cell proportion might be less in effusion specimen of cancer patients which might lead to a faulty interpretation.

Table 8: Comparison of our study on accuracy in cytological diagnosis with other study:

	Accuracy
Ranieri S et al ⁹	95%
Sukladas et al ¹⁰	92%
Our study	99%

The incidence of malignant pleural effusion are more common in males as compared to females. In the present study most common primary site of involvement was lung similar to the study conducted by Cakir et al¹⁴ in Turkey and Ahmed M. Abd El-Aziz et al¹⁵ in Egypt. Alike

Table 9: Comparison of percentage of cellular yield in cell block method with other studies

	Additional yield of cellularity
Rani SSS et al ¹¹	6.66%
Raghuwanshi et al ¹²	12%
Shobha Sn et al ¹³	46.15%
Our study	30.30%

our study the most common neoplasms causing malignant pleural effusions in males and females are those of lung, haemato-lymphoid system, breast, genitourinary tract, and gastrointestinal tract^{5,6,16} with slight male preponderance. The histologic type most commonly seen in malignant effusions is adenocarcinoma⁷ of lung which is similar to our study. The frequency of lung adenocarcinoma is higher in females. In the present study 2 patients with SCC had their initial diagnosis by cytology of pleural fluid. As SCC is compliant to chemo and radiation therapy, it is important to recognize SCC to avoid unnecessary investigation and surgery.

The most common unusual cause of MPE in middle east countries are that of malignant mesothelioma^{17–19} Nonetheless, in our study of North East Indian region, the most common unusual cause of MPE is haematological malignancy without any case of mesothelioma which is similar to a study conducted by Awasthi et al²⁰ in North India. Most cases of haematological malignant pleural effusion reported in the literature are that of acute lymphocytic leukaemia.²¹ In the present study there were two cases of acute lymphocytic leukaemia and two cases of acute myeloid leukaemia. The diagnoses of these cases were confirmed by flow cytometry.

Since lung carcinoma was revealed to be the major cause of malignant pleural effusion, immunohistochemistry (IHC) was done to classify lung carcinoma into small cell lung carcinoma and non-small cell lung carcinoma, further subtyping it into adenocarcinoma and squamous cell carcinoma.

P63 is a member of p53 gene family at 3q27-29. This differentiates squamous cell carcinoma from adenocarcinoma of lung. It is nuclear stain and interpreted as positive if it stains > 50% of the cells.^{22,23}

P40 is Truncated, nontransactivating p63 isoform. It is a nuclear marker with More specific for squamous cell differentiation than p63 in lung carcinoma.²⁴

Thyroid transcription factor 1(TTF-1), also known as NKX2-1 or thyroid specific enhancer binding protein, preferentially expressed in thyroid, lung and brain structures of diencephalic origin. It is a nuclear stain and considered positive on staining > 5% of tumor cells.^{25,26}

5. Conclusion

In an era of molecular diagnosis and cytogenetics, resource restricted hospitals and low-income countries like India, especially the North Eastern region of India, cyto-diagnosis by morphology, using cell block preparation and application of immunohistochemistry will not only help us in accurate diagnosis but also identify the primary sites of the tumors along with subtyping of few tumors causing malignant pleural effusion and thereby reduce the morbidity of advanced staged cancer patients by aiding treating clinician to initiate therapy at the earliest. The cell block method yielded more cellularity with better architectural patterns improving the diagnosis of malignancy. Therefore, it should be used as an adjunct in evaluating fluid cytology along with routine conventional smear method.

6. Conflict of Interest

None.

7. Source of Funding

None.

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