

To Analyse Usefulness of Immunohistochemical Analysis of Cell Block Specimens in Minimally Invasive Procedures of Pulmonary Lesions

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Abstract: Many lung cancers present in an advanced stage and only cytology specimens or small biopsies are available for diagnosis. In modern era therapy for lung cancer is based on subtype of cancer. Immunohistochemistry done on cell blocks of pleural samples help in subtyping of lung cancers. In this study, immunohistochemical analysis on plasma thromboplastin cell blocks of cytology samples of pulmonary lesions received as pleural effusion, bronchial wash and guided FNA of lung lesions were included. A total of 100 cases were studied by plasma thromboplastin method. By cell block technique, cytology specimen were made into mini paraffin embedded biopsy materials which are available for immunohistochemistry. All the 14 cases diagnosed to be malignant on cell block by Hematoxylin and Eosin stain were confirmed and subtyped by immunohistochemistry. A panel of immunohistochemistry markers CK7, CK20, PanCK, Synaptophysin, p63, TTF-1, Calretinin and Estrogen receptor were used. This study showed confirmation of malignancy by immunohistochemistry on cell blocks on all cases (100%) of malignant pulmonary lesions. Subtyping of malignancy could also be done in 11 out of 14 cases (78%)

Keywords: Cell block, Effusions, Immunohistochemistry, Pulmonary Lesions.

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I. Introduction

Many lung cancers present in an advanced stage and only cytology specimens or small biopsies are available for diagnosis (1). Cell blocks can be prepared from cytology samples like pleural fluid, bronchial wash and guided FNAC lung by Plasma Thromboplastin method (2). In modern era therapy for lung cancer is based on subtype of cancer (1,3). Immunohistochemistry done on cell blocks of pleural samples help in subtyping of lung cancers (1). This helps in selecting proper therapy for patients (3). The main subtypes of lung cancer are Small cell carcinoma, Adenocarcinoma, Squamous cell carcinoma and Large cell carcinoma. A small panel of immunohistochemistry, can be used on cell blocks to subtype the lung carcinomas accurately in most of the cases (1). In this study, an attempt is made to analyze the results of immunohistochemistry in cell block specimens of pulmonary lesions and to subtype malignant pulmonary lesions by immunohistochemistry on cell block specimens.

II. Materials and Methods

This was a retrospective study done from January 2013 to August 2014 in the Department of Pathology, Tirunelveli Medical College. The study included total 100 cases. 50 cases were of pleural effusions, 35 cases of bronchial wash and 15 cases of Guided FNAC lung. There were 23 cases of malignancy by cell block in these 100 cases. Material available to run our panel of IHC markers were 14. An analysis of these 14 cases by immunohistochemical markers was done.

The preparation of cell block was done by plasma thromboplastin method (2,4). The samples of pleural effusion and bronchial wash were centrifuged at 2500 rpm for 15 minutes. The supernatant was carefully removed and discarded. The sediment was mixed with two drops of plasma that was kept frozen and brought to room temperature before use. In case of FNAC, saline rinses of syringes and needles were taken and centrifuged before plasma was added. Immediately, four drops of thromboplastin was added and mixed well. The thromboplastin was stored in refrigerator between 2 and 8^o C and brought to room temperature before use. The mixture was left undisturbed for a few minutes until a clot was formed. If there was no clot formation, 4 more drops of thromboplastin was added, until clot formed. The clot was scooped out onto a filter paper and placed in

a cassette. The tissue cassette was then put in 10% neutral buffered formalin for at least 4 hours. Afterwards it was processed along with routine histopathological specimens and paraffin blocks were made. Tissue sections of 4-5 micron thickness were stained with Hematoxylin and eosin. Both the conventional smears and cell blocks were reported by cytopathologist. These blocks were used to do immunohistochemistry for p63,TTF-1,pan CK ,CK 20,Synaptophysin ,calretinin 1 and Estrogen receptor.

III. Results

Most of the patients in our study were in the age group of 51-70yrs. There were more male patients than female patients in our study. 14 cases of malignancy were diagnosed on cell block study which included 6 cases of malignant pleural effusion, 4 cases of malignant bronchial wash and 4 cases of FNA Lung (Figure 1)

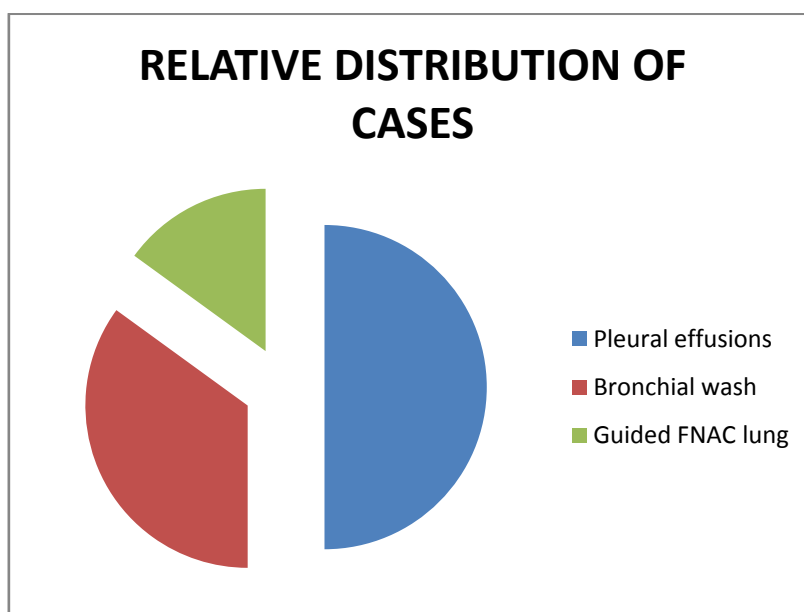


Figure 1: Relative distribution of cases during the study period.

Pleural Effusion :

In the present study, amongst 6 cases of malignant pleural effusion (Table 1& Figure 2) there were 2 cases with primary carcinoma breast (Figure 4) and both these cases showed positivity for Estrogen receptors (Figure 5) and were negative for TTF-1. One case was of primary ovarian carcinoma and immunohistochemistry showed positive CK-7. There were 2 cases of adenocarcinoma lung and both these cases were positive for TTF-1, CK-7 and negative for calretinin and CK20, confirming the diagnosis . In one case, the primary remained unknown (Table 2).

Bronchial wash :

In the present study there were 9 cases of malignant bronchial wash, but adequate material was available for IHC in 4 cases. 3 cases were of squamous cell carcinoma (Figure 6) and all these cases were positive by p63(Figure 7) and PanCK (Figure 8) which was in agreement with the diagnosis. One case could not be subtyped (Table 2).

Guided Lung FNA:

There were 9 cases of malignancy diagnosed by cell block in Guided Fna lung, but material was available for immunohistochemistry in only 4 cases. The panel used were p63, TTF-1, synaptophysin, PanCK. Two cases were of squamous cell carcinoma and were p63 and PanCk positive and negative for TTF-1. One case was reported as poorly differentiated carcinoma by both conventional smear and cell block. It showed synaptophysin positivity (Figure 9) and was p63 negative and was confirmed to be small cell carcinoma (Table 2).

Table 1 : Causes of malignant pleural effusion

S.NO	PRIMARY SITE	NO.OF CASES	PERCENTAGE
1	Breast	2	33.3%
2	Lung	2	33.3%
3	Ovary	1	16.6%
4	Unknown	1	16.6%

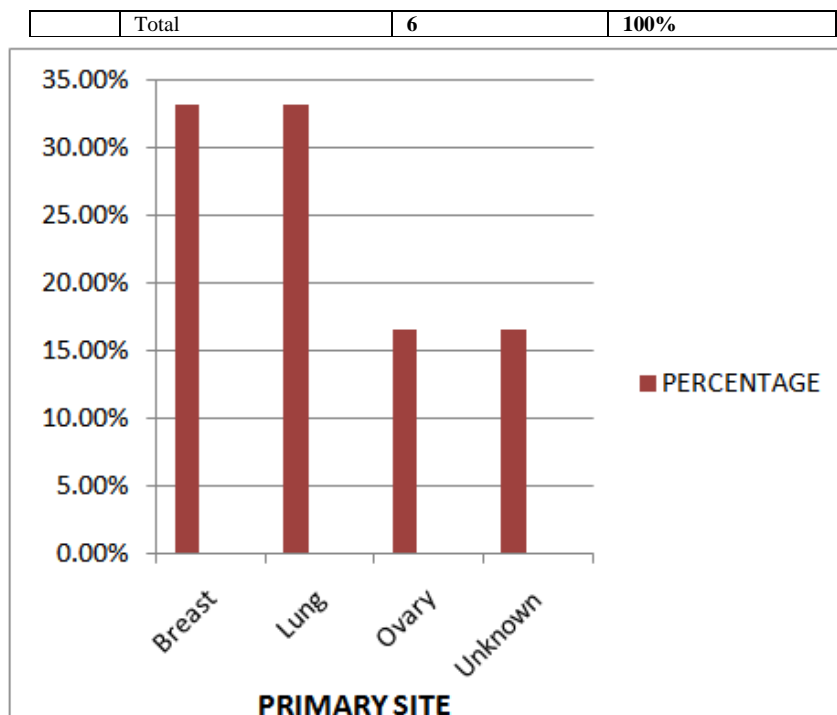


Figure 2: Causes of malignant pleural effusion

Table 2: Type of specimen and diagnosis

TYPE OF SPECIMEN	NO.OF CASES	MALIGNANCY ON CELL BLOCK	MATERIAL AVAILABLE FOR IHC	FINAL DIAGNOSIS AFTER IHC	NO.OF CASES
Pleural effusions	50	6 cases	6/6	Metastatic Breast carcinoma	2
				Metastatic Ovarian carcinoma	1
				Adenocarcinoma lung	2
				Could not be subtyped after IHC	1
Bronchial wash	35	9 cases	4/9	Squamous cell carcinoma	3
				Could not be subtyped after IHC	1
Guided FNA lung	15	9 cases	4/9	Squamous cell carcinoma	2
				Small cell carcinoma	1
				Could not be subtyped after IHC	1

In the present study, of the 14 cases of malignancy diagnosed on cell block study, immunohistochemistry was done. This revealed Squamous cell carcinoma in 5 cases, Adenocarcinoma of lung in 2 cases, metastatic adenocarcinoma in cases and 1 case of small cell carcinoma. 3 cases could not be subtyped (Table 3&Figure 3). A specific diagnosis was possible in 11 out of 14 cases (78%).

Table 3 : Diagnosis Based On Cell Block Vs Immunohistochemistry

CELL BLOCK		IMMUNOHISTOCHEMISTRY	
DIAGNOSIS	NO.OF CASES	DIAGNOSIS	NO.OF CASES
Squamous cell carcinoma	5	Squamous cell carcinoma	5
Adenocarcinoma	5	Adenocarcinoma lung	2
		Metastatic adenocarcinoma	3
Poorly differentiated carcinoma	1	Small cell carcinoma	1
Could not be subtyped			3

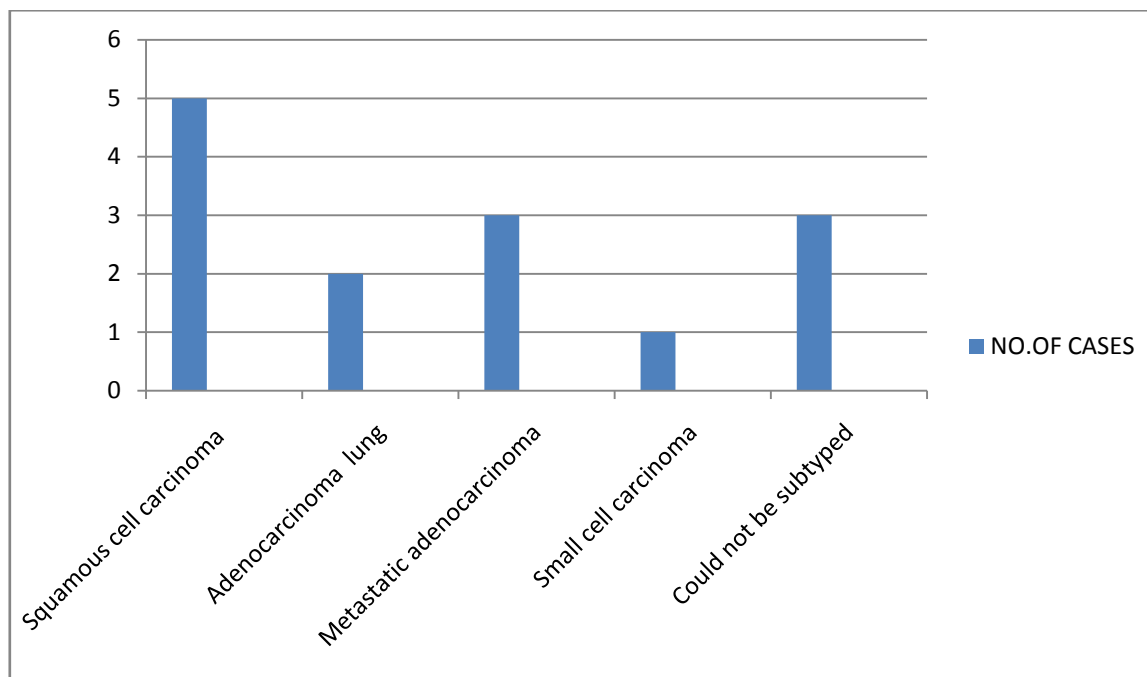


Figure 3: Immunohistochemistry Diagnosis.

IV. Discussion

Fluid samples can be converted to tissue samples by cell block technique and additional diagnosis of cancer can be obtained (5). Cell blocks of specimens of pleural effusion, bronchial wash and Guided lung FNA can be used for immunohistochemistry markers. Malignant pleural effusions has to be differentiated into lung adenocarcinoma, metastatic adenocarcinoma or mesothelioma for treatment and prognosis(1). There is a lack of hundred percent specific marker for mesothelioma (6). In our study we used Calretinin, TTF1, p63, CK 20, Pan CK, Synaptophysin and ER as per clinical data of patient. A panel of markers are frequently used for subtyping pulmonary tumors like calretinin 1, CK 5/6, CK 7, TTF - 1, CK 20. p63, and synaptophysin (1).

In our study there were 6 cases of malignant pleural effusion out of 50 cases (12%). A study by Shivakumaraswamy et al had 10 cases (16%) of malignant pleural effusion out of 60 cases of pleural Effusions(7). A study by Bhanvadia Viral (8) showed 16 malignant pleural effusions out of 79 cases (10%). Subtyping of malignancy with a specific diagnosis was possible in 11 out of 14 cases (78%) in our study. A study by George R Collins et al showed specific tumour diagnosis in 22 out of 29 (76%) cases of malignant bronchial wash specimens (9).

Breast cancer and lung cancer (33.33% each) were the commonest cause of malignant pleural effusion in our study. In a study by Murphy et al, (10) and also a study by George Collins, (9) the most common cause of malignant pleural effusion was breast metastasis. In a study by Dey et al, breast carcinoma was the commonest cause of malignancy in females and lung carcinoma in males.(11) In a study by Gaur et al, adenocarcinoma lung was the commonest cause of pleural effusion.(12) 3 out of 14 cases (22%) could not be subtyped in our study. In a study by Luiesella Right et al, 14% cases could not be subtyped after a panel of immunohistochemistry stain.(13)

All our cases of adenocarcinoma lung were positive for TTF-1. The study by Mihaela Dinu et al also showed TTF-1 positivity in all cases of adenocarcinoma lung (6). In our study all our 5 cases of squamous cell carcinoma were positive for p63 marker. In a study by Mihaela Dinu et al also p63 was positive in all cases of squamous cell carcinoma (6). TTF-1 & p63 were very useful in subtyping tumors in our study. This was also seen in the study done by George Collins et al (9).

This study showed that confirmation of malignancy by immunohistochemistry on cell blocks can be done on all cases(100%) of malignant pulmonary lesions. Subtyping of malignancy could also be done in 11 out of 14 cases (78%).

V. Conclusion

Our study showed that confirmation of malignancy by immunohistochemistry on cell blocks can be done on all cases(100%) of malignant pulmonary lesions. Subtyping of malignancy could also be done in 11 out of 14 cases (78%). However, if immunohistochemistry analysis of cell block is not possible for all aspirations, the technique should be used selectively in cases that are difficult to diagnose. Thus

immunohistochemistry analysis of cell block reports an increase in diagnostic yield and play a significant role in resolving the grey zone that a cytopathologist encounters while determining the nature of cells in cytology.

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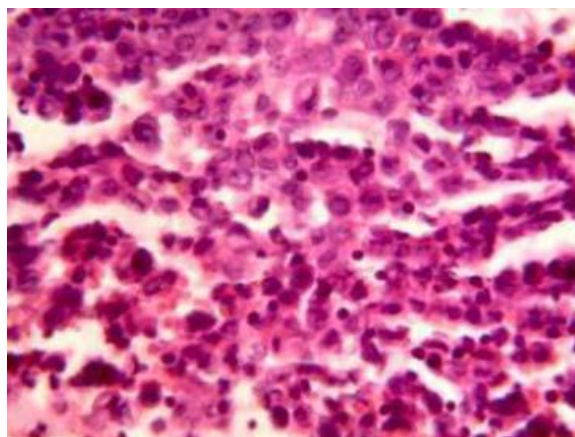


Figure 4 : Cell block showing high cellularity of metastatic breast carcinoma cells in pleural effusion (H & E , 40 x)

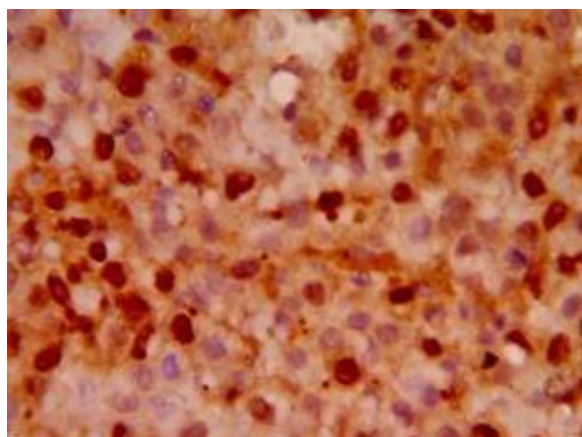


Figure 5 : Cell block showing malignant cells in pleural effusion positive for Estrogen receptor (40 x)

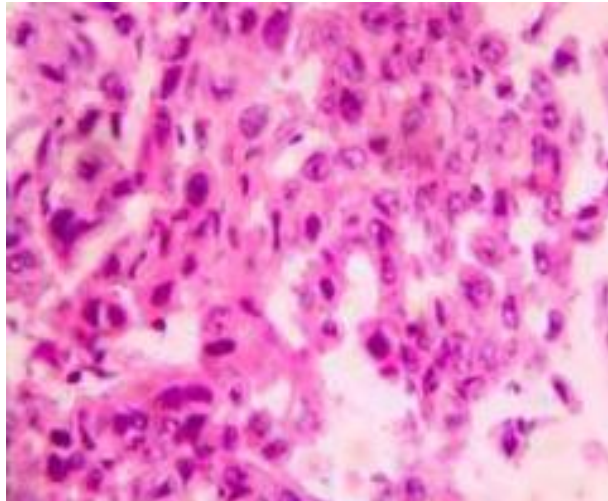


Figure 6: Cell block showing malignant squamous cells with well preserved morphology in bronchial wash (H & E , 40 x)

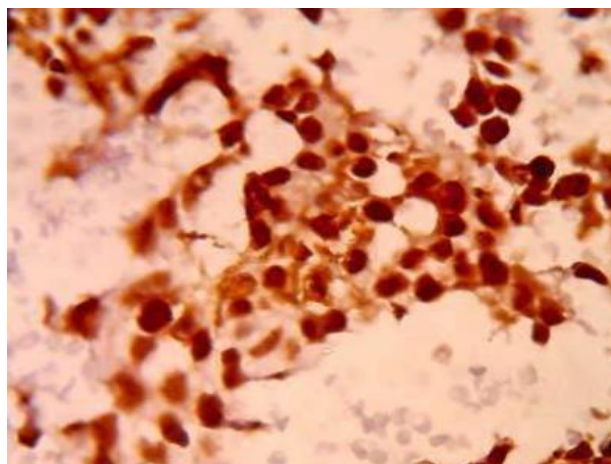


Figure 7 : Malignant squamous cells showing nuclear positivity for p63 in cell block (40 x)

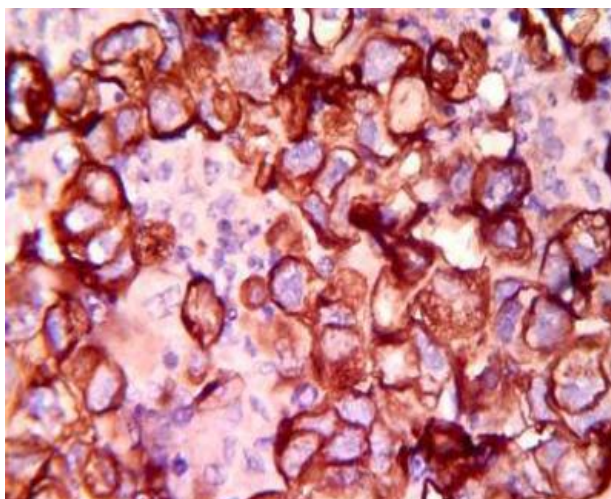


Figure 8 : Cell block showing malignant cells positive for CK 7 in Adenocarcinoma of lung (40x)

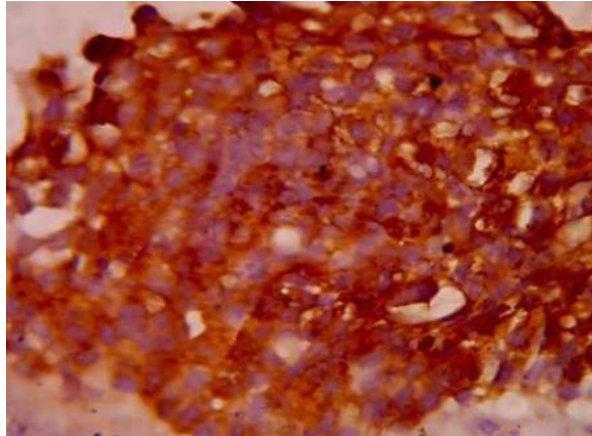


Figure 9 : Cell block showing malignant cells of small cell carcinoma positive for synaptophysin – Image guided FNA Lung (40x)

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