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ROLE OF IMMUNOHISTOCHEMISTRY ON CELL BLOCKS, IN PRIMARY PLEURAL EFFUSION

Pathology

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ABSTRACT

Background: Pleural effusion is a common finding in many malignancies.The present study was undertaken to determine the feasibility of cell block immunohistochemistry in cases presented with pleural effusion and finding the accuracy of the same in diagnosing the primary and finding the subtype.

Methods: In this study, out of 200 cases of malignant pleural fluid effusion, 72 cases with no primary diagnosis were taken up. Based on the cytomorphology of conventional smears and cytoblocks cytomorphological diagnosis was offered. An immunopanel CK7, CK5/6, p63, TTF1, calretinin was applied on cell blocks. Additional immunochemical markers were performed based on clinical details and cytomorphology, wherever indicated.

Results: The mean age of the patients was 50.8 years. The male to female ratio was 3.2:1. Cytomorphology was evaluated in all the 72 cases and IHC was used in 59 cases. After IHC 76.27% cases were confirmed as metastasis from lung primary, 10.1% from ovary, 3.3% from stomach and 10.16% from lymphoma. Other cases include mesothelioma(n=2), small cell carcinoma(n=2).

Conclusion: Judicious use of IHC on cytoblocks of pleural fluid is effective in detection of lung carcinoma and its typing and also for detecting primary site in tumors of extrapulmonary origin especially in cases where obtaining a biopsy is technically challenging.

KEYWORDS

effusion cytology; cytoblock; immunohistochemistry;pleura lfluid; metastasis

INTRODUCTION:

Pleural effusion is a common diagnostic problem, due to the numerous benign and malignant disorders that can cause it. On many occasions pleural effusion is the only manifestation of the underlying disease. Pleural fluid tapping is a relatively less invasive procedure, and may provide a symptomatic relief to the patient and has several diagnostic advantages including easy obtainability and reproducibility. Finding the source of tumor cells in pleural fluid is crucial in diagnosis and treatment of the patients, as it can act as substitute for histopathological examination. Lungs also act as a ground for metastases from malignancies such as gastrointestinal tract, liver, kidney, ovaries, testes and lymphomas, which could have pleural effusion associated with it. Sub-typing of lung cancers into small cell lung cancers (SCLC) and non-small cell lung cancers (NSCLC)(adeno and squamous cell carcinoma) is essential in the present era of personalized treatment. There are only a few studies on the feasibility and reliability of diagnosis and sub-typing of malignancies from the pleural fluid material.1-3 Immunohistochemistry (IHC) can be performed on effusion samples on directly fixed or liquid based cytology (LBC) smears or on the sections obtained from cell block. Cell block is considered the most useful for IHC, because unlike smears, it acts as a mini biopsy, which facilitates multiple and better quality stains. The present study was undertaken to determine the feasibility of cell block. IHC in cases presented with pleural effusion and finding the accuracy of the same in diagnosing the unknown primary and finding the subtype.

MATERIALS AND METHODS:

This was a retrospective study done in the department of pathology GCRI during the year 2017. All diagnostic procedures and interventions were part of the routine care and were not add-ons for the research purpose only and no experimental or new protocols were used. The study was in accordance with the declaration of Helsinki, therefore no separate ethical committee approval was taken.

Total 769 pleural fluid samples were studied during a span of 1 year (Jan 2017- Dec 2017). Out of them 200 cases were found to be positive for malignancy. 259 were new cases , with no definite diagnosis of malignancy before. Morphological criteria including cellularity, arrangement of cells, nuclear and cytoplasmic details were put together and used for categorization of the fluid specimens. Out of 259 undiagnosed cases, 72(N=72) samples tested positive for malignancy, and taken up in our study. Cell block was attempted in all 72 of them. Cell blocks were prepared using acetic acid-formalin technique. Pleural fluid collected in EDTA vacuttainer, centrifuged for 10 min at 600G. Supernatant discarded, 1.5ml methanol is added and left for 15 minutes. Again centrifuged at 600G for 15 minutes, supernatant discarded and 1.5ml of 10% formalin is added and kept for overnight. Next day supernatant is removed and the packed sediment is wrapped in lens paper and put in labeled tissue cassttes which are fixed in 10% formalin. Considering into account the adequacy(cellularity >100cells) of cell block material, only 59(n=59) could be subjected to IHC for further confirmation. Cases were evaluated based on cytomorphology. Further immune-panel comprising of CK7, CK5/6, p63, TTF-1, calretinin, mesothelin and synaptophysin was applied on cell block sections of each case. EMA(epithelial membrane antigen), CEA(carcinoembryonic antigen), CA-125, LCA, CD 20, Bcl2, CD3 were applied in only few cases, selected based on the morphology and clinical details. The immunostaining was assessed semiquantitatively based on the intensity of staining and number of positive tumor cells. Out of 59 cases, 40 patients are under regular follow up, taking chemotherapy regimens. While 10 patients cannot be followed up due to non compliance while 9 patients expired during the course of treatment.

RESULTS:

Out of 259 new samples studied 72 cases tested positive in cytology. None of these 72 were found not to have malignancy subsequently. So true positives were 72 and false positive were 0. Out of 187 new cases tested negative in cytology, 164 patients subsequently found to have malignancy. So number of true negatives were 23 and false negatives were 164.

So the positive predictive value (PPV= True positive / Total positive) of pleural fluid cytology testing was 100%. Negative predictive value (NPV= true negative / total negatives) was 12.2%. Sensitivity (true positives / total people having disease) of pleural fluid cytology testing is 30.5%. Specificity (true negatives / total people without disease) of the test was 100%.

72 patients enrolled under this study, successful IHC could be done in 59(81.94%) patients. Among those, 45 patients were males and 14 were females with a male:female ratio of 3.2:1. Age range was 29-85 years (mean age 50.8 years). Pleural effusion was chief finding in all the patients. Based upon pleural fluid cytomorphology, cases were categorized as metastatic adenocarcinoma(41/59;69.4%), metastatic poorly differentiated carcinoma(9/59;15.2%), malignant tumor with a possibility of adenocarcinoma/mesothelioma(2/59;3.3%), metastatic serous papillary adenocarcinoma(1/59;1.6%) and lastly involvement by Non Hodgkin Lymphoma (6/59;10.1%) After IHC , in 45(76.27%) cases primary site was lung, in 6(10.1%) primary was ovary. Out of 45 cases of lung primaries, 37(82.2%) were metastatic adenocarcinoma, showing strong nuclear positivity for TTF-1 and cytoplasmic positivity for CK7 and negativity for CK5/6, p63 and calretinin. 2(4.44%) cases showed positivity for mesothelin, calretinin and WT1. In these diagnosis of mesothelioma was made. Out of 9 cases of metastatic poorly differentiated carcinoma six cases showed positivity for chromogranin and synaptophysin, hence diagnosed as small cell lung carcinoma.

Based on IHC along with clinicradiological details, 8(13.5%) cases were diagnosed as metastatic adenocarcinoma of extrapulmonary origin including primaries from ovaries and stomach. Out of these 8 cases 3 could be diagnosed as metastatic adenocarcinoma only after IHC, and 1 was diagnosed as serous papillary adenocarcinoma on both cytoblock and IHC. All 8(100%) cases showed ck7 positivity. In 2 cases primary came out to be stomach. In 6(10.16%) cases, pleural fluid was involved by lymphoma. After applying IHC diffuse large B cell lymphoma (DLBCL) was diagnosed in 5 cases showing positivity for LCA, CD20 in all. 1 case showed CD5, CD20, cyclin D1 positivity hence diagnosis of Mantle Cell Lymphoma was made.

DISCUSSION:

The cytological examination of serous fluid has diagnostic, therapeutic and prognostic implications since long time. The malignant cells in the pleural fluid were almost always indicative of metastatic tumors. A positive effusion for malignant cells is an important prognostic indicator in cancer patients. The development of a malignant pleural effusion is a common complication and indication of advanced stages of cancers like lung, stomach, ovary and lymphoma. Thus, the examination of body fluids for the presence of malignant cells has been accepted as a routine laboratory procedure for detection of metastasis of unknown primary origin. Since the introduction of the cell block technique by Bahrenburg nearly a century ago, it has been used routinely for processing fluids.

Upto 1/3rd of the patients with NSCLC have only pleural effusion at the time of diagnosis.4-6 Pleural effusions may be due to pleural seeding of tumor cells, lymphatic obstruction, pulmonary venous obstruction, reactive etiology or metastasis.5 Isolated pleural effusion can be seen in about 4% of the small cell and SCCs and in >20% cases of lung adenocarcinomas.7 Pleural effusion if present has an important role for diagnosis and sub-typing of malignancies. Although no gold standard is available for diagnosing these cases, a combinatorial approach of clinical, radiological, cytological and immunological methods should suffice for a final diagnosis in a large majority of cases. The broad diagnostics offered on cytomorphology such as positive for malignancy, metastatic carcinoma, poorly differentiated carcinoma or suggestive of carcinoma/lymphoma etc can be replaced with a subtype diagnosis of adenocarcinoma or SCC and primary site can also be identified by using IHC. Thus skipping the need of invasive small biopsy procedures. Cell blocks with adequate cellularity (>100 cells) are preferred to perform IHC. One of the most common problem is to distinguish reactive mesothelial cells from metastatic neoplasms. The difficulty is either secondary to marked atypia of mesothelial cells caused by the microbiological, chemical, physical, immunological, or metabolic insults to the serous membranes or to the subtle cytomorphological features of some malignant neoplasm, particularly well differentiated adenocarcinomas.

The immunomarkers to be applied should also be selected judicially in order to preserve tissue. Previous studies have used different immunopanels in order to confirm lung adenocarcinoma cases (Table 2)9-11The common panel of IHC applied on cell block sections of all cases in this study included CK7,CK 5/6,p63,TTF1 and calretinin. Thyroid transcription factor-1(TTF-1) is expressed in epithelial cells of the thyroid and lung. In our study (31/36;86%) lung adenocarcinoma express TTF-1 on cell blocks.

Previous studies have shown 100% diagnostic specificity of TTF-1 for detection of lung adenocarcinoma in pleural fluid samples13,14.No aberrant expression of p63, CK20 can be seen in our study. Aberrant expression of CK5/6 is seen in one case of lung adenocarcinoma which may lead to misinterpretation of tumor as squamous carcinoma. 74.5% pleural samples could be confirmed as metastasis from lung primary in the present study. We added a battery of other immunomarkers such as CEA, EMA, CD 20, mesothelin, LCA, CD20, CD5 and synaptophysin and was able to diagnose small cell carcinoma,malignant mesothelioma and metastatic carcinoma from ovaries and stomach, and do the subtyping of NHL further into DLBCL and Mantle Cell Lymphoma.

This study focuses on use of immunomarkers on cell block sections obtained from pleural fluid samples to delineate the primary site of origin. Morphology on pleural fluid cytology is the initial key to diagnosis. The diagnostic accuracy and reproducibility can definitely be increased using IHC as an adjunct. Thus bypassing the need of invasive biopsy procedure thereby saving time and initiation of treatment without delay.

CONCLUSION:

Pleural effusion cytology is an important diagnostic technique for detection of the lung carcinoma and other metastatic malignancies, with very high PPV and specificity, but low NPV and sensitivity. Therefore negative findings need to be evaluated with repeated cytology/ histopathology testings. Application of IHC on cell blocks prepared from pleural fluid has comparable results with histopathological diagnosis. IHC can also help in determining primary site of extrapulmonary origin. A judicious use of IHC can be an effective procedure for detection and subtyping of lung carcinoma and other metastatic malignancies, especially in cases where obtaining a biopsy is technically challenging.

Table 1 Comparison of the initial cytomorphological & final diagnosis

S. no Cytomorphologicaldiagnosis	final diagnosis afterIHC
1. Metastatic adenocarcinoma (n=42) Metastatic lung adenocarcinoma (n=37)	Metastatic ovarian carcinoma(n=2)
	Metastatic serous papillary ovarian
	Carcinoma (n=1)
	Metastatic carcinoma,stomach origin(n=2)
2.Metastaticpoorlydifferentiated	Metastatic lungadenocarcinoma(n=2)
Carcinoma(n=9)	Small cell lungcarcinoma(n=6)
	Metastatic ovarian carcinoma(n=1)
3.Positive formalignancypossibily	Malignantmesothelioma(n=2)
Mesothelioma or adenocarcinoma	
4.InvolvementbyNHL	DLBCL(n=5)
	Mantle Cell Lymphoma(n=1)

Table2 IHC panels for lung carcinoma used in various studies

Name of study	Antibodies Panel
Kulshreshtha et al	TTF1,CK7,CK20,CEA,CD45,Chromogranin, Synaptophysin
Brown et al	p40,TTF1,NapsinA,CK5
Kargi et al	TTF1,p63,CK5/6
Khayyata et al	p63,CK5/6
Our study	TTF1,p63,CK7,CK5/6,Chromogranin,WT1,CEA EMA and others

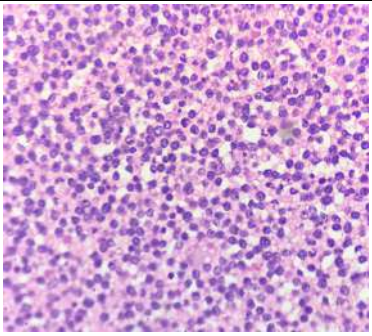

Figure showing metastatic Non Hodgkin Lymphoma having good cellularity pleural fluid cytoblock (H& E stain -40X)

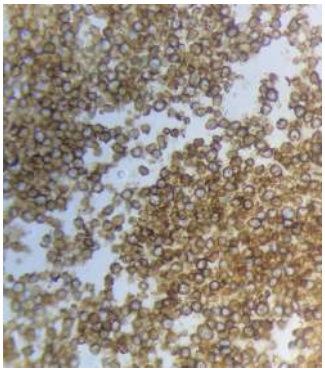

Figure showing strong positivity for LCA in tumor cells in metastatic lymphoma pleural fluid cytoblock(40x)

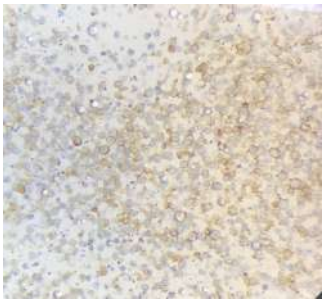

Figure showing cytoplasmic CD 20 positivity by tumor cells in metastatic lymphoma pleuralfluid cytoblock(40X)

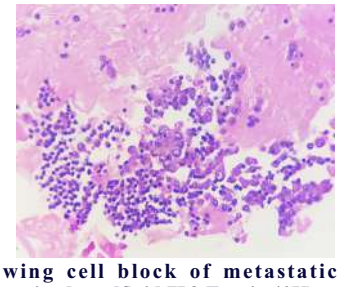

Figure showing cell block of metastatic pulmonary adenocarcinoma in pleuralfluid(H& E stain 40X)

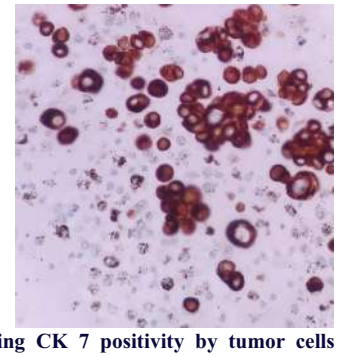

Figure showing CK 7 positivity by tumor cells in metastatic pulmonary adenocarcinoma pleuralfluid cytoblock(40X)

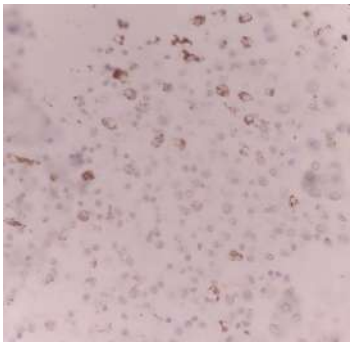

Figure showing focal positivity of CEA in tumor cells in metastatic pulmonary adenocarcinoma pleuralfluid cytoblock(40X)

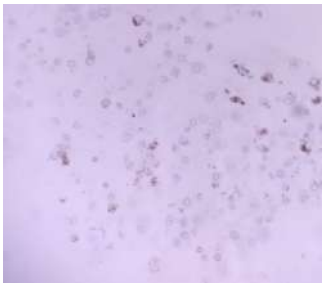

Figure showing CK5/6 negativity in tumor cells in metastatic pulmonary adenocarcinoma pleuralfluid cytoblock

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