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Role of Immunocytochemistry using Cell Block in Cases with Malignant Pleural Effusion

KEYWORDS: Immunohistochemistry, Cell block, Metastatic Adenocarcinoma, Reactive Mesothelial Cells

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ABSTRACT *Cytological examination of serous fluids is of paramount importance in detecting cancer cells. Cytology is more sensitive than blind biopsy for detecting serosal malignancy, presumably because fluid provides a more representative sample. Cell block and immunohistochemistry are essential adjunct to cytomorphology in suspicious cases and substantially improves diagnostic accuracy. Immunohistochemistry is also useful to establish the types of malignant cases.*

A total of 190 pleural fluid samples received for cytopathological examination over a period of 1 year 6 months were analyzed. Cytomorphological features of neoplastic effusions were studied by MGG and PAP staining. Cell block and immunohistochemistry (EMA and Calretinin) were performed to aid the diagnosis in clinico-radiologically malignant cases.

In this study of 190 samples of pleural fluid, 20 cases (10.53%) were found to be malignant and out of which metastatic adenocarcinoma was found to be most common cause of malignant effusion. Reactive mesothelial cells were the most challenging to differentiate from metastatic adenocarcinoma. Immunohistochemical staining was useful to arrive at a definitive diagnosis in difficult cases.

Immunohistochemistry plays a key role to detect malignant effusions. Cell block and immunohistochemical staining must be performed in paucicellular cases and in difficult cases to differentiate metastatic epithelial cells from reactive mesothelial cells.

Introduction:

Cytological examination of serous fluids is of great importance in detecting cancer cells (Grzegorz et al., 2012). Effusions are often the first clinical symptom of malignant tumors or of their metastatic manifestation. In known malignancies effusions are an ominous sign. Admittedly, finding cancer cells in such specimens denotes that the patient has cancer that is not only advanced but also almost always incurable. Thereby cytologic examination may be the first, best or only chance for making the diagnosis of an underlying malignancy, in addition fluid cytology has also clinical significance in the management of patients with malignancy (Elizabeth et al., 1961). Accordingly the purposes of pleural fluid examination are to correctly identify cancer cells and if possible, to identify the tumour types and primary sites when presented with unknown primary tumor sites (El-Sheikh, 2012).

Reporting a cell as malignant or benign reactive mesothelial cell in fluid cytology is an everyday diagnostic problem (Politi et al., 2005). The cell block method is one of the traditional method used for processing cytological material and was described in the literature as early as 1900. Cell blocks are helpful in situations where the cytological abnormalities are ambiguous like in reactive mesothelial cells or in occasional well differentiated adenocarcinoma. Apart from increased cellularity, better morphological details are obtained by cell block method as there is a better conservation of architectural features like arrangement of cells, cytoplasmic and nuclear details (Archana et al., 2003). Immunocytochemistry (ICC) is an essential adjunct to cytomorphology in selected cases and substantially improves diagnostic accuracy.

Cytology is more sensitive than blind biopsy for detecting serosal malignancy, presumably because fluid provides a more representative sample (Nance et al., 1999). C e l l block is a mini formalin-fixed paraffin-embedded (FFPE) biopsy obtained from fluid sediment. Preservation of cytologic material in the cell block for IHC and molecular studies adds to its diagnostic accuracy and enables long-term archiving for future analyses.

Materials and Method:

This study was carried out in the Department of Pathology, Gauhati Medical College, a tertiary level referral hospital situated in Guwahati, North-East India from April 2014 to September 2015. A total of 190 cases of pleural fluid were received from patients attending Pulmonary medicine, General Medicine, Surgery, Paediatrics and O & G wards for cytopathological examination. The study was approved by Institutional Ethical Committee of Gauhati Medical College and Hospital, Guwahati.

A detailed clinical history was taken and physical examination carried out. Patient presenting with unilateral or bilateral pleural effusion were included. 20-30 ml of pleural fluids are collected by thoracocentesis through the back of the chest wall with a wide bore needle of 21G-22G, into a clean, dry container and subjected to physical, chemical and cytological examination.

Aspirated fluid was examined and determination of physical and chemical parameters were done. The fluids received were stained with May-Grunwald-Giemsa stain and Papanicolaou stain for cytological evaluation. In diagnostically difficult cases, to differentiate reactive mesothelial

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cells from adenocarcinoma, cell blocks were prepared. Immunocytochemical test (EMA & Calretinin) were performed in the cell block sections to confirm the diagnosis in selected cases.

In doubtful cases sections were made from the cell block and were stained with Haematoxylin and Eosin stain and if necessary immunocytochemical stains to differentiate between reactive mesothelial cells and adenocarcinoma cells using Epithelial membrane antigen and calretinin.

Results:
In our study of 190 cases, the commonest age group of pleural effusion was found to be 41-50 years with male preponderance. Furthermore, 85.09% cases were found to be exudative type and remaining 14.91% were transudative type. The most common etiology of pleural effusion were found to be tuberculosis accounting for 34.68%of the cases, followed by para-pneumonic and then malignancies.

Out of 33 cases of clinico-radiologically diagnosed malignancy with pleural effusion, 13 cases were diagnosed as metastatic adenocarcinoma, 2 cases were metastatic Squamous Cell Carcinoma (SCC), 10 cases show reactive mesothelial cells and remaining 8 cases were suspicious cytologically which is detailed in Table 1.

Table 1: Distribution of clinico-radiologically diagnosed malignant cases with that of cytological diagnosis of pleural fluid

Clinico-radiologically diagnosed as malignancy	No. of cases	Cytology smears			
		Metastatic adenocarcinoma	Metastatic SCC	Suspicious cells	Reactive mesothelial cells (inflammatory)
Ca lung	22	07	02	06	07
Ca breast	07	03	0	01	03
Ca ovary	02	02	0	0	0
Ca GB	01	01	0	0	0
Mesothelioma	01	0	0	01	0
Total	33	13	02	08	10

EMA positivity was seen in 20 cases out of 33 cases of clinico-radiologically malignant cases. All cytologically diagnosed metastatic adenocarcinoma and SCC showed EMA positivity. 4 suspicious cases also showed EMA positivity. 13 cases were EMA negative.

Out of 33 cases of pleural effusion due to malignancy at various sites, 13 cases showed calretinin positive. All metastatic adenocarcinoma and metastatic SCC were calretinin negative. 3 suspicious cases showed calretinin positive which is detailed in Table 2.

Table 2: Staining status of EMA and Calretinin in clinico-radiologically diagnosed malignant cases

Cytological findings of clinico-radiologically diagnosed malignant cases	Cytology smears	EMA positive but calretinin negative	EMA negative but calretinin positive	Both EMA and calretinin positive
Metastatic adenocarcinoma	13	13	0	0
Metastatic SCC	02	02	0	0
Suspicious cells	08	04	03	01
Reactive mesothelial cells	10	0	10	0
Total	33	19	13	01

Distribution of final cytological findings of all pleural effusion cases after confirmation by IHC show that out of 190 cases, 20 cases (10.53%) were found to be positive for malignant cell. The remaining 170 cases (89.47%) were benign effusion which in detailed in Table 3.

Table 3: Distribution of final cytological findings of all pleural effusion cases after confirmation by immunocytochemistry in clinico-radiologically diagnosed malignant cases

Cytological category	No. of cases	Percentage
Benign effusion (both inflammatory & scanty cellularity)	170	89.47%
Malignant	20	10.53%
Total	190	100%

In our study, out of 20 malignant pleural effusion, metastatic adenocarcinoma is found in 17 cases (85%), metastatic SCC in 2 cases (10%) and mesothelioma in 1 case (5%).

The most common type of malignant pleural effusion was found to be metastatic adenocarcinoma (Figure 1), followed by metastatic SCC and mesothelioma (Figure 2). Further, the most common primary neoplasm in malignant pleural effusion were found to be lung origin (65%) followed by breast origin (15%).

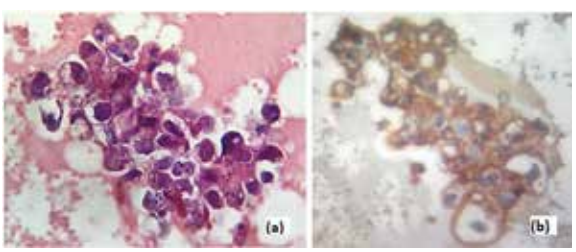

Figure 1: (a) Photomicrograph of metastatic adenocarcinoma cells in pleural fluid (cell block, H & E stain, 10x40X), primary lesion - lung carcinoma. (b)Photomicrograph of metaststic adenocarcinoma cells in pleural fluid showing strong cytoplasmic positivity for EMA (10x40X), primary lesion - lung carcinoma.

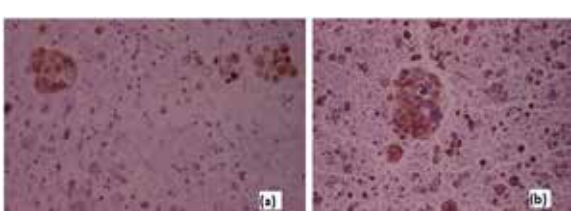

Figure 2: (a) Photomicrograph of malignant mesothelioma cells in pleural fluid showing both nuclear and cytoplasmic positivity for calretinin (10x40X), primary lesion - in pleura. (b) Photomicrograph of malignant mesothelioma cells in pleural fluid showing cytoplasmic positivity for EMA (10x40X), primary lesion in pleura.

Discussion:
Cytological examination of serous fluids is of great importance not only in detecting cancer cells, but it also reveals information regarding various inflammatory conditions of serous membranes. The main purpose of cytological evaluation of effusion samples is to look for presence of malignant cells. Cytoblock prepared from effusion sample can

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be useful adjunct to smear for establishing a more definitive cytopathologic diagnosis (Grandhi et al., 2014; Thapar et al., 2009). Ancillary studies can be done using cytoblock including IHC and various molecular techniques.

In the present study, with conventional smears, 15 cases were diagnosed to be malignant effusion, and 8 cases were interpreted as suspicious. While the use of immunocytochemistry (EMA & Calretinin) has increased the diagnostic yield of malignant effusion to 20 (10.53%) without any suspicious category, which closely resembled with the study done by Dagli et al. (2011) and Grandhi et al. (2014).

Taking into detailed account of clinico-radiological data and considering different cytomorphological features of malignant cell from cell block preparation, primary site could be determined accurately. The combined technique of smear examination as well as cell block preparation help to ascertain the primary site of malignancy (Khan et al., 2005). Cell block technique not only increase the positive result, but also help to demonstrate better architectural pattern which could be of great help in making correct diagnosis of primary site. The cell block technique was also useful for special stain and IHC which can give morphological details by preserving the architectural pattern.

In our study, we have diagnosed various pathological lesions based on the following cytomorphological features:

Metastatic adenocarcinoma can have various architectural pattern, including sheets, three-dimensional cell clusters, papillary clusters, acinar structures, picket fence or drunken honeycomb. Individual tumor cells have homogeneous basophilic cytoplasm that may be granular and foamy and may show cytoplasmic vacuoles. The nuclei are usually eccentrically placed with high N/C ratio, irregular contour and uniform finely granular to coarse hyperchromatic chromatin. Majority of the tumor cells have macro-nucleoli. Metastatic SCC is manifested by three main morphological features: keratinization, intercellular bridges and pearls. Cytoplasm is inky blue color on the MGG stain and dense, opaque. Cells are pleomorphic, nuclei are hyperchromatic with dense homogeneous pyknotic chromatin. Mesothelioma shows moderately cellular specimen, large cell clusters containing more than 50 cells, two- or three-dimensional cell group with knobly outlines. Intercellular windows and peripheral cytoplasmic blabes and nuclear feature of malignancy, central hyperchromatic nuclei with prominent nucleoli are present. Reactive mesothelial cells are moderately cellular specimens, monolayered sheets, cell groups containing less than 20 cells with knobly outlines. Intercellular windows, peripheral cytoplasmic blabes and atypical nuclear features are present. IHC helped in confirmation of diagnosis for EMA +ve and Calretinin -ve in both metastatic adenocarcinoma and SCC whereas EMA -ve and Calretinin +ve in reactive mesothelioma and both EMA and Caretinin are +ve in mesothelioma.

From the results obtained as discussed above it is justified that the diagnosis of metastatic adenocarcinoma, metastatic SCC, mesothelioma and reactive mesothelial cells (reinforced by cytological smear as well as cell block preparation including IHC) are in corroboration with the findings obtained by Johnston et al. (1976), Cibas et al. (2009) and Koss et al. (2006).

In this study, the most common type of malignancy was found to be metastatic adenocarcinoma in 17 cases (85%) followed by metastatic squamous cell carcinoma in 2 cases (10%) and mesothelioma in 1 case (5%) out of 20 cases of malignant pleural effusion. Other malignancies like haematolymphoid malignancy were not found in this study period. This findings are consistent with the findings obtained by Yahya et al. (2013) and Viral et al. (2014). Further, the most common primary neoplasm in malignant pleural effusion were found to be lung origin followed by breast origin which correlates with the study of Viral et al. (2014).

In addition, we evaluated a total of 33 cases of pleural effusion related to malignancy. We found the sensitivity and specificity of conventional cytology in detecting carcinomatous effusion to be 78.57% and 100% respectively which closely resembled to the study done by Pillai et al. (2013) and Yahya et al. (2013). But with the use of ICC in conjunction, sensitivity and specificity increased to 97% and 100% respectively. Thus it is seen that the combination of both cytology and ICC studies using the two markers can greatly enhance the diagnostic accuracy, in malignant effusions, which resembles the study of Yahya et al. (2013).

Conclusion:

From the present study it can be concluded that effusion cytology is a useful tool to distinguish between benign inflammatory and malignant pleural effusions. In the identification of malignant cells in effusion and its differentiation from cells showing reactive and degenerative changes posed diagnostic difficulties in some of the cases. In these problematic cases, additional cytoblock preparation from pleural fluid and immunocytochemical study was undertaken.

Hence immunocytochemistry is an important ancillary diagnostic tool to aid in the diagnosis of morphologically challenging cases and also to establish the types of malignant cases.

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