Cytological evaluation of peritoneal fluid with special reference to malignancy

Ena Dowerah and Sandip Das *

Department of Pathology, Guwahati Medical College and Hospital, Guwahati-781032, Assam

*Correspondence Info:
Dr. Sandip Das
c/o S.C. Das
House number 1418, Tetelia nursery, NH-37 P.O.-Gotanagar PIN- 781033
E-mail: sandipdas116@gmail.com

Abstract
Introduction: Aspiration of serous cavities is a simple and relatively non invasive technique to achieve a diagnosis. Cytological examination of serous fluid is of paramount importance, it reveals as information about inflammatory conditions of serous membrane, infection by bacteria, fungi, virus, finding of cancer cells and also supply evidence of fistulous connection with a serous cavity. Cytological examination not only helps for the diagnosis of cancer but also for staging and prognosis of disease. It is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease.

Methods: A total of 160 peritoneal effusion samples received for cytopathological examination over a 1 year period were analyzed. Cytomorphological features of neoplastic effusions were studied. Special stains and immunocytochemistry (ICC) were performed to aid the diagnosis in difficult cases.

Result: The peritoneal effusion samples comprised of 160 cases out of which adenocarcinoma was found to be the cause of metastatic malignant effusion. Reactive mesothelial cells were the most challenging to differentiate from malignant effusion cytology. Immunohistochemical staining was useful to arrive at a definitive diagnosis in difficult cases.

Conclusion: Cytology is a useful tool to detect malignant effusions. Immunohistochemical staining must be performed in difficult cases to differentiate metastatic epithelial cells from reactive mesothelial cells, which is a benign condition.

Keywords: Immunohistochemistry, metastatic malignant cells, reactive mesothelial cells.

1. Introduction
The peritoneal cavities are lined by a single layer of flat mesothelial cells called the serosa. Normally, these cavities are collapsed and contain only a small amount of fluid, enough to lubricate the adjacent surfaces. In disease states, a greater amount of fluid—an effusion—accumulates. Effusions are classified clinically as transudative or exudative.  

Diagnostic cytopathology is based on two approaches-exfoliative and non-exfoliative. Exfoliative cytology comprises of study of spontaneously exfoliated cells from the surface of mucous membrane, skin as well as serosal lining of the body cavities. In non-exfoliative cytology, cells are scraped from the skin and mucous membrane.

Aspiration of serous cavities is a simple and relatively non invasive technique to achieve a diagnosis. Cytological examination of serous fluid is of paramount importance, it reveals as information about inflammatory conditions of serous membrane, infection by bacteria, fungi, virus, finding of cancer cells and also supply evidence of fistulous connection with a serous cavity. Cytological examination not only helps for the diagnosis of cancer but also for staging and prognosis of disease. It is a complete diagnostic modality which aims at pointing out the etiology of effusion as well as prognosis of disease.  

The first line of investigation of a suspected neoplastic lesion is often the cytological examination of fluid tapped from pleural, peritoneal and pericardial cavity. The aim of the pathologist is (1) to identify the cancer cells accurately (2) to identify the tumor type, and primary site of origin.  

Immunocytochemistry on cytologic material has attained great potential utility. In body fluids it is performed most commonly for the distinction of mesothelial cells from metastatic malignancy. Distinction of malignant mesothelioma and metastatic adenocarcinoma in body fluids is a formidable challenge. Until recently, a variety of negative markers which are present in adenocarcinoma but not in mesothelioma formed the basis for the diagnosis of mesothelioma.  

Aims and Objectives
1. To diagnose the cause of peritoneal effusion.
2. To establish the diagnosis of malignancy and to differentiate its types.
3. To distinguish between benign and malignant effusion by cytological examination, with additional use of immunocytochemical stain in differentiating adenocarcinoma from reactive mesothelial cells.

2. Materials and Method
From July 2012 to June 2013, a total of 160 cases of peritoneal effusion are reported in the Cytology section, in the Department of Pathology, Guwahati Medical College and Hospital, Guwahati.

A detailed clinical history was taken and a physical examination carried out in all patients and recorded in a standard proforma. Aspirated fluid was examined and determinations of physical and chemical parameters were done. The fluids received were stained with May-Grünwald-Giemsa stain and Papanicolaou stain for cytological evaluation. In diagnostically difficult cases, to differentiate reactive mesothelial cells from adenocarcinoma, cell blocks were prepared. Special test like PAS stain and immunocytochemical test (EMA & Calretinin) were performed in the cell block sections to confirm the diagnosis.

A detailed clinical history was taken and a physical examination carried out in all patients and recorded in a standard proforma. Aspirated fluid was examined and determinations of physical and chemical parameters were done. The fluids received were stained with May-Grünwald-Giemsa stain and Papanicolaou stain for cytological evaluation. In diagnostically difficult cases, to differentiate reactive mesothelial cells from adenocarcinoma, cell blocks were prepared. Special test like PAS stain and immunocytochemical test (EMA & Calretinin) were performed in the cell block sections to confirm the diagnosis.

IJBR (2014) 05 (06)
The routine biochemical and hematological investigations were done in all patients. Other investigations namely chest X-ray, USG abdomen, CT-scan abdomen were advised whenever necessary to find out malignancies of primary site and the secondaries.

At least 20-30 ml fluid is collected. The entire fluid tapped should be mixed well so that the cells suspended in it are well dispersed. The specimen of about 10ml is centrifuged at 2000 r.p.m for 10 minutes. The supernatant is used for biochemical tests. The sediment is re-suspended in a drop of fluid and smears are prepared from the centrifuged deposit.

The other half of the specimen is used to prepare slides in a cytocentrifuge machine. Smears are air dried, fixed in methanol and stained with MGG. For PAP stain, smears are wet fixed in absolute alcohol.

For haemorrhagic fluids
Glacial acetic acid technique - In case of haemorrhagic fluid, 1ml of glacial acetic acid is added to 50 ml fluid before centrifugation and smears are made by conventional method.

Cell block
1. Mix sediments/tissue fragments in the above mentioned fixative. If the sediment was bloody glacial acetic acid (1ml) was added per 50 ml of the fluid specimen prior to addition of fixative as a haemolysing agent.
2. Centrifuge the mixture for 10mins at 2000 rpm.
3. Pour off the supernatant and drain tube well by inverting the tube on a paper towel.
4. Packed sediment was wrapped in lens paper and kept in labelled tissue cassette and was processed as tissue sections.

Cytological examination:
Fluids were centrifuged; smears were stained with both MGG and PAP.

The following points were noted:
- Type of predominant cells.
- Characteristics of cytoplasm.
- Nuclear characteristics-nuclear membrane, chromatin pattern, presence of nucleoli, mitotic figures.
- Smear background.

### Table 1: Features for differentiating RMC and Adenocarcinoma cells

<table>
<thead>
<tr>
<th>Features</th>
<th>Reactive Mesothelial Cells</th>
<th>Adenocarcinoma Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Specimen cellularity</td>
<td>Moderate to low</td>
<td>Hypercellular</td>
</tr>
<tr>
<td>2. Cell group</td>
<td>Monolayered and knobbly outline</td>
<td>2- and 3-D and smooth borders (community borders)</td>
</tr>
<tr>
<td>3. Intercellular windows</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>4. Acinar formation</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>5. Cell size variability</td>
<td>Mild variation</td>
<td>Highly variable</td>
</tr>
<tr>
<td>6. Two - zones cytoplasmic appearance</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>7. Nuclear features of malignancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Pleomorphism</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>* Prominent Nucleoli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Atypical mitosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Coarse clumped chromatin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Special stains: PAS stain

Immunohistochemistry:
In diagnostically difficult cases, to differentiate between reactive mesothelial cells and metastatic adenocarcinoma cells, immunocytochemical stains were performed on cell block sections using Epithelial membrane antigen and Calretinin. EMA gave positive results in adenocarcinoma while Calretinin gave positivity for reactive mesothelial cells.

Two step indirect technique was used for immunocytochemistry using:

Primary antibodies:
1. Anticalretinin antibody (polyclonal) - Rabbit polyclonal antibody.
   Ref. No. AR413-5R.
2. Anti EMA antibody - Mouse monoclonal antibody.
   Ref. No. AM057-5M.

Secondary antibody:
1. SS - Polymer HRP Ref. No. HK51906K.

Positive Controls of primary antibodies:
1. Anticalretinin antibody : Brain (neurons).
2. AntiEMA antibody : Lung (epithelium).

3. Results
A total of 160 cases presenting with ascites were evaluated cytologically. Nature of effusion fluid material evaluated for transudate and exudates. Cytological examination was done in all the cases using light microscope. In addition, immunocytochemical studies were also performed in suspicious cases of malignancy to differentiate adenocarcinoma cells from reactive mesothelial cells.

### Table 2: Showing Age distribution of the patients

<table>
<thead>
<tr>
<th>Age group (in years)</th>
<th>No. of cases (N=160)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>2</td>
<td>1.25%</td>
</tr>
<tr>
<td>11–20</td>
<td>7</td>
<td>4.4%</td>
</tr>
<tr>
<td>21–30</td>
<td>27</td>
<td>16.9%</td>
</tr>
<tr>
<td>31–40</td>
<td>26</td>
<td>16.25%</td>
</tr>
<tr>
<td>41–50</td>
<td>31</td>
<td>19.4%</td>
</tr>
<tr>
<td>51–60</td>
<td>39</td>
<td>24.3%</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>28</td>
<td>17.5%</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>100%</td>
</tr>
</tbody>
</table>
4. Discussion

The cell population in the sediment of body fluid represents a much larger surface area than obtained by needle biopsy. Since the introduction of the CB technique by Bahrenburg nearly a century ago, it has been used routinely for processing fluids. Cell blocks prepared from residual tissue fluid can be used as adjuncts to smear for establishing a more definitive cytopathological diagnosis. The technique is simple, safe, cost-effective and reproducible even in resource limited settings.1

Cytological examination of serous fluid has increasingly gained acceptance in clinical practice to such an extent that a positive diagnosis is considered the definitive test and obviates exploratory surgery.1

The malignant cells in the pleural or the ascitic fluids were almost always indicative of metastatic tumours, as primary malignancies which arose from the mesothelial cell lining were uncommon. A positive effusion for malignant cells is an important prognostic indicator in cancer patients.5 The development of a malignant pleural effusion is a common complication and indication of advanced stages of cancers like lung, breast and stomach cancer, while development of malignant ascitic effusion is due to ovary, colon, liver and pancreatic carcinoma. 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.6,7
Of the 160 cases, the most common age group affected by peritoneal effusion was found to be 51-60 years (24.3%) and least commonly age group being 0-10 years (1.25%) to be affected by ascites. Our study is in agreement with Udasmith et al.\textsuperscript{5}

Out of 160 cases, the most common cause of ascites was found to be Liver cirrhosis accounting for 40% of cases and suspicious cases of malignancy accounted for 11%. This is in agreement with Khan\textit{et al} and KP Moore.\textsuperscript{9,10}

In the present study, we evaluated a total of 24 cases of ascites related to malignancy. We found the sensitivity and specificity of conventional cytology in detecting carcinothelial effusion to be 78.57% and 100% respectively. Improved sensitivity of smear in diagnosis of malignant effusion may be due to the use of Cytospin machine that helps to make a monolayered sheet of cells so that morphology of cells can be better studied. Similar findings were also reported by Karoo \textit{et al} and Castaldo \textit{et al}.\textsuperscript{11,12}

In the present study, we used an epithelial marker (EMA) and one mesothelial marker, Calretinin. A total of clinically diagnosed 24 malignant adenocarcinoma were included in our study. Cytologically we found 11 cases suggestive of metastatic adenocarcinoma and 13 cases to be reactive. We then performed immunocytochemistry in cell block sections of all the 24 cases. We got a total number of 14 cases to be metastatic adenocarcinoma which were EMA positive and Calretinin negative. The rest 10 cases were found to be reactive effusion which stained strongly with Calretinin and was negative for EMA. Thus immunocytochemistry is very helpful in distinguishing adenocarcinoma cells from reactive mesothelial cells. Thus diagnostic accuracy of conventional cytology can be improved by use of immunocytochemistry which is simple, should be done in all those cases where cytology on its own is not completely unequivocal and similar findings were noted by Grefte.\textsuperscript{13}

We did not proceed to do immunohistochemical study for other malignancies due to absence of primary tumors as an etiology for ascites.

5. Conclusion
1. The use of cytopathology of peritoneal effusion is gaining popularity in the field of early diagnosis and treatment. The technique is cheap, easy to perform and produces speedy diagnosis.
2. In the identification of malignant cells in effusion and its differentiation from cells showing reactive and degenerative changes there were diagnostic difficulties in some of the cases.
3. Immunocytochemistry is an important ancillary diagnostic tool in effusion cytology.

References