Evaluation of Cell Block Technique in the Cytodiagnosis of Body Fluids

Bansode Shubhada¹, Kumbalkar D², Nayak S³

¹Assistant Professor, Department of Pathology, GMC& H, Latur, Maharashtra, India
²Professor & Head, Department of Pathology, GMC& H, Nagpur, Maharashtra, India
³Assistant Professor, Department of Pathology, GMC& H, Nagpur, Maharashtra, India

Abstract: Background: Conventional smear preparation is a simple procedure but has many disadvantages causing the difficulties in making the definitive diagnosis however cell block are particularly useful when the cytological abnormalities are misleading. Once positive diagnosis is made it is often considered definitive diagnosis and it is paramount important to identify primary site and type of malignancy. This can help obviate the proper surgical management, chemotherapy and radiotherapy so as to increase patients’ survival rate. Aims: To study cell block preparation of all body fluids along with conventional cytology smear and to know primary site of malignancy. This can help to obviate the proper surgical management, chemotherapy and radiotherapy so as to increase patients’ survival rate. Material and Methods: Present prospective study was conducted in tertiary care hospital which includes fresh body fluids minimum of 15 ml in two separate containers (from pleural and peritoneal cavity) from 142 patients with all relevant clinical details of both sexes and all ages. Scanty fluid samples were excluded. Samples were processed by conventional smear method and cell block method. Results: 142 body fluid specimens were evaluated by simultaneous use of smear and cell block technique of which 80% were pleural effusions and 20% were peritoneal fluids. Male: Female was 2:1, most of the patients were in the age group of 41-60 years. In Conventional smear preparation showed diagnostic yield 15% and accuracy 85-90%. In Cell block technique showed increase in diagnostic yield upto 21% and accuracy upto 97%. Conclusion: CB in conjunction with CS increases the accuracy of the fluid reports. The CB method provides high cellularity, better architectural patterns and use of IHC help to identify primary site of malignancy giving more definitive diagnosis.

Keywords: Cell block, conventional smear, IHC

1. Introduction

Cytological examination of body fluids has increasingly gained acceptance in clinical medicine to such extent that once positive diagnosis is made often considered definitive diagnosis and it is paramount important to identify primary site and type of malignancy. This can help to obviate the proper surgical management, chemotherapy and radiotherapy so as to increase patients’ survival rate. Conventional smear is a much simpler procedure than that of cell block technique, it has lower sensitivity because of overcrowding of cells, cell loss, lack of architecture and also, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional smears. Cell block technique is one of the oldest method later on various modifications has been done in this method but the method did not receive much attention due to lack of standardized technique. The method has advantages like it concentrates minimal amount of cellular material in one small area that can be evaluated at a glance with all cells lying in the same focal plane of the microscope, and as it uses of histological techniques it gives better cellular morphology better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin details, preservation of architectural pattern like cell balls, papillae, acini, rosettes and individual cell characteristic, representing its primary site of malignancy. Fragment of this tissue can easily interpreted in a biopsy-like fashion. On the other hand as multiple section of same material can be obtained for special stains and immunohistochemistry. And also the possibility of storing slides for retrospective studies also done, as storage of the conventional smears is a practical problem. For this reason an attempt was made to prepare and analyze both conventional smear preparation and cell blocks from the same specimen by plasma-thromboplastin method to study cellular yield, morphology, and architecture and to know primary site in malignant effusions.

2. Material and Methods

After obtaining the approval from Institutional Ethical Committee the present Prospective Study was conducted in tertiary care hospital. The study included fresh body fluids minimum of 15 ml in two separate containers (from pleural and peritoneal cavity) from 142 patients with all relevant clinical details of both sexes and all ages. Scanty fluid samples were excluded from study. A detailed history regarding age, sex, general examination, systemic examination, relevant investigations clinical diagnosis and the samples was collected in two different containers.

3. Technique of Aspiration

1. After explaining complete procedure, written consent from patient was taken in each and every case before performing any aspiration
2. Pre-medication: patient was sedated with injection atropine 0.6mg and injection calmpose 5mg intramuscularly as and when required
3. Under all aseptic precautions minimum of 15ml fluid were collected in two separate sterile containers
4. After aspiration punctured skin of patient was sealed with tincture benzoin. TPR, BP recorded
5. Fluid was immediately brought to laboratory for further processing.
4. Processing of Fluid

Conventional Smear Preparation:
1) Fluid from one container transferred to centrifuge tube labeled with the specimen identifier and centrifuged for 5 minutes at 2000 rpm
2) Supernatant fluid were discarded and the sediment was taken on the slide with the help of glass rod and spread by thick and thin method
3) Three slides were prepared
4) One dry smear was made and stained with MGG
5) Two slides were fixed in 95% methanol and stained with papanicolaou and hematoxylin and eosin stain

Cell Block Preparation

By Plasma- Thromboplastin method :
1) Plasma (pooled plasma from a blood bank may be used), and thromboplastin reagent are used to prepare cell block. The stability may be checked periodically by adding two drops of thromboplastin reagent to two drops of plasma, which should clot in about 30 seconds.
2) Fluid from second containers transferred into centrifuge tube labeled with the specimen identifier
3) Spin for 5 minutes at 3000rpm
4) Remove centrifuge tube decant the supernatant
5) Mix 2-3 drops of plasma with sediment
6) Add 2-3 drops of thromboplastin reagent and mix by tapping
7) Add 10% buffered formalin
8) Dislodge the clot from tube and let it fix in formalin for 30 minutes
9) The sediment was then wrapped in the filter paper and processed in histokinette as part of routine paraffin section histopathology.
10) Hematoxylin and eosin staining was done. Special staining and immunohistocheimistry was done whenever required.

Criteria for the assessment of quality of smear and cell block

Each individual slide was objectively analysed for background, cellularity, cytoplasmic, and nuclear details (cellular morphology), architecture (acini, papillae, cell balls, and proliferation spheres), using the Miar’s point scoring system as shown in table no.5. According to the criteria mentioned in the table no.5, comments were rendered on the quality of the slides by qualitatively grouping them into three categories.

Quality of Slide

1. Diagnostically unsuitable (0)
2. Diagnostically adequate (1-4)
3. Diagnostically superior (4-8)

These smears were observed and diagnosed on conventional smears and cell block section.

Diagnostic Categories

1. Negative for malignancy
   - Scanty cellularity
   - Acute inflammatory infiltrate rich
   - Lymphocyte rich
2. Suspicious for malignancy
3. Positive for malignancy

Statistical Methods

Descriptive statistics like mean, standard deviation, median and range were obtained for the total scores obtained for conventional smear and cell block methods. Statistical significance of difference in the mean scores by two methods was evaluated using paired t-test. Similar analysis was further performed based on the diagnostic groups i.e. positive and negative malignant groups. To determine if particular criterion differ significantly between methods, Pearson’s Chi-square test was used. Agreement between conventional smear and cell block methods was determined using Kappa statistic. The sensitivity, specificity, positive prediction value, negative prediction value and accuracy were determined for the two diagnostic methods after comparing with final diagnosis (made on the basis of clinico-radiological and cyto-histological investigations). The statistical significance was evaluated at 5% level, and the analysis was carried out using SPSS ver 11.0 and R package. Significance of diagnostic yield was determined by Mc-Naemer’s chi-square test.

5. Results

Study material consisted of pleural and peritoneal effusion samples which were processed by conventional smear preparation and cell block technique. Slides were evaluated individually. Total of 142 fluid samples were processed by both methods.
Table 1: Distribution of cases according to age, sex and type of fluid

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Pleural Male</th>
<th>Pleural Female</th>
<th>Peritoneal Male</th>
<th>Peritoneal Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>9(6%)</td>
</tr>
<tr>
<td>21-40</td>
<td>26</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>37(26%)</td>
</tr>
<tr>
<td>41-60</td>
<td>41</td>
<td>17</td>
<td>12</td>
<td>0</td>
<td>77(54%)</td>
</tr>
<tr>
<td>&gt;61</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>19(13%)</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>30</td>
<td>12</td>
<td>16</td>
<td>142</td>
</tr>
</tbody>
</table>

It is evident from the table no. 1 that 77 (54%) of the patients belonged to age group 41-60 years; while only 9(6%) cases were below age 20 years. As regards gender, 96(68%) of the cases were males and 46(32%) cases were females, cases of pleural effusions were 114 and 28 were peritoneal effusions.

Two methods, Conventional Smear (CS) and Cell Block (CB) were used for scoring the samples based on four different criteria like background, cellularity, cellular morphology and Retention of appropriate architecture. Each sample was interpreted quantitatively with respect to the above features. A total score for both Conventional Smear and Cell Block methods for each sample were obtained independently by summing the scores on each criterion. The distribution of scores for the two methods is shown in figure 3 & 4.

Figure 1: The data revealed 34 (24%) of cases as positive for malignancy, which is shown below.

Figure 2: Distribution of total score for conventional smear

Figure 3: Distribution of total score for cell block methods

It is evident from the figure that in CS method, the range of total score was smaller [0-5] as compared to that of CB which is [0-8]. With CS method, 65 (46%) of the samples had a score of 3 and 30 (21%) of cases had total score greater than 3. With CB, majority i.e. 43 (30%) of cases had score of 3 and 48 (34%) of cases had total score greater than 3.

The analysis was further carried out at each criterion level for both the methods such as CS1 and CB1 for background CS2 and CB2 for cellular yield, CS3and CB3 for cellular morphology and CS4 and CB4 for architecture. The frequency distribution of scores for each criterion was obtained as shown in table 2

Table 2: Frequency distribution for each criterion according to methods

<table>
<thead>
<tr>
<th>Score</th>
<th>Conventional Smear</th>
<th>Cell Block</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS1</td>
<td>CB1</td>
</tr>
<tr>
<td>0</td>
<td>(19.01%)</td>
<td>(18.31%)</td>
</tr>
<tr>
<td>1</td>
<td>(80.28%)</td>
<td>(70.42%)</td>
</tr>
<tr>
<td>2</td>
<td>(0.7%)</td>
<td>(11.27%)</td>
</tr>
</tbody>
</table>

Table 3: Comparison of p-values for each criteria in two methods

<table>
<thead>
<tr>
<th>p-value</th>
<th>CS1 &amp; CB1</th>
<th>CS2 &amp; CB2</th>
<th>CS3 &amp; CB3</th>
<th>CS4 &amp; CB4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0263</td>
<td>0.0010</td>
<td>0.0098</td>
<td>0.0000</td>
<td></td>
</tr>
</tbody>
</table>

To determine if particular criterion differ significantly between two methods, Pearson’s Chi-square test was used with the results shown in Table no. 3. Table No. 3 reveals that there is significant difference in the distribution of scores between two methods for all criteria (p < 0.05).

Table 4: Comparison of conventional smear cell block with final diagnosis

<table>
<thead>
<tr>
<th>Diagnostic criteria</th>
<th>CS</th>
<th>CB (with IHC)</th>
<th>Final Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative for malignancy</td>
<td>104</td>
<td>112</td>
<td>114</td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
<td>19</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Positive for malignancy</td>
<td>21</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Total 114 cases were negative for malignancy and 34 cases were confirmed malignant. Final clinical diagnosis was based on clinical, radiological cyto-histological findings.

In present study, cell block preparation was done by plasma-thromboplastin method, as the method utilizes outdated plasma from blood bank and outdated thromboplastin reagent from hematology laboratory so it becomes cost-effective, and the advantages were it is reproducible and concentrates more cellular material that forms more solid cell button because of formation of clot, better cellular preservation, and also to type the malignancy. Cell blocks provided diagnostic information complimentary or additional to that obtained from an examination of the smears.

Similar analysis was carried out for Cell Block data. Considering suspicious into negative category sensitivity: 0.7352 [0.5534 – 0.8649], specificity: 1.000 [0.9571 – 1.000], PPV: 1 [0.8342 – 1.000], NPV: 0.8239 [0.7491 – 0.8807] and accuracy: 0.936. Considering suspicious into positive category Sensitivity: 0.8823 [0.7160 – 0.9616], Specificity: 0.9444 [0.8781 – 0.9772] PPV: 0.8333 [0.6652 – 0.9303], NPV: 0.9622 [0.9005 – 0.9878] Accuracy: 0.9295

Table 6: Contingency table showing the comparative diagnosis of each method with the final diagnosis

Analysis was carried out for Cell Block with IHC. It resulted into a sensitivity of 0.8823 (95% CI: 0.7160 – 0.9616), specificity of 1.000 (95% CI: 0.9571 – 1.000), PPV of 1.000 (95% CI: 0.8586 – 1.000) and NPV of 0.9642 (95% CI: 0.9056 – 0.9884). The accuracy obtained was 0.9718 (97%).

6. Discussion

In this study due consideration was given to type of cell block technique, age, sex, site of effusion, quality of slides in both methods, clinical and radiological findings and also immunohistochemistry wherever needed to arrive at a final diagnosis and to identify the primary malignant lesion and also to type the malignancy. Cell blocks provided diagnostic information complimentary or additional to that obtained from an examination of the smears.

To obtain sensitivity and specificity of Conventional Smear method, two scenarios were considered for the suspicious samples. In the first, all the suspicious samples were considered as negative by CS method. This resulted into a sensitivity of 0.6176 (95% CI: 0.4362 – 0.7731) and specificity of 1.000 (95% CI: 0.9571 – 1.000). The Positive Prediction Value (PPV) was obtained as 1.000 (95% CI: 0.8075 – 1.000) and Negative Prediction Value (NPV) was 0.8925 (95% CI: 0.8194 – 0.9392). The accuracy obtained was 0.9084. Alternatively, if all the suspicious samples are
cytological details from the cytological perspective is not satisfactory, Nathan et al.10. They prepared cell block by using improvised ethanol-alcohol as fixative.

In present study total 142 fluid samples were subjected to the conventional smear preparation and cell block techniques. Out of which 114 were pleural and 28 cases were of peritoneal effusions. In Sujathan et al.15 out of 85 fluid samples 32 were pleural and 53 were peritoneal effusions. Whereas In Shivkumarswami U. et al17 60 pleural effusions were studied. In Sears et al18 study 61% were pleural and 39% samples were peritoneal effusions. Such difference was because of random selection of cases, which were selected on the basis of inclusion and exclusion criteria.

The age ranged from less than 20 years to more than 60 years with maximum number of cases in 5th decade in male as well as female. In Shivkumarswami U. et al19 age ranged from 18 to 90 years. This difference may be due to random selection of cases. As regards gender, 96(68%) cases were males, and 46(32%) were female, with a ratio of almost 2:1 in favor of males. The figure indicates that the distribution of males and females with age is nearly similar.

In present study interpretation of slide was done on the basis of Mirâ’s point scoring system which was followed by Thaper et al.4 each slide was observed and scored on the basis of background, cellularity, cellular morphology and architecture and p value for each criteria found to be found statistically significant (<0.05) favoring cell block.

Negative for malignancy category, scanty cellularity were seen in cases in congestive cardiac failure (7) effusions present in these cases was transudative having low cellular contents which have correlated with CS and CB findings. cases of post-traumatic effusions (3) showed on hemorrhagic background only, cases with acute inflammatory infiltrate were of exudative effusion and diagnosed as of para-pneumonic effusions, pancreatitis, subacute bacterial peritonitis, tuberculosis clinical, radiological, biochemical, microbiological investigations has helped in the diagnosis, Cases of Para-pneumonic effusions (16) were pleural effusions associated with acute febrile illness and cough and chest radiograph showed pulmonary infiltrates theses cases followed up and responded to antibiotic treatment. High level of serum amylase was found in cases of pancreatitis (6). Cases with Sub acute bacterial peritonitis (3) had culture positivity to microorganism responded well to antibiotics, and cases with lymphocyte rich all the cases were having exudative pleural effusion, with ADA level more than 45 IU and also Mycobacterium Tuberculosis culture positive that confirms the diagnosis of tuberculosis (57). Reactive mesothelial cells along with few inflammatory cells were seen in 10 cases. As reactive mesothelial cells and malignant mesothelial cells are the common diagnostic problem due to overlapping of microscopic features clinicoradiological investigations along with immunohistochemistry which was done on cell blocks excluded the malignancy and cases were diagnosed as cirrhosis of liver (7), congestive cardiac failure (6) and uremia (3).

3 cases negative for malignancy on CS were diagnosed positive for malignancy and were diagnosed as adenocarcinoma of lung (2 cases) by CB method due to scanty cellularity and loss of architecture on CS while CB has advantages like it concentrate more amount of cellular material, increases cellular yield and forms a cell button, and also it maintains architecture.

In suspicious category in CS (19 cases) malignancy was confirmed in 1 case and showed acinar architecture and diagnosed as adenocarcinoma on CB, 11 cases were suspicious on CB too and as IHC which was an added advantage of CB 6 cases were diagnosed as negative and 5 cases were positive for malignancy. In all these malignant cases identification of primary site was also done with the help of radiological investigations, immunomarkers like ck7/ck20, and tumor specific immunomarkers.

In positive for malignancy category 21 cases in CS and CB. Identification of primary site was not possible on CS due to loss of architectural pattern and one of the most significant limitation was inability to evaluate coordinate immunoreactivity since the same cells cannot be present on more than one smear, other limitations like there can be non-specific and unexpected immunoreactivity due to protein rich fluid in which cells are floating, 3-D cell groups show entrapment of immunostains which gives false positive results, and also crushing, degenerated cells while spreading smears show non-specific immunostaining.

On the other hand cell block sections in present study revealed histologic aspect of primary neoplasms with architectural patterns like acini, papillae, rosette-like, squamous pearls, psammoma bodies which has helped to identify possible type or primary site, and commonest tumors were of adenocarcinoma type (18) from breast (5), lung(6), ovary(6) and cervix(2). Papille along with psammoma bodies were seen in a case of papillary serous cystadenocarcinoma of ovary. Rosettes were seen in one case of small round cell tumor, keratin pearls also noted in a case of squamous cell carcinoma of lung. Such type of architectural patterns confirms particular type of malignancy, dilemmas in some cases about identification of primary site was resolved with the help of multiple tumor specific immunomarkers in serial sections and also clinic-radiological correlation.

Table 7: Comparison of cytodiagnosis of serous effusions in present study with other studies

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Study &amp; Year</th>
<th>No. of cases</th>
<th>Negative for malignancy</th>
<th>Suspicious</th>
<th>Positive for malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CS</td>
<td>CB</td>
<td>CS</td>
</tr>
<tr>
<td>1</td>
<td>Takagi et al (1954)</td>
<td>184</td>
<td>145</td>
<td>79%</td>
<td>129</td>
</tr>
<tr>
<td>2</td>
<td>Sujathan et al.(2000)</td>
<td>85</td>
<td>61</td>
<td>72%</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>Bodele et al (2003)</td>
<td>150</td>
<td>118</td>
<td>79%</td>
<td>111</td>
</tr>
<tr>
<td>4</td>
<td>Khan et al (2006)</td>
<td>75</td>
<td>23</td>
<td>51%</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Shivkumarwami et al</td>
<td>60</td>
<td>54</td>
<td>90%</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Present study</td>
<td>142</td>
<td>102</td>
<td>72%</td>
<td>106</td>
</tr>
</tbody>
</table>
In present study most of cases were in negative for malignancy category with 72% on CS while 74% on CB similar findings were seen in study done by Sujathan et al.

In suspicious for malignancy less no. of cases encountered on CB( 8% ), and positive for malignancy maximum no of cases were diagnosed on CB (18%). Similar findings were also noted by Takagi et al.4, Sujathan et al.5, Bodele et al.6, Khan et al.7, and shivkumar swayam et al.7.

In the present study diagnostic yield for malignancy was significantly increased by cell block method. The present study identified additional 6.33%

(9 cases) malignant lesions by cell block method when compared to conventional smear. Additional diagnostic yield was noted in various studies. In study done by Bodele et al, additional 7% (10 cases) of malignant lesions were identified by cellblock method7. Dekkar and Bupp et al study, reported that samples obtained by combined cellblock method and smear technique for malignant lesions were double to that of conventional smear technique only. By using cellblock method tumors were subsequently demonstrated in 38% of the patient who had negative or atypical cytological reports7.

In a study done by Khan et al, additional findings were diagnostic in 16% of malignant cases11. Additional 18 cases for malignant lesions were diagnosed by cellblock method in study done by Takagi F14. Khan et al., in another study titled as usefulness of cellblock verses smears in malignant effusion cases reported that the recovery rate for malignant lesions by cellblock preparation was 20% greater than that obtained for specimen examined in smear only15.

Table 8: Additional yield of malignancy in various studies by cell block

According to various studies additional diagnostic yield for malignancy was noted if conventional smear technique is supplemented by cellblock method.1,2,11 In present study, by using cell block method we diagnosed malignant lesions in 21% of samples, where as in conventional smear method diagnosis for malignant lesion was 15% only. In present study identification of primary site was done in 100% cases. In study done by Khan et al.15 could identify primary site in 81.3% while Thaper et al16 identified the same in 83.3% cases. Clinic-radiological correlation along with immunomarkers for identification of primary site helped to confirm primary site in present study. In present study sensitivity, specificity, PPV, and NPV for CS was 61-79%, 87-100%, 67-100%, 89-93% respectively, and for CB 73-88%, 94-100%, 83-100 and 82-96% and CB with IHC 88%, 100%, 100%,and 97% respectively.

Table 26: Accuracy in different studies in CS and CB

In present study accuracy of CB was 97%, increase accuracy also noted by Thaper et al16 (85%), and Ceelen et al.18 (89%) zemansky et al17.

7. Conclusion

Cell block technique is simple and reproducible and uses routine laboratory reagents and processing. In cell block technique more amount of sample is required for obtaining proper cell button. Cell block technique offers advantage like it concentrates all the cellular material and increases cellular yield. Though cell block show preservation of architectural pattern, yet cellular morphology can be better appreciated on conventional smears. Use of cell block technique eliminated the suspicious for malignancy category giving more definitive diagnosis and shows additional increase in diagnostic yield. In cell block technique multiple sections of the same material can be processed for immunohistochemistry that help to identify primary site of origin in malignant fluids in 100% cases.

Combined approach cell block in conjunction with conventional smear can should be used in suspicious for malignancy cases. Positive results, identification of primary site in malignant effusions and further typing will have an oblivious influence on patient management. Though cell block technique is time consuming causes delay in issuing report. It is balanced by its ability to increase sensitivity and accuracy of final diagnosis

References

fluid effusion Journal of clinical and diagnostic research, 2012 ;September (suppl), vol-6(7):1280-1283


**Author Profile**

**Dr.Shubhada Bansode** is Assistant Professor in Department of Pathology, GMC & H,Latur, Maharashtra,India. She did MBBS – GMC & H, Aurangabad, MD, DNB Pathology – GMC & H, Nagpur.
Photomicrograph 16: cell ball on CS 400X, PAP

Photomicrograph 17: Showing acini, cell balls malignant scattered singal cell population (100X, H&E)

Photomicrograph: showing Cytokeratin 7+ on cell block, IHC – 100X

Photomicrograph: showing Mammoglobin + on cell block, IHC 400X

Case of Metastatic adenocarcinoma – primary site Breast