Original Research Article

# Analysis of Ascitic Fluid Cytology by Cell Block in the Evaluation & Grading of Malignancy with Ki 67 Marker Study in a Tertiary Care Centre

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## Abstract

**Background:** The cell block technique is one of the oldest methods, which is used for the evaluation of body cavity fluids. This method usually increases the yield of cellularity, gives better morphological details, and helps in improving the sensitivity of the diagnosis and grading of malignancy. Multiple sections were obtained from the prepared cell block for special stains and immunohistochemistry studies. **Aim:** Preparation of cell blocks of ascitic fluid in suspicious or confirmed cases of malignancy. Evaluation and grading of visceral malignancy in cell block using Ki 67 immunohistochemical marker by MIB index scoring system. **Method:** Cell blocks were prepared by the plasma thromboplastin method. Sections from the blocks were stained with H&E. Immunohistochemical staining with ki67 was done for selected cases and grading was done. **Results:** Out of 100 samples of ascitic fluid, 14 cases were malignant. The malignancy was graded by applying the ki67 marker by the MIB index scoring system. **Conclusion:** Cell block method is useful for the detection and grading of malignancy by applying immunohistochemistry.

**Keywords:** Cell block, Conventional Smear, Cytodiagnosis, MIB index scoring



## Introduction

The Cell block technique was first described by Bahrenberg in 1896. This is an old method for the evaluation of body cavity fluids. The cell block technique employs the retrieval of cells or small tissue fragments from any body fluids including ascitic fluid, pleural fluid, bronchial wash and image-guided fine needle aspiration cytology specimens.

The cytodiagnosis by conventional smears have got some drawbacks due to overcrowding of cells and cell loss leading to less cellularity [1]. To overcome these drawbacks cell block technique was employed. Cell blocks from fluid specimens can be prepared by using the plasmathrombin or agar method. The cell button formed is formalin-fixed and processed routinely like histopathological specimens. The same material can also be used for special stains and immunohistochemistry studies [2].

Aspiration biopsy material (FNA), sputum, effusions, urine sediment and material from the gastrointestinal tract are all suitable for cell block processing.The most appreciable benefit of the cell block technique is to identify the histologic patterns of disease and architecture of tissue which cannot be correctly identified in conventional smears. There is an increasing need for additional diagnostic techniques such as immunohistochemistry, to define a specific cell lineage on cytology and FNAC specimens [3,4].

Immunohistochemistry is a highly effective ancillary tool that can be used on cell block to distinguish or subclassify malignancies. Ki67 is an s – phase fraction-related antigen which is a proliferative marker. This can be detected by monoclonal antibodies and do not require flow cytometry technique as is required for s phase-related antigen [5]. This is used to establish the growth fraction of tumor cells determined by the number of positive tumor cells among the total number of cells and calculated as index. The index correlates well with the histological grading of the neoplasms.

Measurement of the Ki67 labelling index of a tumour sample provides information beyond that given by other prognostic indicators like tumour size, grade, hormone receptor status and number of positive lymph node. It guides the clinician of the prognostic outcome and avoids the need for adjuvant therapy. Hence the present study was taken to assess the utility of cell block technique in the diagnosis and grading of abdominal visceral malignancy in ascitic fluid.

### Aims and Objectives

1. Preparation of cell blocks of ascitic fluid in suspicious or confirmed cases of visceral malignancy.
2. To compare the diagnostic accuracy of cell block technique with conventional cytology smears.
3. To use immunohistochemistry on cell block for grading of visceral malignancy by applying Ki67 marker by MIB index scoring system.

### Materials and Methods

The present study is a retrospective analytical study. During the period from December 2021 to November 2022, 100 samples of ascitic fluid that were received in the cytology section of Department of Pathology, Madurai Medical College, Madurai were included in the study.

### Inclusion Criteria

- 100 ascitic fluid samples in suspicious or confirmed cases of malignancies received in clinical pathology

### Exclusion Criteria

- All other fluids specimen of body cavity except ascitic fluid.
- The sample processed after 48hours of collection.

The clinical details of patients like name, age, sex, and diagnosis were

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recorded. The conventional smears and cell block were reported under the diagnostic category as benign, suspicious, malignant, and non-diagnostic. A combined evaluation of conventional smear and cell block was done and tabulation of cytomorphological characters was done.Cell blocks were prepared by the plasma thromboplastin method. 5 ml of samples were subjected to fixation for one hour by mixing it with 5 ml of 10% formalin. Centrifuged at 2500 rpm for 15 minutes. The sediment was mixed with 2 drops of pooled plasma. Four drops of thromboplastin was added and the tube was allowed to stand for 5-10 minutes. The resultant clot was sent for processing. Sections from the blocks were obtained for H&E staining and immunohistochemical studies. The proliferation marker Ki67 was applied and the malignant cases were graded from low grade to high grade by applying MIB index scoring system. **(Table 1).** MIB Index = Number of positive cells/Total number of cells counted X 100

**Table 1: MIB Index Scoring**

| Mib Index | MIB Score |
|---|---|
| <10% | Score 0 |
| 1-20% | Score 1 |
| 21-50% | Score 2 |
| >50% | Score 3 |

**Observation and Results**

**Table 2: Comparison of Quality of Conventional SMEAR and Cell Block in Ascitic Effusion**

| Quality | Conventional SMEAR | Cell Block | Inference |
|---|---|---|---|
| Unsuitable | 15 | 6 | Pearson chi square - 0.001 |
| Adequate | 80 | 66 | |
| Adequate & Superior | 5 | 28 | |

**Table 3: Comparison of Cellularity in Ascitic Effusion**

| Cellularity | Conventional SMEAR | Cell Block | Inference |
|---|---|---|---|
| Minimal | 14 | 8 | Pearson chi square - 0.006 |
| Sufficient | 72 | 48 | |
| Abundant | 14 | 44 | |

**Table 4: Comparison of Diagnosis in Ascitic Effusion**

| Diagnosis | Conventional SMEAR | Cell Block | Inference |
|---|---|---|---|
| Benign | 68 | 80 | Pearson Chi square <0.001 |
| Suspicious | 8 | 0 | |
| Malignant | 9 | 14 | |
| Non-diagnostic | 15 | 6 | |

**Table 5: Discrepancies Observed in Ascitic Effusion**

| Conventional SMEAR | | | | Cell Block | | | |
|---|---|---|---|---|---|---|---|
| Benign | Suspicious | Malignant | Non Diagnostic | Benign | Suspicious | Malignant | Non Diagnostic |
| 3 | - | - | - | - | - | 3 | - |
| - | | 1 | - | 1 | - | - | - |
| - | 6 | - | - | 4 | - | 1 | 1 |
| - | - | - | 8 | 8 | - | - | - |

**Table 6: Ki67/ MIB index scoring in the study population**

| S. No | Score | Grade | | |
|---|---|---|---|---|
| | | High | Low | Negative |
| 1 | 0 | | | Negative |
| 2 | 2 | High | | |
| 3 | 2 | High | | |
| 4 | 1 | | Low | |
| 5 | 1 | | Low | |
| 6 | 2 | High | | |
| 7 | 2 | High | | |
| 8 | 1 | | Low | |
| 9 | 2 | High | | |
| 10 | 1 | | Low | |
| 11 | 1 | | Low | |
| 12 | 1 | | Low | |
| 13 | 1 | | Low | |
| 14 | 2 | High | | |

**Table 7: Comparison of Malignancy Yield by Cell Block with Other Studies**

| Diagnosis by Cell Block | Benign % | Suspicious % | Malignant % | Non-Diagnostic % |
|---|---|---|---|---|
| Bhanvadia et al [6] | 78 | 0 | 22 | 0 |
| Richa Nathani et al [7] | 85 | 0 | 15 | 0 |
| Present Study | 80 | 0 | 14 | 6 |

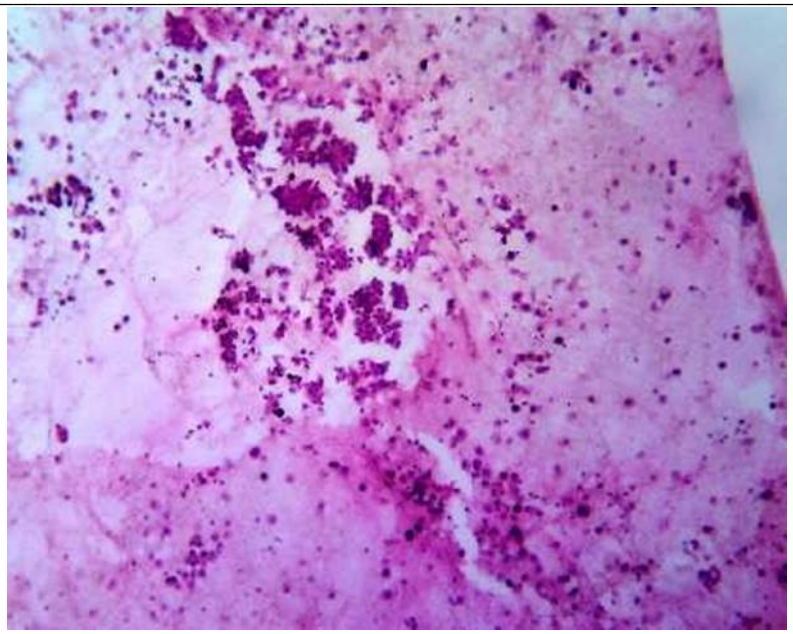

**Fig 1: Cell Block of ascitic fluid shows malignant cells arranged in clusters in a case of carcinoma ovary (H & E, 10X)**

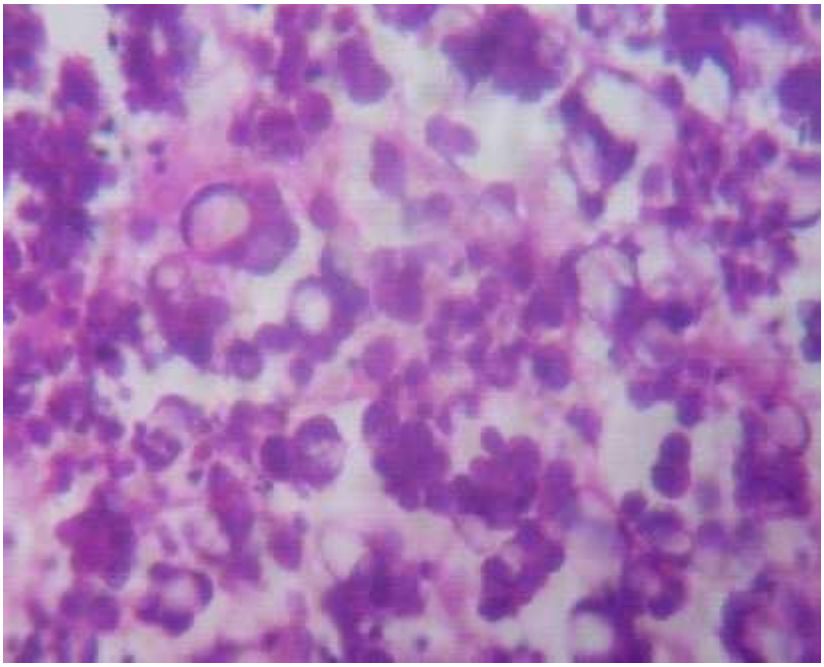

**Fig 2: Cell block of ascitic fluid showing "Signet Ring Cells'' in a case of carcinoma stomach (H&E, 40X)**

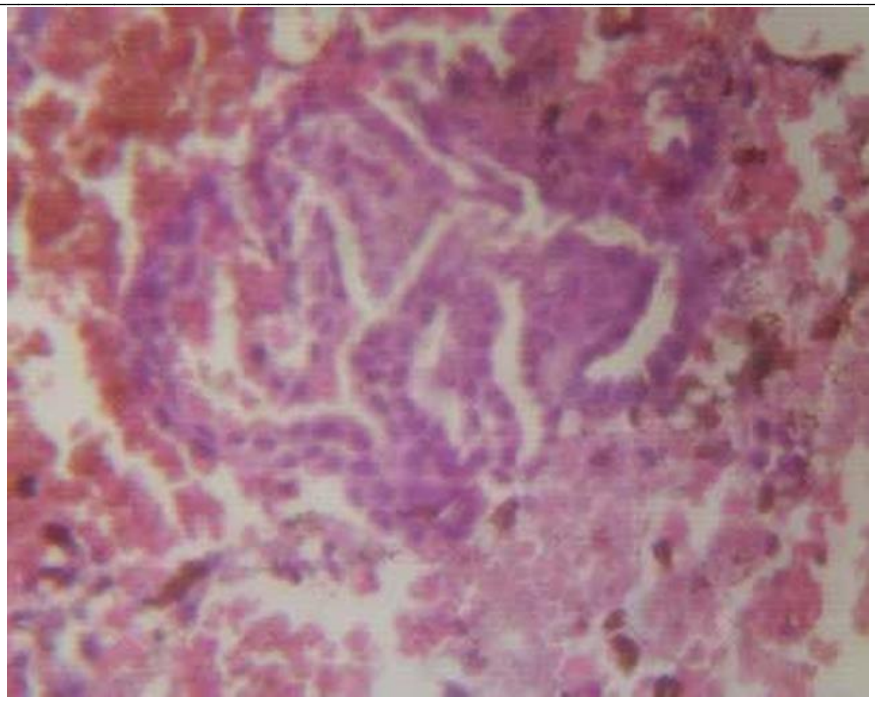

**Fig 3: Cell block showing glandular pattern in a case of invasive carcinoma of breast (H&E, 40X)**

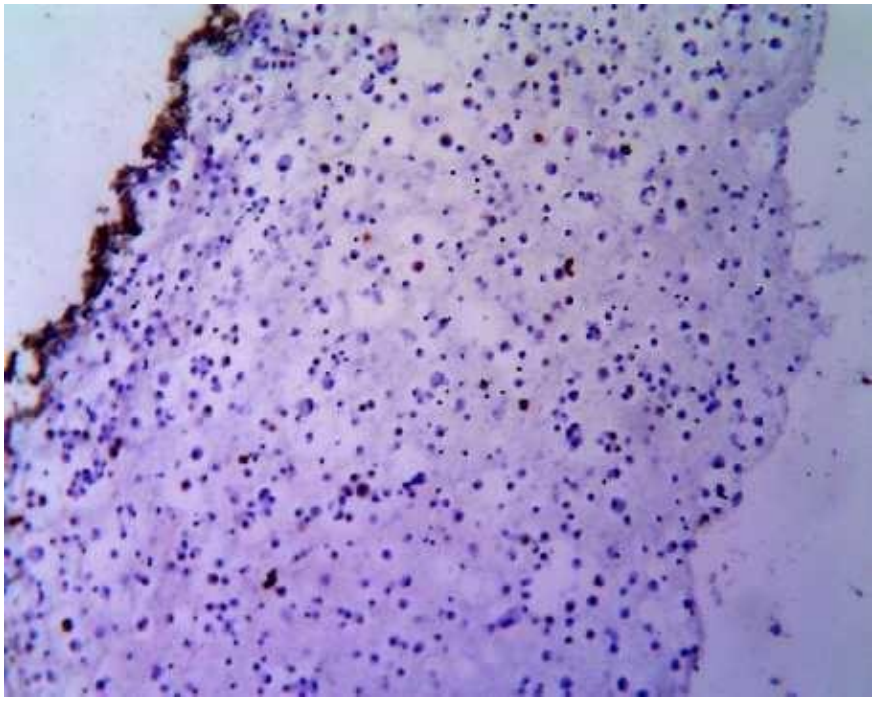

**Fig 4: KI67 marker shows positivity in a case of carcinoma ovary (IHC, 10X)**

**Discussion**

The cell block technique works as an adjunct tool to conventional smears for establishing a definitive cytopathological diagnosis. In this study, routine conventional smears and cell blocks from ascitic fluid were studied for cellularity, cytological preservation of architecture, its diagnostic utility and grading of malignancy.

Of the 100 samples of ascitic effusion, the maximum number of samples was in the age group of 41-50 years accounting for 28% of age distribution.The conventional smear showed 80% of adequacy of which 5% were diagnostically superior. Whereas by cell block method, the adequacy of 66% was observed of which 28% were diagnostically superior.In a study by Richa Nathani et al [7], 25% cases were adequate and diagnostically superior and another study by Thapar et al [8] had higher number of adequate and diagnostically superior cases accounting for 67%.

In the present study, the percentage of diagnostically unsuitable cases by cell block is 6% which is very less when compared to the study by Richa Nathani et al [7] which had 20% and Thapar et al [8] which had

12% of diagnostically unsuitable cases. The cell block showed abundant cellularity in 44% of the cases which is higher than that of conventional smear which shows abundant cellularity in only 14% of the cases and minimal cellularity was seen in 8% of the cases.

In the present study of 100 cases, 80% were benign and 14% were malignant, 77% of the cases had similar diagnosis both in conventional smear and cell block and discrepancies were seen in 18% of the cases. Malignancy was diagnosed in 9% of the cases by CS and in 14% cases by cell block. The cell block yields higher diagnosis of malignancies which were missed by conventional smears. The 'p' value is <0.001 which shows a very significant difference between the two methods.

Cell block has increased the diagnostic yield of malignancy by 5%. In a study by flint et al [9], increase in malignancy yield was 9% & in another study by Calabretto et al [10] was 6.5 %. Among malignant ascitic effusion diagnosed by cell block, ovarian carcinoma was the commonest accounting for 12 cases (78.5%) followed by carcinoma stomach with 1 case (7.14.%) and breast carcinoma with 1 case (7.14%).

Immunohistochemistry study was done in 14 cases of malignancy. We found 7 cases (42.45%) were high grade and 6 cases (50%) were moderate grade and one case (7.15%) was low to negative grade.This is significant when compared to Hasteh et al [11] study which shows only 7.3% of high-grade Ki 67 expression Some benign cases showed the presence of inflammatory cells, including lymphocytes in the background. Ki67 interpretation was estimated only among the epithelial cells excluding the inflammatory cells.[11]

**Conclusion**

The cell block technique by the plasma thromboplastin method is a simple and cost-effective technique. It does not require any special training and there is no need for any special instrument. The main advantage of the cell block is better cellularity, morphology & grading. Multiple sections can be obtained for immunohistochemistry and special stain studies. Grading of malignancy was done by the application of proliferation marker ki67. The accuracy of the diagnosis and yield of malignancy was found to be higher in cell blocks & by IHC on these cell blocks. So, cell block technique can be considered a gold standard and can be used routinely as an adjuvant in all ascitic fluid samples to increase the sensitivity of diagnosis of malignancy.

**Conflict of Interest: Nil Source of support: Nil**

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