

# Role of Cell Block Preparation in Cytopathological Diagnosis

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## ABSTRACT

**Introduction:** Though Fine needle Aspiration Cytology (FNAC) is good method for diagnosis of lesions, sometimes it does not provide precise diagnosis due to inadequate material. Present study was based on use of residual FNAC material to make cell block in order to find out utility of cell block preparation of FNAC.

**Material and Methods:** The two years prospective study carried out in a tertiary Health care hospital of central India, included 360 patients referred for FNAC of the lesions from all body sites. After making the conventional FNAC smears, the remaining material in the needle hub and syringe was obtained by flushing it with 10% alcohol-formalin. After centrifugation the supernatant was decanted and deposit fixed in freshly prepared 10% alcohol-formalin. After half an hour alcohol-formalin was drained, cell button was taken on whatman-filter paper and processed as surgical tissue in automated tissue processing machine. FNAC smears and cell block sections were examined separately for cellularity, reporting done and results correlated with histopathology.

**Results:** Of 360 cases FNAC smears and cell blocks were adequate in 324(90%) and 313(86.94%) cases respectively. Statistical analysis was done in 98 cases in which histopathology available. Sensitivity of FNAC was 88.06%, specificity of 92.86% and accuracy of 89.47%. Sensitivity of cell block was 90.91%, specificity of 92.86% and accuracy of 91.47%. Sensitivity of combined FNAC and cell block technique was increased to 98.53% with specificity of 93.33% and accuracy of 96.94%.

**Conclusion:** Cell blocks as an adjuvant to FNAC smears is very effective technique in cytology to reach the definitive diagnosis.

**Keywords:** Fine Needle Aspiration Cytology (FNAC), Residual FNAC Material, Cell Block

## INTRODUCTION

Fine needle aspiration cytology (FNAC) widely practiced and very effective means for obtaining tissue from different body sites for diagnosis. However sometimes it does not provide precise diagnosis due to inadequate material.<sup>1</sup>

Cell blocks (CB) prepared from residual tissue fluids and fine-needle aspirations can be useful adjuvant to smears for establishing a more definitive cytopathologic diagnosis and for categorization of tumors that otherwise may not be possible from smears themselves.<sup>2</sup>

Dr. Baherenburg was the first pathologist to introduce cell block technique in 1896 on ascitic fluid.<sup>3</sup> His technique consisted of allowing the fluid to stand and clot spontaneously for 24 hours, followed by pouring off the supernatant fluid and allowing the sediment to be hardened by addition of alcohol to obtain a firm and hard tissue mass that was

subsequently embedded in celloidin. The tissue block was then cut and stained similar to histologic sections. After this report, various CB techniques were developed with changes and modification in type of fixatives, processing, and embedding techniques used. Some of the most common techniques include inverted filter sedimentation, collodion bag, thrombin method, simple sedimentation, and Millipore filtration.<sup>4</sup>

Although CB gives additional material to resolve diagnostic difficulty, however the main effectiveness of the CB technique is to provide formalin-fixed paraffin-embedded (FFPE) tissue for a variety of ancillary studies such as immunohistochemical studies and molecular testing.<sup>4</sup>

The present study was based on use of residual FNAC material remaining after making conventional smears to make cell block in order to find out efficacy of cell block preparation of FNA material. Comparison of cytological smears and cell block preparation as diagnostic tool.

## MATERIAL AND METHODS

This was two years prospective study approved by ethical committee of the institution was carried in a tertiary health care hospital of central India. The study included 360 patients attending cytology outpatient department which were referred for fine needle aspiration from other clinical department study also included USG guided FNACS. Patient of all ages and both gender were included in the study. Informed consent was taken from each patient before doing FNAC procedure. After taking complete history, local and systemic examination of the patient was carried out. FNAC was done using all aseptic precautions. Proper position was given to the patient and FNAC site was cleaned with spirit. After the lump was fixed, 23 gauge needle attached to the syringe was introduced through skin. When needle reached the target lump, slight negative pressure was applied and the needle was moved to and fro in all directions. When the material appeared in the needle hub, the needle was withdrawn from the site. The smears were made from the material in the needle and syringe for conventional cytology. Wet fixed

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smear in 95% alcohol were stained with Haematoxylin and Eosin stain and Papanicolau stain, while the air dried smears were stained with May Grunwald Giemsa stain.

Cell block (CB) was prepared from the material remained in the needle hub and syringe after making the conventional smears. Cell block (CB) was prepared by technique described by Koss with slight modifications.<sup>5</sup>

1. The remaining material in the needle hub and syringe was obtained by flushing it with 10% alcohol-formalin (9 parts of 100% ethanol + 1 part of 40% formaldehyde).<sup>6</sup>
2. Entire material was then centrifuged in 10 ml test tube at 3000rpm for 10 minutes to form the sediment.
3. The supernatant fluid was decanted and deposit fixed in freshly prepared 10% alcohol-formalin. Fresh working solution is desired because formalin is capable of oxidizing to formic acid after exposure to air and reacting with blood to form acid haematin pigment artefacts.
4. After half an hour alcohol-formalin was drained and cell button obtained was taken on whatman-filter paper.
5. Then the whatman paper was folded with button inside. This was processed as surgical tissue in automated tissue processing machine.
6. After processing the cell button was removed from whatman paper and embedded in the paraffin to make block.
7. The 4 to 5 microns thickness sections were cut and mounted on the slides.

8. Sections were stained with hematoxylin and eosin stain, special stains and immunohistochemistry was done whenever required.

All the FNAC smears and cell blocks divided into Inadequate Smears: Inadequate smears were those smears, which showed blood or less cellularity for diagnosis.

Adequate smears: All the remaining smears which showed adequate cellularity for reporting were considered as adequate.

FNAC smears were examined separately for cellularity and reporting was done. FNAC smears were reported as per criteria of reporting the various organs smears in the textbook of Orell and textbook of Koss and final diagnosis were given.<sup>5,7</sup>

All the cell block preparations were reported for adequacy; architectural details and final diagnosis were offered. Correlation of cell block preparation was done with cytological smears. Correlation of cytology smears and cell block preparation was done with histopathology smears.

### STATASTICAL ANALYSIS

Statistical analysis was done in 98 cases in which histopathology were available. Sensitivity, specificity and accuracy of FNAC, Cell block and combined FNAC and cell block was calculated and analysed discriptively.

### RESULT

A total of 360 FNAC samples were processed for the cell block preparation. In the study of 360 cases 161 (44.72%)

Sr. No	Site of FNAC	Male		Female		Total cases.	
		N	% of total male cases	N	% of total female cases	N	% of total cases
1	Lymph node	61	37.88	41	20.60	102	28.33
2	Breast	3	1.86	58	29.14	61	16.94
3	Liver	25	15.52	17	8.54	42	11.66
4	Thyroid	11	6.83	29	14.57	40	11.11
5	HNF	20	12.42	18	9.04	38	10.55
6	Joints, limbs and bone	15	9.31	11	5.52	26	7.32
7	Lung, pleura mediastinum	10	6.21	4	2.01	14	3.88
8	Abdomen	7	4.34	6	3.01	13	3.6
9	Pelvis and retroperitoneum	5	3.10	7	3.51	12	3.33
10	Miscellaneous	4	2.48	8	4.02	12	3.33
Total		161	100	199	100	360	100

**Table-1:** Distribution of the cases according to the site.

Sr. No	Site of FNAC	Number of correlated cases	Non correlated diagnosis	Total adequate cases
1	Lymph node	85 (92.93%)	7(7.6%)	92(100%)
2	Breast	55(100%)	0(0%)	55(100%)
3	Liver	30(85%)	5(14.28%)	35(100%)
4	Thyroid	26(89.65%)	3(10.35%)	29(100%)
5	Head, Neck and face	26(89.65%)	4(13.34%)	30(100%)
6	Joint, Limbs and bones	21(95.45%)	1(4.54%)	22(100%)
7	Lung, pleura and mediastinum	8(88.88%)	1 (11.11%)	9(100%)
8	Abdomen	6(85.71%)	1(14.28%)	7(100%)
9	Pelvis and retroperitoneum	8(100%)	0(0%)	8(100%)
10	Miscellaneous	10(100%)	0(0%)	10(100%)
Total		275 (93.6%)	22(6.2%)	297(100%)

**Table-2:** Showing distribution of cases with correlated and non correlated diagnosis between adequate FNAC smears and cell block in percentage according to the site.

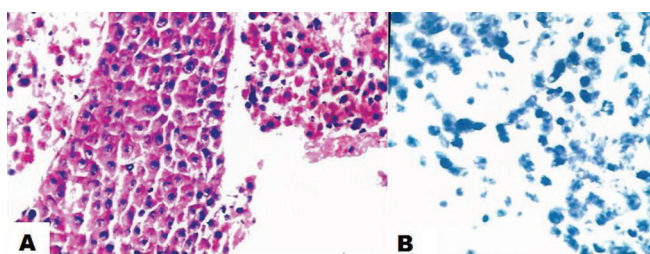
Sr. no	Site of FNAC	No	FNAC smears diagnosis	Cell Block diagnosis	Histopathology diagnosis
1	Lymph node (23)	10	Metastasis of SCC	Metastasis of SCC	Metastasis of SCC
		2	Metastasis of poorly differentiated malignancy	Metastasis of SCC	Metastasis of SCC
		1	Metastasis of poorly differentiated malignancy	Metastasis of adenocarcinoma	Metastasis of adenocarcinoma
		4	Metastasis of IDC	Metastasis of IDC	Metastasis of IDC
		2	TB	TB	TB
		2	Lymph node abscess (FN)	TB	TB
		1	Inadequate	Metastasis of SCC	Metastasis of SCC
		1	Metastasis of SCC	Inadequate	Metastasis of SCC
2	Breast (17)	8	Fibroadenoma	Fibroadenoma	fibroadenoma
		8	IDC	IDC	IDC
		1	Benign breast disease with atypia	Inadequate	Sclerosing adenosis
3	Liver &GBD fossa mass (7)	2	Poorly differentiated malignancy	adenocarcinoma	adenocarcinoma
		1	HCC	HCC	HCC
		1	hepatoblastoma	hepatoblastoma	hepatoblastoma
		1	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma
		1	Inadequate	Adenocarcinoma	Adenocarcinoma
		1	Metastasis of adenocarcinoma	Few Atypical cells seen (FN)	Metastasis of adenocarcinoma
4	Thyroid (16)	6	Multinodular goitre	Multinodular goitre	Multinodular goitre
		1	Colloid goitre (FN)	Follicular neoplasm	Follicular adenoma
		2	PTC	PTC	PTC
		3	Follicular neoplasm	Follicular neoplasm	Follicular carcinoma
		1	Follicular neoplasm (FP)	Follicular neoplasm (FP)	Adenomatoid goitre
		1	FVPTC	Follicular neoplasm (FN)	PTC
		1	FVPTC (FN)	Follicular neoplasm	Follicular carcinoma
		1	Atypia of undermined significance (FN)	Atypical cells seen (FN)	PTC
5	HNF (20)	10	SCC	SCC	SCC
		2	Dysplastic squamous cells (FN)	SCC	SCC
		1	SCC	Dysplastic squamous cells (FN)	SCC
		1	Meningioma	Meningioma	Meningioma
		1	Mucoepidermoid carcinoma	Mucoepidermoid carcinoma	Mucoepidermoid carcinoma
		1	neurofibroma	Inadequate	Neurofibroma
		1	Acinic cell carcinoma	Inadequate	Acinic cell carcinoma
		3	PA	PA	PA
6	Joints limbs bones (5)	2	MRCT	MRCT	Ewings sarcoma
		1	Fibrohistiocytic tumour of low grade malignant potential (FP)	Fibrohistiocytic tumour of low grade (FP)	Fibromatosis
		1	Osteogenic sarcoma	Osteogenic sarcoma	Osteogenic sarcoma
		1	Benign fibrohistiocytic tumour (FN)	Fibrohistiocytic tumour of low grade malignant potential	Dermatofibrosarcoma protuberance
7	Lung, Pleura, Mediastinum (3)	1	Small cell carcinoma	Small cell carcinoma	Small cell carcinoma
		1	Poorly differentiated malignancy	Poorly differentiated malignancy	Adenosquamous cell carcinoma
		1	Squamous cell carcinoma	Dysplastic squamous cells (FN)	Squamous cell carcinoma.
8	Abdomen (4)	1	Inadequate	YST	YST
		2	GIST	GIST	GIST
		1	Adenocarcinoma	Few dysplastic cells (FN)	Adenocarcinoma
9	Pelvis retroperitoneum (2)	1	RCC	RCC/CDC	RCC/CDC
		1	Mucinous tumour of borderline grade	Mucinous tumour of borderline grade	Mucinous carcinoma



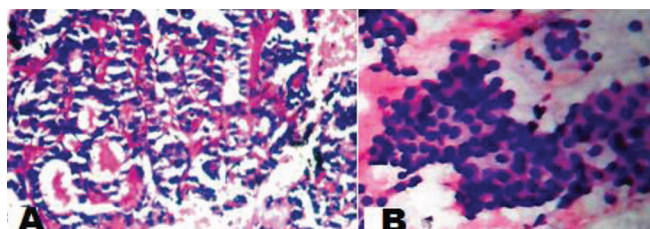
10	Miscellaneous	1	BFH	BFH	BFH
Total Cases		98			
Foot note: BFH-Benign fibrous histiocytoma, CDC- Collecting duct carcinoma, GBD- Gall bladder foss, HNF- Head neck face, FN- false negative, FP- false positive, FVPTC- Follicular variant of papillary thyroid carcinoma HCC- Hepatocellular carcinoma, GIST- Gastrointestinal stromal tumour IDC- Infiltrating duct carcinoma, MRCT- malignant round cell tumour, PTC-Papillary thyroid carcinoma,PA- Pleomorphic adenoma SCC- Squamous cell carcinoma, RCC- Renal cell carcinoma, YST- Yolk sac tumour					
<b>Table-3:</b> Showing correlation between FNAC smears, cell block and histopathology.					

Statistical analysis	FNAC	Cell Block	Combined FNAC and Cell block
True Positive	59	60	67
True Negative	26	26	28
False Positive	2	2	2
False Negative	8	6	1
Inadequate	3	4	-
Sensitivity	88.06%	90.91%	98.53%
Specificity	92.86%	92.86%	93.33%
Accuracy	89.47%	91.47%	96.94%

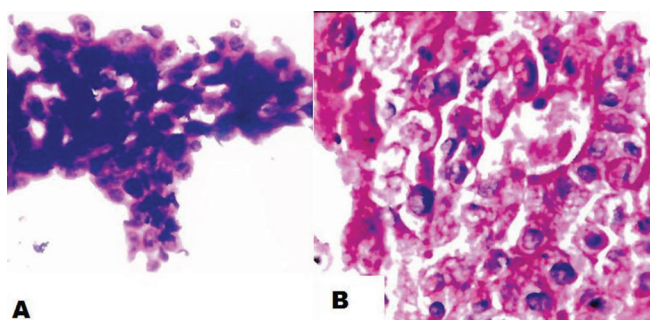
**Table-4:** Showing statistical analysis between cell block, FNAC and combined cell block and FNAC



**Figure-1:** [A] Showing trabecular pattern of hepatocellular carcinoma on cell block (H&E: 400x) and [B] respective FNAC (H&E: 400X)



**Figure-2:** A] Cell block showing thyroid follicles and the lesion diagnosed as follicular adenoma .(H&E: 400X) B] Corresponding FNAC smears (H&E: 400X)



**Figure-3:** A] Lesion diagnosed as undifferentiated malignancy on FNAC [MGG 400X]; B] Same lesion diagnosed as adenocarcinoma on cell block as gland formation seen.(H&E 400X)

were male and 199(55.28%) were female with male to female ratio of 1: 1.23.

The cases were divided into different age group with interval of 10 years. Maximum numbers of the cases (19.4%) were

in the age group 41-50 years, followed by 18.7% cases from 51-60 years age group.

In the present study lymph node was the most common site of aspiration in 102 (28.33%) cases followed by breast in 61 (16.94%) cases. Other sites for aspiration in decreasing frequency are liver 42 (11.66%), thyroid 40 (11.11%) and head, neck and face (HNF) area 38 (10.55%). In male lymph node was the most common site of aspiration in 61 (37.88%) cases followed by liver 25 (15.25%). Whereas in female most common site of aspiration was breast in 58 (29.14%) cases, followed by liver in 41 (20.60%) cases. [Table1]

Of 360 (100%) cases studied, FNAC smears were adequate in 324(90%) and cell blocks were adequate in 313(86.94%) cases. FNAC smears and cell blocks were inadequate in 36(10%) and 47(13.06%) cases respectively. In 297(82.5%) cases both FNAC smears and cell blocks were adequate. In 27 (7.5%) cases only FNAC smears were adequate, in 16(4.5%) cases only cell blocks were adequate and in 20(5.5%) cases both were inadequate.

In 297(82.5%) in which both FNAC smears and cell blocks were adequate divided in 2 categories depending upon correlation of diagnosis between them as

1. Correlated cases (275 cases)
2. Non correlated cases (22cases)

Of 22 non correlated cases maximum 7 cases were from lymph node followed by 5 cases from liver while 100% correlation was found in breast lesion cases. [Table2]

Of 360(100%) of the cases histopathological study was available 98 cases. Of these 22 non correlated cases between FNAC and cell blocks histopathology was available in 17 cases. Cell block was able to give more information in 12 cases and FNAC in 5 cases only. [Table3]

Sensitivity of FNAC was 88.06%,specificity of 92.86% and Accuracy of 89.47%. Sensitivity of cell block block was 90.91%, Specificity of 92.86% and accuracy of 91.47%. While the sensitivity of combined FNAC and cell block technique was 98.53% with Specificity of 93.33% and accuracy of 96.94%.[Table4]

## DISCUSSION

In the present study we have made cell block on the FNAC remaining material. In the study 10% alcohol –formalin (9 parts of 100% ethanol + 1 part of 40% formaldehyde) was used as a fixative. By formalin the proteins are crossed linked hence gel was formed and the firmed pellet was obtained after centrifugation which was not dissolved in any other material used for the processing. In conjunction with alcohol this fixative gives better nuclear and cytological details.<sup>6</sup>

Nathan et al used this fixative for making the cell blocks and found this fixative very effective for obtaining the material and for giving better morphological details.<sup>2</sup>

Various methods for preparing paraffin- embedded cell blocks from FNAC have been reported, which include direct transfer of all centrifuged cellular material wrapped in lens paper, filter paper or embedding in plasma thrombin or agar and then processing as a routine histological specimen.<sup>8</sup> In this study cell block preparation the cellular material was wrapped in whatman filter paper for further histological processing and embedded in the paraffine block. The reagent cost for making the cell blocks by this method is very less as compared to reagent cost for making the cell block by plasma thrombin method and can be done in any modest laboratory. Of 360 (100%) cases studied, FNAC smears were adequate in 324(90%) and cell blocks were adequate in 313(86.94%) cases. While FNAC smears and cell blocks were inadequate in 36(10%) and 47(13.06%) cases respectively. As we were using the material remaining in the needle hub after making the conventional cytology smears may be the cause for the more no of inadequate cases on cell blocks.

However the cytoarchetectural pattern was better appreciated in cell blocks. Trabacular pattern of hepatocellular cellular carcinoma was better appreciated on cell blocks.(figure1) Microfollicular pattern of follicular thyroid neoplasm (figure2) and finger like papillae of papillary carcinoma of thyroid were well seen on cell blocks.

The case was labelled as undifferentiated malignancy on FNAC. While it was diagnosed as adenocarcinoma on cell blocks as the gland formation of adenocarcinoma was better appreciated on cell blocks compared to FNAC samples. This case was confirmed on histology (figure3). Stromal infiltration was well seen on cell block in ductal carcinoma. Monotonous population of atypical lymphocytes was well appreciated on cell blocks. Brownish colloid was retained in colloid goiter on cell blocks. Squamous pearls were well seen on cell blocks. psamomma bodies and whorling pattern of meningioma was well seen on cell block.

There were total 22 non correlated diagnosis between FNAC and Cell blocks. Histopathology was available in 17 cases. Of non correlated cases cell blocks gave additional information in 12 cases for definitive diagnosis. [Table 3]

In the present study Sensitivity of FNAC and cell block was 88.06% and 90.91% respectively, Sensitivity was increased to 98.53% with combine use of FNAC and cell block. Specificity of FNAC and cell block was same as 92.86%. The specificity was increased to 93.33% with combined

FNAC and cell block study, Diagnostic accuracy of FNAC and cell block was respectively 89.47% and 91.47% which was increased to 96.94% with combined FNAC and cell block study.[Table4]

In the cell block of study 148 cases of lesion of various organs by Patil et al , sensitivity & diagnostic accuracy were high in cell blocks in comparison to the smears with the sensitivity of 94.90% in the smears and 98.92% in the cell block. Diagnostic accuracy of FNAC smears & cell blocks were 94.61% & 98.33%, respectively. With the simultaneous use of cell block technique and smear examination sensitivity was increased to 99.02%.<sup>1</sup>

In the cell blocks study of various organs, Basnet et al found were cell block technique superior in diagnosing neoplasm than smears with diagnostic accuracy of 95.91% and 91.8% respectively.<sup>8</sup>

In the cell block study of 46 cases of image-guided fine needle aspiration cytology of abdomino-pelvic and intrathoracic masses by Methew et al, the sensitivity, specificity, and diagnostic accuracy of cell blocks was 71.11%, 100%, and 71.73% respectively. The figures for FNA smears were 62.22%, 100%, and 63.04%, respectively.<sup>9</sup>

Image-guided FNAC and cell block study of 167 cases of intraabdominal lesions by Bhowmik et al cell block had a sensitivity and specificity of 100% and 95.49% respectively, which was clearly better than the conventional smear cytology (100% and 90.09%). Combination of cell block and FNAC attained sensitivity and specificity of 100% and 96.39%, respectively.<sup>10</sup>

This study was conducted in a teaching institute where FNAC is done by residents taking training and who rotate every 6 months. This is true for large number of hospitals attached to medical colleges. In this case, diagnostically important material remains in the needle hub and is wasted. Cell block done by residual FNAC material can give more diagnostic information specially cases inadequate on conventional cytology technique due to low cellularity. In these cases cell block on residual material can aids in diagnosis by providing addition material also the architectural pattern can be well studied. In the study on FNAC, cell block and core needle biopsy of lung masses by Makade al, diagnostic adequacy and test parameters were improved and approached core needle biopsy when both cyto-techniques were combined.<sup>11</sup>

## CONCLUSION

Cell block technique is sensitive and specific tool in diagnostic cytology. Combination of cell block to conventional cytology smears inadequacy of FNAC is reduced, hence unnecessary further biopsies for diagnosis can be avoided.

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