

Utility Of Cell Block In Cytodiagnosis Of FNAC Aspirates

Arpita J. Nishal¹, Sneha R. Patwari², Hinaben H. Kakadiya^{3*}, Krima B. Patel⁴

¹Associate professor, ^{2,3}Senior resident, ⁴Junior resident,

Department of Pathology, Government Medical College, Surat, Gujarat, India

***Corresponding Author:**

Dr. Hinaben H. Kakadiya

Senior Resident, Department Of Pathology, Government Medical College, Surat, Gujarat, India

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Abstract

Background:

Fine needle aspiration cytology of superficial/deep lesion is an increasingly common procedure in diagnosis of neoplastic lesions. Sometimes FNAC does not yield sufficient information for precise diagnosis and the risk of false negative or intermediate diagnosis always exists. In order to overcome these problems, cell block technique has been resorted to make the best use of available material.

Aim of present study was to study utility of cell block method for cytodiagnosis and to compare diagnosis with conventional smear method.

Material and method

Present study was conducted over a period of 21 months enrolling patients attending for FNAC and fluids received in the cytology section. Cell block were made wherever adequate material was available.

Results

A total of 61 cases were subjected to both conventional smear and cell block preparation. Male:Female ratio was 1:1.17. The maximum number of cases were in the age group of 41-60 years(46%). On basis of conventional smear, 61%(37 cases)of total cases are positive for malignancy, 23%(14) are benign, 8%(5) are Inadequate for opinion and 8%(5) are Suspicious of Malignancy. Diagnostic yield of conventional smears was 92%(56/61). Cell block confirms diagnosis in 67% cases whereas 33% were non diagnostic/non contributory. Diagnostic yield increased by 3% by using cell block method.

Conclusion

Cell block method is complementary tool for improving cytodiagnosis. Cell block preparation is an easy, simple and cost-effective method. Cell blocks also prove to be an excellent resource material for ancillary techniques like Immunocytochemistry.

Keywords: Cell block, Conventional smear, Fine needle aspiration cytology

Introduction

Diagnostic cytology is the science of interpretation of cells that are either exfoliated from epithelial surfaces or removed from various tissues. The advantages are, it is non-invasive, simple and helps in faster reporting and is relatively inexpensive.[1]

Cytological examination of lesions is of paramount importance not only for diagnosing cancer but they

may also reveal information regarding various inflammatory conditions, parasitic infestations, infection with bacteria, fungi, or viruses and some immunological conditions.[2]

The accuracy of the cytological examination from any body site depends greatly on the quality of collection, preparation, staining and interpretation of the material.

Sometimes FNA does not yield sufficient information for precise diagnosis and the risk of false negative or intermediate diagnoses always exists.[3] In order to overcome these problems, cell block technique has been resorted to make the best use of the available material. The cell block technique refers to processing of sediments, blood clots or grossly visible tissue fragments from cytological specimens into paraffin blocks that can be cut and stained by the same methods used for histopathology. Various methods for preparing cell blocks from FNAC have been reported. [4]

Though utility of cell block is greatly acknowledged, it is not routinely performed in many institutes including present study setup; hence the present study was undertaken as an attempt to assess the utility of cell block in cytodiagnosis in the cytology section of pathology department.

Aim of present study was to study utility of slides prepared by cell block method for cytodiagnosis and to compare diagnosis of cytological specimens prepared by both cell block method and conventional smear method.

Material And Method

Present study was conducted in the department of pathology in a tertiary care center over a period of 21 months enrolling patients attending for routine FNAC and image-guided FNAC and the fluids received in the cytology section. A total of 61 cases were subjected to both conventional smear(CS) and cell block(CB) preparation.

Aspirates obtained by FNAC of lesions of (all sex and ages) abdominal-pelvic organs, breast, thyroid, lungs, lymph node, soft tissue and body fluids wherever adequate material was available to make cell block.

Cases with inadequate material and abnormal coagulation profile were excluded from study.

Detailed clinical data including radiological details were recorded. Patients were made aware of the procedure and informed consent was obtained.

After preparing conventional smear from FNAC materials and body fluid, remaining material was subjected to cell block preparation. For cell block preparation, residual material from the hub poured in formalin filled test tube. Following formalin fixation for 30-45 mins, clotted aspirate removed from formalin fixation & wrapped in whatman filter paper. For cell block preparation from body fluid, residual sediment wrapped in whatman filter paper by inverting test tube. Then the cell button was processed as routine biopsy specimen and stained with hematoxylin and eosin(HE) staining.

After studying all the available clinical data, based on morphology, cellularity and architecture, conventional smear and cell block were categorized as: Positive for Malignancy, Benign, Inadequate for opinion and Suspicious of malignancy for conventional smear and Non Diagnostic/ No Material, Non Contributory (CS+, CB-), Confirms Diagnosis and Establishes Specific Diagnosis for cell block.

In cases where indicated, Immunocytochemistry was done on formalin-fixed paraffin wax sections with established positive controls wherever it was feasible.

Validity tests were done to find out the sensitivity, specificity and diagnostic accuracy of the cell blocks using software Epi Info Version 7.

Results

A total of 61 cases were subjected to both conventional smear and cell block preparation.

Table 1: Distribution of samples

Site	Count of FNA site /Fluid type	Percentage
Axillary Swelling	2	3%
Breast	5	8%
Gall bladder	2	3%
Liver	15	25%

Lymph node	15	25%
Ovary	2	3%
Peritoneal fluid	1	2%
Pleural fluid	4	7%
Right Gluteal region	1	2%
Submandibular Swelling	1	2%
Swelling behind right ear	1	2%
Swelling in left arm	1	2%
Swelling in right infra-auricular region	1	2%
Swelling over left side of face	2	3%
Thyroid	8	13%
Total	61	

(II) AGE AND GENDER DISTRIBUTION: Out of 61 cases 28 were male(46%) while 33(54%) were female. M: F ratio is 1:1.17.

The maximum number of cases were in the age group of 41-60 years(46%) while minimum number were in age group above 60 years(15%). The age range was 0-above 60 years.(Table 2)

Table 2: Age and Gender Distribution of Cases

Age Range	Female	Male	Total(%)
0-20	4	6	10(16%)
21-40	9	5	14(23%)
41-60	16	12	28(46%)
Above 60	4	5	9(15%)
Total	33	28	61

(III) INTERPRETATION ON BASIS OF CONVENTIONAL SMEAR: After studying all the available clinical data, based on morphology the conventional smear were categorized as:

1. Positive for Malignancy: This category included all the cases where frank malignancy was detected on conventional smear; this made up 61%(37 cases)of total cases in the present study.
2. Benign: This category included all those cases where conventional smear showed benign diagnosis. This made up 23%(14 cases) of all cases.

3. Inadequate for opinion: This category included all those cases where the conventional smear showed only blood or had very low cellularity to come to a specific diagnosis. 5(8%)of cases were categorized under this.
4. Suspicious of Malignancy: This category included those conventional smears which had a malignant diathesis but no pathognomonic feature was found to strongly label it as malignant. 5(8%)of cases were included in it.

Diagnostic yield of conventional smears was 92% (56/61). While 5/61 cases were non diagnostic (8%).

(IV) INTERPRETATION ON BASIS OF CELL BLOCK: To facilitate proper comparison with conventional smear, the cell block conclusion of the same cases was done as follows:

- I. Non Diagnostic/No Material: This included all those cases where the cell block showed only blood and any kind of diagnostic material was unavailable.
- II. Non Contributory: Cell block with sub optimal cellularity were included in this category. The above two category summed to occupy 33% of all cell block diagnosis.
- III. Confirms Diagnosis: In 67% cases, cell block helped to confirm the diagnosis of conventional smear and establish specific diagnosis. This category includes all those cases where the conclusion of cell block correlated with the conclusion of conventional smear, thus helping to establish the correctness of conventional smear.

IV. Establishes Specific Diagnosis: In the present study there were cases where the conventional smear could not help reach a diagnosis while the cell block solely yielded diagnostic material to come to a result surpassing conventional smear. 2(3%) cases were included under this category.

Diagnostic yield of cell block was 67 % (41/61). While 20/61 cases were non diagnostic (33%).

(V) CROSS TABULATION OF CONVENTIONAL SMEAR V/S CELL BLOCK: In present study group, 37 cases were positive for malignancy of which 26 were confirmed by cell block (70.27%). Benign lesions on conventional smear were confirmed in 9 /14 cases (64.28%) cases by cell block. Out of 5 cases which were suspicious for malignancy on conventional smear, cell block established specific diagnosis in one of them while it confirmed the diagnosis of conventional smear in other 4 cases. Cell block established specific diagnosis in 1/5 cases which was inadequate on conventional smear.(Table 3)

Table 3: Cross tabulation of Conventional Smear v/s Cell Block

Conventional Smear Categories	Cell block Categories				
	Non Diagnostic	Non Contributory	Confirms Diagnosis	Establishes Specific Diagnosis	Total
Malignant	7	4	26		37
Benign	3	2	9		14
Inadequate/Non Conclusive	2	2		1	5
Suspicious of Malignancy			4	1	5
Grand Total	12	8	39	2	61

(VI) CROSS TABULATION FOR STATISTICAL ANALYSIS: In present study, conventional smear was positive in 55 cases. Out of these 55 cases, 39 cases were positive on both conventional smear and cell block while in 16/55 cases cell block was not helpful(negative). Conventional smear was negative in 6 cases. Out of these 6 cases, cell block showed independent diagnosis(CB positivity) in 2 cases while 4 cases were inconsequential on both conventional smear and cell block.(Table 4)

Table 4: Cross Tabulation for Statistical Analysis

Cell Block Method	Conventional Smear Method		Total
	Positive	Negative	
Positive	39	2	41
Negative	16	4	20
Total	55	6	61

Statistical Analysis of the study shows that Cell block method has Sensitivity of 71%, Specificity of 66% and diagnostic accuracy of 71%.

Immunocytochemistry: It was performed in the case of liver mass, in which Hep par and CK 7 both were negative which confirms the diagnosis of metastatic adenocarcinoma

Discussion

A total of 61 cases were subjected to cell block formation. Majority of samples were from liver (25%) and lymph node (25%) followed by thyroid (13%) and breast (8%). Among fluids majority were pleural fluid (7%). Least number of samples was comprised by soft tissue swellings. In the study by Nathan Et Al, out of 465 FNAC of solid lesions maximum were from head and neck lesions(59 cases).[5] In the study by Brown Et Al, 57 cases were in the chest(45 lung, four mediastinum, eight pleura-based masses). 16 were retroperitoneal/pelvic biopsies, and 11 were of the liver, pancreas, or kidney.[6] This difference could be attributed to random selection of cases which were selected on basis of inclusion and exclusion criteria.

Age of patients in present Study ranged from 0 to above 60 years; maximum samples were in the age group of 41-60 years. This age range was similar to studies by Brown Et Al, Dekker Et Al, Shobha Et Al & Bansode Et Al.[6-9]

In present study female comprised of 54% of cases while male comprised of 46% of the cases. Similar gender distribution was noted in study by Khan Et Al where females outnumbered males(56.9% v/s 43.1%).[10]

Interpretation on the basis of Conventional Smear after studying all the available clinical data, based on

morphology the conventional smear were categorized as:

1. Positive for Malignancy: This category included all the cases where frank malignancy was

detected on conventional smear; this made up 61% of total cases in the present study.

14/15 liver masses showed malignancy. This made a significant contribution to this category of conventional smear since the major site of aspiration was liver. 8 cases showed features of metastatic adenocarcinoma while 2 cases were of HCC(Hepatocellular Carcinoma). Other malignant cases which were categorized under this were mammary carcinoma, malignant melanoma, pleomorphic round cell tumor etc.

2. Benign: This category included all those cases where conventional smear showed benign diagnosis with complete absence of malignant cell. Benign cases were comprised of proliferative breast disease, benign follicular nodule(TBSRTC Cat II), chronic reactive hyperplasia. This made up 23% of all cases.

3. Inadequate for opinion: This category included cases where the conventional smear showed only blood or had very low cellularity to come to a specific diagnosis. 8% of cases were categorized under this. This may be attributed either to highly vascular nature of the site of aspiration or fibrosis at the site. Also, the technique and the skill of the aspirator were very important for preparation of proper conventional smear. Any flaw in the above may lead to low cellular smears.

4. Suspicious of Malignancy: This category included those conventional smears which had a malignant diathesis but no pathognomonic feature was found to

strongly label it as malignant. Thyroid lesions showing features of suspicious for follicular neoplasm, suspicious for medullary neoplasm, suspicious for

papillary neoplasm etc. were majorly included in this category. 8% of all cases were included in it.

Table 5: Comparison of cytodiagnosis with other studies (Conventional Smear ONLY)

Sr.No	Study	Benign(%)	Suspicious(%)	Malignant (%)
1.	Dekker and Bupp. (Fluid)[7]	74%	4.6%	16.2%
2.	Nathan Et al. (FNAC+Fluid)[5]	2%	0.4%	17.5%
3.	Thapar Et al. (Fluid)[11]	63%	-	26.3%
4.	Udasimath Et al. (Fluid)[12]	90%	8.3%	1.66%
5.	Ghosh Et al.(Fluid)[13]	-	-	36.6%
6.	Sumitha Et Al (Fluid)[14]	86.8 %	1.8%	8.2%
7.	Liu Et Al (FNAC+Fluid)[15]	30%	8.9%	55.4%
8.	Present Study (FNAC + Fluid)	23%	8%	61%

The findings of present study were comparable with the study by Liu Et Al due to similar inclusion criteria while the comparison may not be valid with other studies due to inclusion of only fluids in those studies.(15)

The overall diagnostic yield of conventional smear in the present study was 92% which was comparable to the study conducted by Nathan Et Al where the diagnostic yield of conventional smear was 86%.(5)

Interpretation on the basis of Cell Block

To facilitate proper comparison with conventional smear, the cell block conclusion of the same cases was done as follows:

I. Non Diagnostic/ No Material: This included cases where the cell block showed only blood and any kind of diagnostic material was unavailable.

II. Non Contributory: Cell block with sub optimal cellularity were included in this category.

The above two category summed to occupy 33% of all cell block diagnosis. The reason may be attributed to exhaustion of entire cellular material in conventional smear preparation, leaving no diagnostic useful cellularity in the residual clot for making cell block.

III. Confirms Diagnosis: In 67% cases, cell block helped to confirm the diagnosis of conventional smear and establish specific diagnosis. This category includes cases where the conclusion of cell block correlated with the conclusion of conventional smear, thus helping to establish the correctness of conventional smear. In metastatic adenocarcinoma liver the glandular pattern was better appreciated on cell block.(figure 1) The predominant spindled cell in syncytial pattern can be significantly noted in the cell

block of suspicious of medullary carcinoma of thyroid.(figure 2) In BFN Cat II bland looking thyroid follicular cells are seen in cohesive clusters in cell

block. Cell block showed malignant pleural effusion having reactive mesothelial cells, malignant cells and polymorphonuclear cells.(figure 3)

Figure 1: CS and CB of metastatic adenocarcinoma Liver (HE,40x)

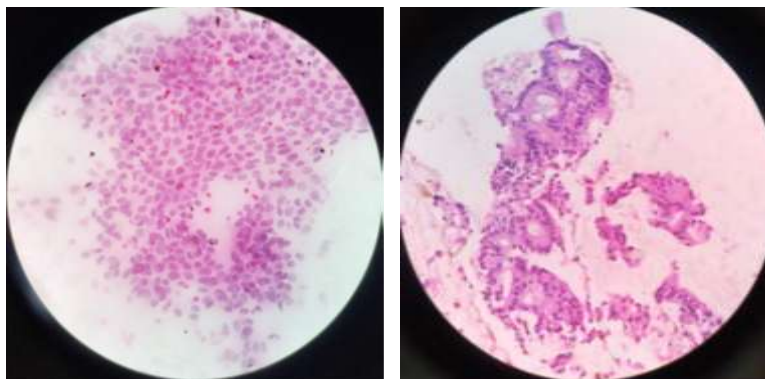


Figure 2 : CS(giemsas) and CB(HE) of Suspicious Medullary Carcinoma Thyroid (40x)

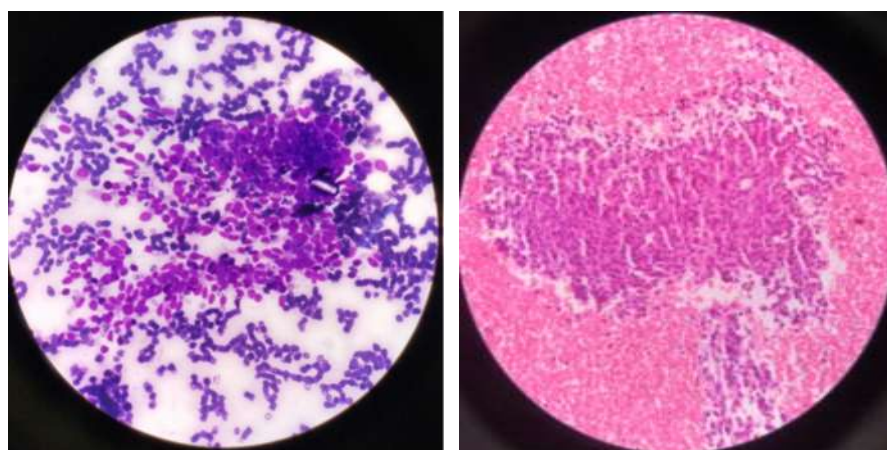
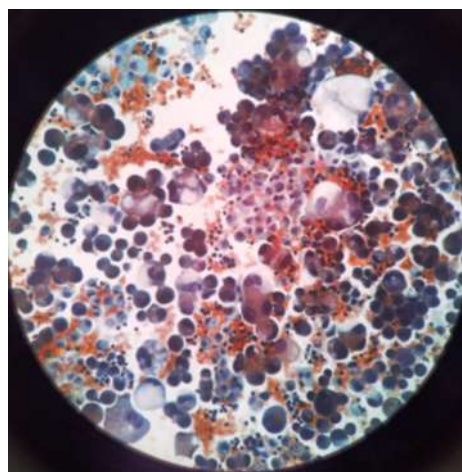


Figure 3: CB of Malignant Pleural effusion



IV. Establishes Specific Diagnosis: In the present study there were cases where the conventional smear could not help reach a diagnosis while the cell block solely yielded diagnostic material to come to a result surpassing conventional smear. In a case cell block of aspirate from cervical lymph node showed features of SCC whereas

the corresponding conventional smear was suspicious of malignancy. According to Yamamoto et al.[16], tumor morphology, which could not be identified on cytology, could be better appreciated on cell blocks. According to De Bore et al. [17] and Nathan et al. [5], cell blocks may give a better idea of tissue architecture. In another case, cell blocks showed features of chronic reactive hyperplasia where the conventional smear showed only blood.(figure 4) This could be due to entrapment of diagnostic material in the clot. Orell [18] observed that when samples are heavily admixed with blood, smears may show only blood and a few distorted cells caught in blood clots, and, yet, good tissue fragments may be found in sections of cell blocks. Rofaga et al. [19] found that cell block samples were particularly useful in cases with aspiration of thyroid and recurrent carcinoma. The above cases showed that the diagnostic yield increased by 3.3% by using cell block method.

Figure 4: CB showing Lymphocytes in varying stages of maturation in chronic reactive hyperplasia(HE, 40x)

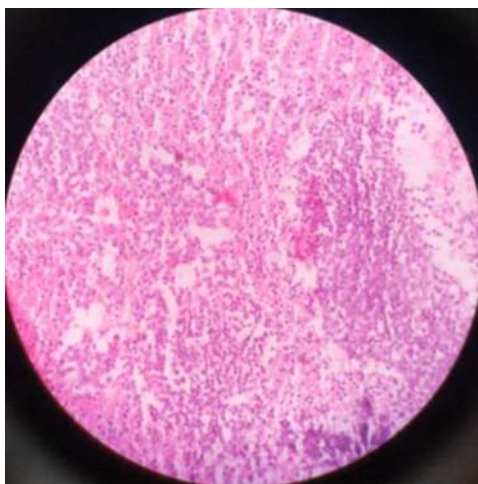


Table 6: Comparison of cytodiagnosis with other studies (Cell Block ONLY)

Sr.No	Study	Benign(%)	Suspicious(%)	Malignant (%)
1.	Dekker and Bupp. (Fluid)[7]	54.5%	6.2%	32%
2.	Nathan et al. (FNAC+Fluid)[5]	1.8%	-	17.1%
3.	Thapar et al. (Fluid)[11]	63.4%	-	31.5%
4.	Udasimath et al.(Fluid)[12]	83.3%	-	16.6%
5.	Ghosh et al.(Fluid)[13]	-	-	76.6%
6.	Sumitha Et Al (Fluid)[14]	26.2 %	-	6.5%
7.	Liu Et Al (FNAC+Fluid)[15]	17%	4.5%	33.1%

8.	Present Study (FNAC + Fluid)	15%	8%	44.3%
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The present study was comparable to Liu Et Al. Different procedures of cell block formation, inclusion criteria and random selection of cases in various studies may account for some of the reported variability with the present study.[15]

The additional yield by use of cell block of the present study(3.3%) showed concordance with studies by Bodele Et Al(7%), Richardson Et Al(5%) and Bansode Et Al(6.33%).[20,21,9] Dekker and Bupp et al and Khan et al have larger increase in diagnostic yield 38% and 20% respectively by cell block as compared to present study which can be attributed to their larger sample size in their study.[7,10]

According to Nathan et al., cell blocks are adjuvant to FNAC smears for establishing a more definite cytopathologic diagnosis. They observed improvement in 15.2% cases when cell blocks were studied with smears.[5]

The statistical analysis of the present study showed that cell block method had Sensitivity 71%, Specificity 66% and Diagnostic Accuracy 71%. The present study was concordant with the study by Axe Et Al[22]. The diagnostic accuracy of present study was concordant with the study by Khastriya Et Al[23] and Liu Et Al[15].

Immunocytochemistry

Immunocytochemistry was performed in the case of liver mass, in which Hep par and CK7 both were negative which confirms the diagnosis of metastatic adenocarcinoma. Hence, cell blocks were also instrumental for the performance of immunocytochemistry.

Conclusion

FNAC is a simple, safe and reliable procedure for diagnosis of various lesions. Smears prepared from aspirates and fluids help in detecting lesions of various organs. Hence to make best possible use of aspiration, smears should be combined with cell block preparation that in turn gives better morphological and histological details, thus refining the diagnosis.

Cell block method is an excellent complementary tool for improving cytodiagnosis. Cell block preparation is

an easy, simple yet reliable and cost-effective method hence can be incorporated into routine cytology laboratory of any setup with limited expertise. Cell blocks also prove to be an excellent resource material for ancillary techniques like Immunocytochemistry.

Future research in the department would be benefited by conducting more studies with more sample size and optimized cell block formation to understand its utility better.

Ethical approval: The study was approved by the Institutional Ethics Committee

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