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Original Article

Diagnostic Efficacy of Cell Block Technique as a Complementary Adjunct to Fine Needle Aspiration Cytology

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Abstract

Background: Though fine needle aspiration cytology is the most routinely performed and widely accepted diagnostic procedure for an initial diagnosis in any swelling, we often come across difficulties in diagnosis, if the material obtained is hemorrhagic or fluid in nature. In such cases cell blocks can be prepared from the residual tissue fluids or the aspirated material. Cell blocks then be sectioned, stained and studied. Cell block study can be an useful adjunct to smears for establishing a more definitive cytopathologic diagnosis. Hence this study was conducted to evaluate the efficacy of cell block with FNAC and to compare the findings of cell block and FNAC with histopathology as gold standard. *Methodology:* The study was conducted in the department of Pathology at Adichunchanagiri Institute of Medical Sciences and Research Centre. All the cystic/ solid lesions sent for fine needle aspiration which yielded sufficient amount of material for the cell block were studied after taking a detailed clinical history. *Results:* Out of total 66 cases of FNAC and cell block, 35 cases were sent for histopathology. Mean age of the patients was 41.36 years and female patients were more in number (73%). Benign lesions (71.4%) were more than malignant ones (29.6%). Cell block section had more number of thyroid lesions (31%). The diagnostic accuracy of FNAC was found to be 94.28% while that of cell block was 97.14% *Interpretation and Conclusion:* Though FNAC is the first line of investigation for mass lesions, it has its disadvantages due to fluid and hemorrhagic aspirates. Cell block provides an excellent complement with FNAC in diagnosis as the diagnostic accuracy increases when FNAC and cell block are combined.

Keywords: CB-Cell block; FNAC-Fine Needle Aspiration Cytology; Histopathology.

Introduction

Fine Needle Aspiration Cytology (FNAC) is the study of cells in the view of identifying diseases due to infection, inflammation and neoplasia. FNAC can identify many neoplasms including benign and malignant ones. These neoplasms may be primary, recurrent or metastatic, or their precursor lesions [1].

Though fine needle aspiration cytology is the most routinely performed and widely accepted diagnostic procedure for an initial diagnosis in any swelling, we often come across difficulties in diagnosis, if the material obtained is scanty, hemorrhagic or fluid in nature. Another constraint of the conventional FNAC smear is the limited material available for adjuvant diagnostic investigations including immunocytochemistry [2].

The cell block technique is a technique which employs the retrieval of small tissue fragments from a FNA specimen which are processed to form a paraffin block. It is widely accepted that this method of analysis increases the cellular yield and improves diagnostic accuracy [3]. The ability to obtain numerous tissue sections allows for multiple immunostains and other studies to be performed akin to paraffin sections produced in histopathology [4].

In FNAC, at times, quality of smears is also variable as one would encounter thick clumps of cells or poor cell distribution In such times cell block surmounts over FNAC. Cell block also provides additional insights by assigning patterns which can provide additional clues for the diagnosis. Keeping this in view, the present study was conducted to evaluate the role of FNAC and cell block with histopathology as gold standard.

Aims and Objectives of the Study

1. To evaluate the efficacy of cell block technique as a diagnostic aid.

2. To evaluate the combined efficacy of cell block technique and FNAC.

3. To compare the findings of cell block technique and FNAC with histopathology as gold standard whenever possible.

Materials and Methods

Selection of specimens for Cell blocks (CBs)

FNAs yielding good amount of material or bloody aspirates can all be used for the preparation of CBs. An initial assessment of cellularity and indication for CB can be ascertained by rapid on-site staining or by examination of routine cytological preparations in the laboratory to encourage the judicious use of CBs. CBs are also of use in cases in which some aspirated sample or blood is seen in the hub of the aspirating needle. An extra pass may be required in cases in which scant or no material is seen in the hub of the needle [11].

Cell blocks offer the opportunity to examine the histological structure besides allowing the use of ancillary tests. Important residual material is salvaged which is generally not available in cytology smears. Although the technique of cell block is not new, handling of the specimens in a conventional way may lead to considerable loss of material and is inconvenient.

According to Orell, [6] Cell blocks help pathologists by giving a better idea of tissue architecture and allow multiple sections for panels of immune markers with controls. But cell blocks are relatively time consuming and costly compared to routine smears. Hence cell blocks should be used selectively, especially if there is a need for immunocytochemistry. Cell blocks also aid if samples are heavily admixed with blood. Good tissue fragments are often found in a cell block even when smears show only blood.

Various methods illustrated in literature for cell blocks

The earliest method was simple sedimentation technique but had a major disadvantage of being less cellularity. Hence, an additional step of centrifugation was introduced to enhance the cellularity of CBs [11]. Fixation of the sediment was a major difficulty as it may lead to the disintegration of cell clusters and fragments because of friability or crumbling into many minute pieces during dehydration or paraffin-embedding steps. The basic steps of all the CB preparations comprise of fixation, centrifugation of the aspirate and later cell sediment which is formed is treated for paraffin embedding.

Most simplified method as explained by Orell by using cell button technique: Here aspirates obtained from cell rich tissues like lesions from lymphnode and cellular neoplasm were used [6]. Those lesions which yield creamy aspirates were placed on a slide and were not smeared instead the drops were allowed to stand on the slide for few seconds and the aspirates would adhere to the slide. The slides were carefully placed into the jar containing 90% ethanol. The sample remained stuck to the slide as a drop ('button'). Alcohol fixation, unlike formalin, used to hold the sample together. After fixation the cell button which is formed was carefully taken out of the slide using a scalpel. And the cell button was treated as a small biopsy.

Normal saline needle rinse method: The commonly used method of cell block where aspiration is rinsed with 20-30 ml of normal saline and the same is

centrifuged immediately. It has been recommended by The British Society for Clinical Cytology (BSCC) code of practice that needles have to be rinsed in balanced saline to provide flexibility for ancillary tests, such as flowcytometry or microbiology, prior to fixation for CBs [12]. Also 10 ml of formalin or paraformaldehyde or 50% ethanol alone can be used for rinsing cell block material [13-16].

Tissue coagulum clot method (TCC): To increase the cellularity in CB sections, the TCC method is used where dilution with normal saline is avoided and clot of tissue and blood mixture is formed in the lumen of the needle. When this clotted material streams out of the needle it is collected on a piece of filter paper and allowed to air dry. In order to preserve cellular morphology the clot is slightly air dried. The tissue coagulum is thentransferred into a formalin container and subsequently processed in the histology laboratory [17–19]. TCC method is superior to traditional aspiration needle rinse method in the persistence of cellular material and resisting the loss of diagnostic material.

Plasma thrombin method: After all of the smears/ slides have been made, the remaining material of the sediment is mixed with plasma which is available from the blood bank or hematology laboratory. For every one ml of sample four ml of plasma is added and together mixed with 4 drops of thrombin [20]. Few commercially available thromboplastin prepared from rabbits lung or brain contain epithelial cells which may lead to difficult interpretation. The uneven distribution or concentration of cells can be avoided by agitating the tube during clot formation which disperses the cells evenly in the fibrin mesh. This is allowed to sit for few minutes till a semi solid area develops. This will be fixed and placed inside the blotting paper and processed for histopathology with a cassette.

The albumin method: The albumin method is a simple method for making cell blocks. This method uses 30% bovine albumin which is usually present in the blood bank. This method is avoided if the patient is taking certain anticoagulant therapy or on blood thinners which can prevent the clot formation. The centrifuged material is mixed with 2-3 drops of 30% bovine albumin and to this mixture 95% alcohol is added which creates a soft ball and the same should be placed inside the cassette, if the mass doesn't hold up together then it is wrapped inside the lens paper later put in the cassette for paraffin embedding [20].

The agar method: Agar is a jelly like substance obtained from algae. Agar exhibits hysteresis that is it melts at 85 degree celsius and solidifies at 40 degree celsius. Time consuming but this has been compensated by the producing excellent blocks. After centrifugation, the sediment material is placed in a small pool of the glass slide or petridish where melted 3% agar is placed and mixed with the exposed parts of the sediment material allowed to mix [21]. If the material is scanty the melted agar is directly added into the test tube of the sediment. After the agar hardens, the agar button is gently removed and cut into half and put in a tissue cassette for processing.

Simplified cell block technique as explained by Krogerus and Anderson, [22] the technique is being unique that all the processes are carried out in the sample tube ensuring minimal cell loss. No transfer of sediment to cassette is necessary which avoids the needfor wrapping paper, thrombin or agar. The procedure involves 50 ml conical plastic centrifuge tube, the material aspirated is placed inside the conical tube and centrifuged for 7 minutes and supernatant is poured of. The cell pellet is allowed to stand with 3 ml of acetone for 10 minutes, the sample is again centrifuged for 10 minutes and acetone is poured of. The tube is placed onto the warm plate (not more than 60 degree celsius) and melted paraffin is added to the dry, warm cell pellet. After the paraffin is solidified, the bottom of the tube is tapped to remove the cell block and the conical end is sectioned as regular histopathology technique.

Method of Collecting Data

Fine Needle Aspiration: After taking a detailed clinical history, the procedure was explained briefly to the patient and informed consent was taken. The swelling was palpated (in cases of palpable lesions) and the area cleansed with a spirit swab. The swelling was immobilized with the non-dominant hand. A 23-25 gauge needle attached to a 10 ml syringe was introduced into the lesion. The plunger of the syringe was pulled back to create a negative pressure and the needle is moved back and forth. Negative pressure will be released when the needle remains in the swelling. After 8-10 rapid passes the needle was withdrawn and the syringe detached. The aspirate was blown on to glass slides from the syringe. The smears was fixed in 95% ethanol and stained with Haematoxylin–Eosin or Papanicolau stain. The air dried smears was stained with May Grunwald Giemsa stain and studied. ZiehlNeelsen staining was done to look for acid fast bacilli if they are suspected to be present.

Methods of cell block preparation

3% Bacterial agar cell block

Centrifuged specimen with solid sediment: After the direct smears have been prepared, the remaining sediment was re-suspended in an equal volume of 10 percent buffered formalin or other tissue fixative. If the specimen is prefixed in alcohol, 80% alcohol was used instead of formalin.

It was centrifuged at 1500 rpm for 10 minutes and allowed to fix for two hours. Samples with scanty material or very small tissue fragments may fix within 1 hour. Supernatant fluid was decanted by draining the last drops on a paper towel point up. With a scalpel, the apex of the packed sediment was cut through. On a used glass slide, a small pool of melted agar was spread. The tube of agar was melted in water broth, as agar melts at approximately 60 degree Celsius. The unused agar was discarded. The cut side of the packed sediment was placed on the pool of agar.

The entire specimen was covered with melted agar. The exposed areas of packed sediment was covered with melted agar and after ensuring that there are no bubbles. The agar should harden in a few minutes. Excess agar was trimmed as close to the packed sediment as possible, forming a rectangular square. The agar button was placed in tissues capsules, from the histology department for processing of the material into a paraffin block. The tissue capsule was labeled and placed in a jar of fixative. The fixative was be the same one that was used originally to fix the sediment.

The agar button which is ready shall go to an automatic tissue processing machine for dehydration and paraffin infiltration. It was embedded and sectioned just as a tissue biopsy would be.

Excessively bloody or turbid specimen

If the specimen to be processed is excessively bloody or turbid, the entire specimen was poured through a fine wire mesh. Any tissue fragments present was picked off the mesh and placed in a fixative to be embedded in agar. The filtrate was centrifuged for smear and cell block preparations. This ensures that minute fragments will not be lost in the large volume of sediment. It alleviates the necessity of sectioning the entire paraffin block.

Centrifuged sediment which is blood

Bloodwashemolysed prior to centrifugation by adding 0.1 normal HCL to the specimen until a uniform clear brown colour appears. The specimen shall then be centrifuged and the supernantant fluid discarded. It is a good idea to then wash the sediment with physiologic saline to remove the HCL. The specimen was centrifuged again and the packed sediment treated as directed previously. If the blood is not hemolysed prior to centrifugation, the formalin or alcohol fixed packed sediment may not solidify completely. If this happens, the fixative was carefully decanted and the entire sediment transferred gently into a petridish. With a forceps, all the solid particles were oriented close together on the pool of melted agar.

Specimen with fibrin clots

If a fluid contains fibrin clot, a forceps was used to remove the clot from the container and placed in a petridish. The clot was twisted with the forceps until all the fluid is squeezed out. The clot was placed in a membrane fixative. After fixation it was embedded in agar.

Fluid aspirates with less solid sediment

The supernatant fluid was decanted from the centrifuged specimen which has been fixed for one hour in 10% buffered formalin. The last drop was drained from the tip of the tube into a paper towel. The tube was agitated to disperse the material evenly. Several drops of melted agar was added. The agar was allowed to run down the slide. The tube was agitated to mix thoroughly. The agar was allowed to harden. After the agar is hard, the agar button was gently dislodged from the bottom of the tube the button was cut with a scalpel. The cut agar button was placed into a tissue capsule, labeled and placed in a jar with fixative. The agar button is now ready to go on automatic tissue processing machine for dehydration and paraffin infiltration. It was embedded and sectioned just as a tissue biopsy would be. Sections were submitted for processing, slides were made with 5 µm thickness of tissues and stained with haematoxylin and eosin. The microscopic features were analyzed. Apart from agar, plasma was made to mix the sediment after which thrombin wasused to harden the sediment. Rest of the procedure was same as agar method.

Staining Procedure

Haematoxylin and Eosin

1. Bring sections to water.
2. Stain in Harris haematoxylin for 15-20 minutes.

3. Remove excess of haematoxylin by keeping section in running water for 10 minutes followed by differentiation in 1% acid alcohol.
4. Decolorizing acid is then removed by keeping in running tap water followed by bluing in Lithium carbonate.
5. Stain in Eosin for 2 minutes.
6. Dehydrate, clear and mount.

Results

Nucleus appears blue and cytoplasm appears pink.

Statiscal Data: All the collected data parameters were analyzed statistically using:

Standard deviation ± 0.5

Unpaired't' student test

Chi square and Z test.

The data thus collected was tabulated and analyzed systematically by the use of appropriate statistical methods and results were obtained. They were compared to similar studies done previously and conclusions were drawn which are presented here.

Table 1: Showing age wise distribution in all the lesions

Age group	Number	Percentage
1-10	1	1.5%
11-20	5	7.5%
21-30	12	18.8%
11-20	5	7.5%
21-30	12	18.8%
31-40	14	21.2%
41-50	20	30.3%
51-60	7	10.6%
61-70	5	7.5%
71-80	1	1.5%
81-90	1	1.5%
	Total-66	100%

Table 2: Showing sex wise distribution in all the lesions

Sex	Number	Percentage
Female	48	73%
Male	18	27%
Total	66	100%

Table 3: Showing organ wise distribution according to age group in all the lesions

	Age Group									
Organ	1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	>80	Total
Thyroid	1	1	2	5	4	3	0	1	0	17
Salivary Gland	0	0	0	2	2	0	1	0	0	5
Lymph Node	0	1	0	0	4	1	3	0	1	10
Skin	0	1	3	3	3	2	0	0	0	12
Soft tissue	0	2	2	0	1	0	0	0	0	5
Breast	0	0	5	5	5	1	0	0	0	16
Miscellaneous	0	0	0	0	0	1	0	0	0	1
Total	1	5	12	15	19	8	4	1	1	66

Table 4: Showing organ wise distribution according to sex of the patient in all the lesions

Organ	Female	Male	Total	%
Thyroid	16	1	17	25.7%
Salivary Gland	2	3	5	7.5%
Lymph Node	3	7	10	15.1%
Skin	7	5	12	18.1%
Soft tissue	4	1	5	7.57%
Breast	16	0	16	24.2%
Miscellaneous	0	1	1	1.5%
Total	48	16	66	100%

Cyto-diagnostic Categories:

Table 5: Showing distribution of cases according to cyto-diagnostic categories in both FNAC and cell block

Cyto-diagnostic categories	FNAC	Cell block
Benign	51 (77.2%)	46(69.6%)
Malignant	14 (21.2%)	16(24.2%)
Inadequate	1 (1.5%)	4(6%)
Total	66	66

Table 6: Showing comparison between Cell block and Histopathology diagnosis

	Cell block		
Nature of the lesion	Consistent	Non consistent	Histopathology
Benign	25 (71.4%)	0	25(71.4%)
Malignant	9 (25.7%)	1 (2.8%)	10(28.5%)
Total	34 (97.1%)	1 (2.8%)	35

Table 7: Showing organ wise comparison of Cell block Diagnosis with histopathology

Site	Consistent with HP diagnosis	Non Consistent with HP diagnosis
Skin	4	0
Lymph node	2	0
Thyroid	10	1
Breast	9	0
Salivary gland	5	0
Soft tissue	4	0
Total	34	1

Table 8: Showing cytological, cell block and histopathological Diagnosis correlation in all the lesions

S. No	Site	FNAC consistent	FNAC non-consistent	Cell block consistent	Cell block Non consistent	HP
1	*Thyroid*					
	Thyroglossal cyst	2	0	1	0	2
	Colloid goiter with cystic change	4	0	4	0	4
	Hashimoto's thyroiditis	3	0	3	0	3
	Suspicious papillary ca	1	0	0	0	0
	Papillary ca	2	1	2	1	3
2	*Salivary Gland*					
	Pleomorphic adenoma	1	0	1	0	1
	Warthin's tumor	3	0	3	0	3
	Mucoepidermoid carcinoma	0	1	1	0	1
3	*Lymph Node*					
	Metastatic SCC	2	0	2	0	2
4	*Skin*					
	Keratinous cyst	4	0	4	0	4
5	*Soft tissue*					
	Lymphatic cyst	1	0	1	0	1
	Ganglion	1	0	1	0	1
6	Breast					
	Fibrocystic change	2	0	2	0	2
	Fibroadenoma	2	0	2	0	2
	Phyllodes tumor	1	0	1	0	1
	Carcinoma Breast	4	0	4	0	4
Total		33	2	34	1	35

Table 9: Showing sensitivity, specificity, and positive predictive value, negative predictive value and accuracy of cell block in 35 cases where histopathology correlation was available.

Sensitivity	90%
Specificity	100%
Positive Predictive Value	100%
Negative Predictive Value	96.15%
Accuracy	97.14%

Discussion

FNAC is well established tool for treating patients with mass lesions, as to medical versus surgical line of management. However at times, it is difficult to arrive at a precise diagnosis at FNAC, where the major drawback being that the relation of cells to each other is often lost. Similarly when the material obtained is fluid or hemorrhagic in nature, pathologist is confronted with a diagnostic dilemma. Cell block is a technique wherein all these issues can be taken care off. A cell block prepared from the FNAC material will reveal architectural pattern marginally similar to that seen in tissue sections. Also it has further advantages of concentration of material and preserving material for ancillary techniques like IHC as well. With this background, the present study was undertaken to compare the role of FNAC and cell block technique in diagnosis of mass lesions with histopathology as gold standard.

Type of study: Prospective study

Place of study: Department of Pathology, Adichunchanagiri Institute of Medical sciences.

Duration: Study conducted over a period of 18 months (October 2016 to March 2018)

In the present study which included 66 cases, benign lesions (77%) were common than malignant lesions (17%). Inflammatory lesions accounted for remaining 6% of the cases. Thyroid (25.7%) and breast (24.2%) were the most common organ aspirated. In the thyroid lesions, colloid goiter was the predominant one while in breast it was carcinomabreast.

Histopathological correlation was available in 35 lesions.

Comparative studies

Thyroid Lesions Cell Block

In the present study had one case of thyroglossal cyst was confirmed by cell block. In Sanchez N et al. [63] study there were more number of Hashimoto's thyroiditis diagnosed by cell block while in the study by Basnet S et al. [35] papillary carcinoma was more commonly diagnosed by cell block technique. In the present study, maximum number of colloid goitre (Table 10).

Lymphnode

FNAC, Cell Block & Histopathology (Table 11)

Table 10: Showing comparative study of thyroid lesions in cell block:

Lesions in FNAC	Sanchez N et al. (2006) [63]	Basnet S et al. (2012) [35]	Present study
Thyroglossal cyst	-	-	1 (5.2%)
Colloid goiter	-	-	8 (42%)
Hashimoto's thyroiditis	5 (41.6%)	-	4 (21%)
Follicular neoplasia	1 (8.3%)	2 (20%)	1 (5.2%)
Papillary carcinoma	3 (25%)	8 (80%)	4 (21%)
Suspicious for malignancy	1 (8.3%)	-	0
Non diagnostic	2 (16.6%)	-	1 (5.2%)
Total	12	10	19

Table 11: Showing comparative study of lymph node in FNAC, cell block and histopathology:

Diagnosis	FNAC		Cell block		Histopathology	
	Basnet S et al. (2012) [35]	Present study	Basnet S et al. (2012) [35]	Present study	Basnet S et al. (2012) [35]	Present study
Metastatic Squamous cell carcinoma	5 (33%)	8(80%)	5 (38%)	8 (89%)	5 (33%)	2 (100%)
Metastatic Adenocarcinoma	4 (27%)	-	4 (31%)	-	4 (27%)	-
Metastatic Malignant Melanoma	1 (7%)	-	1 (8%)	-	1 (7%)	-
Lymphoma	5 (33%)	-	3 (23%)	-	5 (33%)	-
Granulomatous Lymphadenitis	-	2(20%)	-	1 (11%)	-	-
Total	15	10	13	9	15	2

Fnac Versus Cell Block

Table 12: Showing comparison study between cell Block and FNAC

Site	Thyroid	Breast	Lymph node
Basnet S et al. (2012) [35] FNAC	12	10	13
Present study FNAC	19	15	11
Basnet S et al. (2012) [35] Cell block	12	10	15
Present study Cell block	18	15	11
Basnet S et al. (2012) [35]Z value	NA	1.49	1.47
Present study Z value	0.2	NA	NA
Basnet S et al. (2012) [35] P value	NA	>0.05	>0.05
Present study P value	>0.05(0.4)	NA	NA

Table 13: Showing true positive, true negative, false positive and false negative of combineduse of FNAC and cell block with histopathology as gold standard in different studies:

Remarks	Raafat A et al. (2014) [39]	Present study
True positive	12 (14.1%)	9 (25.7%)
True negative	65 (76.4%)	25 (71.4%)
False positive	1 (1.1%)	0
False negative	2 (2.3%)	1 (2.8%)
Total	85	35

Sensitivity, Specificity and Diagnostic Accuracy

Table 14: Showing comparison of sensitivity, specificity, and positive Predictive value, negative predictive value and accuracy of cell block in Different studies:

Statistical Data	Raafat A et al (2014) [38]	Present study
Sensitivity	94%	90%
Specificity	98%	100%
Positive Predictive Value	94%	100%
Negative Predictive Value	98%	96.15%
Accuracy	98%	97.14%

Raafat A et al. (2014) [38] study had higher sensitivity, negative predictive value and accuracy of cell block compared to present study. While present study had higher specificity and positive predictive value as in shown in table no 26.

Summary

The present prospective study was carried out in the Department of Pathology, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, a rural Tertiary Care Hospital, Teaching Institute and Research Center between October 2016 and March 2018 (18 months).

During this study period 66 cases of cell blocks were made from FNAC. Out of these 66 cases, 35 cases were sent for histopathology examination.

Lowest age and highest age of the patient was 10 years and 87 years respectively. The mean age was 41 years. Highest number of patients were included in the age group between 41 and 50 years.

Female patient was more in number (72%) than males (28%). Benign lesions (71%) were more in number than malignant (29%). Highest number of cell blocks was done for thyroid lesions (25.7%) followed by breast (24.2%), skin (18.1%), lymph node (15.1%), salivary gland (9.25%), soft tissue (7.57%), and miscellaneous (1.5%).

In the thyroid lesions colloid goiter was the most common lesion. Out of 66 cases, cell block had 4 inadequate sections. On the other hand one inadequate breast sample in FNAC, the cell block of the same had few ductal epithelial cell in acinar formation and one suspicious for papillary carcinoma in FNAC was diagnosed with cell block as papillary carcinoma

FNAC when compared with histopathology, there were two false negative cases (5.7%), benign cystic lesion and colloid goiter which were later diagnosed as mucoepidermoid carcinoma.

Cell block when compared with histopathology, had the same result in benign lesions but better results in diagnosing malignant ones. Cell block when used along with FNAC yields higher diagnostic accuracy.

Conclusion

Though fine needle aspiration cytology is the most routinely performed and widely accepted diagnostic procedure for an initial diagnosis in any swelling, we often come across difficulties in diagnosis, if the material obtained is scanty, hemorrhagic or fluid in nature.

In such cases cell blocks can be prepared from the residual tissue, fluids or the aspirated material.

Cell block study is an useful adjunct to smears for establishing a more definitive cytopathologic diagnosis.

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