# **Original Article**

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# Cell Block: A Tool to Improve Cytopathologic Diagnostic Value of Fine Needle Aspiration Material

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#### **ABSTRACT**

**Background:** The various advantages of Fine-needle aspiration (FNA) biopsy/ cytology can be limited due to inadequacy of the specimen and expertise required for diagnosis. Cell blocks (CBs), though routinely used in cytology of body fluids, can also be used in FNA material with considerable increase in diagnostic accuracy. Aims & objectives: To compared FNA & CB as diagnostic tool and to evaluate whether cell block adds to the diagnostic accuracy of FNA.

**Methods:** This is a two years prospective study carried out in the pathology department of a tertiary heath care hospital and medical college of South India which included the cases undergoing the FNAC, and followed by the biopsy. CB was prepared using Tissue coagulum clot (TCC) method. The smears and tissue sections were assessed for cellularity and adequacy for diagnosis. CBs were grouped into four diagnostic categories. FNA & CB were compared with histopathology (HPE) diagnosis to calculate Sensitivity, Specificity and Diagnostic Accuracy.

**Result:** A total of 195 cases were included. Cellularity and adequacy for diagnosis were higher in CB. They were diagnostically better (superior) in 13.84% (27) cases. The sensitivity (95.77%) and diagnostic accuracy (94.87%) of CB was found to be considerably higher than FNA (78.84% and 78.46% respectively).

**Conclusion:** In cases of suspicious/ intermediate diagnosis or diagnosis discordant with clinical ones, by FNA; should be followed with the CB to improve the diagnostic yield & to facilitate accurate diagnoses. TCC method is a simple CB method which has higher diagnostic accuracy than FNA.

Keywords: FNAC, FNAB, Cell Block, Cytology, Cytopathology, Diagnostic Accuracy.

### Introduction

Fine-needle aspiration (FNA) biopsy/ cytology is an easy, OPD basis, minimally invasive and cost-effective technique with good accuracy and efficiency in routine cytopathology. However, the inadequacy of the specimen can be as high as upto 30%. [1] Associated is, always a risk of false negative diagnosis or other situations requiring use of experience and expertise for a confidant diagnosis which itself may be limiting the result. [2, 3] However, the advantage of histopathology (HPE) over the cytological examination can be obtained by using all the remaining material of the FNA for making cell blocks (CBs) which gives a better morphological and histological detail. Thus, the diagnosis may be refined. Also, the benefit of availability of the multiple sections for special stains and IHC form cell block makes it a desirable practice in the set up with required resources. Both techniques together can improve the diagnostic accuracy, eliminate the need of repeat FNA for non diagnostic cases and avoid the trauma and risks of biopsy. [4]

In the present study, we compared these two methods as a diagnostic tool and evaluated whether cell block adds to the diagnostic accuracy of FNA.

# **Materials and Methods**

The present study was two years prospective study carried out in the pathology department of a tertiary heath care hospital and medical college of South India. The cases undergoing the FNAC, and followed by the biopsy were included.

FNAC was performed under aseptic condition using 21, 22 or 23 gauge needles attached to the 10 ml disposable syringes with or without aspiration as required. Smears were prepared without delay taking care to avoid the coagulation of the sample. All of them were immediately alcohol fixed in 95% ethyl alcohol for Papanicolaou (Pap) stain & hematoxylin and eosin (H & E) stain except a few which were air dried for May Grunwald – Giemsa (MGG) stain and in the suspected cases of Tuberculosis (TB), Zeihl – Neelson stain (ZN stain) for demonstrating Acid Fast Bacilli.

The method used for preparation of the CB was Tissue coagulum clot (TCC) method. The material remaining in the needle hub and attached syringe was allowed to clot, later left overnight to fix in the mixture of 10% formalin and 95% alcohol (1 part each). The material thus obtained was processed as routine biopsy specimen and the tissue sections were stained with H & E stain.

The smears and tissue sections were assessed for cellularity and adequacy for diagnosis. All the cases were categorized as having abundant, moderate, scant and no cellularity on smears as well as cell block. Again, they were divided into adequate and inadequate. The diagnoses on FNAC were given according to criteria of reporting the various organs smears in the textbook of Orell and textbook of Koss. The cell blocks were also reported separately. They were grouped into 1) Non-Diagnostic/ Diagnosis Discordant with FNA & HPE 2) Diagnostically equal (same diagnosis as of FNA and HPE), 3) Diagnostically aiding (confirming a suspicious/ possible/ intermediate FNA diagnosis, which was confirmed by same diagnosis on HPE) and 4) Diagnostically Superior (giving a different diagnosis than one of FNA, or giving a specific neoplastic lesion in cases suspicious of malignancy - same diagnosis on HPE). HPE diagnosis was taken as gold standard and the diagnosis of FNA & CB were compared with it to calculate Sensitivity, Specificity and Diagnostic Accuracy. The statistical analysis was done after recording the complete data on Excel worksheet.

#### Result

A total of 195 cases were included in this study with FNA and HPE both samples available. The age of patients ranged from 5 years to 85 years. Thyroid was the most common site for FNA (32.8%) followed by lymph node, breast, and soft tissue & bone (Table 1).

Cellularity was better marginally in CB (Table 2). The adequacy for diagnosis was also higher in CB (94.87%) by

a little difference than FNA (92.3%) (Table 3). Out of the 15 cases found to be inadequate for diagnosis by FNAC, eight cases were adequate by CB. But three of the adequate FNAC cases were inadequate by CB.

On comparison with the HPE diagnosis, CB was found to be diagnostically better (superior) in 13.84% (27) cases and aiding the final diagnosis in 12.83% (25) cases than FNA (Table 4). Most of the cases in the former category were from lymph nodes and thyroid. The latter category had maximum cases of suspicious for malignancy diagnosis on FNAC.

The discordant cases on FNA with respect to HPE belonged to lymph nodes (nine cases) and thyroid (seven cases) mainly (Table 1). CB was found to be diagnostically superior in all of these, giving the diagnosis same as HPE. In lymph nodes both neoplastic and non neoplastic lesions were found to have discordant cases, but in all the neoplastic cases of these, the CB gave correct diagnosis. There were three cases proved to be metastatic deposits of squamous cell carcinoma by CB and HPE, of which two were diagnosed as suppurative lymph node and another as keratinous cyst on FNA. Similarly, in three breast FNAs, malignancy was correctly diagnosed on CBs, as compared to fibroadenoma with atypia and inconclusive diagnosis given by FNA. Correct typing of the malignancy was evident by CB in the cases diagnosed as malignancy or suspicious of malignancy from lung masses (five cases) and thyroid (two cases). Also, two cases diagnosed as goiter by FNA were found to be follicular neoplasm on CB, later confirmed to be follicular adenoma. A case of goiter, simple cyst breast and a parasitic cyst each were inconclusive due to lack of cellularity on CB when FNA was able to give correct diagnosis.

The sensitivity (95.77%) and diagnostic accuracy (94.87%) of CB was found to be considerably higher than FNA (78.84% and 78.46% respectively) (Table 4).

Table 1: Sources of FNA/CB with the site-wise discordant cases.

Sr.	Site	No of cases	FNA diagnosis		_	<b>HPE Diagnosis</b>	No of	No of
no.			n	Diagnosis	in discordant cases/ ones with superior diagnosis	in discordant cases/ ones with superior diagnosis	discordant cases of FNA vs CB & HPE	discordant cases of CB vs FNA & HPE
1	Soft tissue,	ue, 30 (15.38%)	13	Lipoma				
	bone.		1	Reactive I n	Neurofibroma	Neurofibroma	1	
			6	Keratinous cyst				
			2	BNST				
			6	Abscess				
			1	Parasitic cyst	Inconclusive	Parasitic cyst		1
			1	Cystic lesion - bone	Giant cell lesion	Giant cell tumour	1	

A-368 Cell Block in Cytology

Sr.	Site	No of	FNA diagnosis		CB diagnosis	<b>HPE Diagnosis</b>	No of	No of
no.		cases	n	Diagnosis	in discordant cases/ ones with superior diagnosis	in discordant cases/ ones with superior diagnosis	discordant cases of FNA vs CB & HPE	discordant cases of CB vs FNA & HPE
2	Lymph nodes	43 (22.05%)	13	Tb In	Suppurative In (2 cases)	Suppurative In (2 cases)	2	
					Suppurative In (1 case)	Tb In (1 case)		1
			9	Reactive In	TB In (1)	TB In (1)	1	
			4	Mets – SCC				
			1	? Keratinous cyst	Mets SCC	Mets SCC	1	
			1	Lepromatous In				
			4	Suppurative In	Mets SCC (2 case) TB In (2 case)	Mets SCC (2 case) TB In (2 case)	4	
					Abscess (1 case)	Suppurative In (1 case)		1
			3	Chronic non- specific In				
			1	Hodgkin disease				
			2	NHL				
			5	Inconclusive	Reactive In (1 case) Mets (1 case)	Reactive In (1 case) Mets (1 case)	2	
3	Breast	32 (16.41%)	13	Fibroadenoma	Ca breast (2 cases)	Ca breast (2 cases)	2	
			3	Breast abscess				
			2	Gynecomastia				
			8	Ca breast	Atypical Fibroadenoma (1 case)	Atypical Fibroadenoma (1 case)	1	
			3	Simple cyst	Inconclusive (1 case)	Simple cyst (1 case)		1
			3	Inconclusive	Ca breast (1 case)	Ca breast (1 case)	1	
4	Thyroid	61	3	Neoplastic	PCT ( 2 cases)	PCT ( 2 cases)	2	
		(31.28%)	3	PCT				
			3	Follicular neoplasm		Follicular adenoma		
			32	Goiter	Follicular neoplasm (2 cases)	Follicular adenoma (2 cases)	2	
					Inconclusive (1 case)	Goiter (1 case)		1
			7	Thyroiditis	Inconclusive (1 case)	Thyroiditis (1 case)		1
			9	Benign cystic lesion	Thyroiditis (1 case)	Thyroiditis (1 case)	1	
			4	Inconclusive	Goiter (1 case) Thyroiditis (1 case)	Goiter (1 case) Thyroiditis (1 case)	2	

Sr.	Site	No of	FNA	diagnosis	CB diagnosis	<b>HPE Diagnosis</b>	No of	No of
no.		cases	n Diagnosis		in discordant cases/ ones with superior diagnosis	in discordant cases/ ones with superior diagnosis	discordant cases of FNA vs CB & HPE	discordant cases of CB vs FNA & HPE
5	Salivary gland	10 (5.13%)	1	Myoepithelioma	Pleomorphic adenoma	Pleomorphic adenoma	1	
			5	Pleomorphic adenoma				
			3	Sialadinitis	Abscess (1 case)	Sialadinitis (1 case)		1
			1	Inconclusive	Pleomorphic adenoma	Pleomorphic adenoma	1	
6	Skin subcut	6 (3.08%)	1	Keratinous cyst				
			1	Keloid				
			2	SCC				
			1	Inconclusive	? Spindle cell lesion	Fibrotic lesion	1	
7	Lung, pleura	10 (5.13%)	4	Koch's				
			5	Malignant	SCC (3 cases), Adenocarcinoma (2 cases)	SCC (3 cases), Adenocarcinoma (2 cases)		
			1	Inconclusive	s/o Malignancy	SCC	1	
8	Oral cavity	3 (1.54%)	3	SCC				
Total		195					27	7

Table 2: Cellularity in FNA vs CB.

Cellularity	FNA	СВ		
Abundant	41 (21.10%)	46 (23.52%)		
Moderate	104 (53.34%)	104 (53.34%)		
Scant	42 (22.16%)	41 (21.10%)		
No cellularity	7 (3.4%)	4 (2.04%)		
Total cases	195 (100%)	195 (100%)		

Table 3: Distribution of adequacy between FNAC & CB.

		FNAC	Total	
		Adequate	Inadequate	
Cell Block	Adequate	177 (90.77%)	8 (4.1%)	185 (94.87%)
	Inadequate	3 (1.53%)	7 (3.6%)	10 (5.13%)
Total		180 (92.3%)	15 (7.70%)	195 (100%)

 $\label{thm:continuous} \textbf{Table 4: Diagnostic categories of CB and Statistics.}$ 

Diagnostic categories of CB		n (%)
Diagnostically Superior		27 (13.84%)
Diagnostically aiding		25 (12.83%)
Diagnostically equal		129 (66.15%)

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A-370 Cell Block in Cytology

Diagnostic categories of CB		n (%)				
	Non-Diagnostic (HPE diagnostic)/ CB Diagnosis Discordant with FNA & HPE	14 (7+7), (7.18%)				
Diagnosis		FNA [n (%)]	CB [n (%)]			
True Positive	Offered Diagnosis same as HPE	149 (76.41%)	181 (92.82%)			
True Negative	Inconclusive, HPE as well Inconclusive	4 (2.05%)	4 (2.05%)			
False Negative	Offered Diagnosis different from HPE	29 (14.87%)	2 (1.03%)			
	Inconclusive, HPE Diagnostic	11 (5.64%)	6 (3.08%)			
False Positive	Diagnosis offered, HPE inconclusive	2 (1.03%)	2 (1.03%)			
Sensitivity		78.84%	95.77%			
Specificity		66.67% 66.67%				
Diagnostic Accuracy		78.46% 94.87%				

Table 5: Comparison of adequacy, sensitivity, specificity and diagnostic accuracy of FNA & CB in recent Indian works.

Author	Site – if specific	Adequacy		Sensitivity		Specificity		Diagnostic Accuracy	
		FNA	СВ	FNA	СВ	FNA	СВ	FNA	СВ
Wadhwa et al.[18]	HNF			88.8%	96%		100%	92%	97.67%
Parate et al.[19]		90%	86.94%	88.06%	90.91%	92.86%	92.86%	89.47%	91.47%
Mathew et al.[20]	GFNA	100%	100%	62.22%	71.11%	100%	100%	63.04%	71.73%
Barsagade et al.[21]		81.85%	79.25%						
Patil et al.[22]		87.83%	81.08%	94.90%	98.92%	93.75%	96.30%	94.61%	98.33%
Present study		92.3%	94.87%	78.84%	95.77%	66.67%	66.67%	78.46%	94.87%

HNF – Head Neck Face, GFNA – Guided FNAs.

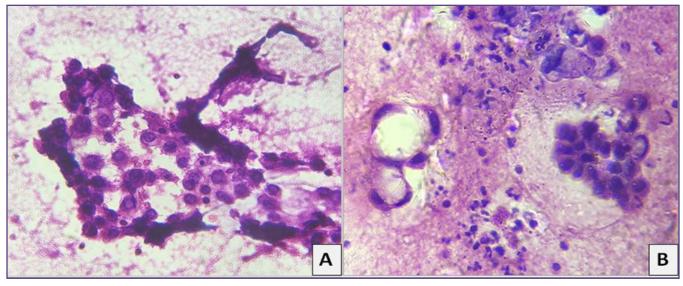


Fig. 1 a - Smear prepared from FNAC lung mass showing loosely cohesive malignant epithelial cells. (400x MGG). Other fields showed doubtful signet ring cells as well. Figure 1 b - corresponding cell block showing acini and signet ring cells. (400x H & E).

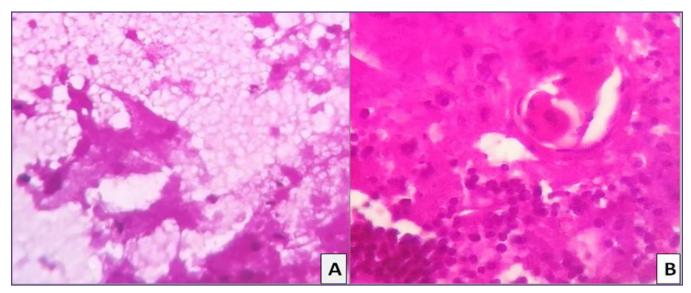


Fig. 2 a - Smear prepared from FNAC right submandibular lymph node. Shows few anucleate and nucleated squamous cells only, with occasional dysplastic squamous cells and mononuclear inflammatory infiltrate. (400x H & E). Figure 2 b - corresponding cell block showing keratin pearl with surrounding lymphocytes. (400x H & E).

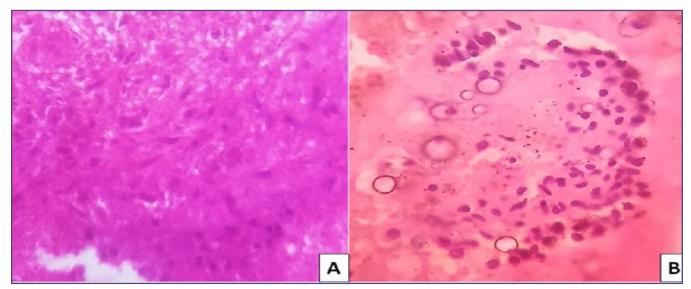


Figure 3 a & b - Cell block showing ill formed epitheloid cell granuloma formation. (400x H & E). The corresponding FNAC smears showed only scant cellularity comprising of few lymphocytes with occasional epitheloid cell.

#### **Discussion**

The history of FNA for the diagnosis of the neoplastic lesion goes back to 1847, then described as a "new instrument for the diagnosis of tumors" by Kun. Afterwards it was applied by various practitioners to different sites.

Cell block technique was first used almost a century ago by Bahrenburg (in1896), approximately five decades later to FNA initial use, as an alternative to the preparation of conventional smear from ascitic fluid.<sup>[5]</sup> Since then, other than various fluids, it has also been prepared from

hemorrhagic aspirates.<sup>[6, 7]</sup> To the various advantages of FNA, it adds diagnostic architecture just as a histological diagnosis.<sup>[8]</sup> Another very important virtue is that multiple sections can be taken from the cell block to be used for special staining and IHC other than routine staining.<sup>[9, 10]</sup>

CBs have been popularly prepared from the material of FNA by paraffin- embedding the direct transfer of all centrifuged cellular material wrapped in lens paper,<sup>[11, 12]</sup> or embedding in plasma,<sup>[13, 14]</sup> or agar.<sup>[15, 16]</sup> Afterwards it is processed as a routine histological specimen. The Tissue

A-372 Cell Block in Cytology

coagulum clot (TCC) method has an advantage over the conventional aspiration needle rinse in the recovery of cellular material and prevention of the loss of diagnostic material. Also, there is no requirement of any special equipment or reagents in TCC method.<sup>[17]</sup> This makes it feasible method in any modest laboratory.

Less dispersal of the cells in the CBs, may result in improved adequacy and ease at microscopic examination. The adequacy of the CBs has been found to be 79.25% to 100% by the recent works, with concordant finding in the present one (94.87%) as well (Table 5). [18-22] It is little less than or equal to that of FNA in all, but we found the adequacy to be a bit higher than that of FNA. The possible reason may be the high dependence of FNA on the expertise of the person performing the aspiration. Also, if the blood appears in the needle hub, the diagnostic material may be sucked in syringe; as a result it will be processed in CBs, against hemorrhagic FNA smears. [23] A common finding is increase in adequacy when the two methods are combined. [21, 22]

The CBs are known to show additional diagnostic information along with architectural patterns, than FNAC smears aiding in increased diagnostic accuracy with them and confirmation of many suggestive/ suspicious/ intermediate diagnosis by FNA.<sup>[21, 22]</sup> The present work has same finding. The architectural features better appreciated in CB included gland formation of malignant cells in adenocarcinomas in lung aspirate (Figure 1), keratin pearl formation in lymph node (Figure 2) and lung lesion aspirates in SCC. The epitheloid granulomas were seen in a lymph node aspirate CB when only necrotic material and scant chronic inflammatory infiltrate seen in smears (Figure 3).

As widely known, the CB is contributory in only upto one third of cases in the diagnosis of thyroid lesions. One of the main causes is the low cellularity, and yet another cause may be hemorrhagic nature of the thyroid aspirates. [24, 25] The case inconclusive by CB in our work was due to very low cellularity and colloid being better appreciated in the smears. The neoplastic lesions could be confirmed and subtyped due the architectural details available from CB including papillary arrangement in PCT. The findings are similar to some recent works showing better CB diagnosis than FNA from thyroid. [18, 22]

In lung carcinoma CB is important in the subtyping of by morphology,<sup>[26]</sup> and also in IHC for squamous and adenocarcinoma markers.<sup>[27]</sup> Endobronchial ultrasound-guided transbronchial needle aspirates (EBUSTBNAs)

samples' CBs have been found to yield adequate tissue for cytological analysis in over 97% of cases. [28, 29] Similarly, in the present study and yet another recent one, [22] correct typing of the malignancy was done in the lung masses by CB, where FNA gave diagnosis of malignancy.

Another site with noticeable role of CB in this research was breast lesions where they confirmed, ruled out or diagnosed malignancy against suspicious malignancy cases, negative ones in FNA or inadequate ones respectively. This is concordant with other Indian works. [21, 22] The possible cause may be better appreciation of cellular and architectural details of the cellular material entangled in the hemorrhagic aspirate. Also, in lymph nodes the cases of malignant deposits either missed or misdiagnosed on the FNA were correctly diagnosed in CB, as in other studies. [21, 22]

The sensitivity, specificity and diagnostic accuracy of CB has been found to be higher than FNA in the recent works (Table 6).<sup>[18-22]</sup> The present study found the sensitivity and diagnostic accuracy of CB more than FNA; and same specificity of CB and FNA. Concordant finding is present in another work.<sup>[19]</sup> If only neoplastic diagnoses are considered, then the specificity is found to be very high.

Despite all the above positive findings, CBs were found have longer processing time, due to histopathology processing to be used in them and the cost would increase due to additional method. However, in cases where the FNA has not been diagnostic should be followed with the CB to increase the diagnostic rate and accuracy.

# Conclusion

The less frequently used technique of Cell block actually allows the recovery and use of even minimum amounts of diagnostic cellular material, thus facilitating the correct diagnosis when FNA is not conclusive or does not give correct diagnosis. TCC method being a simple method, not requiring special reagents or expertise to handle the specimen; is feasible in any resource limited laboratory. CBs give advantage of HPE from the cytology sample. Thus, cases where the FNA has given inaccurate/ suspicious/ intermediate diagnosis should be followed with the CB to improve the diagnostic yield & to facilitate accurate diagnosis.

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# **Competing Interests**

None

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A-374 Cell Block in Cytology

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