

Cell Block or Centrifuged Smear: A Comparative Study from a Tertiary Care Center

Sumitha M P¹, Niveditha S R¹, Tejaswi Krishnamurthy¹

¹Department of Pathology, Rajarajeswari Medical College & Hospital, Bengaluru.

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ABSTRACT

Background: Cellblock technique (CB) is one of the oldest techniques of preparing materials for microscopic examination by paraffin embedding of sediments of fluids. CB enables retrieval of small tissue fragments from fluids, improving the cellular yield and diagnostic accuracy. The main advantage of CB is the potential to make many sections for special stains and ancillary techniques like IHC. **Objective:** To compare the efficacy of cellblock vs. centrifuged smear examination in effusions and fluids from cystic lesions. **Methods:** A total of 220 samples which included effusions (pleural, peritoneal, pericardial and synovial fluids), CSF, BAL fluids and fluids from cystic lesions were studied and categorized by both routine centrifuged smear and cellblock preparation by fixed sediment method. For CB categorization, volume of fluid and presence of pellet were given due importance. **Results:** Majority of the samples were pleural fluids (34.1%) followed by peritoneal fluids (29.1%) and fluids from cystic lesions (22.1%). Male to female ratio was 1.2:1 with peak age between 40-70 years. On CS 86.8% were benign, 1.8% were suspicious for malignancy, 8.2% were positive for malignancy and 3.3% were inadequate for opinion. CBs were non-contributory in 65.9%, in 27.2%, CBs confirmed CS diagnosis and in 4.5% they established a specific diagnosis. (Sensitivity 63%, specificity 71.7%, PPV 80%, NPV 94.6%)(Kappa value = 0.175, p value=0.001). Volume of the fluid did not matter in malignant effusions and material on CB was seen in majority of these fluids with the presence of pellet formation. Among the non-neoplastic fluids material on CB was seen with volume >10ml with a good pellet formation. **Conclusion:** CBs were complementary to CS in the overall categorization of benign and malignant groups. However, they appeared to be more useful in diagnosis of malignancy by better-preserved architectural patterns and provided material for ancillary techniques like histochemistry and IHC. In CB, presence of pellet after centrifugation may be an indicator for the availability of material.

Keywords: Centrifuged Smear, Cellblock, Fixed Sediment Method.

INTRODUCTION

Serous inflammation is marked by the outpouring of a thin fluid that may be derived from the plasma or from the mesothelial cells lining the peritoneal, pleural or pericardial cavities. Accumulation of fluid other than blood in these cavities is called an effusion, which in the abdomen is called ascites.^[1] Serous effusions are accumulation of fluid in excess of the normal small amount in serous cavities.^[2] These effusions are classified into two types- transudates and exudates. The transudates are clear, straw-colored fluids characterized by a low specific gravity often-below 1.010 and low protein content (Usually below 3g/dL). It is due to increased venous pressure as in congestive heart failure or cirrhosis of liver and decreased oncotic intravascular pressure as in hypoproteinemia, nephrotic syndrome.

The exudates are characterized by relatively high protein content (Usually above 3 g/dL) and therefore a high specific gravity more than 1.015.^[2,3]

There are Several Causes of Exudates

- Inflammatory condition that are usually, but always caused by infectious processes in the organ enclosed by the membrane,
- Tumors that may be primary or metastatic
- Miscellaneous causes.^[2]

Cytologic examination of pleural, peritoneal & pericardial effusions reveals information about inflammatory conditions of the serous membranes, parasitic infestations and infections with bacteria fungi or viruses. It can also supply evidence of the presence of a fistulous connection with a serous cavity.^[3] Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions.^[2]

Cytological examination of serous fluids is one of the commonly performed investigations.^[2] The accurate identification of cells either as malignant or reactive mesothelial cells is a diagnostic problem in

Name & Address of Corresponding Author

Dr. Sumitha M P,
Department of Pathology,
Rajarajeswari Medical College & Hospital,
Kumbalagodu,
Bengaluru.-560074.

conventional cytology smears. The cytodiagnosis by conventional smears have got lower sensitivity due to overcrowding of cells, cell loss and different laboratory processing methods.^[4]

Smears stained with the Papanicolaou technique generally have good definition of malignant cellular changes. However, in certain conditions cytological findings of fluids on smear preparation can be misleading, for e.g. differentiating reactive mesothelial cells from a mesothelioma, differentiating an exuberant reactive mesothelial hyperplasia from peritoneal metastasis etc. The cellblock technique of examining the fluids, along with concomitant use of smears has shown an added advantage in such cases.^[5]

Various studies have shown that the cytological examination of fluids by means of smears, however carefully prepared, leaves behind a large residue that is not further investigated but that might contain valuable diagnostic material.^{4,6} This residual material can be evaluated in a simple and expedient fashion by treating it as a cell block, embedded in paraffin and examined in addition to the routine smears.^[4,6]

Many a times, a cyst may be encountered while aspirating a mass such as thyroid, salivary gland, breast or a metastatic tumor with cystic degeneration. The fluid thus obtained is subjected for cytological evaluation to determine whether it is benign or malignant.^[7]

Viscous synovial fluid, which lubricates the joints, is secreted by synovium. The volume of synovial fluid may increase after trauma or inflammation and this fluid can be aspirated for cytological analysis.^[8]

Cerebrospinal fluid samples sent to the laboratory are obtained by spinal tap and because these samples are usually small (Rarely >10 ml) impeccable laboratory techniques are essential for cytological evaluation.^[8]

In 1882, Quincke first published a detailed description of cancer cells in abdominal and pleural fluids using cell films prepared from sediment that was formed when fluids were allowed to stand.^[9]

Bahrenburg first introduced cellblock technique or paraffin embedding of sediments of fluids in 1896. In this method, fluid sediment is fixed, processed and embedded in paraffin.

Cell Blocks Offer Multiple Advantages such as-

- Better morphology of cells and architecture (especially in neoplastic lesions).
- Scope for multiple sections for special stains and other ancillary studies like Immuno- Histochemistry etc.
- It bridges the gap between cytology and histology.^[6]

Hence the present study was planned to evaluate the utility of cellblock preparation in increasing the sensitivity of cytodiagnosis of serous effusions and other fluids received in our laboratory.

Objectives of the study

To compare the efficacy of cellblock versus centrifuged smear examination in effusions and fluids from cystic lesions.

MATERIALS AND METHODS

This diagnostic evaluation study was carried out in the Central Laboratory, department of pathology, in a tertiary care hospital, Bangalore.

A total of 220 fluids comprising of various effusions (pleural, peritoneal, pericardial, synovial fluid), cerebrospinal fluid and fluids aspirated from cystic lesions were studied. Data was collected in a pretested proforma. Sample size was taken based on the convenience of the study.

All fluids, irrespective of volume, received and aspirated in the laboratory were processed at the earliest. Due to technical reasons, if the fluids could not be processed immediately, they were stored in a refrigerator at 4°C and processed later.

The fluids were examined grossly for volume, color and appearance and findings were noted. Got approval from IEC.

Processing of Fluids

The fluids were divided into two equal parts. One part was kept for conventional cytology (centrifuged smear – CS) and the other part for cellblock (CB).^[2]

Centrifuged Smear

For conventional smear, the fluid was centrifuged at 2500 rpm for 10 minutes (REMI CENTRIFUGE) in plastic test tubes and supernatant decanted. Minimum of two thin smears were prepared from the sediment according to the method of Papanicolaou. PAP and H&E staining was done.

Cell block technique:

The other portion of fluid specimen was processed by Fixed Sediment Method,^[2] of Cellblock according to Nathan et al. The fluid specimens were fixed in ethanol formalin fixative (9 parts absolute alcohol & 1 part 10% formalin) in the ratio of 1:1 for one hour. After fixation the specimen was centrifuged at 2500 rpm for 10-15mins. The Supernatant was poured off and sediment drained by inverting the tube on Whatman filter paper (No: 52, WR BALSTON LTD, 11cm disc). The sediment was then wrapped in the same filter paper and processed in histokinette as routine histopathological specimen. Multiple thin sections of 4-5 micron thickness from paraffin blocks were obtained, stained with Haematoxylin and Eosin stain and examined microscopically.

Special stains like Ziehl-Neelsen and Periodic Acid Schiff (PAS) were performed wherever necessary. Immunohistochemistry was done in feasible cases.

Interpretation of Conventional Smear Versus Modified Cellblock

After studying all the available clinical data, based on morphology, the CS and CB were categorized as:^[10]

Centrifuged Smear	Cell Block
1. Positive for malignancy	Non diagnostic/ no material
2. Benign diagnosis	Non contributory (CS+, CB-)
3. Inadequate for opinion	Confirms the smear diagnosis
4. Suspicious for malignancy	Establishes a specific diagnosis

Comparative evaluation of conventional smear versus cellblock was done and tabulation of cytomorphological characters was studied with special importance to the volume of fluid and pellet formation for cellblock.

Since this is a Comparative Study, for Statistical Purposes the CS and CB Categories were grouped as-

- CS= 0 (Positive for malignancy & Suspicious for malignancy)
- CS=1 (Benign diagnosis & Inadequate for opinion)
- CB=0 (Non diagnostic/ Noncontributory)
- CB=1 (Confirms/ Establishes diagnosis)

Volume of fluids were categorized as-

- 1 = <10ml
- 2 = 10-100ml
- 3 = >100ml

Pellet formation after centrifugation was categorized as-

- 0 = no pellet present
- 1 = pellet present

Statistical Analysis

Binomial distribution was performed to assess the comparison between conventional smear and cellblock. SPSS 20.0 for Windows software package (SPSS Inc., Chicago, IL, USA) was used for analysis by Chi-square test.

$P < 0.05$ was considered to be statistically significant.

RESULTS

The study comprised a total of 220 samples, which included effusions of body cavities (pleural, peritoneal, pericardial), synovial, cerebrospinal fluid (CSF) and fluids aspirated from cystic lesions. All fluids were studied in the cytopathology section, Department of Pathology, in a Tertiary care hospital, Bangalore.

Distribution of Cases [Table 1]

Majority (75/220) of the samples were pleural fluids (34.1%), followed by peritoneal fluids (29.1%) and cystic lesions (22.7%). Pericardial and synovial fluids were the least (0.9 % each) among the study group.

Table 1: Distribution of Samples

Type of Fluid	Frequency (n)	Percent (%)
Pleural fluid	75	34.1
Peritoneal fluid	64	29.1
Pericardial fluid	2	0.9
CSF	7	3.2
Synovial fluid	2	0.9
Fluid from Cystic lesions	50	22.7
BAL fluid	20	9.1
Total	220	100

Sex Distribution: [Table 2]

Males comprised 55% of cases with a number of 121 and females were 45% with 99 cases. Males predominated in pleural, peritoneal and BAL fluids (64%, 59.4 and 75% respectively), while females predominated in CSF and Cystic lesions (71.4 and 68% respectively). Pericardial and synovial fluids had equal distribution between both sexes.

Table 2: Distribution of Fluids among the Sexes

Type of Fluid	Male -n (%)	Female - n (%)	Total
Pleural	48(64)	27(36)	75
Peritoneal	38(59.4)	26(40.6)	64
Pericardial	1(50)	1(50)	2
CSF	2(28.6)	5(71.4)	7
Synovial	1(50)	1(50)	2
Cystic lesions	16(32)	34(68)	50
BAL	15(75)	5(25)	20
Total	12(55)	99(45)	220

Distribution of Cases According to Centrifuged Smear Categories [Table 3]

Among the 220 fluids, 18(8.2%) were positive for malignancy, 191 (86.8%) cases were given a benign diagnosis, 4(1.8%) were suspicious for malignancy, and remaining 7(3.2%) were inadequate for opinion.

Table 3: Distribution of Cases According to Centrifuged Smear Categories

Centrifuged Smear Categories	Frequency (n)	Percentage (%)
Positive for Malignancy	18	8.2
Benign Diagnosis	191	86.8
Inadequate Sample	7	3.2
Suspicious for Malignancy	4	1.8
Total	220	100

Table 4: Distribution of cases according to cell-block categories

Cell block categories	Frequency (n)	Percentage (%)
Non diagnostic	145	65.9
Non contributory	5	2.3
Confirms diagnosis	60	27.3
Establishes diagnosis	10	4.5
Total	220	100

Distribution of Cases according to Cell-block Categories: [Table 4]

Of the 220 fluids, Cell Block (CB) confirmed the smear diagnosis in 60 cases (27.2%). In 10 (4.5%) cases CB established a specific diagnosis by improved architecture and with the help of histochemical stains and also by

Immunohistochemistry (IHC). CB was non-diagnostic in 65.9% cases and non-contributory in remaining 5 cases (2.2%).

Comparison of Centrifuged and Cell Block Categories [Table 5]

Of the 22 cases, which were diagnosed as positive for malignancy/suspicious for malignancy on centrifuged smear, 14 were confirmed by cell block, out of which a specific diagnosis could be established in 8 cases. (Sensitivity 72%, specificity 75%, positive predictive value 92%)

Of the 18 positive cases for malignancy by smear, 13 cases were confirmed by cell block (72%), and a

specific diagnosis was established in 7 of them (50%).

Of the 4 smears, which were suspicious for malignancy, cell block established the diagnosis in one case (25%) and in the remaining 3 cases cell blocks were non-diagnostic.

Of the 191 cases, which were diagnosed as benign by smear, cell block confirmed/established the smear diagnosis in 56 cases. (Sensitivity 29%, specificity 100%, positive predictive value 100%).

Seven cases (7/220) cases were inadequate for opinion on smear and were not included in cellblock categorization.

From the above results it appears that CBs were of utility in confirming/establishing a diagnosis in fluids in malignancy.

	Centrifuged Smear Categories	Cell	Block	Categories	Total
	Non diagnostic	Non contributory	Confirms diagnosis	Establishes diagnosis	
Positive for malignancy	0	5	6	7	18
Benign	135	0	54	2	191
Inadequate	7	0	0	0	7
Suspicious	3	0	0	1	4
Total	145	5	60	10	220

Table 6: Cross tabulation of CS vs CB (n=213)

		Cellblock	Method	Total
		Non Diagnostic/ Non Contributory	Confirms/ Establishes Diagnosis	
Centrifuged smear	Benign	135 (70.7%)	56 (29.3%)	191(100%)
Method	Malignant/ Suspicious	8 (36.4%)	14 (63.6%)	22 (100%)
Total		143 (67.1%)	70 (32.9%)	213 (100%)

Symmetric Measures

Measure of Agreement	Kappa	Value	Asymp. Std. Errora	Approx. Tb	Approx. Sig.
N of Valid Cases		213	0.061	3.245	0.001
a. Not assuming the null hypothesis.					
b. Using the asymptotic standard error assuming the null hypothesis.					

Table 7: Statistical analysis of 220 fluids:

Cell Block Method	Centrifuged	Smear Method	Total
	Positive	Negative	
Positive	14	56	70
Negative	8	142	150
Total	22	198	220

Sensitivity: 63%
Specificity: 71.7%
Positive Predictive value: 80%
Negative Predictive value: 94.6%

The measure of agreement among the two methods (Kappa value) is 0.175 with a significant statistical value of $p=0.001$ [Table 7]

Statistical analysis in our study with 220 fluids shows that cell-block method has a sensitivity of 63%, specificity of 71.7%, positive predictive value of 80% and negative predictive value of 94.6%.

Types Of Fluid	No Pellet	Pellet Seen	Total
Pleural Fluid	50	25	75
Peritoneal Fluid	48	16	64
Pericardial Fluid	1	1	2
Csf	7	0	7
Synovial Fluid	1	1	2
Fluids From Cystic Lesions	17	33	50
Bal	16	4	20
Total	140	80	100%

DISCUSSION

Diagnostic cytology is the scientific art of interpretation of cells from the human body that exfoliate or are removed from their physiologic milieu. The cytologic study of fluids represents the cell population from a much larger surface area than that obtained by needle biopsy.^[11-13] Cytology has a greater opportunity than needle biopsy to retrieve malignant cells in the presence of malignant deposits.^[14]

Cytological examination of serous effusions is of paramount importance in the diagnostic algorithm and has therapeutic as well as prognostic implications. Reactive mesothelial cells, abundance of inflammatory cells and paucity of representative cells contribute to considerable difficulties in making conclusive diagnosis on conventional centrifuged smears.^[3,15]

Thus, in our study, an attempt was made to compare and analyze both smears and cellblock from the same fluid specimen. Due consideration was given

to age, sex, site of effusion, clinical findings and biochemical investigations to arrive at a final diagnosis

In this study, we have used ethanol formalin fixative (NATHAN alcohol formalin substitute) consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde. This preparation offered cytomorphologic features corresponding closely to cells in PAP stained smears with optimal preservation of histochemical and immunocytological properties similar to Nathan et al and Shobha et al.^[16,12]

We received 220 samples of fluids from different sites of which majority were pleural fluids (34%) followed by peritoneal fluid samples (29.1%). Similar findings were noted by Nathan et al,^[16] Meenu Thapar et al,^[4] FootNc et al,^[17] and Murphy et al.^[18] Cystic fluids from various lesions,^[19-24] have been studied under individual organ series but all fluids aspirated from cystic lesions during routine FNA have not been studied together as in our study.

Specimen Type	Foot NC et al (1955) ^[17]	Murphy et al (1972) ^[20]	Nathan et al (2000) ^[14]	Thapar M et al (2009) ^[4]	Present Study (2013)
Pleural fluid	1301	72	182	88	75
Peritoneal fluid	700	45	108	92	64
Pericardial fluid	28	-	8	10	2
Peritoneal washing	-	-	30	-	-
Bronchial washing	-	-	107	-	-
BAL fluid	-	-	63	-	20
Sputum	-	-	2	-	-
Synovial fluid	17	-	11	-	2
Ovarian cyst fluid	-	-	15	-	13
Urine	-	-	12	-	-
CSF	-	-	4	-	7
Brain cyst fluid	-	-	2	-	-
Cystic lesions of FNA	-	-	-	-	37
Hydrocele	3	-	-	-	-
Total	2049	117	544	190	220

Comparison of cytodiagnosis with other studies (CS ONLY)

SL no.	Study & Year	No. of cases	Specimen	Benign (%)	Suspicious(%)	Malignant(%)
1.	Dekker and Bupp.1978	173 (CS only)	Serous effusions	128 (74%)	8(4.6%)	28(16.2%)
2.	Nathan et al. 2000	544 (436 grouped as No malignant cells)	Washings and exfoliative cytology	11(2%)	2(0.4%)	95(17.5%)
3.	Thapar M et al. 2009	190	Serous effusions	120(63%)	-	50 (26.3%) (70 cases of HPE/ FNA proven malignancy)
4.	Udasimath S et al. 2012	60	Pleural fluids only	54(90%)	5(8.3%)	1(1.66%)
5.	Ghosh I et al. 2012	60 (56 confirmed by all other modalities)	Suspected malignant pleural effusions only	-	-	22(36.6%)
6.	Present study 2013	220	Effusion fluids,CSF,BAL,fluids from cystic lesions	191(86.8%)	4(1.8%)	18(8.2%)

In our study on CS examination, 86.8% cases were benign, 1.8% were suspicious for malignancy, 8.2%

were positive for malignancy. This finding is concordant with most of the studies as seen above.

Comparison of cytodiagnosis with other studies (CB ONLY)

S.no.	Study & Year	No. of cases	Specimen	Benign (%)	Suspicious (%)	Malignant (%)
1.	Dekker & Bupp.1978	178(CS &CB)	Serous effusions	97(54.5%)	11(6.2%)	57(32%)
2.	Nathan et al. 2000	544	Washings and exfoliative cytology	10(1.8%)	-	93(17.09%)
3.	Thapar M et al. 2009	190	Serous effusions	120(63.4%)	-	60(31.5%)
4.	Udasimath S et al. 2012	60	Pleural fluids only	50(83.3%)	-	10(16.6%)
5.	Ghosh I et al. 2012	60	Suspected malignant pleural effusions only	-	-	46(76.6%)
6.	Present study 2013	213	Effusion fluids,CSF,BAL,fluids from cystic lesions	56(26.2%)	-	14(6.5%)

In our study, CB by fixed sediment method was attempted on all fluids diagnosed on CS(n=213).CBs confirmed/ established diagnosis in 31.8% (27.3%+4.5%) cases while in 68.2% (65.9%+2.3%) cases CBs were non diagnostic/ non-contributory. The overall efficacy of CBs in our study shows a sensitivity of 63%, specificity of 71.7% and PPV of 80%.All fluids irrespective of the volume and site,were subjected to the CB procedure. Various authors have suggested various advantages of CB over CS in effusions, however the overall percentage of CB showing material (especially when a variety of fluids ie., both benign and malignant are included) has not been recorded.

CONCLUSION

CB method is an excellent complementary tool for improving cytodiagnosis in effusions and cystic fluids. CB preparation by Fixed Sediment Method is an easy, simple yet reliable and cost-effective method, hence can be incorporated into routine cytology laboratory with limited expertise. Though CBs were complementary to CS in the overall categorization of benign and malignant groups, they appeared to be more useful in diagnosis of malignancy by better preserved architectural patterns, as seen in corresponding histopathology sections. Thus CBs appeared to bridge cytology and histopathology. CBs proved to be an excellent resource material for ancillary techniques like special stains and IHC, thereby establishing the primary site of malignancy. Presence of pellet after centrifugation was an indicator for the availability of material on CB.

Limitations of the study

- ❖ CBs by Fixed Sediment method does take a minimum of 12-18 hours for routine processing, thereby delaying the report.
- ❖ The material on CB method is directly proportional to the pellet formation.

Future recommendations

- ❖ Studies in larger numbers are essential.
- ❖ Faster processing techniques like microwave processing could be attempted in processing of CBs

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