

Research Article

Role of Cell Block Technique by Fixed Sediment Method in Fluid Cytology

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Abstract

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 (CS) especially in recognition of malignant effusions. The cell block (CB) technique of examining fluids along with concomitant use of smears has shown an added advantage in study of effusions, where the residual material can be evaluated in a simple, expedient fashion by paraffin embedding.

Aim: To study the efficacy of CS vs. CB by fixed sediment method (FSM) in effusions (pleural, peritoneal, pericardial, synovial), CSF and Broncho alveolar lavage (BAL).

Materials and methods: A total of 170 fluids (pleural, peritoneal, pericardial, CSF, BAL and synovial) received in the cytology section of a tertiary care hospital in south India, were included in the study. The fluids were examined grossly and were divided into two equal parts. One part was used for CS and the other part for CB by FSM of Nathan et al. Role of volume and degree of pellet formation was also studied. Comparison of CS and CB was studied by Chi-square test and kappa test. A p value of < 0.05 was considered significant.

Results: Majority (57%) of the fluids were exudates from pleural cavities. Males predominating, the peak age was between 40-70 years. CB gave an improved diagnosis in 75% of malignant cases, both in pleural and peritoneal effusions compared to 25.7% and 18.9% of benign cases respectively. Among the CB of BAL fluids, 16 cases were non-diagnostic & 4 cases confirmed the diagnosis given on CS. CSF samples were 7 in number, out of which none of them yielded material on CB. CB confirmed the diagnosis in 50% of pericardial effusions. Statistical analysis by Chi-square test showed a p value of 0.000264. Kappa test showed fair degree of agreement between CS and CB (kappa value = 0.2119).

Conclusions: CB preparation by FSM is an easy, simple yet reliable and cost-effective method, which can be incorporated into routine cytology laboratory. CBs were complementary to CS in the overall categorization of benign and malignant groups. CBs appeared to be more useful in diagnosis of malignancy by a good pellet formation, preserved architectural patterns, thereby bridging cytology and histopathology.

Keywords

- Effusions
- BAL
- Cell block
- Fixed sediment method

INTRODUCTION

Serous cavity effusions are relatively simple to drain and hence collected for both therapeutic and diagnostic purposes. Cytologic techniques have been universally recognized as the most important diagnostic tool in the recognition of malignant tumors in effusions [1]. Accurate identification of the exact nature of cells (benign/ malignant/reactive) is often a practical problem in conventional cytology smears (CS), due to overcrowding of cells, cell loss and different laboratory processing methods [2]. The cell block (CB) technique of examining fluids along with concomitant use of smears has shown an added advantage in study of effusions [3]. Where the residual material can be

evaluated in a simple, expedient fashion by paraffin embedding [4].

Quincke in 1882, first published detailed description of cancer cells in abdominal and pleural fluids using cell films from sediment [5] while Bahrenburg first introduced cell block technique or paraffin embedding of sediments in 1896 [4]. Many techniques for CB are described like the plasma thromboplastin method [6] bacterial agar method, [1,6] simplified cell block technique [6,7] compact cell block technique [8] histogel technique [9] and Fixed sediment method (FSM) [6]. Of all these, the FSM of CB by Mandelbaum (modified by Nathan et al) [10] is easy, economical and technically reproducible with little expertise.

MATERIALS AND METHODS

This observational study was carried out in the cytopathology division, department of pathology, Kempegowda Institute of Medical Sciences (KIMS), Bengaluru. A total of 170 fluids i.e., pleural, peritoneal, pericardial and synovial effusions, cerebrospinal fluid (CSF) and bronchoalveolar lavages (BAL) were studied.

All fluids received (irrespective of volume) in the laboratory were processed at the earliest. If the fluids could not be processed immediately, due to technical reasons they were stored in a refrigerator at 4°C and processed later. The fluids were examined grossly for volume, color, appearance and findings were noted.

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) by fixed sediment method of Nathan et al. [10]. For CS, the fluid was centrifuged at 2500 rpm for 10 minutes (REMI CENTRIFUGE) in plastic test tubes and supernatant decanted. Pellet formation after centrifugation was categorized as 0 when no pellet was present and 1 when pellet was present. A minimum of two thin smears were prepared from the sediment and attained with routine PAP and H&E stains.

The fluid specimen reserved for CB was fixed in ethanol formalin fixative (9 parts absolute alcohol & 1 part 10% formalin) in the ratio of 1:1 for one hour, followed by centrifugation at 2500 rpm for 10-15mins. 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 a histokinette and embedded in paraffin. Multiple thin sections of 4-5 micron thickness from paraffin blocks were obtained, stained with H and E and examined microscopically.

After studying all the available clinical data, based on morphology, the CS and CB were categorized as: [11]

CENTRIFUGED SMEAR	CELL BLOCK
Positive for malignancy	Non diagnostic / no material
Benign diagnosis	Non-contributory (CS+, CB-)
Inadequate for opinion	Confirms the smear diagnosis
Suspicious for malignancy	Establishes a specific diagnosis

Since this was 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/ Non-contributory)

CB=1 (Confirms/ Establishes diagnosis)

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, kappa test. P< 0.05 was considered to be statistically significant.

RESULTS

A total of 170 effusions were studied both by CS and CB. Four cases were inadequate for opinion on CS and hence not included in CB categorization. Majority of the fluids were pleural 75/170(44%), irrespective of gender (Table 1). However among females both pleural and peritoneal effusions were almost equally distributed (Table 2). On centrifugation 27.64% of fluids (47/170) showed pellet formation i.e., score 1, with none of the CSFs showing pellet formation (Table 3). Benign effusions, contributed to 91.76% (156/170), which were grouped into transudates (43%) and exudates (57%). About 10 effusions were grouped under suspicious/malignant on CS (4 each from pleural and peritoneal and 1 each from pericardial and BAL) (Table 4).

Table 1: Distribution of Samples.

Type of fluid	Frequency (n)	Percent (%)
Pleural	75	44.1
Peritoneal	64	37.6
Pericardial	2	1.1
CSF	7	4.1
Synovial	2	1.1
BAL	20	11.7
Total	170	100

Table 2: Sex distribution.

Type of fluid	Male	Female	Total
Pleural	48(64%)	27(36%)	75
Peritoneal	38(59.4%)	26(40.6%)	64
Pericardial	01(50%)	01(50%)	02
CSF	02(28.6%)	05(71.4%)	07
Synovial	01(50%)	01(50%)	02
BAL	15(75%)	05(25%)	20
Total	105(61.7)	65(38.2)	170

Table 3: Type of fluid vs Pellet formation.

Type of fluid	No pellet (score 0)	Pellet seen (score 1)	Pellet Formation (%)	Total
Pleural	50	25	33.30%	75
Peritoneal	48	16	25%	64
Pericardial	1	1	50%	2
CSF	7	0	0	7
Synovial	1	1	50%	2
BAL	16	4	20%	20
Total	123	47	100%	170

Table 4: Distribution of benign and malignant cases (CS).

Type of fluid	Benign	Malignant/suspicious for malignancy
Pleural	70	4
Peritoneal	58	4
Pericardial	1	1
CSF	6	0
Synovial	2	0
BAL	19	1
Total	156	10

(n=166, 4 cases were inadequate for opinion)

Pleural fluid

Pleural fluids constituted about 44.1% (75/170), of which 70 cases were benign, 3 were malignant, and 1 each was suspicious & inadequate on CS. Among the benign, maximum number were exudative in nature with tuberculous etiology being the commonest followed by syn-pneumonic effusions. Congestive cardiac failure, nephrotic syndrome and anemia with hypoproteinemia contributed to transudates. CB was non-diagnostic/non-contributory (mostly from transudates) in 70% cases. In 21 cases (30%) CB confirmed the CS diagnosis.

CB yielded material in all 4 malignant/suspicious cases. Identification of the primary as lung was possible in two cases with sheets of atypical cells and PAS positive bull's eye inclusions (Figure 1). Of the two, one case was suspicious for malignancy on CS, but CB established the diagnosis with atypical cells showing acinar pattern (table 5). Lymphoma/leukemia spillover (Figures 2a & 2b) and metastatic carcinoma from breast contributed to the remaining 2 cases. CB gave an improved diagnosis in malignant cases (75%) when compared to benign cases (25.7%).

Peritoneal fluid

Peritoneal fluids constituted 37.6% (64 cases) of the total samples. Of these, 58 cases (90.6%) were given a benign

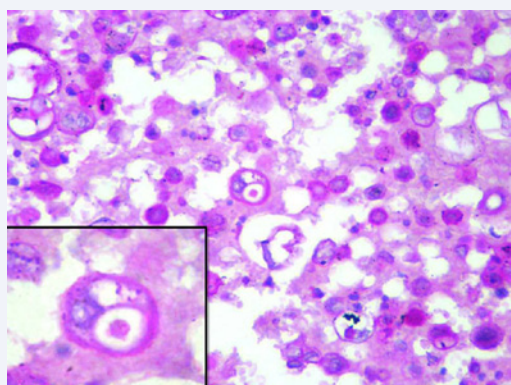


Figure 1 PAS stain on CB showing Bull's eye inclusions - PAS stain 10X (inset 40X).

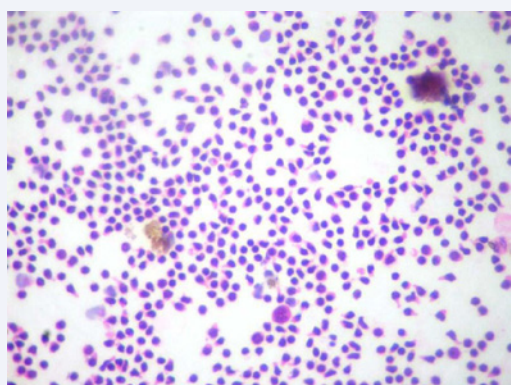


Figure 2a CS showing atypical Lymphocyte lymphoma/leukemia - H&E 40X.

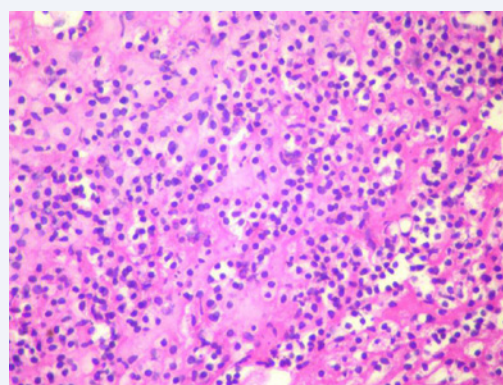


Figure 2b CB on pleural fluid showing sheets of atypical lymphocytes (H&E 40X).

diagnosis, 4 (6.2%) were positive for malignancy & 2 (3.2%) were inadequate for opinion on CS. CB in 78.2% (50/64) cases were non diagnostic/non-contributory whereas in 21.8% (14/64) cases either confirmed or established a diagnosis. Cirrhosis was the commonest cause followed by tuberculosis and CCF.

CB yielded material in 75% (3/4) cases, which were positive/suspicious for malignancy by CS. Better architecture on CBs like glandular pattern (Figures 3a & 3b) and papillary fragments helped in establishing a specific diagnosis of primary in gastrointestinal tract and serous cyst-adenocarcinoma of ovary in 2 cases respectively. However a third case diagnosed as suspicious for malignancy, showed a well formed granuloma with AFB positivity (Figures 4a & 4b), confirming tubercular etiology thereby shifting the diagnosis from malignant to benign. Hence among peritoneal fluids, CB gave an improved diagnosis in 75% of malignant and 18.9% of benign cases.

BAL fluids

Bronchoalveolar lavage fluids were 20 (11.7%) among the total number. Out of these, 19 were benign on CS & 1 was suspicious for malignancy. In CB, 16 cases were non-diagnostic & 4 cases confirmed the benign diagnosis given on CS (2 cases of intra-alveolar hemorrhage - (Figures 5a & 5b) and 2 cases suggestive of COPD)

CSF

CSF samples were 7 in number, out of which none of them yielded material on CB.

Pericardial fluid & synovial fluid

These samples were 2 each in number with CB confirming diagnosis in 50% of them (1 out of 2 cases). One pericardial fluid which was positive for malignancy on CS was a known case of breast carcinoma (Figures 6a & 6b). Synovial fluid showing only proteinaceous material was a case of rheumatoid arthritis.

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

Table 5: summary of malignant effusions.

Sl no.	Type of fluid	Volume (ml)	Pellet formation	CS	CB	Morphology	Special stain
1	Pleural	>100	+	1	4	Sheets of atypical cells, Bull's eye inclusions	PAS +
2	Pleural	10-100	+	1	3	Cell balls	-
3	Pleural	10-100	+	1	2	-	-
4	Pleural	>100	+	4	4	Acinar pattern	PAS +
5	Peritoneal	10-100	+	1	3	Papillary cluster	-
6	Peritoneal	10-100	+	1	4	Granuloma	AFB +
7	Peritoneal	>100	+	1	2	-	-
8	Peritoneal	10-100	+	1	4	Acinar pattern	PAS +
9	Pericardial	10-100	+	1	4	Cell balls, 3D clusters	-

*CS Categories: 1=Positive for malignancy, 2=Benign diagnosis, 3=Inadequate for opinion, 4=Suspicious for malignancy

**CB categories: 1=Non diagnostic/no material, 2= Non contributory, 3= Confirms smear diagnosis, 4=Establishes specific diagnosis

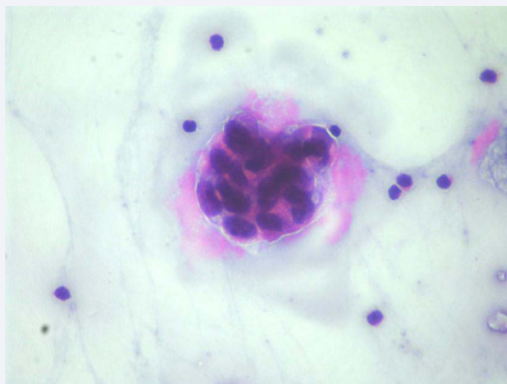


Figure 3a CS of ascitic fluid showing an occasional cluster of atypical cells PAP stain 40X.

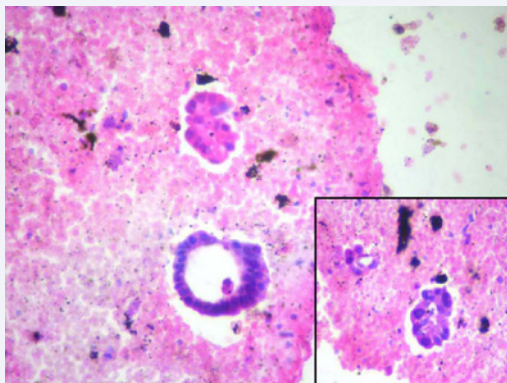


Figure 3b CB on ascitic fluid showing glandular pattern – H&E 40X (inset-gland).

obtained by needle biopsy [12-14]. Cytology has a greater opportunity than needle biopsy to retrieve malignant cells in the presence of malignant deposits [15].

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 with paucity of representative cells contribute to considerable difficulties in making conclusive

diagnosis on conventional centrifuged smears [1,16].

In this study, we have used ethanol formalin fixative, consisting of 9 parts of 100% ethanol and 1 part of 40% formaldehyde which offered cytomorphologic features corresponding closely to cells in PAP stained smears with optimal preservation of histochemical and immunocytological properties similar to Nathan et al. [10], and Shobha et al. [13].

Majority were pleural followed by peritoneal effusions similar

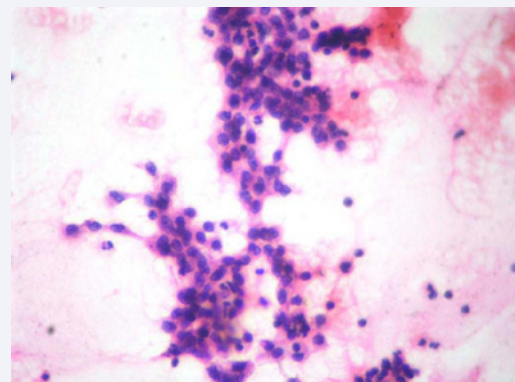


Figure 4a CS on ascitic fluid showing? Atypical cells – H&E 40X.

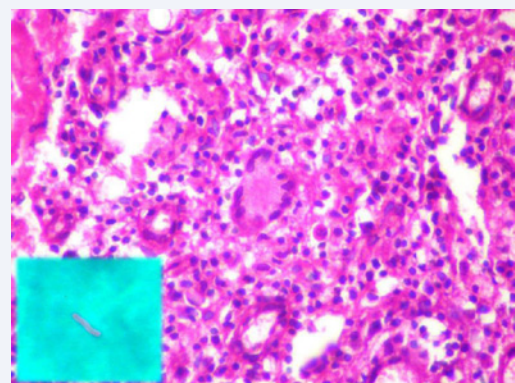


Figure 4b CB on ascitic fluid showing well formed granuloma with AFB positive (inset)-H&E 40X.

to other studies [2,10,12,17,18]. However in contrast to Khan et al. [14], males outnumbered females in our study. Maximum samples were in the age group of 40-70 years similar to Dekker et al. [3], and Shobha et al. [13], (majority in the age range of 51-60 years).

CB vs CS in non-neoplastic effusions

Benign effusions contributed to 91.76% and is at a higher

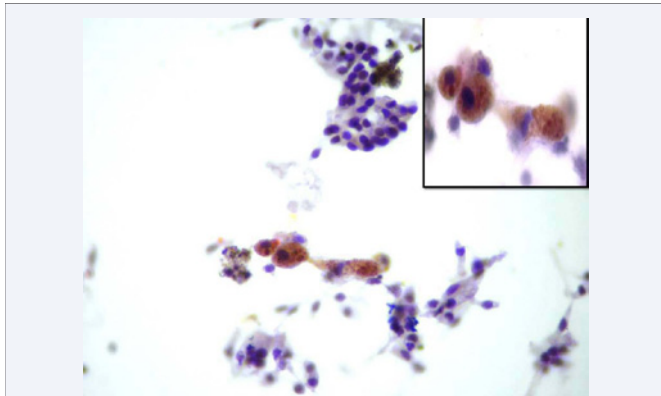


Figure 5a CS from BAL fluid showing scattered hemosiderin laden macrophages – PAP stain 40X.

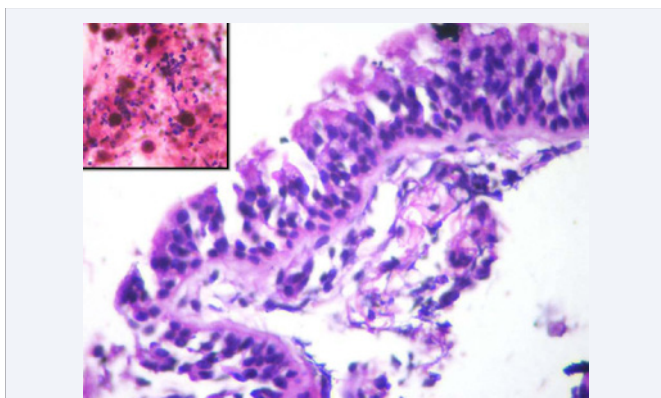


Figure 5b CB from BAL fluid showing respiratory lining epithelium – PAP 40X, inset – collection of hemosiderin laden macrophages.

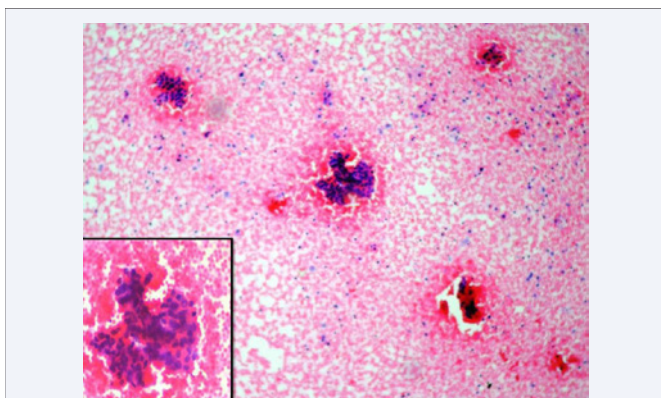


Figure 6a CS on pericardial fluid showing groups of atypical cells – H&E 10X (inset 40X).

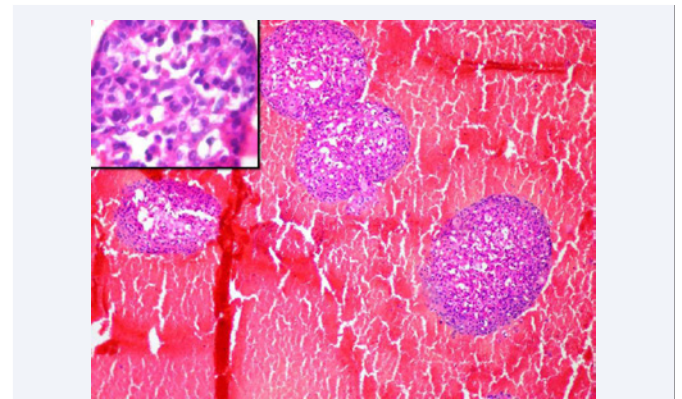


Figure 6b CB on pericardial fluid showing cell balls and 3D clusters of atypical cells – H&E 10X (inset 40X).

rate compared to Dekker [3] (74%), Thapar et al. (63%) [2], and Udasimath et al. (90%), [19]. This, we attribute to the different types of fluids in our study whereas the above said studies are related to pleural [19] and or peritoneal [2,3] effusions only. CB yielded material in 23.17% of benign cases predominantly from exudates which comprised 57% of all the non-neoplastic effusions. Among transudates majority of the cases did not yield material on CB. Thapar M et al. [2], Udasimath S [19] and Shobha et al. [13], have recorded the contribution of CB in 54.5%, 83.3% and 63.4% of benign effusions respectively.

CB showed material in 38.6% of exudates confirming the smear diagnosis. Lymphocyte predominance was seen in 32% suggesting tuberculosis supported by radiological and biochemical findings, however ZN stain did not reveal any acid fast bacilli. Among the transudates cirrhosis was the commonest cause followed by congestive cardiac failure similar to Luse et al. [21], Thapar M et al. [2], and Shobha et al. [13]. In addition to serous cavity effusions, our samples included fluids from BAL and CSF [7] which did not yield material on CB. However all these samples were hypocellular with a cell count ranging from 0-5 cells/cu mm. Synovial fluids comprised 0.9% (2 cases) of the total sample size. Both were diagnosed as benign on CS, and in one case CB showed scanty proteinaceous material. Nathan et al. [10], studied a variety of fluids including CSF, synovial fluids and BAL but have not commented on efficacy of CB in these fluids. Due to the small sample size of CSF and Synovial fluids, efficacy of CB could not be assessed in our study.

Flint A et al. [26], studied bronchial washings from suspected bronchial neoplasms and found CBs to improve the diagnosis by 9%. Our study included predominantly BAL fluids from benign lesions and hence cannot be compared.

Many authors suggested that CBs helped in non-neoplastic effusions with identical morphology as in CS and hence, though role of CB appears complementary in diagnosis of non-neoplastic effusions their role in subcategorizing non-neoplastic effusions does not seem encouraging. CSs along with clinical, radiological and biochemical findings were able to diagnose and subcategorize the benign effusions in majority of cases. Therefore attempting the laborious process of CB in non-neoplastic effusions may not be worthy.

CB vs CS in Malignant fluids

In our study, among the 9 cases diagnosed as positive for malignancy on CS, all 9/9 cases yielded material on CB (Table 5). Among them, 5/9 established a specific diagnosis, 2/9 confirmed the diagnosis and the remaining were non-contributory (one each from pleural and peritoneal effusion). Thapar M et al. [2], recorded 65.7% positivity for malignancy on CB while in Nathan et al. [10], study CBs confirmed malignancy in 92.7% of cases.

Some studies have shown additional cases of malignancy on CB by increasing the diagnostic yield (Udasimath et al. [19], by 9% and Thapar M et al. [2], by 20%). However we did not come across any such additional cases of malignancy in our study. One of our cases reported as positive for malignancy on CS, showed well formed granulomas with Langhans type giant cells on CB with Acid-Fast Bacilli on ZN stain. Florid mesothelial hyperplasia on CS had led to the erroneous diagnosis. An occasional study [13] has demonstrated well-formed granulomas in peritoneal effusions, attributing it to tuberculosis. However unlike our study, confirmation of tubercular etiology was not done either by culture or ZN stain.

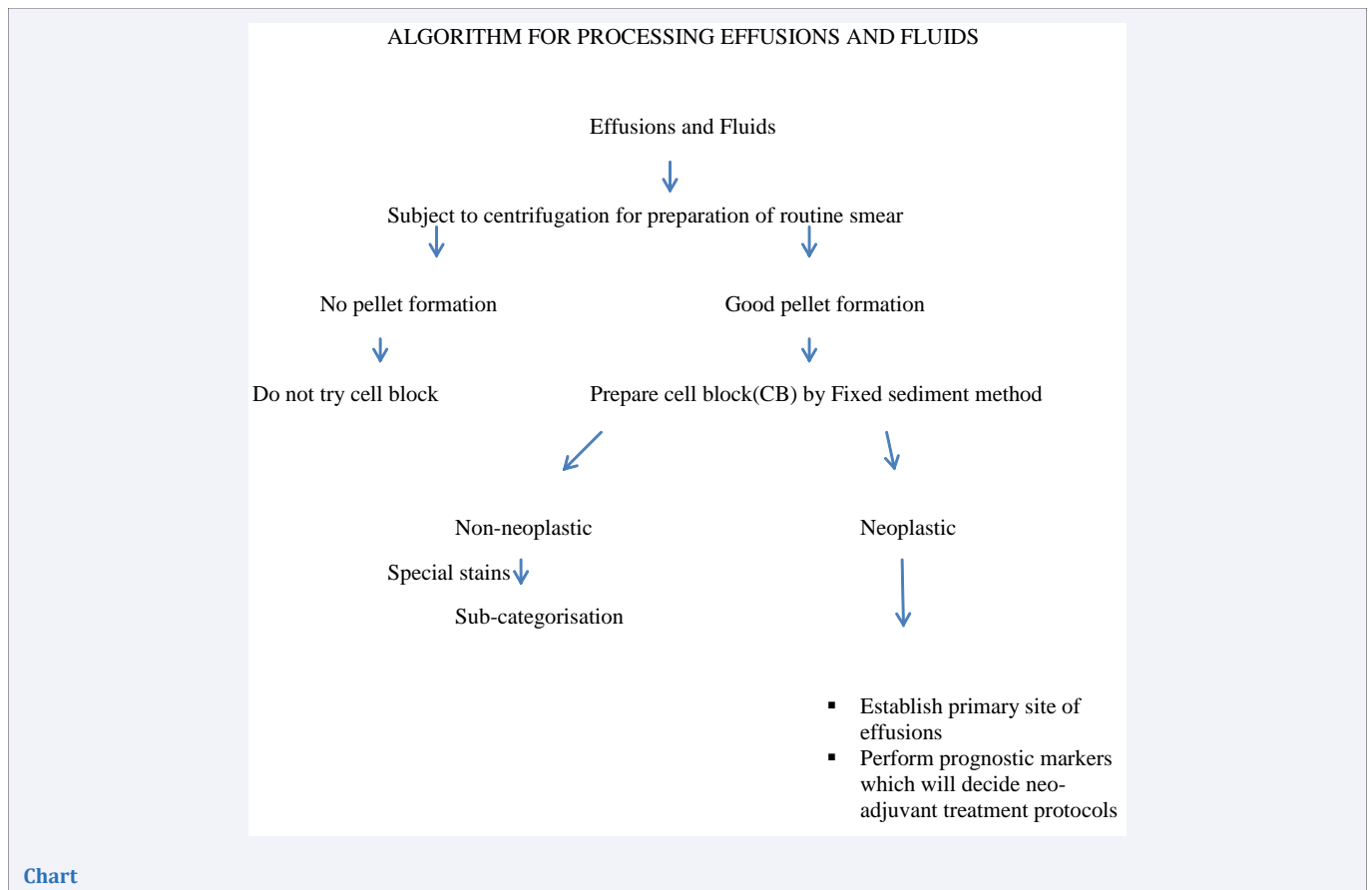
We noted better morphological details on CB such as preservation of architectural patterns like three dimensional clusters, presence of cell balls, acini, papillary fragments and better cytoplasmic features. Nuclear morphology was better appreciated in CB when compared to CS. Similar findings were noted in various studies [2,3,19,23]. Bull's eye inclusions (PAS

positive) which are described as a body mass at the center of a cytoplasmic vacuole in neoplastic cells were observed in two cases of malignant pleural effusions suggestive of metastatic adenocarcinoma similar to studies by Udasimath et al. [19], and Kumar P et al. [24].

Though most authors suggested additional advantages of CB when compared to CS, Khan et al. [25], raised doubts about the same. They studied 58 malignant effusions with both cytospin and cell block preparations. Cytospin alone in correlation with clinical, radiological and cytological features, could accurately detect the primary site in 89.7% cases, while in 10.3% cases cytologic features were inconclusive. Additionally the cells in CB were shrunken with loss of cytoplasm. Hence they were of the opinion that, though cytospin and CB preparations were complementary, compared to the time and labour for CB preparation, cytospin was a better choice.

CB vs. CS in cases suspicious of malignancy

In our study, 2 cases on CS were suspicious for malignancy (1 pleural, 1 BAL). Of these, CB from one case of pleural effusion confirmed and established a specific diagnosis of primary adenocarcinoma lung, while in the BAL there was no material on CB. Shobha et al. [13], studied 100 pleural effusions, and reported 6 cases as suspicious for malignancy on CS. All the 6 cases were confirmed as malignant on CB. Udasimath et al. [19], found 8.33% of pleural fluids to be suspicious which were later confirmed by CB. Our study was concordant with the above said studies.



Volume of fluids, pellet formation and CB

In our study, we have processed all the fluids irrespective of the volume. In malignant cases, CB yielded enough material to come to a conclusion even in fluids with volume <10 ml, on the contrary in benign cases, CBs were non diagnostic in cases with volume of fluids <10 ml (81.4%). We are of the opinion that in malignant cases, CB would have yielded material due to high cellularity even in small volumes, when compared to benign cases.

CB yielded material when pellet formation was seen on centrifugation. In cases where pellet did not form, the yield on CB was very poor. A note of special mention here is that pellet formation was appreciated better in malignant cases Dekker A and Bupp PA [3] in their study had selected cases forming ample sediment (exudates) for combined cell block-smear preparation and those cases with slight sediment (transudates) were chosen for centrifuged smear study only.

After evaluating the results of our study, we suggest the following algorithm in studying fluids in cytology.

CONCLUSION

CB preparation by FSM is an easy, reliable and cost effective method which aids in improving the diagnostic accuracy in fluid cytology. CBs were complementary to CS in overall categorization of both benign and malignant effusions, but however good pellet formation with preserved architectural patterns was observed in malignant effusions. Pellet formation can act as a guide to attempt CB preparation, both in benign and malignant effusions.

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