

Assessment by Mair Scoring System for the Cytodiagnostic Preparations of Conventional Centrifuge and Cytospin for Body Fluids.

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Abstract

Background: The quality of preparation forms the foundation of diagnostic sensitivity of body fluids. The preparations of conventional centrifuge (CC) and cytospin (CS) are qualitatively different when stained for diagnostic microscopy. The Mair scoring system is the one that determines the overall quality of cytologic preparations at diagnostic interpretations.

Objectives: the present study is carried out with the objectives to compare the diagnostic cell yield and efficacy of CC and CS by Mair scoring system.

Material and Methods: The 120 preparations of 60 samples each run for CC and CS of 30 samples each of pleural and peritoneal fluids. The preparations were assessed by Mair scoring system upon the stains of Papanicolaou and Giemsa of these cytologic preparations. Chi² test was applied for comparison.

Results: The mean scores for CC for pleural fluids for the feature of background was 1 ± 0.83 , 0.97 ± 0.85 for cellularity, 1.03 ± 0.85 for morphology and 1 ± 0.74 for distribution; and 1.03 ± 0.81 for background, 1.1 ± 0.80 for cellularity, 0.93 ± 0.83 for morphology and 1.1 ± 0.76 for distribution for peritoneal fluid. Pleural fluid sample mean scores were 1.3 ± 0.70 for background, 1.17 ± 0.79 for cellularity, 1.2 ± 0.76 for morphology and 1.73 ± 0.52 for distribution for CS preparations; and 1.16 ± 0.79 for background, 1.2 ± 0.76 for cellularity, 1.07 ± 0.78 for morphology and 1.13 ± 0.82 for distribution for peritoneal fluid. The CS preparations worked well as compared to CC method for the parameters of background and distribution in pleural fluid samples, and background and morphology in peritoneal fluid samples with significant p-values (<0.01).

Conclusion: The Mair scoring system appears to be ideal scoring system for comparisons of qualitative parameters between the two methods of cytologic preparations. The CS appears to be more sensitive for diagnostic yield than CC over the body fluid preparations with increased diagnostic sensitivity.

Keywords: Body fluids, Cytospin, Mair scoring system.

INTRODUCTION

The cytological examination of body fluids is a common practice and of distinct value in confirming diagnosis.^[1] Cytological assessments of serous effusions have been done for nearly a century.^[2] It is not only important for diagnosis of malignancies but has helped in staging and prognosis of malignant tumors and also gives information regarding various inflammatory conditions like parasitic infestation, infection with bacteria, fungi and viruses and some immunological conditions.^[3,4]

The cytological study of body effusions is a diagnostic modality which indicates the etiology of the effusion as well as in certain cases is a means of prognostication of the disease process.^[2] Adenocarcinomas, well differentiated squamous cell carcinomas, small cell carcinomas, malignant melanomas, large cell lymphomas and acute leukaemias are accurately classified when present in effusions. Although a positive diagnosis is often considered as definitive diagnosis, a negative result does not rule out a malignant cause.^[4-6]

The diagnostic performance of cytologic study of the effusions may be attributable to the fact that the exfoliated cell population present in the sediment accumulate from all surface lining, representing the entire serous cavity than that obtained by a needle biopsy, as focal lesion on a serous surface may be missed by a biopsy, giving false negative results.^[3,4]

The percutaneous access of pleural and peritoneal spaces are relatively simple and general cytological examinations can be performed easily, quickly and inexpensively.^[5] The technique still widely used in most of Indian laboratories is the centrifugation and sedimentation smear preparation. With this technique, collection of relatively fewer cells from any fluid and keeping them on the slide during staining is responsible for large number of unsatisfactory smears^[2]. The lower sensitivity is mainly attributable to bland morphological details of cells.^[7]

The lower diagnostic sensitivity in the cytological preparation of conventional centrifuge (CC) material is due to suboptimal cellularity and the crowding and overlapping of the cells. The study of Deshpande et al^[1], Joshi et al^[2] and Mahajan et al^[3] concluded that the cytospin (CS) preparation of the fluids are better than the CC method as the CS should be used preferably for serous and haemorrhagic fluids regularly to minimise the non-representative cytology.

Joshi et al^[2] did a study with the objective to determine the sensitivity of CS. Their study concluded through their descriptive statistics that the cell block followed by CS have a minimum qualitative variation as compared to samples processed by CC. They adopted the scoring system suggested by Mair et al, which includes the features of background, cellularity, cell morphology and cell distribution. The Mair scoring system taking in account these four parameters and the scores are distributed across 0 to 2+. The comparative statistics when applied to these three cytological methods, the CS and cell block were found to work well on these parameters.

With the backdrop of published literature, there are variable observations attributable to advantages and disadvantages of CC preparations versus CS preparation in evaluation of the suspected malignant serous effusions.

The present study explores this technical aspect of fluid processing in cytology and compares the diagnostic yield of the cells by using Mair's scoring system.

AIM AND OBJECTIVES

Aim: To compare the techniques of conventional centrifuge and cytopsin for diagnostic cell yield in cytological evaluation of peritoneal and pleural fluid specimens.

OBJECTIVES

1. To compare the diagnostic cell yield (cellularity) obtained by conventional centrifuge and cytopsin technique applied over the samples of pleural fluid and peritoneal fluid.
2. To study the qualitative difference between the preparations of the conventional centrifuge and cytopsin at staining of PAP and Giemsa on the samples of Pleural and Peritoneal fluids.
3. To evaluate the diagnostic efficacy of conventional centrifugation and cytopsin preparation by Mair scoring system.

MATERIALS AND METHODS

This prospective and analytical study was carried out in the department of Pathology, J.N.M.C., Sawangi (Maharashtra). A total of 60 randomly taken samples of peritoneal and pleural fluids aspirated from admitted patients of medical, surgical or gynaecological wards. All other fluids were excluded from the present study.

Freshly aspirated fluid samples collected in clean, dry, rubber stoppered, labelled glass containers were used in this study. The fluids were equally divided and simultaneously centrifuged in CC and the CS. The fluids were centrifuged at 15,000 rpm for 15 minutes for CC smears. The CS preparations were made by standard methods utilising the guidelines subscribed in the literature of Thermo Cytospine. The smears from both methods were wet and dry fixed for Pap stain and Giemsa stain respectively. The Pap stain and Giemsa stain were carried out by standard methods.^[8] No fixatives or anticoagulants were used to process the samples.

The smears obtained by each of the above techniques were evaluated for features such as background, cellularity, cell morphology and cell distribution and were scored from 0 to 2+ scale according to the Mair et al^[2-4] scoring system (Table-1).

Mair Scoring System^[2-4]

The following Mair Scoring System was used for reporting by both the methods.

Table 1: Mair Scoring System

Parameter	Quantitative Description	Point Score
1. Background blood or proteinaceous	1. Large amount, great compromise in diagnosis. 2. Moderate amount, diagnosis possible.	0 1

material	3.Minimal,diagnosis easy.	2
2.Amount of cellular material	1. Minimal to absent,diagnosis not possible. 2. Sufficient for cytodiagnosis. 3.Abundant,diagnosis simple.	0 1 2
3.Cell morphology,cellular degeneration and trauma	1.Marked cellular degeneration,diagnosis not possible. 2.Moderate cellular degeneration,diagnosis possible. 3.Minimal cellular degeneration,diagnosis easy.	0 1 2
4.Distribution of cells	1.Totally in the periphery or sparsely distributed. 2.Combination. 3.Evenly distributed.	0 1 2

The statistical methods were applied for comparison between the CC and CS preparation for the results of Mair Scoring System by Chi² test and calculating the value of significance (p-value). P-value of ≤ 0.01 was considered as significant at C.I. of 95%.

RESULTS

The pleural and peritoneal effusions as observed were distributed across all age ranges. However, as per the data collected the maximum number of pleural fluid cases were found to be in the age range of 51 to 60 years and for peritoneal fluid, it was 41 to 50 years as shown in table 1.

Table 2: Distribution of age and gender for pleural and peritoneal fluids.

Age	Male (%)	Female (%)	Total	Pleural Fluid	Peritoneal Fluid
0-10	1 (3.33)	1 (3.33)	2	1	1
11-20	1	0	1	1	0
21-30	4 (13.3)	3 (10)	7	4	3
31-40	9 (30)	1 (3.33)	10	6	4
41-50	5 (16.67)	7 (23.3)	12	3	9
51-60	7 (23.3)	6 (20)	13	7	6
61-70	1 (3.33)	9 (30)	10	3	7
71-80	2 (6.67)	3 (10)	5	5	0
81-90	0	0	0	0	0
Total	30	30	60	30	30

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Irrespective of the type of fluid and gender, 33.33% (20 cases) belonged to less than 40 years of age and 66.67% (40 cases) were beyond the age of 40 years.

The male to female ratio observed for pleural and peritoneal fluids together was 1 : 1. The cytological diagnosis for pleural and peritoneal fluids processed by CC method and by CS method is depicted in table 3.

Table 3: Distribution of cytodiagnosis for pleural and peritoneal fluid.

Fluid	Conventional Centrifuge				Cytospin			
	Total	Benign		Malignant	Total	Benign		Malignant
		S*	I†			S*	I†	
Pleural	30	11	16	03	30	10	16	04
Peritoneal	30	20	03	07	30	20	03	07

S* – Serous Effusion and Serous Effusion with Mesothelial Reaction

I† – Inflammatory Reaction

The smears of CC method in a single case missed the detection of malignancy, which was diagnosed on CS preparation. The cytodiagnosis of serous fluid and serous fluid with mesothelial reaction or hyperplasia as well as exudative effusions did not vary much, except for a case as described above, but it varied for characterisation when microscopic parameters for cellularity, morphology, distribution of the cells and background were considered. The obscuring of cellular morphological features by the background material was found to be a matter of microscopic limitation for the smears of CC when compared with CS smears.

The qualitative evaluation of cytological preparations of CC and CS preparations have shown the marked differences by Mair scoring system.

The Mair scoring system uses parametric scoring of 0, 1 and 2 for the background of the smear, cellularity of the smear, the preservation of morphology of cells and uniformity of distribution of the cells.

A total of 60 cases which underwent a comparative evaluation for these qualitative parameters on smears of CC and CS are shown in table 4 and 5.

Table4: Distribution of samples for parameters by conventional centrifuge and cytopsin

Parameters		Conventional Centrifuge			Cytospin		
		0	1	2	0	1	2
Pleural Fluid	Background	10	10	10	04	13	13
	Cellularity	11	09	10	07	11	12

	Morphology	09	11	10	06	12	12
	Distribution	08	14	08	01	06	23
Peritoneal Fluid	Background	09	11	10	07	11	12
	Cellularity	08	11	11	06	12	12
	Morphology	11	10	09	08	12	10
	Distribution	07	13	10	08	10	12

Of all the 4 parameters, irrespective of the type of fluid, have shown that less number of cases in the scores of 0 (figure 1 and 2) for qualitative assessment of background, cellularity, morphology and distribution for CS preparations.

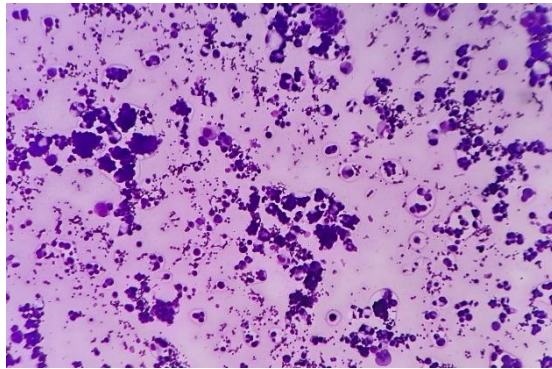


Figure 1: Peritoneal fluid – Conventional Centrifuge preparation

Diagnosis – Infiltrate of Adenocarcinoma

Mair Score: Background 0, Cellularity 2, Morphology 1, Distribution 0
(Giemsa stain, 10x)

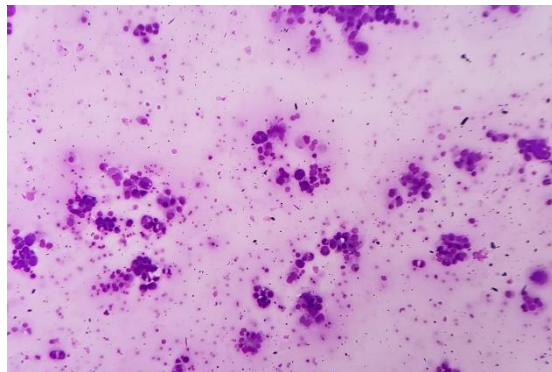


Figure 2: Peritoneal fluid – Cytospin preparation

Diagnosis – Infiltrate of Adenocarcinoma

Mair Score: Background 2, Cellularity 2, Morphology 2, Distribution 2
(Giemsa stain, 10x)

Table 5: Mean score for qualitative parameters of conventional centrifuge and cytopsin preparations

Concentration Method	Parameter	Pleural Fluid	Peritoneal Fluid
Conventional	Background	1 ± 0.83	1.03 ± 0.81

Centrifuge	Cellularity	0.97 ± 0.85	1.1 ± 0.80
	Morphology	1.03 ± 0.85	0.933 ± 0.83
	Distribution	1 ± 0.74	1.1 ± 0.76
Cytospin	Background	1.3 ± 0.70	1.16 ± 0.79
	Cellularity	1.17 ± 0.79	1.2 ± 0.76
	Morphology	1.2 ± 0.76	1.07 ± 0.78
	Distribution	1.73 ± 0.52	1.13 ± 0.82

The statistical test applied upon these parametric Mair score was Chi square test and the value of significance. The results of statistical test for Mair scoring system over CC smear versus CS smears microscopy is depicted in table 6.

Table 6: The results of comparison between conventional centrifuge and cytopsin preparations by Chi² test.

Parameters		Conventional Centrifuge			Cytospin			Chi ²	p-Value
		0	1	2	0	1	2		
Pleural Fluid	Background	10	10	10	04	13	13	21.230	0.000
	Cellularity	11	09	10	07	11	12	12.694	0.013
	Morphology	09	11	10	06	12	12	9.247	0.055
	Distribution	08	14	08	01	06	23	13.443	0.009
Peritoneal Fluid	Background	09	11	10	07	11	12	14.872	0.005
	Cellularity	08	11	11	06	12	12	10.909	0.028
	Morphology	11	10	09	08	12	10	15.597	0.004
	Distribution	07	13	10	08	10	12	13.090	0.011

The processing of pleural fluid, when compared for background between CC smears and CS smears showed a significant p-value, so also the uniform distribution of the cellular material (0.000 and 0.009 respectively). The comparison between CC and CS smears of the peritoneal fluid for the features of background was found to be significant with a p-value of 0.005. It was significant for the morphology also with a p-value of 0.004. However, the comparison for other two features of cellularity and morphology for the two different ways of processing pleural fluid was found to be insignificant. The processing of peritoneal fluid when compared between CC and CS smears for the features of cellularity and distribution were found to have insignificant p-values (>0.01).

DISCUSSION

The pathological collection of fluid in the potential spaces is the commonest clinical presentation of several underlying causes. Therefore, their cytological assessment for diagnosis and therapeutic implications are of immense importance. The processing of tissue fluids matters a lot for diagnostic cell yield. There are several approaches used for cell concentration methodologies to obtain the representative diagnostic cellularities for the microscopy. The studies picked up for the present work that compared the various techniques of cell concentration such as conventional centrifuge^[1-4,6,9], cytopsin^[1-4,11-13], cell block^[2,4,6,12,13] and Thinprep^[9,11].

Pleural and peritoneal fluids submitted to cytopathology laboratories for diagnostic workup belonged to all age group individuals and therefore their comparisons for the age ranges is unwarranted until the malignancy is suspected clinically. The males suffering more of pleural and peritoneal effusions than females have been documented in the studies of Joshi et al^[2], Singh et al^[4], Gaur et al^[5] and Jadhav et al^[7] reviewed for the present work. However in the present study, the ratio of 1 : 1 was observed for males and females suffering from plural and peritoneal pathologies.

The multiple underlying causes have been reported for pleural and peritoneal fluids.^[1-5,7,12,13] The present study also observed similar kind of underlying pathologies of pleural and peritoneal fluids as described by other authors such as serous effusions, serous effusions with reactive mesothelial cells, inflammatory effusions and malignancy.

Irrespective of fluid type (plural or peritoneal) benign pathologies outnumbered the malignant pathologies in the present study. Similar such observations for the pathologies in the fluids have been recorded by Deshpande et al^[1], Joshi et al^[2], Mahajan et al^[3], Singh et al^[4], Jadhav et al^[7], Mulkalwar et al^[12] and Qamar et al^[13].

The cellular yield and maintenance of morphology of the cells of cytological preparations determines the diagnostic sensitivity of the effusions. The reports in the literature have compared the smears of CC and that of CS for their advantages.^[1-4] The comparisons were qualitative based on the features of (i) cellularity, (ii) morphology, (iii) background and (iv) distribution of cells.^[2-4,6,10]

The Mair system has been documented that ideally compares the cell concentration techniques for the above qualitative methods.^[2-4] The studies those have compared the diagnostic potential for the preparations of CC and CS methods includes Deshpande et al^[1], Joshi et al^[2], Mahajan et al^[3] and Singh et al^[4].

The present study has observed that overall qualitative assessment of pleural fluid and that of peritoneal fluid based on the Mair system that CS preparations works well for the diagnostic cellular yield and morphology as compared to the CC preparations as one additional case of malignancy could be picked up by the CS preparations. Similar findings for picking up the malignancy on CS preparations as compared to the CC preparations have been observed in the studies of Deshpande et al^[1] (2 cases), Joshi et al^[2] (5 cases), Mahajan et al^[3] (4 cases) and Singh et al^[4] (9 cases).

Under the mayor scoring system, CS appears to work well on qualitative parameters as seen by the mean values for background (1.3 ± 0.70 and 1.16 ± 0.79), cellularity (1.17 ± 0.79 and 1.2 ± 0.76), morphology (1.2 ± 0.76 and 1.07 ± 0.78) and distribution (1.73 ± 0.52 and 1.13 ± 0.82) of cells when compared with CC smears [background (1 ± 0.83 and 1.03 ± 0.81),

cellularity (0.97 ± 0.85 and 1.1 ± 0.80), morphology (1.03 ± 0.85 and 0.933 ± 0.83) and distribution (1 ± 0.74 and 1.1 ± 0.76) for pleural and peritoneal fluid, respectively]. The studies of Joshi et al^[2] and Mahajan et al^[3] obtained similar observations as the present study, where performance was judged for the parameters by statistical mean values and were higher in CS as compared to CC.

The Chi² test was applied and the value of significance (p-value) were drawn for the individual features of background, cellularity, morphology, and distribution of Mair scoring system on the observations of the present study. The present study has shown that there exists a significant p-value of 0.000 and 0.005 respectively for pleural and peritoneal fluids for the CS preparations when compared with CC smears for the features of background. For the features of distribution, a significant p-value was observed of 0.009 for CS preparation when compared with CC smear assessment for pleural fluid cytology. The CS worked much better at eliciting the morphology when compared to the preparations of CC method of peritoneal fluid with the p-value of 0.004 (significant). The comparable reports as reviewed for the present study of Joshi et al^[2], Mahajan et al^[3] and Singh et al^[4].

Joshi et al^[2] have found the significant p-values of <0.0001 for all the fluids compared for the preparations of CC and CS on all parameters of Mair scoring system of background, cellularity, morphology and cell distribution. Mahajan et al^[3] also compared the CC and CS preparations for all the fluids and found the difference to be significant (<0.05) for all parameters of background, cellularity, cell morphology and cell distribution. Singh et al^[4] compared the methods of fluid preparations and found the difference to be significant (<0.0001) between CC and CS smears for all the four parameters of Mair scoring system.

The overall performance of CS preparations by Mair scoring system was high as compared to smears of CC of pleural and peritoneal fluids.^[5-7,9-13] Few of the related studies were reviewed [14-16].

CONCLUSION

Fluid cytology is most sought after diagnostic investigation in clinical practice. The adequacy (cellularity), morphological preservations, less background hinderances and uniform distribution of the cells augments the sensitivity, specificity, and accuracy of the fluid cytology. The observations made in the present study concludes that CS preparations are far better preparations than CC smears at reporting the cytological abnormalities and diagnosis of pleural and peritoneal fluids when assessed on Mair scoring system.

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