

**Research Paper****Interobserver Agreement in Pap Stained Smear and Cell Block Preparation in Pleural Fluid Cytology**

Authors

**Dr Soumya T R^{1*}, Dr Sheela Varghese², Dr Sankar Sundaram³,
Dr Abhilash K K⁴, Dr Deepa S⁵**¹Postgraduate student, Department of Pathology, Government Medical College, Kottayam²Additional Professor, Department of Pathology, Government Medical College, Kozhikode³Professor and Head of the Department, Department of Pathology, Government Medical College, Kottayam⁴Assistant Professor, Department of Pathology, Government Medical College, Kottayam⁵Assistant Professor, Department of Pathology, Government Medical College, Kottayam

*Corresponding Author

Dr Soumya T R

Postgraduate student, Department of Pathology, Government Medical College, Kottayam, India

Abstract

Background: *The cytological examination of pleural fluid is a simple, inexpensive and non-invasive method which helps in rapid diagnosis. A positive diagnosis is often considered as definite criteria for even an explorative surgery. It helps not only in the diagnosis but also in the staging and prognosis of malignancy. However it is difficult for even an experienced pathologist to reliably distinguish benign and malignant effusions.*

Objective: *To analyse the interobserver agreement in pap stained smear and cell block preparation in pleural fluid cytology.*

Methods:

Study Design: *Cross Sectional Study –Interobserver Agreement.*

Sampling Procedure: *Continuous sampling.*

Study Population: *Study sample include first 100 pleural fluid samples received in department of pathology, Govt. medical college, Kottayam during the study period.*

Study Procedure: *Cytological smear and cell block were interpreted by two different pathologists each.*

Analysis: *Interobserver agreement in cytological smear and cell block, each were interpreted with kappa value.*

Results and Discussion: *The mean age of the present study population is 57 years .Minimum age is 7 years and maximum is 85 years. Majority belong to the age group 51-60 years (23%). Cytological smear shows substantial agreement with kappa value of 0.642 and cell block preparation also shows substantial agreement with kappa value of 0.783.*

Conclusion: *Both cytological smear and cell block preparation shows substantial agreement with cell block preparation being superior than cytological smear for diagnosis with a higher interobserver agreement.*

Keywords: *Cytological smear, cell block, interobserver agreement.*

Introduction

Pleural effusion is the accumulation of fluid in pleural cavity, that is, in between the visceral and parietal layers of the pleura and is one of the major causes of pulmonary mortality and morbidity. The cytological examination is the easiest and the least invasive procedure in detecting both benign and malignant conditions. The involvement of pleural cavity by malignant neoplasms has important therapeutic and prognostic implications.

The development of malignant pleural effusion is a common complication of cancers like pulmonary and gastric carcinomas. Malignant neoplasms, especially lymphoid neoplasms, represent a major cause of death in children and in these cases cytological examination is very useful in its management.

The most common reason to submit pleural fluid for cytological examination is to determine whether it contains malignant cells or not. The presence of malignant cells is not only important for its diagnosis but also for its staging and prognosis. Cytological examination of pleural fluid is a cost effective and non-invasive method. But it is difficult even for an experienced pathologist to reliably distinguish reactive mesothelial cells from malignant cells in cytological smear. Reactive mesothelial cells shows high nuclear: cytoplasmic ratio, mitosis and multinucleation resembling a malignant cell.

Along with cytological smear, cell block can also be made from the fluid. The advantages include it is cost-effective, more cellular, preserves histological architecture and multiple sections can be made and special stains and immunohistochemistry can be done. Thus the cell block preparation when used as an adjunct to cytological smear has got increased diagnostic yield.

This study was carried out to evaluate interobserver agreement among pathologists in assessing both smears and cell block preparations made from pleural fluid.

Objective

To analyse the interobserver agreement in pap stained smear and cell block preparation in pleural fluid cytology.

Materials and Methods

Study Design: Cross Sectional Study Interobserver Agreement.

Study Period: 18 months AFTER IRB approval (Dec 2017 to May 2019).

Study Setting: Department of Pathology, Govt. Medical College, Kottayam.

Sample size: 100

Study Population: Study sample include first 100 pleural fluid samples received in department of pathology, Govt. medical college, Kottayam during the study period.

Inclusion Criteria: All pleural fluid samples received in department of pathology.

Exclusion Criteria: Cases with inadequate data (if age, gender of the patient not mentioned).

Cases with inadequate amount of fluid (at least 10ml) to make cytological smear and cell block.

Study Tools:

1. Detailed proforma for each case.
2. Reagents for pap stained smear preparation.
3. Reagents for cell block preparation.
4. Instruments for making cell blocks and cutting thin sections from it.

Study Procedure: Details of cases were recorded in the proforma. Pleural fluid samples were first examined by naked eye for physical characteristics, measured and then divided into two halves. Half of the specimen was centrifuged at 2000 rpm for 10 mins. The supernatant fluid was then pipetted out and the sediment is transferred to a slide and then fixed in 95% alcohol for a minimum period of 15mins and stained with Papanicolaou stain. If the fluid is bloody, before fixing, smear was placed in Carnoy's fixative (95% alcohol, chloroform and glacial acetic acid in the ratio 6:3:1).

The other half of the fluid specimen was fixed in a solution of alcohol: formalin (9 parts of 90% alcohol and 1 part of 7.5% formalin) for one hour. After fixation, the specimen was centrifuged at 2500

rpm for 10-15mins. The supernatant was poured off and a further 3ml of fresh alcohol-formalin is once again added to the sediment and was kept for one day. Next day the sediment was completely drained off by inverting the tube over filter paper. The sediment was then wrapped in the same filter paper and processed as part of routine paraffin section histopathology.

The samples were studied in detail taken into account the available clinical data, and morphological details. Both Pap stained smear and cell block were interpreted by two different pathologists each independently and was categorized as inconclusive, benign, suspicious for malignancy, or malignancy.

Data management and Analysis

The data was entered in Microsoft excel and further statistical analysis was done and interobserver agreement for cytological smear and cell block were calculated with kappa value.

Personnel responsible for data collection:

Dr.Soumya T R

Personnel responsible for data analysis:

Dr.Soumya T R

Funding agency: Self

Results

The present study was conducted in the 100 pleural fluid samples received in the department of Pathology, Government Medical College, Kottayam during the study period. In this study, pap stained cytological smear and cell block were prepared. Firstly cytological smear was interpreted by two different pathologists of equal experience and competency, independently. Each smear was studied for 4 minutes and categorized into inconclusive, benign, and suspicious of malignancy and malignancy. Then another two pathologists, of equal experience and competency, independently interpreted cell block preparation. Each slide was studied for 4 minutes and categorized into inadequate, benign, suspicious of malignancy and malignancy. The observers who seen the cytological smear have not seen the cell block preparation and vice versa.

Age

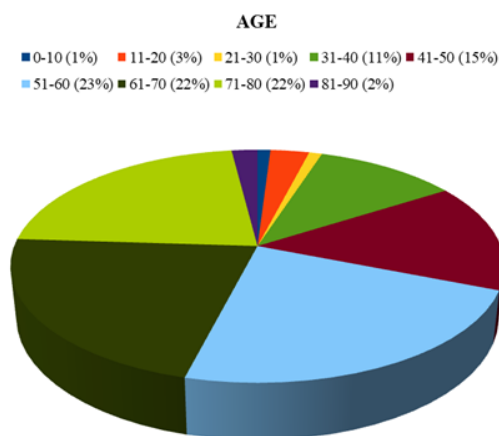


Figure 1: Age wise distribution of patients whose pleural fluid samples received

The mean age of the present study population is 57 years .minimum age is 7 years and maximum is 85 years. Majority belongs to the age group 51-80 years (67%).

Gender distribution:

The males were more (61%) in the present study compared to females.

Gross appearance

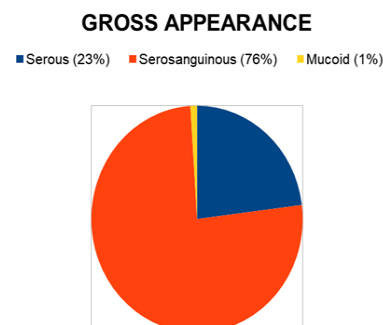


Figure 2: Gross appearance of pleural fluid samples received

The gross appearance of the samples was analysed and it was found that majority were exudates (77%) and serosanguinous.

Amount of pleural fluid

AMOUNT IN ml

- 0-200 (50%) ■ 201-400 (5%) ■ 401-600 (15%) ■ 601-800(5%)
- 801-1000(17%) ■ 1001-1200 (1%) ■ 1201-1400 (0%) ■ 1401-1600 (3%)
- 1601-1800 (1%) ■ 1801-2000 (3%)

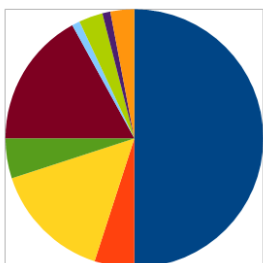


Figure 3: Amount of pleural fluid received in ml (n=100)

All the fluid samples were measured. Half of the samples received had less than 200ml of fluid.

Interobserver agreement in cytological smear

Table 1: Interobserver agreement in cytological smear

Cases	Kappa value	Agreement	Significance
Inconclusive-Benign	.862	Almost perfect	Significant
Benign-Suspicious of malignancy	.418	Moderate	Significant
Suspicious of malignancy-Malignancy	.250	Fair	Non-significant
Benign-Malignancy	.661	Substantial	Significant
Overall	.642	Substantial	Significant

Interobserver agreements in cell block preparation

Table 2: Interobserver agreement in cell block preparation

Cases	Kappa value	Agreement	Significance
Inadequate-Benign	.876	Almost perfect	Significant
Benign-Suspicious of malignancy	.851	Almost perfect	Significant
Suspicious of malignancy-Malignancy	.290	Fair	Non-significant
Benign-Malignancy	1	Almost perfect	Significant
Overall cases	.783	Substantial	Significant

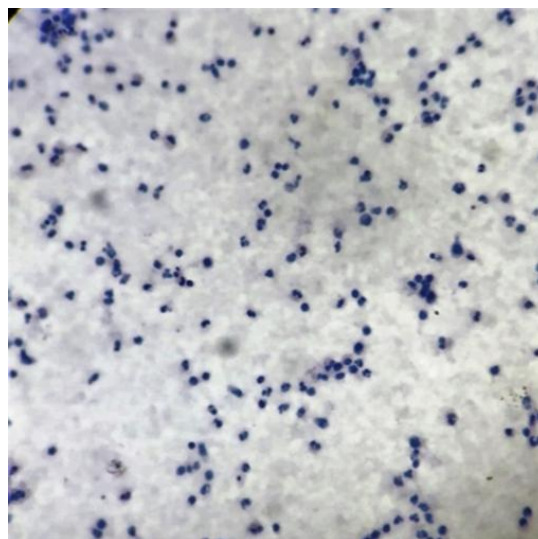


Figure 4: Case 1 Suppurative effusion (pap stained cytological smear) 400X

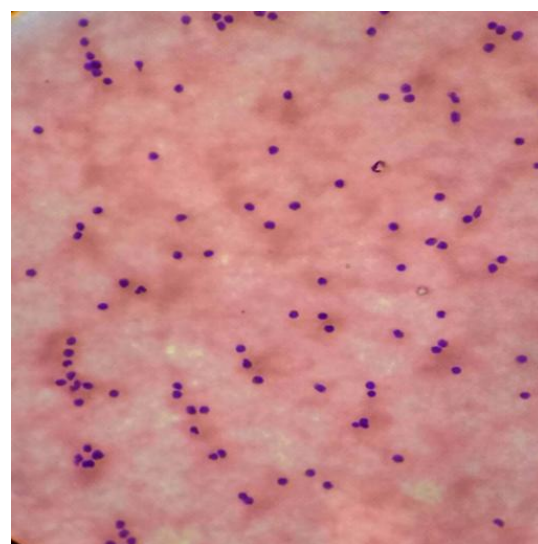


Figure 5: Case 2 Lymphocytic effusion (pap stained cytological smear) 400X

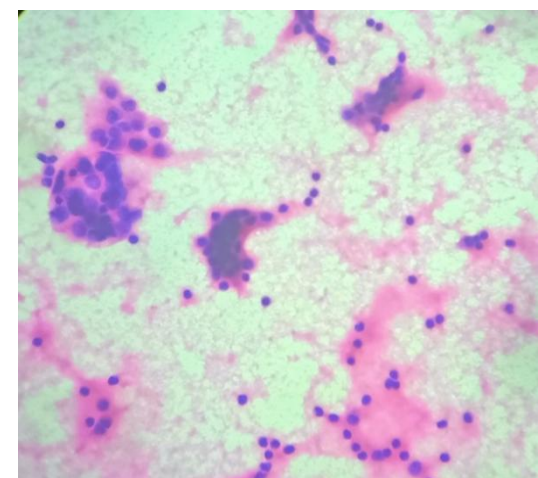


Figure 6: Case 3 Suspicious of malignant cells and reactive mesothelial cells in a lymphocytic background (pap stained cytological smear) 400X

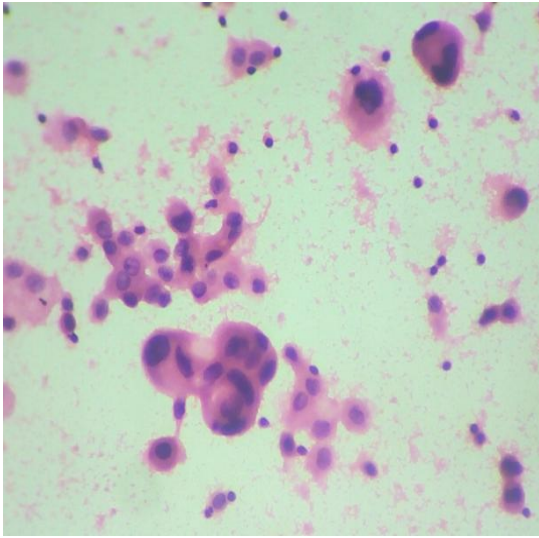


Figure 7: Case 4 Malignant cells (pap stained cytological smear) 400X

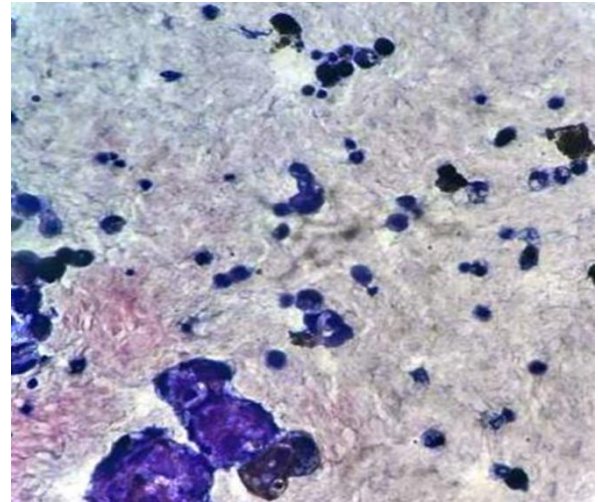


Figure 10: Case 5 Malignant melanoma metastasis in pleural fluid (Pap stained cytological smear) 400X

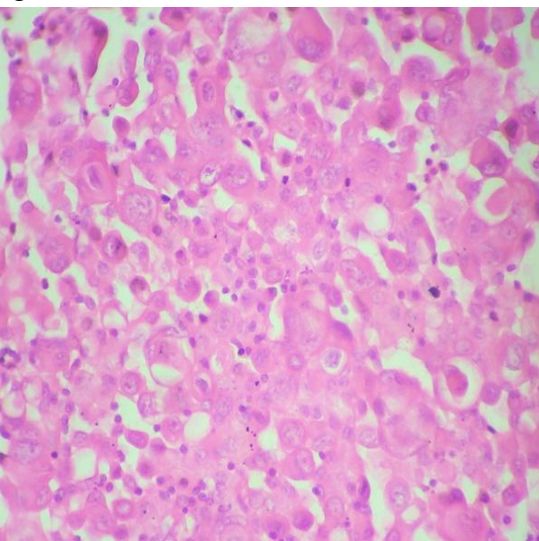


Figure 8: Case 4 Malignant cells in cell block (H & E stained) 400X

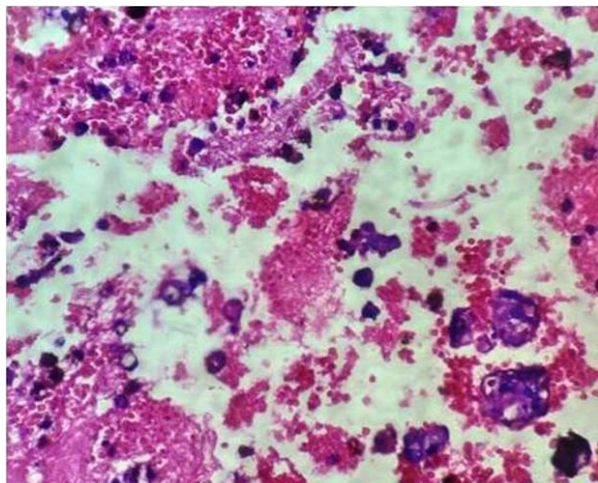


Figure 11: Case 5 Malignant melanoma metastasis in pleural fluid (H & E stained Cell block preparation) 400X

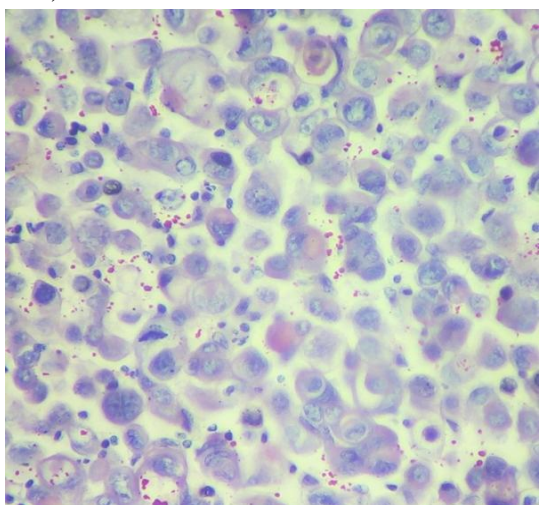


Figure 9: Case 4 Malignant cells are mucicarmine positive. Suggestive of mucoepidermoid carcinoma metastasis to pleural fluid. Cell block section- Mucicarmine stain 400X

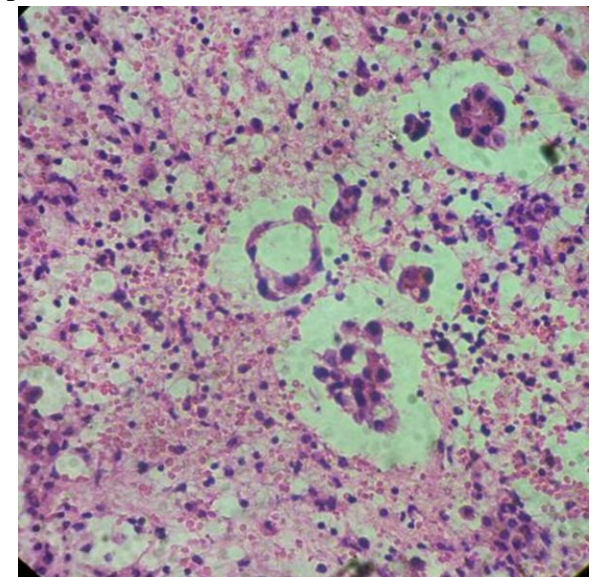


Figure 12: Case 6 Malignant cells (H & E stained Cell block preparation) 400X

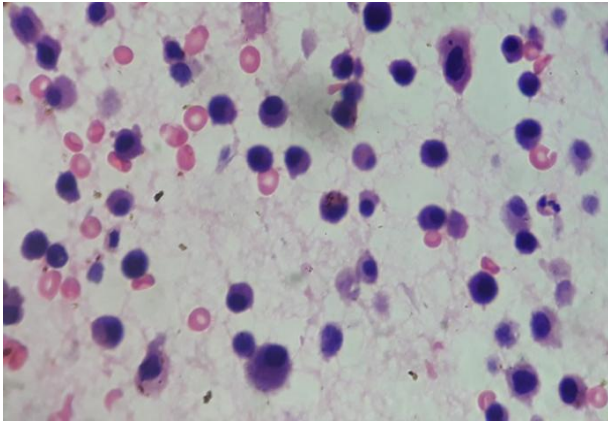


Figure 13: Case 7 Myelomatous pleural effusion. (Pap stained cytological smear) 400X

Conclusion

- 1) Interobserver agreement in cytological smear showed substantial agreement with a kappa value of 0.642.
- 2) Interobserver agreement in cell block preparation showed substantial agreement with a kappa value of 0.783.
- 3) Cell block preparation is superior to pap stained cytological smear.

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