



### Original Research Article

## A Qualitative Comparison of Conventional Cytosmears and Thromboplastin-Plasma Cell Blocks from Liver Space Occupying Lesions

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### Abstract

**Introduction:** Most of the radiologically evident hepatic lesions are quite easily approachable using Fine Needle Aspiration (FNA). However, limited material and less architectural details are few of the drawbacks frequently encountered with FNA. Thromboplastin-Plasma cell block technique is cost effective, simple, reproducible and can provide improved cytomorphological features. This study applies Mair et al. point scoring system in comparing conventional cytospins and thromboplastin-plasma cell blocks from liver space occupying lesions.

**Methodology:** This was a prospective study over a period of 1 year (from January 2018 to December 2018), comprising of a total of eighty cases with liver space occupying lesions. A comparison between the cellularity, morphological preservation, architectural preservation and background was performed on both conventional smears and cell blocks based on the point scoring system described by Mair et al.

**Results:** On comparing overall quality, cellularity, extent of cellular degeneration, amount of obscuring background and architecture and applying Fischer exact test, cell blocks were of better quality with p-value for superior quality being 0.009 which was statistically significant; cell blocks provided minimal obscuring background with p-value 0.01 which was statistically significant. The architecture analysis in cell blocks showed excellent resemblance to histology in 14 cases (17.5%) from liver SOL aspirates, the p-value for excellent architecture being 0.003, which was statistically significant.

**Conclusion:** Thromboplastin-Plasma cell blocks prepared from liver SOL aspirates reveal better architecture and less obscuring background, when compared to conventional cytospins and cell blocks should be prepared whenever possible to aid to the cytospin diagnosis.

### Introduction

Most of the radiologically evident hepatic lesions are quite easily approachable using Fine Needle Aspiration (FNA). Compared to conventional core biopsy, FNA is less expensive, less invasive and

approachable to wide area. However, limited material and less architectural details are few of the drawbacks frequently encountered with FNA.<sup>1</sup> Concomitant preparation of cell blocks with cytospins provides additional architectural

details and provision for application of immunocytochemical stains which help not only in confirmation but also subtyping of malignancy. Thromboplastin-Plasma cell block (TP-CB) technique is cost effective, simple, reproducible and can provide improved cytomorphological features.<sup>2</sup> It is also suitable for performing immunocytochemical studies as antigenic epitopes are well preserved with formalin being the fixative instead of alcoholic fixatives.<sup>3</sup>

This study applies Mair *et al.*<sup>4</sup> point scoring system in comparing conventional cytospins and TP-CBs from liver space occupying lesions (SOLs).

### Methodology

This was a prospective study over a period of 1 year (from January 2018 to December 2018), comprising of a total of eighty cases with SOLs, clinico-radiologically suspicious for malignancy and referred to our department for FNAC under USG guidance.

Inclusion criteria of the study were i) patients clinico-radiologically suspicious of liver malignancy and ii) Patients with normal coagulation profile.

Cases in which sample for cell block preparation was kept for fixation after 1 hour of collection, were excluded from study.

Preliminary details of every patient including name, age and gender were noted. The chief complains along with essential clinical details, radiological findings and serological data were noted on a structured proforma for the study.

An informed consent was taken from each patient prior to performing the FNA procedure. An ultrasound guided FNAC was performed using a 20 gauge lumbar puncture needle, fitted to a 20-ml disposable syringe. The skin entry site was sterilized and infiltrated with 2% lignocaine. One to two passes were made to get adequate aspirates. Direct air dried smears were prepared for routine Giemsa stain and few smears were immediately fixed in 95% Ethyl alcohol (15 minutes) for Hematoxylin and Eosin (H & E) stain. Diagnostic

criteria described by Orell *et al.*<sup>5</sup>, 2012 were followed while analyzing cytospins.

After preparation of the cytospins, the remaining material from the aspirate was rinsed using normal saline and the material was taken in a conical tube. A dedicated needle pass was made for cell block, in case the patient consented. This material was used for cell block preparation by Thromboplastin-Plasma method. Needle rinse samples were centrifuged at 2500 rpm for 15 minutes. Following the centrifugation, supernatant was removed and discarded. The remaining sediment was mixed with 4 drops of pooled plasma that was kept and brought to room temperature before use. Following this, two drops of thromboplastin (Neoplastine<sup>TM</sup>) at room temperature were added and mixed. The tube containing the above mixture was agitated and then kept undisturbed for 15-20 seconds or until a clot was formed. If no clot formation could be appreciated, 2 more drops of thromboplastin were added until clot appeared. The formed clot was scooped out using a spatula, placed on a filter paper and kept in cassette. The tissue cassette was then fixed in 10% neutral buffered formalin overnight and processed along with routine histopathological specimens. Cell blocks were made and tissue sections of 3 micron thickness were taken and stained with routine H & E for morphological evaluation.<sup>6</sup>

A comparison between the cellularity, morphological preservation, architectural preservation and background was performed on both conventional smears and cell blocks based on the point scoring system described by Mair *et al.*<sup>4</sup> According to the criteria mentioned in Table No.1, comments were rendered on the quality of the cytospins and sections from cell blocks, subsequently classifying them into three categories:

- a. Diagnostically unsuitable (score 0-2)
- b. Diagnostically adequate (score 3-6)
- c. Diagnostically superior (score 7-8)

Data were analysed using Fischer-exact test. The p-value of < 0.05 was taken as statistically significant.

**Results**

On comparing overall quality, cellularity, extent of cellular degeneration, amount of obscuring background and architecture and applying Fischer exact test, p-value for superior quality was derived to be 0.009 which was statistically significant (Table no.2), p-value for minimal cellularity was derived as 0.079 which was statistically insignificant (Table no.3), p-value for minimal cellular degeneration was derived as 0.062 which

was statistically insignificant (Table no.4), p-value for minimal obscuring background was derived as 0.01 which was statistically significant (Table no.5). The architecture analysis in cell block showed scanty cells in 15 cases (18.75%), cellular arrangement (acini, papillae, sheets, clusters) in 51 cases (63.75%) and excellent resemblance to histology in 14 cases (17.5%) from liver SOL aspirates. The p-value for minimal architecture was derived as 0.445 which was statistically insignificant, whereas the p-value for excellent architecture was derived as 0.003, which was statistically significant (Table no.6).

**Table No.1** Mair *et al.* point scoring system

Criteria	Qualitative description	Point Score
1) Volume of obscuring background blood or proteinaceous material	Large amount: Diagnosis Greatly Compromised	0
	Moderate Amount: Diagnosis Possible	1
	Minimal amount: Diagnosis easy	2
2) Amount of diagnostic cellular material present	Minimal or absent : diagnosis not possible	0
	Sufficient for diagnosis	1
	Abundant : diagnosis simple	2
3) Degree of cellular degeneration and cellular trauma.	Marked : diagnosis impossible	0
	Moderate: diagnosis possible	1
	Minimal: good preservation	2
4) Retention of appropriate architecture and cellular arrangement	Minimal to absent: non- diagnostic	0
	Moderate: some preservation example: follicles, papillae, acini, syncytia or single cell pattern.	1
	Excellent architectural display, closely reflecting histology; diagnosis obvious	2

**Table No.2** Comparison of quality of cytosmears and cell blocks

Quality	Cytosmear	%	Cell Block	%	P-value*
Unsuitable	15	18.75	15	18.75	0.009
Adequate	62	77.5	51	63.75	
Superior	3	3.75	14	17.5	
Total	80	100	80	100	

\*Fischer exact test, significance level 0.05

**Table No.3** Comparison of cellularity of cytosmears and cell blocks

Cellularity	Cytosmear	%	Cell Block	%	P-value*
Minimal	10	12.5	3	3.75	0.079
Sufficient	44	55	43	53.75	
Abundant	26	32.5	34	42.5	
Total	80	100	80	100	

\*Fischer exact test, significance level 0.05

**Table No.4** Comparison of extent of cellular degeneration in cytosmears and cell blocks

Cellular Degeneration	Cytosmear	%	Cell Block	%	P-value*
Minimal	4	5	12	15	0.062
Moderate	70	87.5	60	75	
Marked	6	7.5	8	10	
Total	80	100	80	100	

\*Fischer exact test, significance level 0.05

**Table No.5** Comparison of amount of obscuring background in cytospin smears and cell blocks

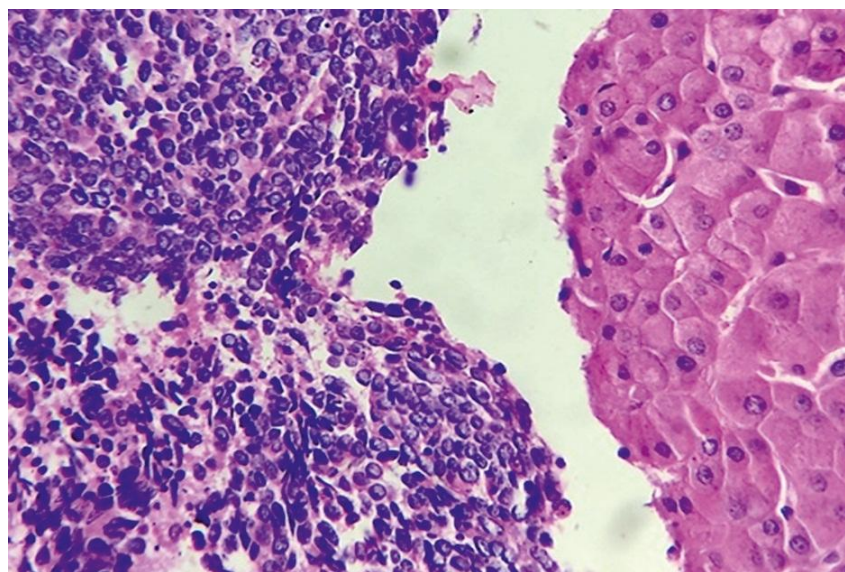
Obscuring background	Cytospin smear	%	Cell Block	%	P-value*
Large amount	8	10	7	8.75	
Moderate amount	67	83.75	56	70	
Minimal amount	5	6.25	17	21.25	0.01
Total	80	100	80	100	

\*Fischer exact test, significance level 0.05

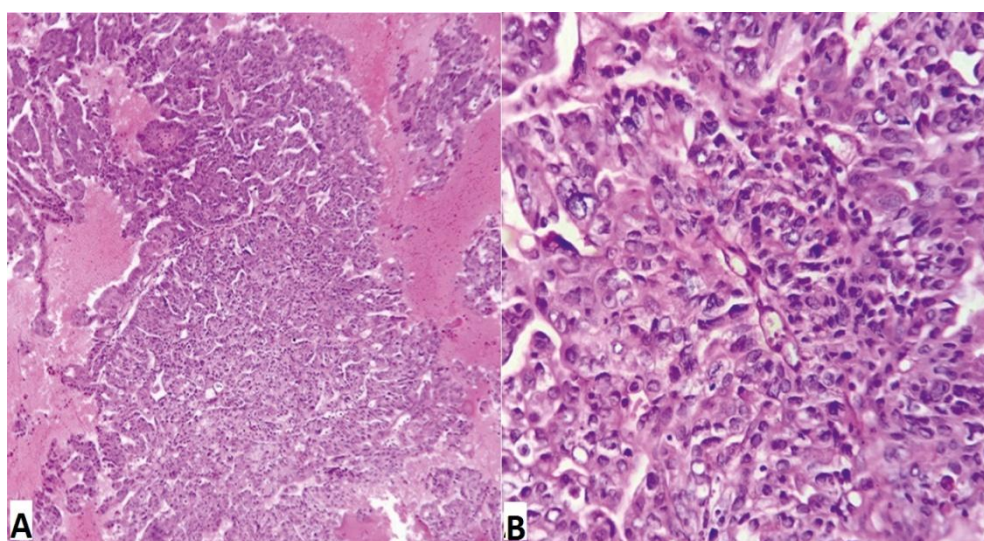
**Table No.6** Comparison of architecture in cytospin smears and cell blocks

Architecture	Cytospin smear	%	Cell Block	%	P-value*
Minimal	20	25	15	18.75	0.445
Moderate	58	72.5	51	63.75	
Excellent	2	2.5	14	17.5	0.003
Total	80	100	80	100	

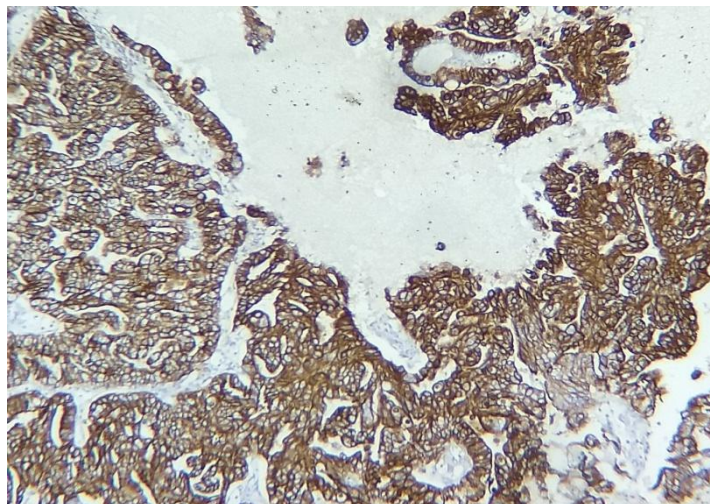
\*Fischer exact test, significance level 0.05



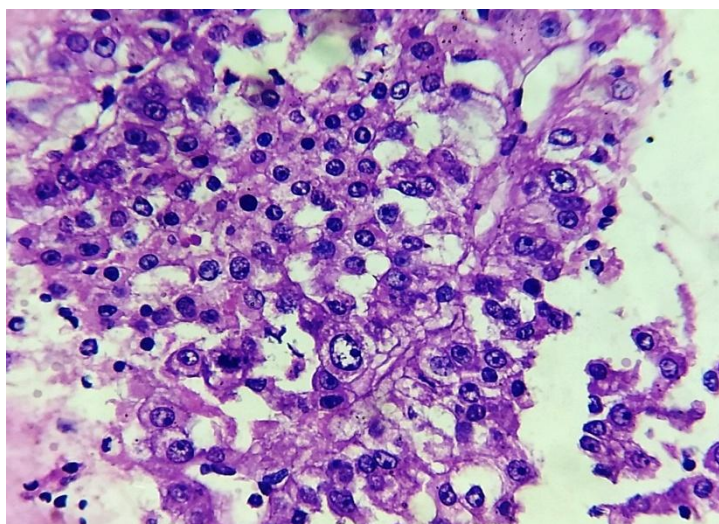
**Figure 1:** Cell block section from metastatic small cell carcinoma showing malignant small cells, apoptotic bodies, mitotic figures, and benign hepatocytes in excellent morphology. (Cell block, H & E stain, 400X)



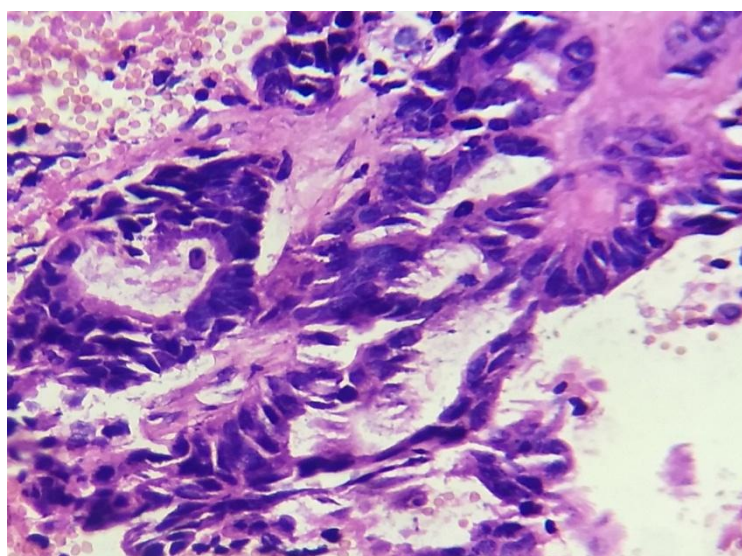
**Figure 2:** Metastatic cholangiocarcinoma. *A*, Glandular differentiation is apparent (Cell block, H & E stain, 100X). *B*, the tumor cells are in crowded sheets. Haphazard cellular arrangement and glandular differentiation are apparent. Anisonucleosis is seen. (Cell block, H & E stain, 400X)



**Figure 3:** Metastatic cholangiocarcinoma. Immuno staining with CK19 reveals prominent cytoplasmic and membranous positivity. (Cell block, 100X)



**Figure 4:** Moderately differentiated Hepatocellular carcinoma. Neoplastic hepatocytes showing increased N/C ratio and nuclear pleomorphism. (Cell block, H & E stain, 400X).



**Figure 5:** Metastatic adenocarcinoma from jejunum, cell block revealing excellent glandular architecture and atypical, hyperchromatic nuclei. (Cell block, H & E stain, 400X)

## Discussion

Although, FNAC has proved to be a reasonably efficient tool in diagnosis of liver neoplasms, there is tendency in a pathologist, whenever in doubt, not to subtype a malignancy solely based on cytospreads. Whenever there are limitations in diagnosis like poor differentiation of tumor, ancillary techniques like immunocytochemistry become mandatory for subtyping or classifying the malignancy. Many studies have shown that concomitant preparation of cell blocks in addition to cytospreads provide more cellularity, better morphology, tissue architecture and reduced obscuring background.

In our experience, cytospreads with cellular overlapping, obscuring background due to presence of blood and lack of proper tissue architecture posed a diagnostic dilemma. Also, difficulties arose in classifying poorly differentiated neoplasm into primary or metastatic. Preparation of TP-CB helped us immensely to overcome these diagnostic difficulties. Privileged with cell blocks, we could render more confident diagnoses and further subtyping of metastatic carcinoma.

In our study of 80 cases of liver SOLs, males (67.5%) outnumbered females (32.5%) with a male to female ratio of 2.076. The age of our patients ranged from 32 to 81 years. This was comparable to the study carried by Sheefa H. *et al.*<sup>7</sup> in which male to female ratio was 1.5 and the age of patients ranged from 32-90 years.

On applying Mair *et al.*<sup>4</sup> point scoring system, we noted statistically significant difference in overall diagnostic quality of cell blocks and cytospreads with 14 cell blocks (17.5%) being of superior quality in comparison to only 3 (3.75%) cytospreads. This implies that TP-CB method, when appropriately executed, provides better quality of sections when compared to cytospreads. 15 (18.75%) cell blocks and cytospreads each were non diagnostic due to suboptimal quality.

While analyzing the cellularity, the conventional smear showed minimal cellularity in 10 (12.5%) cytospreads in comparison to only 3 (3.75%) cell

blocks. This difference however was not statistically significant. Further, 26 (32.5%) CS and 34 (42.5) CB achieved a cellularity score of 2, thereby, providing an additional increase of 10% by preparing CB. This was in concordance with the study of Thapar *et al.*<sup>8</sup> who acquired increased cellularity in 13% cases. Lack of cellularity in cytospreads might be due to spreading of cells on a larger area of a slide while in a cell block, cells are concentrated over a smaller area. Another important factor might be the fact that most of the diagnostic material in acquired in the hub of the needle which sometimes gets stuck and cannot be utilized on a cytospread. The pressure of a needle rinse may get these stuck tissue fragments in the cell block.

In our study, no statistically significant difference was found in extent of cellular degeneration between cytospread and cell block. However, minimal cellular degeneration was found in 12 (15%) cell blocks in comparison to only 4 (5%) cytospreads. Another notable point was the marked cellular degeneration in 6 (7.5%) cytospreads and 8 (10%) cell blocks. This again emphasizes that cell blocks and cytospreads are complimentary to each other and are best utilized when assessed together.

On observing the amount of obscuring background, cell blocks had minimal amount of obscuring background with a score of 2 in 17 (21.25%) cases, which reduced to 5 (6.25%) in cytospreads. We experienced that obscuring background was less in cell blocks due to paraffin processing and cell wash during cell block preparation.

While comparing the architectural details in cell blocks and cytospreads in our study, excellent architectural preservation was found in 14 (17.5%) cell blocks compared to only 2 (2.5%) cytospreads. This difference was found to be statistically highly significant. Excellent architectural preservation in cell blocks is acquired as cellular fragments and clusters are fixed in formalin and embedded in paraffin. This results in better preservation of architecture as

seen in tissue obtained from biopsy. In cytosmears, fragments get folded and spread on slides making different pattern. Crushing artifacts in making smear also alters the original architecture to certain extent.

A possible explanation for non-diagnostic cell blocks might be the patient not consenting for a dedicated needle pass for cell block preparation. A dedicated pass was made in only 2 of the 15 non diagnostic cell blocks whereas in 24(70.6%) of the 34 cell blocks with a cellularity score of 2, we made a dedicated needle pass after obtaining consent. In our experience, a separate needle pass for cell block preparation could be made in 40 cases out of which 24 (60%) had abundant cellularity, 14(35%) had adequate cellularity and 2(5%) were non diagnostic. This correlated with the study of Shehnaz khan *et al.*<sup>9</sup> who achieved improved cellularity after making a dedicated needle pass for preparing cell block. Hence, we emphasize that a dedicated needle pass for cell block preparation improves cellularity and should be made if patient consents.

### Conclusion

From our experience, Thromboplastin-Plasma cell blocks prepared from liver SOL aspirates reveal better architecture and less obscuring background, when compared to conventional cytosmears. Preparation of cell block by Thromboplastin-Plasma method is cost effective but demands skill. On the other hand, conventional cytosmears are easy and quick to prepare and are known to have reasonable sensitivity in diagnosing malignancy. We conclude that cell blocks and cytosmears are best utilized when assessed together and cell blocks should be prepared whenever possible to aid to the cytosmear diagnosis.

**Conflicting Interest** (If present, give more details): None

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