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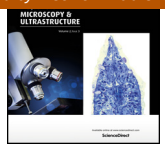

Original Article

Implementation of routine thromboplastin-plasma cell block technique in the evaluation of non-gynecologic specimens: A methodologic comparison with conventional cytology

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ABSTRACT

The cell block is an ancillary technique used in cytology to increase the diagnostic accuracy in the analysis of effusions and aspirations. In our laboratory, we implemented the routine use of the Thromboplastin-Plasma Cell-Block (TP-CB) technique because it is simple, reproducible and has low cost. The aim of this prospective study was to proof the utility of performing routine cell blocks in non-gynecologic cytology by comparing the diagnostic concordance, cellularity, and contribution to diagnosis from paired TP-CB and Conventional Cytological (CC) preparations. For this, all non-gynecologic specimens including effusions, body fluids and aspirations, were collected for an 8-month period. A total of 179 TP-CBs were prepared from the remaining fluid following CC preparations. Absolute concordance was found in 81.6% cases between both techniques ($\kappa = 0.56$). The cell block aided the diagnosis in 28% of cases and ICC studies were done in 12%. The use of routine TP-CB complements and enhances the diagnostic accuracy of CC, allows the performance of ancillary studies and improves the diagnostic approach and treatment.

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1. Introduction

For more than two decades the cell block has been used as an ancillary technique in cytology to increase diagnostic accuracy [1] in the analysis of effusions and aspirations. This technique enables small tissue fragments to be retrieved in a fluid specimen to form a paraffin block [1], which concentrates the cells in a limited field without loss of cellular material [2] and preserves tissue architecture [3]. Furthermore, additional sections can be obtained from a cell block to perform ancillary tests such as histochemistry, immunocytochemistry (ICC) or molecular studies (i.e., fluorescent in situ hybridization – FISH) [4].

Cell blocks can be prepared by several different methods; however, no ultimate technique has been established yet. In our laboratory, we decided to implement the use of the Thromboplastin-Plasma Cell-Block (TP-CB) technique as per routine for fluids and fine needle aspiration (FNA) specimens, following the procedure described by Kulkarni et al. [2], because it is simple, can be used in different types of specimens and has low cost.

This study was done prospectively, performing Conventional Cytological (CC) smears and TP-CB in every fluid or FNA available for an 8-month period. We described

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the characteristics of each sample, compared the diagnostic categories, and determined the degree of agreement between both techniques.

2. Materials and methods

All non-gynecologic effusions, body fluids, and aspirations were collected for cytological evaluation, from November 2011 until July 2012. We received a total of 179 samples, including fluids and FNAs (Table 1).

Either CC smears or cytospins were prepared from each sample. From the remaining fluid, 10 mL were centrifuged. In the case of aspirations, rinses of syringes and needles were collected in normal saline and then centrifuged [2]. The supernatant was carefully removed and the sediment was mixed with two drops of pooled plasma that was kept frozen and brought to room temperature before use.

Subsequently, four drops of thromboplastin were added and mixed again. The thromboplastin used for the TP-CB was the same as the one used for the thromboplastin test, and it should be stored in the refrigerator between 2 and 8 C° and brought to room temperature before use.

The tube was allowed to stand for 5 min and the resultant clot was slid into a premoistened with formalin filter paper, wrapped, and put in a cassette. The tissue cassette was then fixed in buffered formalin for at least 4 h. Afterwards, the sample was processed as usual for histological techniques [2].

Table 1
Cell block specimen source.

Specimen	No.
Body fluid, effusion or washing	
Peritoneal effusion	66
Pleural effusion	47
Cerebrospinal fluid	4
Bronchoalveolar lavage	3
Urine	2
Bronchial washing	2
Amniotic fluid	1
Pericardial fluid	1
Fine needle aspirations	
Ovary	12
Soft tissue and bone	11
Thyroid	9
Pancreas	6
Central nervous system	6
Breast	5
Lymph node	1
Liver	1
Parotid	1
Adrenals	1
Total	179

When there was no clot formation, four more drops of thromboplastin were added until a grossly visible clot appeared; then, it was processed as described above.

All cases were studied and interpreted by a pathologist and the following items were noted for both techniques: diagnostic category (non-diagnostic – ND, reactive/benign

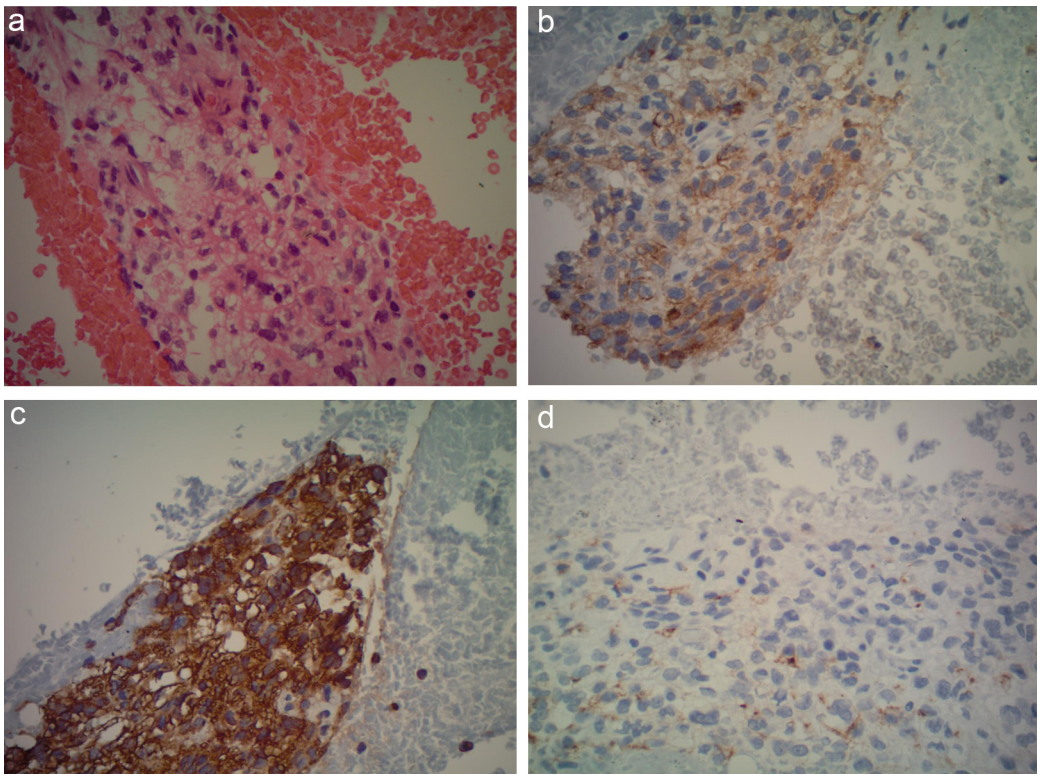

Fig. 1. (a) Cell block of a pancreas FNA showing a cluster of neoplastic cells with clear cell features. CC was acellular. Immunocytochemical showed positivity for EMA (b), vimentin (c), and focally for RCC (d). The case was signed as positive for malignancy, compatible with compromise by clear cell renal cell carcinoma and subsequently confirmed on surgical excision.

Table 2
Cellularity comparison between Conventional Cytological (CC) and Thromboplastin-Plasma Cell-Block (TP-CB) techniques.

Cellularity	CC (%)	TP-CB (%)
Acellular	6 (3.4)	12 (6.7)
Scant	58 (32.4)	55 (30.7)
Moderate	72 (40.2)	59 (33)
Abundant	43 (24)	53 (29.6)
Total	179	179

Table 3
Comparison of diagnostic categories for Conventional Cytological (CC) vs. Thromboplastin-Plasma Cell-Block (TP-CB) techniques.

	CC (%)	TP-CB (%)
ND	8 (4.5)	16 (8.9)
RB	135 (75.4)	122 (68.2)
A	10 (5.6)	9 (5)
M	26 (14.5)	32 (17.9)
Total	179	179

Abbreviations: ND: non-diagnostic; RB: reactive/benign; A: atypical; M: malignant.

– RB, atypical – A, or malignant – M), cellularity (acellular, mild, moderate, or abundant), contribution of the cell block to the diagnosis (yes, no or diagnosis remained the same), and immunocytochemistry studies performed (yes or no).

3. Results

The proportion of cellularity was similar for both techniques; however, TP-CB had a larger number of acellular cases (Table 2).

The diagnostic categories by both techniques were compared (Table 3). The diagnostic concordance between CC and TP-CB was moderate (kappa value, κ of 0.59) with 81.6% concordant cases (146 cases), and 18.4% of discrepant cases (33 cases). The proportion of cases was similar by both techniques, but CC had a larger proportion of RB than TP-CB (75.42% vs. 68.16%). Even though TP-CB had more ND specimens when compared with CC (8.94 vs. 4.47%), the number of malignant diagnosis was higher with TP-CB (17.88% vs. 14.52%). TP-CB allowed the reclassification of 4 ND cases as malignant (Table 4) (Fig. 1).

Among discordant cases, in the RB category with CC, TP-CB was able to reclassify 4 cases as atypical and 6 as malignant. Four of the 10 cases cataloged as atypical by the CC were correctly classified as RB (3 cases) and malignant (1 case) on the TP-CB.

Cell block aided diagnosis in 28.5% (51) of the cases; the diagnosis remained the same in 55.3% (99), and did not give any additional information in 16.2% (29) cases.

Immunocytochemistry studies were performed in 21 (11.73%) cases. Of these, 17 received a more accurate diagnosis with the use of ICC (Figs. 2 and 3). The markers used in order of frequency were: calretinin, MOC 31, CEA, D2-40,

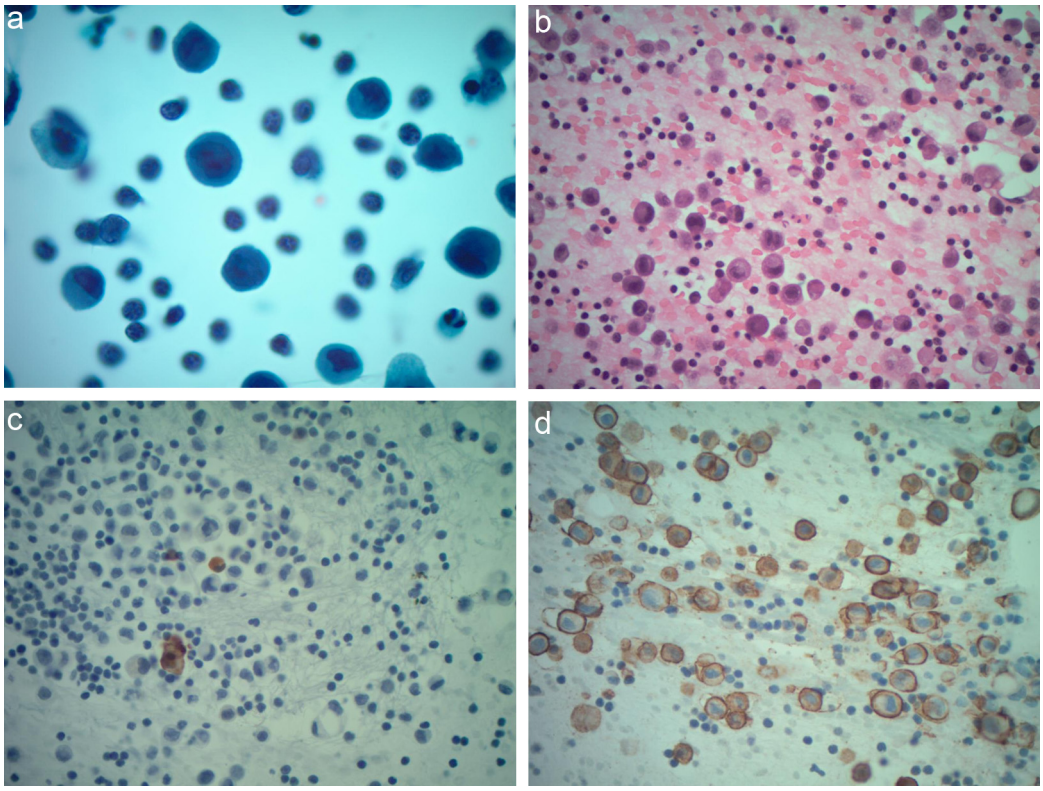

Fig. 2. (Pap stain) CC of an ascitic fluid with increased number of single epithelioid cells that resemble mesothelium in an inflammatory background (a) also noted on the cell block (b). ICC studies on TP-CB were negative for calretinin (c) and positive for MOC31. The effusion was positive for malignancy and compatible with history of signet ring cell adenocarcinoma of the stomach.

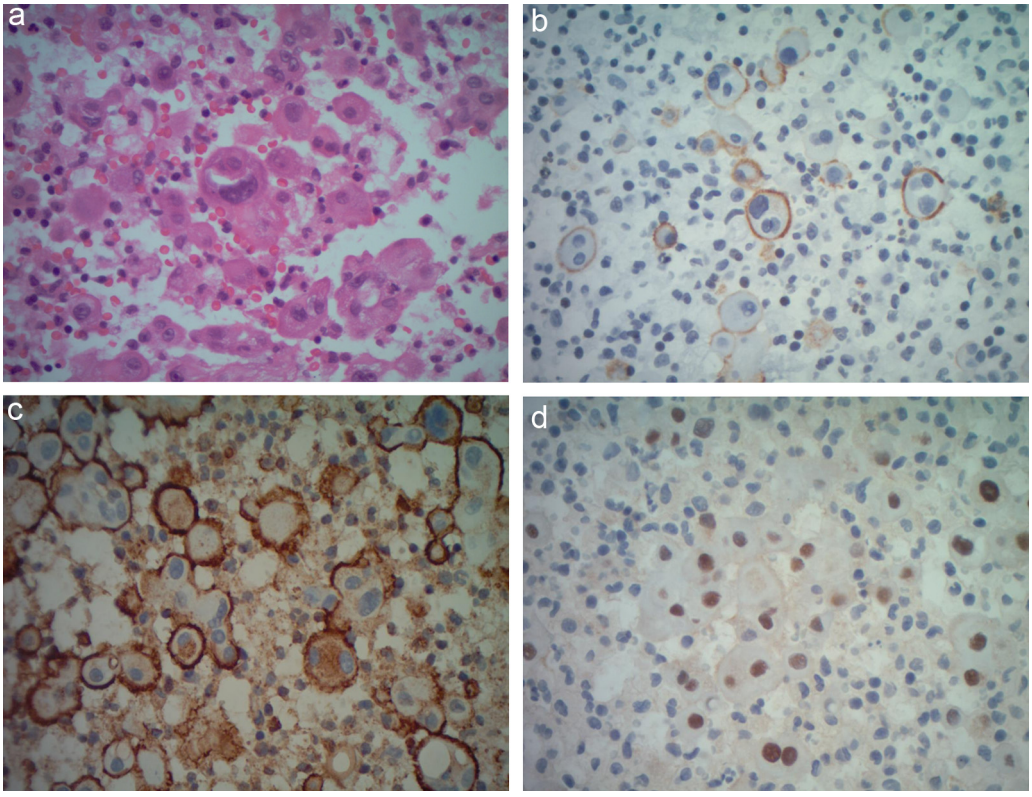

Fig. 3. TP-CB of a pleural effusion with highly atypical epithelial cells (a). These cells were positive for D2-40 (b), calretinin (c) and WT1 (d); and were negative for MOC31 and CEA. ICC findings confirmed mesothelial origin of this malignant neoplasia.

BRST-2, p53, CD68, TTF1, desmin, WT1, CDX2, vimentin, CK7, CK20, AE1/AE3, CD10, RCC, RE, and CA125.

4. Discussion

The use of cell-block as an ancillary technique for cytological evaluation can assist and increase the diagnostic yield from effusions, body fluids and washings, when used in conjunction with CC smears.

The cytological examination has increasingly gained acceptance in clinical medicine, as it aids in the diagnosis, staging and prognosis [3] of multiple diseases, now even more with the surge of minimally invasive procedures. Cell blocks work as adjunct tools to CC smears for establishing a definitive cytopathologic diagnosis [5].

Our objective was to proof the utility of the routine use of TP-CB by assessing the concordance between diagnostic categories, cellularity, contribution to diagnosis and possibility to perform ancillary studies comparing both techniques CC vs. TP-CB.

As described in previous studies, we found that with the TP-CB technique the cellular elements were preserved and concentrated in a small area, making their evaluation less time-consuming [6] and could be used in different types of samples, providing an accurate diagnosis. Our concordance (81.6%) was slightly lower than the reported in the literature (94%) [2]; but the kappa index was within acceptable limits (moderate agreement; $\kappa = 0.56$).

Table 4
Agreement matrix for diagnostic categories for Conventional Cytological (CC) smear and Thromboplastin-Plasma Cell-Block (TP-CB) techniques.

CC Categories	TP-CB Categories				
	ND	RB	A	M	Total
ND	3	1	0	4	8
RB	8	117	4	6	135
A	1	3	5	1	10
M	4	1	0	21	26
Total	16	122	9	32	179

Abbreviations: ND: non-diagnostic; RB: reactive/benign; A: atypical; M: malignant.

Of the 33 discordant cases, 24 were explained by sampling (9 of them sample non diagnostic on the CC but diagnostic on the TP-CB, and of them 15 sample non diagnostic on the TP-CB but diagnostic on the CC). The remaining 9 cases were considered Atypical either in the CC smear or the TP-CB, among this cases 7 of them had follow up histology positive for malignancy (4 CC smears, 3 TP-CBs).

The main advantages of cell blocks are: the possibility of obtaining multiple sections for ancillary tests as special stains and ICC [3], and the improvement or contribution to CC original diagnosis; in our study 28.5% of the cases

obtained a more accurate diagnosis. Therefore, it can be a valuable diagnostic adjunct to CC [7], especially those cases with no clear definitive diagnosis, those in which the morphological characteristics are similar and those where additional studies are required [1].

5. Conclusion

Cell block is considered and aid that enhances the use of available material in non-gynecologic specimens concentrating cells in a limited area permitting for an easier, more detailed, less time consuming and sometimes more accurate microscopic evaluation and can be used for ancillary techniques, such as special stains and inmunocytochemistry. Therefore we consider that its routinely use in non-gynecologic specimens improves the diagnostic approach of noninvasive samples.

Conflicts of interest

None declared.

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