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Cell block technology: bridgingcytology and histology

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Introduction

Diagnostic cytology is an amazing science that allows us to interpret cells from the human body, such as those found in fluids or through fine needle aspirates.This residual material can be evaluated quickly and easily by forming a cell block, embedding it in paraffin, and examining it in addition to the routine cell smears (CS). It has been observed in numerous studies that the cytologic examination of fluids and fine needle aspirates by means of smears leaves behind a significant residue that is not further investigated and that may contain valuable diagnostic material. In addition to traditional cytomor phology, immunocyto chemistry is being utilized to diagnose fine needle aspirates.

However, smears have significant disadvantages, such as a small number of smears for testing, non-specific staining, and a lack of parallel samples of the same cells for further or control tests. Additionally, the price is increased by using large volume of antibodies to cover a vast region of less cellular smears. In order to analyze such materials, cytological smears are frequently used. This review compiles information on cell block (CB) technology in the field of pathology.

Definition of cell block

Cell block (CB) is the term used to describe the collection of sediment, blood clots, or visibly large pieces of tissue from cytologic specimens that are processed into paraffin blocks and stained primarily with the stain known to all pathologists especially, hematoxylin-eosin(Jain et al., 2014).It combines the fields of cytology and histology.

History of cell block

First, there is a lack of an ideal cell block approach that produces reproducible high cellularity, is not labor or skill intensive, doesn't demand for a particular type of media, and is affordable. Second, technology in various facets of medicine is always being developed and improved.

Since 1986, Bahrenburg first developed the CB technique, it has been widely applied to the processing of fluids. Mandelbaum developed a method for CB preparation in 1917. Zemansky came to the conclusion that the CB approach was superior than the CS technique in 1928 and that it was unreliable to examine materials other than pleural and ascitic fluids. Early CB preparation techniques did not get much attention, presumably because there was no defined process. The potential of material loss during preparation is, in fact, the primary issue with CB preparation.The use of a centrifuge in 1901 was the following significant development. CB for Serous effusion modified by Chapman and Whalen in 1947, changed the course of CB research.Agar usage was reported by Shackelford and Jones in 1959, and the plasma-thrombin technique was first used by Kaltenbach et al. in 1973. In 2007, Hologic Inc. (Marlborough, Massachusetts) unveiled the Cellient Automated Cell Block System, which to our knowledge was the first automated cell block preparation system.

Disadvantages of CS

- Indistinct morphological details
- Overlapping or overcrowding of cells
- Abundance of inflammatory cells
- Paucity of representative cells
- Cell losses or changes
- Lack of architectural patterns
- Improper smear, fixation, and staining techniques

Advantages of cell block

The advantages of the CB procedure include:

1. Recognition of histological patterns of diseases that sometimes cannot be identified reliably in conventional smears.

2. Possible to study multiple sections by routine staining, special staining and immunocytological procedures.

3. Less cellular dispersal, which permits easier microscopic observation than do traditional smears.

4. Less difficulty in spite of background showing excess blood on microscopic observation.

5. Possibility of storing slides for retrospective studies. Storage of the CS is a practical problem.

6. CB provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin.

The nucleoli do not display as prominently in the CB as they do in the CS, and when pseudoacinar or acinar structures are present, they can be seen more clearly. The CB successfully places both characteristics in their proper perspective. The concentration of cellular material in a condensed region that can be quickly assessed with all cells in the same focal plane of the microscope is another benefit of CB(Shivakumarswamy et al., 2012).

Cell block preparations using liquid specimens

The preparation of CBs generally has the potential to impede the rapid flow of cytological diagnostics. Any kind of cytological material is acceptable, including FNAs, liquid-based preparations, brushings, bodily fluids, and scraped cells. The cells in cytological material are intact and unprocessed.

Buffered formalin, neutral buffered formaldehyde solution, Bouin solution, picric acid fixative, Carnoy fixative, and ethanol are few histologic fixatives that have been utilised for CB. From the perspective of cytologists, formalin produces the least desirable results when trying to distinguish nuclear features. Alcohol formalin fixative was first introduced by Nathan. Its benefit is the preservation of antigenicity by alcohol and the minimization of cell destruction by formalin (Nathan et al., 2000). By lysing red blood cells and solubilizing

protein, the CytoRich Red Preservative, which contains isopropanol, methanol, ethylene glycol, and formaldehyde, enhances cellular yield without changing the shape of cells (Krogerus & Kholová, 2018).

The ideal formalin solution for CB is 7.5% buffered formalin, and a shorter xylene exposure time is preferred. Nevertheless, xylene significantly shrinks cells. Fixation and processing of the samples must be changed in order to enhance the utility of a cell block such that its morphology resembles that of the usual paraffin slices of surgical specimens(Shivakumarswamy et al., 2012).

Diagnostic accuracy: Sensitivity, specificity

Success of any diagnostic method vested with sensitivity and specificity. In that context, few research have emphasised the aspects mentioned above that make CB a superior diagnostic tool.

Combining FNACs and cell blocks yielded NPV and diagnostic accuracy of 98.27%, 100%, 100%, 92.85%, and 98.59%, respectively. On cell blocks, the sensitivity, specificity, PPV, NPV, and accuracy were, respectively, 98.18%, 93.75%, 98.18%, 93.75%, and 97.18%. Additionally, the study demonstrates that cell blocks have higher diagnostic precision than FNAC.A study found that the combined FNAC and cell blocks had sensitivity, specificity, PPV, NPV, and accuracy values of 98.27%, 100%, 100%, 92.85%, and 98.59%, respectively. The combined FNAC and cell block sensitivity was 98.27%. Higher sensitivity, specificity, PPV, NPV, and diagnostic accuracy were achieved whenboth FNAC and cell blocks was interpreted(M et al., 2022).

CB were more accurate than CS in diagnosing pancreatic tumours by 61% to 85.2% (P 0.001), 100% to 93.1%, 100% to 98.4%, 36% to 55.1% (P 0.046), and 68% to 86.5% (P 0.001). Compared to cytological smears, the cell block approach displayed improved sensitivity, negative predictive value, and accuracy(Bhowmik et al., 2018).CS and CB provided similar diagnostic yield (48.7% and 49.9%). However, combination of both gave a higher yield (57.2%)(Assawasaksakul et al., 2017).

Similarly, CB had a 67.1% sensitivity rate, while only 32.3% of the results in CS were positive(Nair & Manjula, 2021).Comparing cytological smears, cell blocks indicated greater diagnostic accuracy. Out of 70 cases, malignant cells could be found in 46 (65.7%) of the cases. Diagnostic precision varied between 50.4% (lymphoma) and 71.4% (malignant ovarian

neoplasm). Cell block approach raised the same diagnostic yield by 20% (from 65.7 to 85.7%), but cytological smear examination increased the diagnostic yield for malignant cells by 17.14% (from 54.28 to 71.42%). The cell block method not only improved the beneficial results, but also served to show superior architectural patterns that might be very helpful in approaching the accurate diagnosis of the primary site. Cell blocks and smears were used to identify the main location of cancer in 83.3% of the patients.Diagnostic accuracy was improved by 13.0% when the cell block approach and smear examination were used simultaneously(Mishra et al., 2009).Lung malignancies were evidenced in CB at sensitivity of 96% and specificity of 92.5%(Sutanto et al., 2021).

High Cellular yield

When compared to the cellular yield obtained using the CS approach, the CB method produced a higher yield.Comparatively to the single scattered cells, glands, and cell clusters identified in the CS findings, architectural patterns such glands, three-dimensional cell clusters, cell balls, and sheets were frequently observed in the CB approach(Singh et al., 2018).For the CB preparation, a 10% alcohol-formalin fixative was utilised. This improved cellularity because formalin reduced cell loss by generating protein cross linkages and gels that were resistant to the various chemicals employed in processing(Shivakumarswamy et al., 2012).

In 120 cases of reactive effusion, there was scant cellularity in 40 (33.3%) cases, inflammatory infiltrate in 26 (21.7%) cases, blood in eight (6.7%) cases, mixed cellularity with moderate cell count in 24 (20.0%) cases, lymphocyte predominance in 16 (13.3%) cases, and mesothelial cells in six (05.0%) cases(Mishra et al., 2009).Additionally, there were no FNA samples (0/47) that had a CB cellularity score of zero. About 60% (18/30) of the 30/47 cell block samples that were aspirated directly into the vial containing Shandon's Formal-Fixx fixative demonstrated sufficient cellularity (score 2 and 3). This indicates that higher yield may be achieved with a special needle aspiration for cell blocks(Khan et al., 2012).

A high-quality cell blocks accounting for51.8% are with high cellularity terminal FNA. This emphasis the good collection of aspirates directly contributes to the high-quality blocks. Performing additional sections in low cellularity aspirates is likely to yield medium quality blocks(Collins et al., 2015).

Superior cellular morphology

Reactive mesothelial cells, an overabundance of inflammatory cells, and a lack of representative cells all contribute to the CS method's significant challenges in reaching a definitive diagnosis. Reactive alterations in the mesothelial cells, such as cytomegaly, nucleomegaly, multinucleation, mitotic figures, and a high N/C ratio, may be present. There are some restrictions in the diagnosis of malignant lesions due to the conventional cytology's (40%–70%) lower sensitivity compared to cell block preparation. Due to cell crowding or cytological atypia brought on by different processing techniques, the cytomorphological traits might occasionally be difficult to diagnose(Singh et al., 2018).

Better immunocytology

The higher preservation of nuclear and cytoplasmic features made the Pap-stained FNA smear the preferred method for routine diagnostics, but the cell block technique was better suited to immunocyto chemical analysis. According to recent research, cell block samples work best as a support for ICC rather than as a stand-alone tool for cytological diagnosis(Khan et al., 2012).

Differentiating the stages of cancer

By using the cell block method, we reported three additional cases of cancer that had been flagged as suspected of cancer using more traditional techniques. It was determined that the CB approach was significantly more useful than the CS method for the cytodiagnosis of malignant effusions.CS is important not only in diagnosis but also in staging and guiding treatment for malignancy.Malignant primaries made up the majority of the 102 cases (100%), with 40 cases (39.21%), followed by metastatic cases with 29 instances (28.43%). About 16 cases (15.68%) were inflammatory, and 16 cases (15.68%) were benign(M et al., 2022).The findings were classified as benign, possibly malignant, and malignant.

In general, 45 malignant lesions on FNAC were divided into carcinoma (36 instances), lymphoma (6 cases), sarcomas (2 cases), and melanoma (3). (1case). 42 (95.45%) of the 44 "malignant" fluid cases were classified as carcinomas, and 2 (4.55%) were lymphomas(“Role of Cell Block in Diagnostics-a New Paradigm in Cancer Diagnosis,” 2015).The CB technique demonstrated a 100% sensitivity in the diagnosis of cancer. By using the cell block approach, a further nine cases—or 15% more diagnostic yield for malignancy—were identified as malignant. These samples were classified as benign or suspected for

cancer(Shivakumarswamy et al., 2012).A malignant diagnosis was rendered in 35% (132, 132/372), neoplasm in 5% (17, 17/372), 2% suspicious (9, 9/372). Thirteen percent were with descriptive of benign findings.A better diagnostic yield can be evidenced by positive, negative and accuracy of CB versus CS in diagnosing pancreatic tumors were 85.2% vs 61%, 100% vs 98.4% and 68% vs 86.5% respectively("Role of Cell Block in Diagnostics-a New Paradigm in Cancer Diagnosis," 2015).

In lung cancer patients, the most common lesions identified by CB was 64% adenocarcinoma and 29.4% neuroendocrine tumors.Results showed 12% positives in all techniques CB, bronchial washing smears and smears. Sensitivity of CB technique for bronchial washing smears was 85.7% while specificity was 75%(Sutanto et al., 2021).

Conclusion

High cellularity, superior architectural patterns, morphological details, and an increased yield for cancerous cells are all provided by the CB approach. Cell block studies should therefore be performed frequently in addition to traditional techniques for better diagnosis.

Combined use of smears and cell blocks increases sensitivity remarkably in cytologic diagnosis. In 'malignant'cases, cell blocks are superior to smears in showing positivity in higher percentage of cases.In 'suspicious' lesions cell blocks again are superior to smears for giving a definitive diagnosis and categorization of lesion. Cell block preparations, of FNAC fixed in 10% formalin and paraffin embedded are suitable and reliable for application of Immunomarkers. Multiple sections from cell blocks can be obtained for application of multiple IHC antibodies for purpose of differential diagnosis.Cell block technique is simple, inexpensive and reliable adjuvant to smears and it is recommended for routine cytologic diagnosis and application of immunomarkers.

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