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CYTOSCAPE CELL BLOCK IMPROVES CYTOLOGICAL EVALUATION OF FINE NEEDLE ASPIRATION SMEARS AT KENYATTA NATIONAL HOSPITAL (NAIROBI, KENYA): A CROSS-SECTIONAL STUDY

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CYTOSCAPE CELL BLOCK IMPROVES CYTOLOGICAL EVALUATION OF FINE NEEDLE ASPIRATION SMEARS AT KENYATTA NATIONAL HOSPITAL (NAIROBI, KENYA): A CROSS- SECTIONAL STUDY

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ABSTRACT

Background: There are more than nine methods used to prepare cell-blocks. Cytoscape cell-blocks have been found to be effective for haemorrhagic and overly thick smears. Our study utilized the direct formalin method for the preparation of cytoscape cell-blocks.

Objective: to assess the utility of cytoscape cell-blocks for evaluation of fine needle aspiration (FNA) smears at Kenyatta National Hospital, Nairobi, Kenya.

Methodology: This was a cross-sectional (laboratory-based) study involving 55 smears of breast and axillary lymph node aspirations reported as 'suspicious' or 'malignant'. Scrapings from fine needle aspiration smears were processed as a micro-biopsy. The cytoscape block findings were compared with smear findings, and cyto-histological correlation was made whenever available. Data were analyzed using Microsoft Excel 2019 and Cohen's Kappa test for agreement. Categorical data were summarized, and Kappa was calculated at a 95% CI. P-values <0.05 were considered statistically significant.

Results: cellular material was successfully recovered in 85.2% (47 of 55) of smears. Architectural picture was improved in 58.5% (n=16) of cases, providing additional information. The overall agreement between the cytoscape technique and conventional smears was 72.3% with a kappa score of 0.43 (95% CI; p<0.001). Cytohistological correlations were performed in 20 cases. The agreement between the cytoscape technique and histology was 85%, with a Kappa score of 0.69 (95% CI; p = 0.006).

Conclusion: The cytoscape technique could enhance the utility of cytology in diagnostic workflows. It is an essential tool for secondary analysis of conventional specimens when FNA slides are the only available specimens.

INTRODUCTION

Cell-block preparation has been an integral component of cytology practice for over a century¹⁻³. It is an excellent adjunct to conventional smears, particularly for ancillary tests (immunocytochemistry and molecular studies)^{1,4}. It provides improved architectural preservation comparable to histological specimens and allows for multiple sections to be obtained for further diagnostic evaluation^{2,5-7}. Fine needle aspirates (FNA), body cavity fluids, washings, brushings, and gynaecological specimens are among the cytology specimens on which cell blocks can be prepared¹. The introduction of the cytoscape technique (scraped material from cytology smears) in the year 2000 has expanded the utility of cytology⁸. This technique was designed to maximize diagnostic yield by enabling the recovery of diagnostic material from previously prepared FNA smears, particularly in cases where no additional specimen is available or where a cytology slide is the only available specimen^{5,8,9}. Its effectiveness has been demonstrated, including its utility in haemorrhagic and overly thick smears^{5,6,9}. Over time, cell-block⁸ preparation has evolved, with approximately nine preparation methods currently described among which the Plasma Thrombin (PT) method is the most commonly used^{1,3,4}. Despite these advancements, conventional cytoscape cell-block preparation methods, such as agar-based and fixed sediment techniques, remain labour-intensive, time-consuming, and technically demanding, limiting its widespread adoption in routine clinical practice^{1,8}. Additionally, challenges such as loss of cellular material during processing and inadequate yield in hypocellular samples continue to affect diagnostic accuracy^{9,10}. Although simplified approaches, including the direct formalin

method, have demonstrated feasibility in fluid specimens and FNA needle rinses, their application in cytoscape-derived material remains under-explored^{1,3,4}. This represents a critical gap in optimizing cytological specimen utilization, particularly in areas where histology is not available or where repeat sampling may not be feasible.

This study, therefore, aimed to evaluate the effectiveness of a simplified direct formalin cytoscape cell-block technique in improving cytological evaluation of FNA smears at Kenyatta National Hospital, Nairobi, Kenya. Specifically, the study sought to assess whether this approach enhances cellular yield and preserves tissue architecture, thereby improving its overall utility compared to conventional smear-based evaluation.

METHODOLOGY

This was a descriptive cross-sectional study of 55 fine needle aspiration (FNA) smears of breast lesions and axillary lymph nodes screened between January 2017 and December 2020. The study was conducted at Kenyatta National Hospital (KNH), the largest teaching and referral hospital in Kenya. The hospital is located along Hospital Road in the Upper Hill area of Nairobi City. Data collection, smear retrieval, and processing were conducted at the histology and cytology laboratories between November 2023 and April 2024.

Technique and ethical consideration

Eligible for inclusion were Haematoxylin and Eosin (H&E)-stained smears of breast and axillary lymph node FNA reported as 'suspicious for malignancy' or 'malignant' within the study period. Only cases with more than one slide available in the archive were included to ensure an extra slide was retained at the laboratory archives for purposes of future reference. Extensively degraded smears, broken slides, and cases

that lost diagnostic material during processing were excluded.

Processing was done using the initial procedure described, bypassing the gelling step ⁸. Briefly, coverslips and mounting media were removed from the slides by dipping in xylene for thirty (30) minutes. The H&E-stained smears were passed through descending grades of alcohol for rehydration, de-stained in 1% acid alcohol, and thoroughly rinsed in running tap water. A scalpel blade was then used to scrape the smears entirely into a biopsy paper, wrapped tightly, and placed in a tissue cassette. The material was fixed in 10% buffered formalin overnight before being transferred into the automatic tissue processor. Four- micrometres thick sections (Cytosrape sections) were cut from the paraffin wax block for H&E staining. They were then evaluated and compared with the aspirate smear findings. Cyto-histological correlation was done for cases whose histology findings were available.

Ethical approval to conduct the study was obtained from Kenyatta National Hospital-UoN Ethics and Research Committee (KNH-UoN ERC), approval no. P679/10/2023.

Data Management: Data were entered and cleaned in Microsoft Excel 2019, and Cohen's Kappa statistic was used to test for agreement. Measures of central tendency were used for numerical continuous variables (e.g., age). Categorical data were summarized, and the summaries were presented in tables. Kappa values were calculated for the categorical data at a 95%

confidence interval and p-values <0.05 were considered statistically significant.

Quality assurance: all procedures (destaining, processing, and staining) were performed as stipulated in the Standard Operating Procedures (SOPs) for reproducibility. The smears were mounted with a drop of water to keep them wet for ease of scraping, preserve specimen integrity, and minimize loss of diagnostic material. When embedding in paraffin, the side of the biopsy paper containing the scraped material was placed facing down in the tissue mould. Only specialized biopsy papers were used for the study since the standard coffee-style filter papers can sometimes leave fibres that interfere with sectioning. As in standard practice, the stains were changed regularly and filtered before use to minimize artifacts during the microscopic evaluation.

RESULTS

A total of fifty-five (55) fine needle aspiration (FNA) cytology smears were included in the study: 39 cases of breast FNA and 16 auxiliary lymph node FNAs. Of these, 47.3% (n =26) were suspicious for malignancy, while 52.7% (n =29) were malignant, as shown in Table 1. The mean age of patients was 46.4 (SD±12.4) years, with 60% (n =33) of them aged less than 50 years. The minimum age was 12 years, and the maximum age was 81 years. Most of the patients were female, 96.4% (n =53).

Table 1

Preamble of FNA cytology smears included in the study and processed for analysis (n=55)

	Frequency	Percentage (%)
Breast		
Malignant	19	34.5
Suspicious for Malignancy	20	36.4
Lymph node metastases		
Malignant	10	18.2
Suspicious for Malignancy	6	10.9

Cytoscape cell blocks (CSB) were prepared from all 55 FNA smears and sections from each examined. CSB retained diagnostic material in 85.5% (n=47) of cases, providing useful clinical information. The staining quality of the sections with Haematoxylin and Eosin (H&E) stains was of excellent quality, with distinctive morphological

features for categorization. Of these, 58.2% (n=32) were diagnosed as malignant, 14.5% (n=8) suspicious for malignancy, and 12.8% (n=7) as benign. Cytoscape sections provided a better architectural picture, providing additional information in 58.5% (n=16) of cases. Eight cases (14.5%) lost cellular material during processing.

Table 2

Summary of the diagnoses on cytoscape cell blocks (CSB) (n=55)

	Frequency (n)	Percent (%)
Breast		
Malignant	24	43.7
Benign	7	12.8
Suspicious for malignancy	8	14.5
Lymph node metastases		
Malignant	8	14.5
Lost cellular material	8	14.5

We compared the modified cytoscape cell block (CSB) with fine needle aspiration cytology diagnoses, as shown in Table 3. The two techniques had an agreement of 68.8% (n = 22) for malignant diagnoses (Table 3).

On Cohen's Kappa, the two techniques revealed an overall agreement of 72.3% with a kappa score of 0.43 (95% confidence interval, p<0.001).

Table 3

Comparison of cyto-scape cell blocks with conventional smears diagnoses (n=47)

		<u>Cytoscape Cell Block Technique</u>	
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<u>Conventional Cytology</u>	Benign n (%)	Suspicious n (%)	Malignant n (%)	Agreement (%)	Kappa	Std. Err.	Z	P value
Suspicious	6 (100)	6 (66.7)	10 (31.2)					
Malignant	0 (0)	3 (33.3)	22 (68.8)					
Overall Agreement				72.3	0.43	0.139	1.139	<0.001

Cyto-histological correlation was performed in 20 cases whose histological diagnoses were available. All cases that were malignant on FNAC smears were histologically confirmed as malignant, while six (6) cases categorized as "suspicious" on FNAC were

benign on histology. All five (100%) benign and 11 (73.3%) malignant histology cases were correctly identified by CSB. Three (3) of the malignant histology cases were categorized as suspicious on CSB, as summarized in Table 4.

Table 4*Cyto-histological correlation of cyto-scrape cell block and fine needle aspiration diagnoses (n=20)*

Histology diagnosis	Lesion categories	FNA cytology smear		Cytoscrape cellblock		
		Suspicious n (%)	Malignant n (%)	Benign n (%)	Suspicious n (%)	Malignant n (%)
	Benign	6 (54.5%)	0	5(100%)	0	0
	Malignant	5 (45.5%)	9 (10%)	0	3 (100%)	12 (100%)

The agreement between FNAC smears and histology was 75% with a kappa score of 0.52 ($p = 0.014$ at 95% confidence interval). The agreement between CSB and histology was

85%, with a kappa value of 0.69- substantial agreement ($p = 0.006$ at 95% confidence interval) (Table 5).

Table 5*Level of agreement of the two cytology techniques with histology (n=20)*

Method	Agreement (%)	Kappa	Std. Err.	Z	P value
Conventional cytology smears	75.0	0.52	0.196	2.65	0.004
Cytoscrape cell blocks	85.0	0.69	0.212	3.24	0.006

DISCUSSION

This was a cross-sectional study involving 55 fine needle aspiration cytology (FNAC) smears from breast and axillary lymph node lesions at Kenyatta National Hospital. From this study, the modified cytoscrape technique improved cytological evaluation of the FNA smears. Useful material in most cases was retained, enhancing architectural detail and showing better cyto-histological agreement compared to conventional smears. This finding aligns with the increasing role of cell block preparations as an adjunct to routine cytology, especially where tissue architecture and confirmatory ancillary studies are required^{5,8,10}.

The primary site of malignancy was the breast (63.6%), with significant number of cases with metastases to the axillary lymph nodes (36.4%). This distribution reflects the common pathways of metastasis in breast cancer, where the axillary lymph nodes are often the first site of metastatic spread¹¹. The mean age of patients was 46.4 (SD±12.4) years, with a significant portion (60%) under

the age of 50. Notably, the youngest patient was 12 years old, demonstrating that the disease can manifest across all age groups. Comparable age bracket of patients has been reported in previous studies^{11,12}.

A major finding of our study was that CSB recovered diagnostic cellular material in 85.5% of cases and produced readable slides. These findings are consistent with previous studies that demonstrated preservation of cellular material ranging from 91.6% to 100%^{1,5,8,10}. Notably, loss of cellular material was almost entirely observed in smears that had scant/thin material to scrape. Slides with thicker smears should therefore be selected for preparation of cytoscrape cell blocks whenever possible⁸. The formalin method is also more susceptible to loss of material during processing compared to cell button-based approaches^{1,8}. Extra caution is recommended during processing when using this approach.

Morphological features were well preserved from the smears, providing valuable information for categorization. The staining quality of the sections with Haematoxylin

and Eosin (H&E) stains was excellent and readable in all the cases. Architectural picture was improved in 58.5% (n=16) of cases, providing additional information. Specifically, this was frequently observed in smears that were initially categorized as 'suspicious for malignancy'. This finding is comparable with previous studies that reported a better capacity for CSB to provide clearer information for enhanced categorization of lesions compared to FNA smears^{1,8,10}. One study, however, reported that smear diagnoses correlated well with the findings on CSB with no additional information⁵.

On comparing the diagnoses of the formalin-based cyto-scrabe technique with conventional smears, the two tests had an agreement of 72.3% (kappa = 0.43, with 95% confidence interval), which is comparable with previous findings¹³. The fact that 68.8% of patients with malignancy were identified by both CSB and conventional cytology indicates a substantial level of consistency between the two methods but also highlights areas for potential improvement^{8,13}.

Our study also performed a cytohistological correlation by comparing the two cytology techniques (conventional cytology and cyto-scrabe cell block) with histology as the gold standard. CSB demonstrated greater architectural clarity compared to conventional smears providing clearer information for categorization, particularly in cases with morphological overlap. This has been demonstrated in previous literature^{1,8-10}. The higher agreement between CSB and histology (kappa = 0.69, p=0.006) compared to conventional smears (kappa = 0.52, p=0.004) highlights that CSB could be valuable in enhancing the utility of fine needle aspiration smears. This has been supported by previous research^{4,14-18}.

The overall agreement between conventional cytology and CSB was moderate, with a kappa score of 0.43. Although this indicates only moderate

concordance, it also shows that the two techniques are not simply duplicative; rather, CSB provides incremental information that changes interpretation in a meaningful subset of cases. This is consistent with the purpose of cell block preparation in cytology, which is to complement smear-based diagnosis rather than replace it^{2,8,10,18}. More importantly, CSB showed superior agreement with histology than conventional smears. In the cases with histological follow-up, CSB achieved 85% agreement and substantial agreement with histology (kappa 0.69), compared with 75% agreement for conventional smears (kappa 0.52). This improved cyto-histological correlation suggests that CSB may reduce diagnostic uncertainty in lesions where smear morphology alone is not sufficient for definitive interpretation^{5,6,9}.

Limitations of the study

This study does have limitations. The sample size was small, histological confirmation was available only for a subset of cases, and the work was performed in a single institution, which may limit generalisability. In addition, the study focused on breast and axillary lymph node lesions, so performance in other specimen types cannot be assumed. However, despite these limitations, the findings are strengthened by the direct comparison of conventional smears, CSB, and histology.

CONCLUSION AND RECOMMENDATIONS

The cytoscrabe technique produced high-quality sections and readable slides when stained with Hematoxylin and Eosin stains. This resulted in preserved diagnostic material, improved architectural assessment, and better cytohistological agreement than conventional smears. These findings support its incorporation into routine cytology practice as an adjunct to breast and lymph node aspirations,

particularly when fine-needle aspiration slides are the only available specimens.

Given the study's limitations, including a small sample size, it is recommended that larger, prospective studies with enhanced cytohistological correlation be conducted. Additionally, further research should evaluate the suitability of the modified cytosrape cell block for molecular applications.

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AUTHOR CONTRIBUTIONS

Bonnie A. Walumbwa conceived, designed the study, interpreted the results, drafted the manuscript, and carried out the data analyses. All authors (Bonnie A. Walumbwa, Josephine N. Rioki, Mary Mungania & Lucy W. Muchiri) participated in writing and revising the manuscript. All authors revised the final manuscript. All authors read and approved the final manuscript.

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