DETERMINING THE SUITABILITY OF BIO-SPECIMENS OBTAINED BY FINE NEEDLE ASPIRATION BIOPSY AT A TERTIARY HOSPITAL IN MALAWI FOR IMMUNOCYTOCHEMICAL ASSESSMENT

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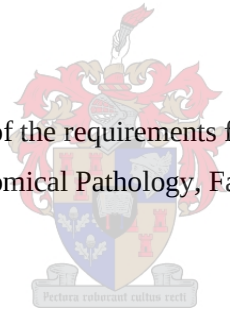

"Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Cytopathology in the Division of Anatomical Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University".

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April 2022

“DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: April 2022

ABSTRACT

Fine-needle aspiration biopsy (FNAB) is a quick, economical, least invasive and easy to perform a minor surgical procedure. In resource-limited settings, FNAB is of utmost importance in providing a rapid diagnosis that facilitates timely and correct institution of treatment. The FNAB smear preparation provides an opportunity for either rapid on-site evaluation or routine diagnosis if ancillary tests are necessary to establish a specific diagnosis. Cell blocks (CB) prepared from FNAB specimens improve the diagnostic yield, increase the sensitivity and reduce false-positive interpretations of detecting a malignant neoplasm. In addition, CB allow for additional morphological evaluation with a better architectural pattern, enable the performance of numerous ancillary diagnostic studies, including immunocytochemistry and molecular studies and offer the storage of material that can be used for future research studies.

Delays in fixing the cell block have been challenges in various cell block preparatory techniques. However, a special alcohol-based fixative, commercially available solution called CytoRich Red® (CRR) has been described to be comparative to liquid-based cytology due to its effectiveness in lysing red blood cells, reducing background material, and improving staining qualities of the nucleus and cytoplasm in routine preparations of non-gynaecological material in suspension or fluids. Despite this breakthrough, there is a paucity of data on the suitability of CRR cell blocks for immunocytochemical and DNA assessment from FNAB material obtained from solid tumours. This study aimed to establish and confirm the suitability of CytoRich Red® Cell Blocks and FNAB biospecimens obtained and prepared at Kamuzu Central Hospital, Lilongwe, Malawi, for cytomorphological and immunocytochemical assessment.

This study analysed 144 cell blocks and 128 FNAB smears. It is one of the first within sub-Saharan Africa to describe diagnostic efficacy from FNAB specimens obtained from various superficial and deep masses fixed in CRR. It describes the advantage of using an alcohol-based fixative immediately to reduce pre-fixation time lag. This study showed that CRR-fixed cell blocks improve sensitivity and architectural preservation, and immunocytochemical staining characteristics of the aspirate compared to routine FNAB smears. It is envisioned that CRR-fixed cell blocks will be a source of extractable, stable and usable DNA that supports research in biorepositories and biobanks.

Key terms: Fine-needle aspiration biopsy, cell block, CytoRich Red® solution, pre-fixation time lag, sensitivity, morphological, architectural, immunocytochemical staining.

OPSOMMING

Fyn naald aspirasie biopsie (FNAB) is 'n vinnige, koste doeltreffende, minimaal indringende en maklike prosedure. FNAB is veral belangrik in die verskaffing van 'n vinnige diagnose wat tydige en korrekte instelling van behandeling fasiliteer. Die FNAB-smeervoorbereiding bied 'n geleentheid vir 'n vinnige roetine diagnose by die pasiënt en die besluit of aanvullende toetse nodig is om 'n spesifieke diagnose te vestig. Selblokke (SB) wat uit FNAB-monsters voorberei is, verbeter die diagnostiese opbrengs, verhoog die sensitiwiteit en verminder vals positiewe interpretasies van die opsporing van maligne neoplasmas. Daarbenewens maak SB voorsiening vir addisionele morfologiese evaluering met 'n beter argitektoniese patroon, wat die uitvoer van talle aanvullende diagnostiese studies moontlik maak, insluitend immunositochemie en molekulêre studies en die berging van materiaal wat gebruik kan word vir toekomstige navorsingstudies.

Vertragings in die fiksering van die selblok is bekend as een van die uitdagings in verskillende selblok voorbereidende tegnieke. 'n Spesiale alkohol gebaseerde fikseermiddel is kommersieel beskikbaar as 'n oplossing genaamd CytoRich Red® (CRR). Hierdie oplossing vergelyk goed met vloeibare gebaseerde sitologie omdat dit rooibloedselle doeltreffendheid liseer, agtergrondmateriaal verminder en kleurkwaliteite van die kern en sitoplasma verbeter in roetine-voorbereidings van nie-ginekologiese materiaal in suspensie of vloeistowwe. Ten spyte van hierdie deurbraak is daar gebrekkige inligting oor die geskiktheid van CRR-selblokke vir immunositochemiese en DNA-assessering van FNAB-materiaal wat uit soliede neoplasmas verkry word. Die doel van hierdie studie was om die geskiktheid van CytoRich Red® Cell Blocks en FNAB monster wat by die Kamuzu Sentrale Hospitaal, Lilongwe, Malawi verkry en voorberei is, te evalueer vir sitomorfologiese en immunositochemiese assessering.

Hierdie studie het 144 selblokke en 128 FNAB-smere ontleed. Dit is een van die eerste studies om die diagnostiese doeltreffendheid te beskryf van FNAB monsters, verkry uit verskeie oppervlakkige en diep massas, wat in CRR fikseer is. Die resultate bevestig die voordeel om onmiddellik 'n alkohol gebaseerde fikseermiddel te gebruik om pre-fiksasie vertragings te verminder. Hierdie studie het getoon dat, in vergelyking met roetine FNAB-smere, die CRR-gefikseerde selblokke die sensitiwiteit, morfologiese en argitekturele gehalte, asook die immunositochemiese kleuringseienskappe van die aspiraat verbeter. Daar word voorsien dat CRR-gefikseerde selblokke 'n bron bied vir die ekstraksie van stabiele en bruikbare DNA wat navorsing in biobanke kan ondersteun.

Sleutelterme: Fyn naald-aspirasie biopsie, selblok, CytoRich Red® -oplossing, voor-fiksasie tydvertraging, sensitiwiteit, morfologie, argitektureel, immunositochemiese kleuring.

DEDICATION

This work is dedicated to my wife, Chisomo, for the physical, emotional and spiritual support that has enabled me to reach this far and our children, Maureen, Nathan and Ivan. I love all of you.

ACKNOWLEDGEMENTS

I would like to thank God almighty for the gift of life and the opportunity He granted me thatI should further my studies.

My utmost gratitude also goes to my wife, Chisomo, for the physical, social and emotionalsupport. I don't know what I could have been without you.

I would also like to thank my supervisor, Prof J Schneider, and co-supervisor, Prof P Schubert, for their guidance and untiring effort that has enabled me to complete this research assignment. The financial support rendered to me through the training, including purchasing reagents for this study, cannot go unmentioned. May God continue to bless you.

My special thanks also go to the Hospital Director and ethics committee of Kamuzu CentralHospital and Malawi National Health Sciences Research Council for permitting me to conduct the study and all the pathology laboratory technicians who participated in the study.

I would also like to extend my gratitude to Dr Yambanso Manda for the much initial input inthis study and for accepting the change of principal investigator. Special mention should also goto Dr Maganizo Chagomerana for the data analysis process.

Finally, my appreciation also goes to Dr Steve Kamiza, Dr Tamiwe Tomoka, Dr Micheline Sanderson and Greta Neethling for their support and encouragement.

May God Almighty richly bless you all.

LIST OF ABBREVIATIONS

CAP: College of American Pathologists

CB: Cell block

CD45: Lymphocyte common antigen

CLIA88: Clinical Laboratory Improvement Act Amendments of 1988

CRR: CytoRich Red®

DAB: 3, 3'-diaminobenzodine tetrahydrochloride

DFSP: Dermatofibrosarcoma protuberans

DNA: Deoxyribonucleic acid

ER: Oestrogen receptor

FN: False negative

FNA: Fine needle aspiration

FNAB: Fine needle aspiration biopsy

FP: False positive

FTA: Flinders Technology Associates

H&E: haematoxylin and eosin

HREC: Health Research Ethics Committee

KCH: Kamuzu Central Hospital

MNF116: Cytokeratin MNF116 antibody

NHSRC: Malawi National Health Science Research Committee

NOS: Otherwise not specified

NPV: Negative predictive value

PPV: Positive predictive value

RNA: Ribonucleic acid

SSA RBR: Sub-Saharan African Regional Biospecimen Repository

TN: True negative

TP: True positive

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INTRODUCTION

Fine-needle aspiration biopsy (FNAB) is a quick, economical, minimally invasive and easy to perform minor surgical procedure. FNAB is performed on superficial or deep-seated lesions with imaging guidance in the latter[1]. Adequate training and experience of health care workers performing FNAB are essential to ensure better patient compliance and satisfactory results[2]. In resource-limited settings, FNAB is of utmost importance in providing a rapid diagnosis that facilitates timely and correct patient referral and institution of treatment[3].

The FNAB smear preparation allows rapid on-site evaluation or routine diagnosis if ancillary tests are necessary to establish a specific diagnosis. Cell blocks (CB) prepared from FNAB specimens improve the diagnostic yield, increase the sensitivity and reduce false positive interpretations of detecting a malignant neoplasm. Also, CB allow for additional morphological evaluation with a better (micro-) architectural pattern, enable the performance of ancillary diagnostic studies including immunocytochemistry, in-situ hybridisation and molecular studies and offer archival material that can be used for future research studies[4-6].

FNAB is a valuable tool in clinical medical practice for stratifying palpable soft tissue masses into benign and malignant categories. In the clinical management of breast masses, FNAB uses a smaller needle and, therefore, is unlikely to cause hematoma and other rare complications such as pneumothorax compared to core-needle biopsies. In addition, FNAB is used as a first-line investigation in evaluating palpable head and neck masses.

Delays in fixing the cell block have been challenges in various cell block preparatory techniques. This pre-fixation time lag contributes to degeneration of cells and loss of nuclear and cytoplasmic characteristics[4]. Different fixatives, including alcohol, formalin and heavy metal fixatives, have been used in cell block preparation. Challenges have been reported in each of these fixatives, including inhibition of certain immunostains (e.g. S100 protein and hormone receptors), poor discrimination of nuclear and cytological details and lack of DNA preservation, respectively[4]. However, a commercially available, alcohol-based fixative called CytoRich Red® (CRR) solution has been described to be comparative to liquid-based cytology[7] due to its effectiveness in lysing red blood cells, reducing background material, and improving staining qualities of the nucleus and cytoplasm in routine preparations of non-gynaecological material in suspension or fluids[8, 9]. Despite this breakthrough, there is a paucity of data on the suitability of CRR cell blocks for immunocytochemicaland DNA assessment from FNAB material obtained from solid tumours.

Despite the lack of a standardised method for cell block preparation, cell blocks allow the performance of more extensive ancillary testing such as immunocytochemistry, in-situ hybridisation and molecular characterisation, which are increasingly important in the era of personalised medicine[4].

Molecular diagnostic tests that require deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) from tumour specimens play a crucial role in the accurate classification, prognosis, and treatment of tumours[10]. Cryopreservation is preferred for preserving FNAB specimens for molecular testing, especially RNA and DNA[11]. However, high cost and logistic challenges pertaining to the collection and preservation of frozen FNAB cells have prompted the development of alternative methods such as FTA cards for preserving unfixed cytological material for high-throughput molecularanalysis[12].

Biospecimens have been transported between African and western countries for research purposes and preservation in biobanks, often without appropriate consideration of ethico-regulatory requirements and best practices for biobanking[13-15]. The rapid growth in global biobanking and the more recent establishment of biobanks in Africa emphasised the need for high-quality biospecimens and data sets to support research that will address major health problems to improve people's health globally, including Africa[16, 17]. However, there is little information about preserving the integrity of samples obtained by FNAB after transportation across African countries for diagnostic and research purposes. In addition, ethico-regulatory challenges pertaining to biobanking inSub-Saharan Africa remain largely unresolved regarding the ethical issues involved in the entire process of transporting specimens across these countries[16, 17].

This study aims to establish and confirm the suitability of CytoRich Red® Cell Blocks and FNAB biospecimens obtained and prepared at Kamuzu Central Hospital, Lilongwe, Malawi, for cytomorphological and immunocytochemical assessment. The hypothesis is that it is possible to obtain biospecimens by fine needle aspiration biopsy (FNAB) in a resource-limited setting suitable for immunocytochemical and DNA assessment.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Health Research Ethics Committee at Stellenbosch University, South Africa (S17/01/023) and the Malawi National Health Science Research Committee (Protocol #1756), as well as the Research and Ethics Committee of Kamuzu Central Hospital (KCH), Malawi. The cases were recruited at Casualty and Radiology Departments of KCH.

Inclusion criteria included all adults (18 years and above) with palpable superficial or deep masses or lymphadenopathy more than or equal to 1cm in diameter, with known HIV status and signed informed consent from the patient.

The exclusion criteria included patients younger than 18 years, superficial or deep masses or

lymphadenopathy of less than 1cm in diameter, unknown HIV status or unavailability of informed consent from the patient.

Research Participants

All outpatients presenting with masses attending the surgery department were screened if they satisfied the inclusion criteria. Other patients were being referred from the oncology ward. Depending on the location and size of the mass, the FNAB were done in the radiology department (ultra-sound guided) or the casualty department (without ultrasound guidance). Participants were not selected based on race and minorities. All the patients were given the option to withdraw from the study. An anatomical pathologist performed all the FNABs.

All the collected samples received patient identification. Clinicians could access the diagnostic results needed for treating the patients via the result database at Kamuzu Central Hospital, Malawi. The information obtained for this study was deidentified and kept on a password protected excel spreadsheet accessible to only the approved researchers involved in this study. Patient identity remained anonymous to ensure patient privacy and protect patients' confidentiality.

FNAB specimen collection

Two fine needle aspiration (FNA) passes were performed on palpable masses or lymph nodes using a 23-gauge needle.

The first pass was smeared onto 2 glass slides, of which one was air-dried and the other fixed with alcohol. The air-dried and alcohol-fixed smears were stained with Giemsa and Papanicolau stains, respectively. An anatomical pathologist assessed these smears as part of the routine diagnostic work-up of the specimen.

The second pass was directly deposited into a tube containing CRR Collection Fluid, and the needlewas rinsed with the CRR Collection Fluid to ensure complete collection of the aspirated material. This pass was used for cell block preparation.

In most circumstances, one pass yielded adequate material for preparing an air-dried smear and an alcohol-fixed smear for collection into the CRR fluid.

Cell Block Preparation, Processing, staining procedures and evaluation

The cells preserved in CRR were transported and stored at room temperature. The aspirated material remained in the CRR Collection Fluid for a minimum of 6 hours and a maximum duration of 48 hours. The de-identified specimen was labelled with an "F" number. The preserved cells were decanted into test tubes and centrifuged at 1500 rpm for 10 minutes at room temperature. The

supernatant was then decanted back into the original tube. Three to four drops of plasma were added to the remaining cell pellet and mixed thoroughly with a plastic pipette. Three to four drops of Dade® Innovin® (working solution) were added and mixed thoroughly by pipetting with a plastic pipette. A clot was formed and placed in a biopsy bag into the cassette and labelled with the corresponding "F" number. The cassette was placed into a specimen container filled with 10% buffered formalin that contained a few drops of eosin to highlight the cell block clot. The cell blocks remained in formalin for 12 to 18 hours, routinely processed in a tissue processor and sectioned with a microtome according to routine histological techniques.

Cell block sections were stained with haematoxylin and eosin (H&E). The "fitness for purpose" was assessed for immunocytochemistry using a manual immunohistochemistry platform with 3, 3'-diaminobenzodine tetrahydrochloride (DAB) as chromogen and hematoxylin as a counterstain. The antibodies that were assessed included MNF-116 (pancytokeratin), oestrogen receptor (ER), vimentin and CD45 (lymphocyte marker). The nature of the cytological diagnosis determined the selected antibody or antibodies to be applied to a particular sample, e.g. ER and MNF-116 on cases of breast carcinoma, CD45 and vimentin on cases of lymphoma, and vimentin only on cases of soft tissue lesions. Table 1 details the panel of primary antibodies used in this study.

The evaluation of ER, MNF-116, vimentin and CD45 on CRR cell block sections was performed in routine cytopathology or histopathology practice.ER positive result was interpreted as >1%of neoplastic cells showing nuclear staining. Vimentin and MNF-116 were interpreted as positive when most tumour cells showed cytoplasmic brown staining. Positive CD45 staining requiredmembranous brown staining in the majority of lymphoid cells.

The FNA smears and cell blocks for the malignant cases were graded according to the morphological and architectural preservation using the grading system depicted in Table 2[18]. The intensity of the immunocytochemical staining and the presence of background staining were graded using the same grading system[18]. Immunocytochemical staining and background staining were graded using six-tiered and four-tiered grading systems, respectively. The higher the grading system, the better the scrutiny and the smaller the margin of error in the final score. The higher grading systems for immunocytochemical staining are practical in research settings. In clinical/routine practice, the three-tiered grading system takes little time or effort and is simple and easily reproducible.

Table 1: Panel of primary antibodies used in this study

Primary antibody	**Source/catalogue or Lot number**	**Dilution**	**Antigen retrieval method**	**Enhancement**
Anti-vimentin	Dako/M7020	1:200	Proteinase K solution	Nil
Anti-CD45	Novocastra/6054053	1:100	Microwave	BD retrievagen A (pH 6.0)
Anti-MNF	Dako/M0821	1:100	Proteinase K Solution	Nil
Anti-ER	Novocastra/6069100	1:50	Microwave	BD retrievagen A (pH 6.0)

Table 2: The grading system

Score	**Description**
Morphological preservation	Examination of presence or absence of crisp, clear nuclear chromatin, nuclear margin, cytoplasm contents and cytoplasmic membrane
0	Poorly preserved
1+	Well preserved
Architectural preservation	Examination of presence or absence of tissue architecture as evidenced by a cellular relationship with each other, e.g. honeycomb arrangement of adenocarcinoma or moulding in neuroendocrine carcinomas.
0	Absent
1+	Present
Immunocytochemistry	Focal staining refers to positive staining of cells concentrated to a specific area/s of the sample; diffuse staining refers to positive staining of cells spread throughout the sample.
0	Negative/absent staining
1+	Focal weak intensity <10% of tumour cells showing positivity
2+	Focal moderate intensity 10-50% of tumour cells showing positivity
3+	Focal strong intensity >50% of tumour cells showing positivity
4+	Diffuse weak intensity <10% of tumour cells showing positivity
5+	Diffuse moderate intensity 10-50% of tumour cells showing positivity
6+	Diffuse strong intensity >50% of tumour cells showing positivity
Background staining	Examination of presence or absence of background staining in relation to smear/section.
0	No background
1+	Mild background staining (<10% of smear/section)

2+	Moderate background staining (10-50% of smear/section)
3+	Severe background staining (>50% of smear/section)

Tissue biopsy specimens

Diagnostic accuracy evaluates the diagnostic utility of protocols, including those that produce tissue for cytopathologic interpretation. Traditionally, the diagnostic accuracy in cytology is evaluated by comparison with a gold standard which in anatomical pathology is the histopathologic diagnosis obtained from tissue biopsies[19]. If the tissue biopsies are not available, the diagnostic accuracy is ascertained by clinical follow-up[20].

Our study measured the diagnostic accuracy by comparing the cytology diagnosis (smears and/or cell block) with the histopathologic diagnosis from the routinely obtained tissue biopsies from the same patient. The histopathological diagnoses are available in the KCH pathology database, and this study's protocols did not require the acquisition of new biopsies from the study participants. The patients' names were completely anonymised.

Data analysis

This study determined sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for cell blocks using the following formulas and 2 by 2 tables.

PPV=TP/(TP +FP), where TP (true positive) is the number of cases diagnosed with malignancy or suspicious for malignancy on both cell block and tissue biopsy.

NPV=TN/(TN+FN), where TN (true negative) is the number of cases diagnosed negative for malignant cells on the cell block and tissue biopsy.

Specificity=TN/(TN+FP) where FP (false positive) is the number of cases diagnosed with malignancy or suspicious for malignancy on cell block but negative for malignant cells on tissue biopsy.

Sensitivity=TP/(TP+FN), where FN (false negative) is the number of cases called negative on cell block but with a diagnosis of malignancy on the tissue biopsy.

In accordance with CLIA88 Final rule[21,] concordance and discordance were calculated as part of cytologic-histologic correlation. We calculated sensitivity, specificity,positive, and negative predictive values using the paired cytologic-histologic values. These discrepancy values were calculated for cell block vs tissue biopsy, FNA smears versus tissue biopsy, and cytology (combined cell block and smears) versus tissue biopsy.

Validation of a new test requires comparison with a prior or subsequent testing of the same tissue with a validated protocol and may be done in the same laboratory. In our setting, we compared the diagnostic accuracy of cytology diagnosis based on combined CRR cell block and smears with tissue biopsy and evaluated the improvement in sensitivity, specificity, positive and negative predictive values.

RESULTS

This study recruited 128 research participants with paired FNA smears and cell blocks. In addition, nine participants had FNA smears taken at a different hospital, and they consented to a repeat FNA for CB preparation only and not to repeat FNA smears. The original FNA smears for the nine participants were not available. The study, therefore, had 128 FNAs and 137 cell blocks.

The 9 participants were first managed at a private hospital, and that's where the initial/original FNAs were taken and evaluated at a private pathology laboratory. These 9 participants did not meet the study's exclusion criteria, and they could not be left out. These cases were not included in the analysis of discrepancies and levels of agreement between FNA and CB. They were only included in the evaluation of the immunocytochemical staining profile.

The cell blocks were distributed as follows: liver 26; lymph node 22; head and neck 7; breast 40; lung 3; extremities(lower and upper) 28; and lesions from the trunk 11 as indicated in Tables 3. Thirty-five of the 128 research participants were HIV positive (27.3%). Seventy-four participants had tissue biopsy or resection specimens available at the time of data analysis for histopathological evaluation and comparison with the corresponding cytological diagnoses. The participants were referred to the surgical clinics for FNAB for various reasons. Some of the participants had excision biopsies following malignant FNAB diagnoses.

Table 3: List of diagnoses made on cell block, FNA smears and tissue biopsies.

CODE	CB DIAGNOSIS	SMEAR DIAGNOSIS	TISSUE DIAGNOSIS	SITE
F1	Blood only	Blood, fibrous tissue and chronic inflammation only	Hemangioma	Lower extremity (leg)
F2	Blood only	Subcutaneous tissue or adipocytic lesion	No biopsy was received	Lower extremity (knee)
F3	Suspicious for malignancy	Suspicious for malignancy	Malignant neoplasm favour chondrosarcoma	Upper extremity (forearm)
F4	Blood only	Blood only	Normal cartilage only. Likely not representative	Lower extremity (hip)
F5	Blood only	Colloid nodule	No biopsy was received	Neck (Thyroid)
F6	Blood only	Keratinous material favour epidermoid cyst	No biopsy was received	Upper extremity (shoulder)
F7	Suspicious for malignancy	Duct carcinoma	An invasive carcinoma, NST	Breast
F8	Blood only	Malignant cells present	No biopsy was received	Lymph node (Groin)
F9	Ductal carcinoma	Duct carcinoma	No biopsy was received	Breast
F10	Reactive lymph node	Blood only	Hemangioma, pleomorphic/spindle cell lipoma, cannot exclude liposarcoma	Lower extremity (leg)
F11	Blood only	Blood only	No biopsy was received	Upper extremity (arm)
F12	Fibroadenoma	Fibroadenoma	Fibroadenoma	Breast
F13	Breast ducts and fibrous tissue only	Benign ductular cells, stroma with inflammatory cells favouring part of fibrocystic breast disease	Gigantomastia	Breast
F14	Subcutaneous tissue or lipomatous lesion.	Subcutaneous tissue or lipomatous lesion. Biopsy advised.	No biopsy was received	Upper extremity (arm)
F15	Melanoma	Melanoma	Malignant melanoma	Lower extremity

				(foot)
F16	Blood only	Blood only	Phyllodes tumour	Breast
F17	Hepatocellular carcinoma	Hepatocellular carcinoma	No biopsy was received	Liver
F18	Malignant cells	Malignant cells favour carcinoma	No biopsy was received	Liver
F19	Suspicious for malignancy	Blood only	Duct carcinoma in-situ.	Breast
F20	Spindle cell neoplasm	Blood only	Dermatofibrosarcoma protuberans (DFSP)	Trunk (chest)
F21	Blood only	Blood only	Kaposi sarcoma	Lower extremity (foot)
F22	Papillary lesion	Suspicious for malignancy	Duct carcinoma in-situ	Breast
F23	Malignant cells favouring duct carcinoma	Blood only	No biopsy was received	Breast
F24	Melanoma	Blood only	Melanoma	Lower extremity (Foot)
F25	Squamous cell carcinoma	Suspicious for malignancy	An invasive squamous cell carcinoma	Trunk (perianal area)
F26	Fibroadenoma with lactational changes	Fibroadenoma	No biopsy was received	Breast
F27	fibroadenoma	Fibroadenoma	No biopsy was received	Breast
F28	Invasive duct carcinoma	Blood only	No biopsy was received	Breast
F29	Invasive duct carcinoma	Duct carcinoma	No biopsy was received	Breast
F30	Spindle cell lesion favouring sarcoma	Malignant spindle cell neoplasm	No biopsy was received	Trunk (flank)
F31	Reactive lymph node	Lymphoproliferative lesion cannot exclude lymphoma	No biopsy was received	Lymph node (neck)
F32	Sarcoma, NOS	Blood only	Pleomorphic sarcoma	Trunk (iliac bone)
F33	Metastatic carcinoma	Blood only	Metastatic carcinoma	Lymph node (neck)
F34	Fibrous tissue and scattered inflammatory cells	Blood only	Benign fibroblastic lesion. Material less than optimal for definitive diagnosis	Breast

F35	Benign epidermal cells and blood	Blood only	No biopsy was received	Lower extremity (leg)
F36	Blood only	Blood only	Kaposi sarcoma	Lower extremity (leg and foot)
F37	Necrotic debris and blood	Blood only	An invasive adenocarcinoma	Trunk (perianal area)
F38	Suspicious for malignancy	Malignant cells favour carcinoma	No biopsy was received	Breast
F39	Gynaecomastia	Gynaecomastia	Gynaecomastia	Breast
F40	Scattered inflammatory cells only	Blood only	Kaposi sarcoma	Lymph node (groin)
F41	Blood only	Negative for malignant cells	Lipomatous lesion	Lower extremity (thigh)
F42	Fibrous tissue and scattered inflammatory cells	Blood only	Ulcerated skin with dermal granulation tissue response	Lower extremity (thigh)
F43	Ductal carcinoma	Malignant cells	No biopsy was received	Breast
F44	Hepatocellular carcinoma	Blood only	Hepatocellular carcinoma	Liver
F45	Hepatocellular carcinoma	Malignant cells favour carcinoma	No biopsy was received	Liver
F46	Malignant neoplasm favouring carcinoma	Malignant cells favour carcinoma	No biopsy was received	Trunk (flank)
F47	Malignant cells favouring adenocarcinoma	Malignant cells	No biopsy was received	Liver
F48	Fibroadenoma	Fibroadenoma	No biopsy was received	Breast
F49	Malignant neoplasm favouring melanoma	Blood only	No biopsy was received	Lymph node (Groin)
F50	Blood and stromal fragments only	Suspicious for malignancy	Diffuse Large B-cell Lymphoma	Lymph node (neck)
F51	Duct carcinoma	Duct carcinoma	Infiltrating duct carcinoma, NST	Breast
F52	Fat necrosis and chronic mastitis	Chronic mastitis and fat necrosis	Chronic mastitis	Breast
F53	Duct carcinoma	Duct carcinoma	Invasive duct	Breast

			carcinoma, NST	
F54	Benign salivary gland ducts. No malignancy	Benign salivary gland cells	No biopsy was received	Neck (salivary gland)
F55	Spindle cell neoplasm. Differential; sarcoma & melanoma	Malignant spindle cell neoplasm favouring sarcoma	High grade sarcoma	Lower extremity (knee)
F56	Blood only	Blood only	Kaposi sarcoma	Lower extremity (foot)
F57	Negative for malignant cells	Malignant cells	No biopsy was received	Liver
F58	Hepatocellular carcinoma	Hepatocellular carcinoma	No biopsy was received	Liver
F59	Suspicious for malignancy	Fibroadenoma	Benign breast tissue	Breast
F60	Negative for malignant cells	Blood only	Fibrofatty breast tissue with fibrosis only	Breast
F61	Duct carcinoma	Duct carcinoma	Invasive duct carcinoma, NST	Breast
F62	Metastatic carcinoma	An adenocarcinoma	Metastatic infiltrating duct carcinoma	Lymph node (neck)
F63	Infiltrative duct carcinoma	Malignant cells favour carcinoma	No biopsy was received	Breast
F64	Duct carcinoma	Malignant cells favour carcinoma	No biopsy was received	Breast
F65	Carcinoma, NOS	Malignant cells	No biopsy was received	Liver
F66	Adenocarcinoma	Malignant cells favouring carcinoma	Necrosis only	Lung
F67	Malignant neoplasm favouring carcinoma.	Malignant neoplasm.	No biopsy was received	Neck (salivary gland)
F68	Duct carcinoma	Duct carcinoma	An invasive duct carcinoma, NST	Breast
F69	Fibroadenoma	Benign ductal cells only	No biopsy was received	Breast
F70	Fibroadenoma	Fibroadenoma	Normal breast tissue	Breast
F71	Duct carcinoma	Blood only	No biopsy was received	Breast
F72	Adenocarcinoma	Malignant cells	Adenocarcinoma	Liver
F73	Malignant cells	Malignant cells	No biopsy was	Lung

	favouring carcinoma	favour adenocarcinoma	received	
F74	Invasive squamous cell carcinoma	Malignant cells	An invasive squamous cell carcinoma	Trunk (flank)
F75	Lymphoproliferative lesion cannot exclude leukaemia	Lymphoproliferative lesion cannot exclude lymphoma	Leukemic cell infiltration	Head (submental)
F76	Sarcoma, Synovial sarcoma is a differential diagnosis		Sarcoma, NOS	Lower extremity (thigh)
F77	Invasive carcinoma, favouring ductal	Ductal carcinoma	No biopsy was received	Breast
F78	Mixed inflammatory cells	Scattered benign inflammatory cells only	Hodgkin Lymphoma	Lymph node (Neck)
F79	Fibroadenoma	Fibroadenoma	No biopsy was received	Breast
F80	Blood only	Benign ductal cells only	Breast tissue with fibrosis only	Breast
F81	Malignant tumour favouring carcinoma with metastasis to the neck	Metastatic carcinoma to the neck	No biopsy was received	Lymph node (neck)
F82	Kaposi sarcoma	Blood only	Kaposi sarcoma	Lower extremity (leg)
F83	Duct carcinoma	Duct carcinoma	No biopsy was received	Breast
F84	Blood only	Blood only	An invasive carcinoma favouring hepatocellular carcinoma	Liver
F85	High grade Non Hodgkin B-cell lymphoma	High grade lymphoma	No biopsy was received	Liver
F86	Duct carcinoma	Duct carcinoma	Infiltrating duct carcinoma, NST with lymph node metastasis	Breast
F87	Hepatocellular carcinoma	Blood only	Hepatocellular carcinoma	Liver
F88	Blood only	Blood only	No biopsy was received	Liver
F89	Negative for		Myxoid spindle cell	Lower

	malignant cells		neoplasm	extremity (thigh)
F90	Tuberculosis	Blood, inflammatory cells and necrotic debris.	No biopsy was received	Lymph node (neck)
F91	Fibroadenoma		No biopsy was received	Breast
F92	Carcinoma, NOS		No biopsy was received	Liver
F93	Lymphoproliferative lesion cannot be excluded on cytology		No biopsy was received	Lymph node (neck)
F94	Hepatocellular carcinoma	Hepatocellular carcinoma	No biopsy was received	Liver
F95	Melanoma on both the foot lesion and inguinal lymph node	Melanoma	Melanoma	Lower extremity (foot)
F96	Duct carcinoma	Duct carcinoma	Infiltrating duct carcinoma, NST	Breast
F97	Blood only	Blood only	Burkitt lymphoma	Breast
F98	Blood only		No biopsy was received	Breast
F99	Hepatocellular carcinoma		No biopsy was received	Liver
F100	Blood only	Suspicious for malignancy	Hepatocellular carcinoma	Liver
F101	Endometriosis	Blood only	No biopsy was received	Trunk (abdominal wall)
F102	Malignant neoplasm favouring sarcoma		No biopsy was received	Lymph node (neck)
F103	High grade Non-Hodgkin Lymphoma		No biopsy was received	Lymph node (neck)
F104	High grade Non-Hodgkin Lymphoma		No biopsy was received	Lymph node (neck)
F105	High grade Non-Hodgkin Lymphoma	Lymphoproliferative lesion, cannot exclude lymphoma	Non-Hodgkin B-cell Lymphoma	Liver
F106	Blood only	Scattered inflammatory	No biopsy was received	Lung

		cells		
F107	Malignant epithelioid neoplasm	Blood only	No biopsy was received	Neck (Salivary gland)
F108	Hepatocellular carcinoma	Hepatocellular carcinoma	No biopsy was received	Liver
F109	Reactive lymph node	Reactive lymph node	No biopsy was received	Lymph node (Groin)
F110	Kaposi sarcoma (HHV8 positive)	Blood only	No biopsy was received	Lymph node (Groin)
F111	Reactive lymph node	Reactive lymph node	No biopsy was received	Lymph node (Neck)
F112	Blood only		No biopsy was received	Lymph node (Neck)
F113	Hepatocellular carcinoma	Hepatocellular carcinoma	No biopsy was received	Liver
F114	Duct carcinoma	Blood only	An invasive carcinoma, NST	Breast
F115	Hepatocellular carcinoma	Blood only	No biopsy was received	Liver
F116	Hepatocellular carcinoma	Hepatocellular carcinoma	No biopsy was received	Liver
F117	Negative for malignant cells		No biopsy was received	Lymph node (axilla)
F118	Hepatocellular carcinoma		No biopsy was received	Liver
F119	Malignant neoplasm, Sarcoma, Carcinoma	Suspicious for malignancy	Sarcoma, NOS	Liver
F120	Suspicious for heamatolymphoid neoplasm	Blood only	Non-Hodgkin lymphoma, high grade	Lymph node (Groin)
F121	Blood only	Blood only	No biopsy was received	Neck (submandibular gland)
F122	Blood only	Blood only	No biopsy was received	Liver
F123	Carcinoma, NOS	Malignant cells	Skin and macrophages	Lymph node (Groin)
F124	High grade Non-Hodgkin Lymphoma	Scattered inflammatory cells	Large B-cell lymphoma	Lymph node (neck)
F125	Blood only	Blood only	Kaposi sarcoma	Lower extremity (thigh)
F126	Blood only	Blood only	Verruca vulgaris	Lower extremity

				(ankle)
F127	Plantar lesion—Scattered inflammatory cells and blood only	Plantar lesion—Blood only Inguinal mass—abscess wall and contents	Plantar lesion—Melanocytic naevus Inguinal mass—abscess wall and contents	Lower extremity (foot)
F128	Blood only	Blood only	No biopsy was received	Lower extremity (leg)
F129	Blood only	Blood only	No biopsy was received	Trunk (flank)
F130	Epidermoid cyst	Epidermoid cyst	Epidermoid cyst	Trunk (Back)
F131	Blood only	Blood only	An invasive carcinoma, NOS	Upper extremity (forearm)
F132	Necrotic material only	Blood only	No biopsy was received	Lower extremity (thigh)
F133	Malignant cells	Blood only	An invasive adenocarcinoma.	Trunk (chest)
F134	Necrotic material only	Blood only	Kaposi sarcoma	Lower extremity (Foot)
F135	Duct carcinoma	Blood only	No biopsy was received	Breast
F136	Blood and benign hepatocytes only	Blood and a few benign hepatocytes.	No biopsy was received.	Liver
F137	Lymphoproliferative lesion with abundant cells showing plasmacytoid differentiation	Lymphoproliferative lesion with abundant cells showing plasmacytoid differentiation	Plasmablastic lymphoma	Head (maxilla)

The evaluation of the validity of diagnostic tests used in this study is summarised in Table 4. This table compares sensitivities, specificities, positive and negative predictivevalues of cytopathological diagnoses based on FNA smears, cell blocks and combined smears and cell blocks compared to the corresponding histopathological diagnoses made on tissue biopsy or resection specimens. The cases whose histology were not available were not excluded from the study, and they were not included in the evaluation of the validity of diagnostic tests calculated.

Table 4: Evaluation of validity of diagnostic tests used in this study

Smears vs tissue biopsy		**CB vs tissue biopsy**	**Combined smears and CB vs tissuebiopsy**
Sensitivity	45.83%	64%	74%
Specificity	89.4%	85%	86.36%
PPV	91.66%	91.66%	92.5%
NPV	39.53%	48.57%	59.38%

For data analysis, all paired FNA smears and cell blocks were categorised into 4 main groups based on the final diagnosis: malignant, suspicious for malignancy, benign, and non- diagnostic. On analysing the discrepancies between smears and cell block technique (Table 5), the maximum outcome of the non-diagnostic category for smears was 43.7%, followed by malignancy (32%),benign (16.4%) and suspicious for malignancy (7.8%). However, in the cell block technique, the non-diagnostic category significantly reduced to 30.47%, malignancy increased to 46.09%, suspicious formalignancy reduced to 6.25%, and the benign category increased to 17.18%.

Table 5: Analysis of discrepancies between paired smears and cell block technique

Categories	**Paired FNA smears and cell block**	
	Smears	Cell block
Malignant	41 (32%)	59 (46.09%)
Suspicious for malignancy	10 (7.8%)	8 (6.25%)
Benign	21 (16.4%)	22 (17.18%)
Non-diagnostic	56 (43.7%)	39 (30.47%)
Total	**128**	**128**

Table 6 shows the analysis on the level of agreement between paired FNA smears and cell block caseson the diagnostic categories, morphological and architectural preservation. The percent agreement for the diagnostic categories was 68%, with a kappa statistic of 0.5445. This moderate level of agreement was statistically significant (p=0000). Although percent agreement on morphological and architectural preservation varied considerably, the level of agreement (kappa statistic) is consistently none. This finding is also statistically significant.

Table 6: Analysis on the level of agreement between the paired FNA smears and cell block cases.

1. Diagnostic categories

	Cell block				
FNA smear	Malignant	Suspicious	Benign	Negative	**Total**
Malignant	35	2	0	2	39
Suspicious	4	3	1	2	10
Benign	0	0	17	4	21
Negative	18	3	4	33	58
Total	57	8	22	41	128

Agreement	Kappa	Std. Err.	Z	Prob>Z
68.75%	0.5445	0.0559	9.74	0.0000

2. Morphological preservation

	Cell block		
FNA smear	**0**	**1**	**Total**
0	2	21	23
1	6	41	47
Total	8	62	70

Agreement	Kappa	Std. Err.	Z	Prob>Z
61.43%	-0.0488	0.0971	-0.50	0.6924

3. Architectural preservation

	Cell block		
FNA smears	0	1	Total
0	23	30	53
1	3	14	17
Total	26	44	70

Agreement	Kappa	Std. Err.	Z	Prob>Z
52.86%	0.1673	0.0875	1.91	0.0279

The immunocytochemistry was done on 73 cell blocks with an initial diagnosis of malignancy or suspicious for malignancy (table 7). Table 8 shows the staining intensity and background staining for MNF-116, ER, Vimentin and CD45, respectively. MNF-116 was performed on 64 CBs, 47 of which had a cytological diagnosis of carcinoma.

MNF-116 was positive (confirmed the diagnosis) in 43 CBs. The MNF-116 negative epithelial neoplasms included two hepatocellular carcinomas, one lung adenocarcinoma and one case from a groin mass. These lung and groin masses had the final diagnosis changed to non-epithelial neoplasm and sarcoma, respectively. The two MNF-116 negative hepatocellular carcinomas were signed out as a malignant neoplasm, favouring hepatocellular carcinoma. We could not perform other immunocytochemistry (e.g. HepPar 1, Arginase) to completely exclude hepatocellular carcinoma.

Five CBs had a diagnosis of suspicious for malignancy, and MNF116 was used for diagnostic immunocytochemical workup. Three of these 5 CBs were positive with MNF-116 and had a final diagnosis of epithelial neoplasm. Four melanoma cases were negative for MNF-116. MNF-116 was also done on 8 other CBs with broad initial diagnosis (spindle cell neoplasm, Sarcoma NOS, Malignant neoplasm). MNF-116 was also used for diagnostic/immunocytochemical workup in these eight cases. Three of the eight CBs had the final diagnosis modified to malignant epithelial neoplasm favouring carcinoma due to positive MNF-116. One CB had positive MNF-116 and Vimentin, and the final diagnosis was synovial sarcoma. There were 50 positive CBs, and moderate to strong diffuse staining intensity with MNF-116 was seen in 38 CBs (76%). Twelve positive MNF-116 CBs had focal weak (4 CBs), focal moderate (5 CBs) or focal strong(3 CBs) staining intensity. Of all 64 MNF-116 stained CBs, only 4 had severe background staining. The rest had moderate (27 CBs), mild (25 CBs) or absent (9 CBs) background staining.

ER was done on 23 breast CBs, 22 of which were invasive carcinoma. Twelve of the 22 cases (54.5%) were ER positive, and 10 were ER negative. One of the 23 CBs had an initial diagnosis ofsuspicious for malignancy, and it was ER negative. Out of the twelve ER positive CBs, ten (83.3%)had ≥focal moderate intensity (10-50% of tumour cells showing positivity). Two had focal weak intensity involving <10% of tumour cells showing positivity. There was no moderate or severe background ER staining observed in this study since 19 CBs and 4 CBs showed no background staining and mild background staining, respectively.

Vimentin was done on 18 CBs, eight of which confirmed the initial diagnosis of a spindle cell neoplasm. Six CBs had an initial diagnosis of either epithelial neoplasm or lymphoma and vimentin stained negatively. This finding means that 14 of the 18 CBs (77.8%) were correctly labelled with vimentin. One CB had an initial diagnosis of suspicious for malignancy and was positive for vimentin, suggesting a spindle cell neoplasm. Another case with an initial diagnosis of non-Hodgkin lymphoma was CD45 negative and vimentin-positive. The final diagnosis was high grade malignant neoplasm, and tissue biopsy was advised. One melanoma CB was also negative for vimentin. One CB had the initial diagnosis changed from carcinoma to sarcoma, NOS due to a positive Vimentin and negative MNF-116. All the11 vimentin-positive CBs (100%) had moderate to strong diffuse staining intensity. 16.7% of the CBs (3/ 18) had no background staining for vimentin. The remaining 15 CBs had moderate (12 CBs) and mild (3 CBs) background staining for vimentin. There was no CBs that had severe background staining.

CD45 was done on 15 CB, eight of which confirmed the initial diagnosis of haematolymphoid neoplasm. Although six CBs were negative for CD45, haematolymphoid neoplasm could not be excluded, and tissue biopsies were advised for further evaluation. Therefore, 14 of the 15 CBs (93.3%) were correctly labelled with CD45. One CB with an initial diagnosis of non-Hodgkin lymphoma was CD45 negative, but vimentin-positive CB had the final diagnosis of high grade malignant neoplasm. All eight CD45 positive CBs showed a moderate to strong diffuse staining intensity. Twelve of the 15 CBs (80%) showed no to mild background staining with CD45. Moderate background staining for CD45 was noted in 20% (3/15) of all the cell blocks. Table 9 lists CBs with the initial diagnosis modified or changed after a panel of immunocytochemistry. Immunocytochemistry modified/changed the initial diagnosis in 13 of the 73 CBs (17.8%).

Table 7: Summary of immunocytochemical staining pattern

CODE	SITE	DIAGNOSIS	MNF-116	ER	VIMENTIN	CD45
F3	Forearm	Suspicious for malignancy	Negative		positive	
F7	Breast	Suspicious for malignancy	Positive	negative		
F9	Breast	Ductal carcinoma	Positive	Positive		
F15	Foot	Melanoma	Negative			
F17	Liver	Hepatocellular carcinoma	Negative			
F18	Liver	Malignant cells	Positive			Negative
F20	Chest wall	Spindle cell neoplasm.	Negative		Positive	
F22	Breast	Papillary lesion	Positive	Positive		Negative
F23	Breast	Malignant cells favouring duct carcinoma	Positive	negative		
F24	Foot	Melanoma	Negative			
F25	Perianal	Squamous cell carcinoma	Positive			
F28	Breast	Ductal carcinoma	Positive	Positive		
F29	Breast	Ductal carcinoma	Positive	Positive		
F30	Groin	Spindle cell lesion favouringsarcoma			positive	
F32	Flank	Sarcoma, NOS	Negative		positive	
F33	Neck	Metastatic carcinoma	Positive			
F38	Breast	Suspicious for malignancy	Positive	negative		
F43	Breast	Ductal carcinoma	Positive	negative		
F44	Liver	Hepatocellular carcinoma	Positive			

F45	Liver	Hepatocellular carcinoma	Positive	
F46	Flank	Malignant neoplasm favouring carcinoma	Positive	
F47	Liver	Malignant cells favouring adenocarcinoma	Positive	
F49	Groin	Malignant neoplasm favouring melanoma	Negative	
F51	Breast	Duct carcinoma	Positive	Positive
F53	Breast	Duct carcinoma	Positive	negative
F55	Ankle	Spindle cell neoplasm. Differential; sarcoma & melanoma	Negative	Positive
F58	Liver	Hepatocellular carcinoma	Positive	
F59	Breast	Suspicious for malignancy	Positive	Positive
F61	Breast	Duct carcinoma	Positive	Positive
F62	Neck	Metastatic carcinoma	Positive	Negative
F63	Breast	Duct carcinoma	Positive	negative
F64	Breast	Duct carcinoma	Positive	negative
F65	Liver	Carcinoma, NOS	Positive	Negative
F66	Lung	Adenocarcinoma	Negative	

F67	Neck	Malignant neoplasm favouring carcinoma. Differential diagnoses include mucoepidermoid carcinoma, adenoid cystic carcinoma, adenocarcinoma NOS	Positive	
F68	Breast	Duct carcinoma	Positive	Positive
F71	Breast	Duct carcinoma	Positive	negative
F72	Liver	Adenocarcinoma	Positive	
F73	Lung	Malignant cells favouring carcinoma	Positive	
F74	Groin	Invasive squamous cell carcinoma	Positive	
F75	Neck	Lymphoproliferative lesion, cannot exclude leukaemia.		Positive
F76	Thigh	Sarcoma, Synovial sarcoma is a differential diagnosis	Positive	positive
F77	Breast	Invasive carcinoma, favouring ductal	Positive	negative
F81	Neck	Malignant tumour favouring carcinoma with metastasis to the neck	Positive	Negative
F82	Leg	Kaposi sarcoma		positive
F83	Breast	Duct carcinoma	Positive	Positive
F85	Liver	High grade non-Hodgkin B-cell lymphoma		positive
F86	Breast	Duct carcinoma	Positive	Positive

F87	Liver	Hepatocellular carcinoma	Positive		
F92	Liver	Carcinoma, NOS	Positive		
F94	Liver	Hepatocellular carcinoma	Positive		
F95	Foot and groin	Melanoma on both the foot lesion and inguinal lymph node	Negative	positive	
F96	Breast	Duct carcinoma	Positive	negative	
F99	Liver	Hepatocellular carcinoma	Positive		
F102	Neck	High grade Non-Hodgkin Lymphoma	Negative	Positive	Negative
F103	Axilla	High grade Non-Hodgkin Lymphoma		Negative	Positive
F104	Neck	High grade Non-Hodgkin Lymphoma		Negative	Positive
F105	Liver	Non-Hodgkin Lymphoma			Positive
F107	Neck	Malignant epithelioid neoplasm	Positive		Negative
F108	Liver	Hepatocellular carcinoma	Positive		
F110	Neck	Kaposi sarcoma, HH8 positive		Positive	
F113	Liver	Hepatocellular carcinoma	Negative		
F114	Breast	Duct carcinoma	Positive	Positive	
F115	Liver	Hepatocellular carcinoma	Positive		
F116	Liver	Hepatocellular carcinoma	Positive		
F118	Liver	Hepatocellular carcinoma	Positive		

F119	Liver	Malignant neoplasm favour sarcoma	Negative		Positive	
F120	Groin	Suspicious for Hematolymphoid neoplasm (CD45 positive, Ki-67 high	Negative		Negative	Positive
F123	Groin	Carcinoma, NOS	Negative		positive	negative
F124	Neck	High grade Non-Hodgkin Lymphoma	Negative			positive
F133	Chest wall	Malignant cells	Positive	negative	Negative	Negative
F135	Breast	Duct carcinoma	Positive	Positive		
F137	Maxilla	Lymphoproliferative lesion with abundant cells showing plasmacytoid differentiation				Positive

Table 8: Intensity and background staining

1. MNF-116

	background				
intensity	0	1	2	3	Total
0	6	2	5	1	14
1	1	2	1	0	4
2	0	2	3	0	5
3	0	1	2	0	3
4	0	0	0	0	0
5	0	4	3	0	7
6	1	14	13	3	31
Total	8	25	27	4	64

2. ER

	background				
intensity	0	1	2	3	Total
0	9	2	0	0	11
1	2	0	0	0	2
2	2	1	0	0	3
3	1	0	0	0	1
4	1	0	0	0	1
5	3	1	0	0	4
6	1	0	0	0	1
Total	19	4	0	0	23

3. Vimentin

intensity	background 0	1	2	3	Total
0	1	1	5	0	7
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0
4	0	0	0	0	0
5	1	0	1	0	2
6	1	2	6	0	9
Total	3	3	12	0	18

4. CD45

intensity	background 0	1	2	3	Total
0	5	1	0	0	6
1	0	0	0	0	0
2	0	1	0	0	1
3	0	0	0	0	0
4	0	0	0	0	0
5	0	2	0	0	2
6	1	2	3	0	6
Total	6	6	3	0	15

Table 9: The role of immunocytochemistry in modifying/changing the final diagnosis

CB Number	Initial CB diagnosis	MNF116	Vimentin	CD45	Final CB diagnosis
F3	Suspicious for malignancy	-	Positive	-	Spindle cell neoplasm
F7	Suspicious for malignancy	Positive	-	-	Epithelioid neoplasm
F17	Hepatocellular carcinoma	Negative	-	-	Malignant neoplasm favour hepatocellular carcinoma
F18	Malignant cells	Positive	Negative	Negative	Carcinoma, NOS
F38	Suspicious for malignancy	Positive	-	-	Epithelioid neoplasm
F59	Suspicious for malignancy	Positive	-	-	Epithelioid neoplasm
F66	Lung adenocarcinoma	Negative	-	-	Non-epithelioid neoplasm
F76	Sarcoma, synovial sarcoma favoured	Positive	Positive	-	Synovial sarcoma
F102	High grade Non-Hodgkin Lymphoma	Negative	Positive	Negative	Sarcoma, NOS
F113	Hepatocellular carcinoma	Negative	-	-	Malignant neoplasm favour hepatocellular carcinoma
F120	Suspicious for hematolymphoid neoplasm	Negative	Negative	Positive	Hematolymphoid neoplasm
F123	Groin carcinoma, NOS	Negative	Positive	Negative	Sarcoma, NOS
F133	Malignant cells	Positive	-	-	Carcinoma, NOS

DISCUSSION

This series is one the first within sub-Saharan Africa to describe diagnostic efficacy from FNA specimens and CRR-fixed CB obtained from various superficial and deep masses. This study shows that CRR-fixed cell blocks add sensitivity, morphological and architectural preservation, significantly improving FNA specimens' diagnostic value and yield. Also, this study demonstrated the feasibility of using CRR-fixed CB for immunocytochemical staining, thereby increasing the diagnostic accuracy and sensitivity of FNA specimens in a LMIC.

Various authors have described the advantages of cell blocks, but there is still no agreement on the processing techniques and fixative that produce optimal results[22]. Although there are several commonly used cell block preparations, the plasma thrombin/thrombin clot method has been identified as a simple, low-cost option with easy availability of reagents and produces optimal cytomorphology[4]. Despite the advantages of this method, the possible introduction of foreign proteins, and by implication, genetic material may compromise future molecular genetic testing of these CB samples.

The other big challenge is the choice of fixative employed in preserving the FNA aspirate. Although 10% buffered formalin is a widely used universal fixative for morphology and immunohistochemistry, attempts to extract usable DNA from formalin-fixed tissues have been variably successful[4]. Alcohol-based fixatives (CRR) have also been used. However, laboratories need to validate the CRR appropriately[4]. In a study by Veena VS et al. [9], cell blocks prepared from samples homogenised in CRR solution significantly increased the diagnostic efficacy. A combined analysis of smears and cell blocks improved the sensitivity. This study, however, was on sputum and not from FNA aspirate. Although CRR contains 0.4% formaldehyde, storing cell pellets made from lung adenocarcinoma yielded higher amounts of DNA and better stability of the extracted DNA[23]. In a study by La Fortune K et al. [24], a Cell-Gel method consistently yielded abundant cellular material. This method used CRR as a hemolytic fixative and disposable base moulds to reduce cell block failure rate by approximately 67%. However, the CB failure rate was calculated by comparing it with another CB processing method called HistoGel Tube method. There was no comparison made between Cell-Gel method and the plasma thrombin/thrombin clot method. In this series, CRR was used as a fixative for FNA specimens from several organs, and its results were analysed.

There was no significant difference in specificity and positive predictive test for FNA smears and cell blocks (table 4). The only significant difference between smears and cell block is on sensitivity. Sensitivity is the ability of a diagnostic test to classify a lesion correctly. Our finding of cell block sensitivity of 64% is within the expected ranges. The sensitivity of cell blocks varies from 60% to 86%depending on sampling type and size, type of specimens, aspiration techniques[4,] and cell block

preparatory techniques.

There was an improvement in sensitivity when both smears and cellblocks were evaluated together (74%). The contribution of cell blocks to the final cytological diagnosis emphasises that CB should be considered in all FNA specimens[25]. Table 10 compares the sensitivity of cell block preparation techniques in various studies. In most previous studies that calculated sensitivity values, there were no tissue or resection biopsies and analysis of the data was limited to smears and cell block studies only. Calculation of sensitivity depended on counting the number of CBs that had diagnostic cellular material[22, 25]. This definition falls short of the true meaning of sensitivity of a diagnostic test. The histologic outcome is a gold standard against which cytologic interpretation should be measured[19].

Table 10: Comparison of the sensitivity of cell block preparation in various studies

Studies	**FNA smear diagnosis**	**CB diagnosis**	**FNA+CB diagnosis**
Khan N et al. [29]	56%	72%	85.3%
Keyhani-Rofaga S et al[30]	55%	60%	86%
Richardson HL et al. [31]	28%	68%	82%
Present study	45.83%	64%	74%

Cell blocks in our study improved sensitivity by 18.17% (from 45.83% to 64%). This increased sensitivity is one of the highest among some studies that have compared smears and cell blocks. This finding indicates that FNAB smears are associated with increased false negative diagnoses and can be reduced by supplementing with cell block evaluation. Table 11 shows the increment of sensitivity between smears and cell blocks in previous studies.

Table 11: Comparison of increment of sensitivity by cell block preparation in various studies

Study	**Year of the study**	**Percentage**
Grandhi B et al. [26]	2014	5%
Bhanvadia V et al. al[27]	2014	10%
Katti R et al. [28]	2016	15.5%
Sharma et al. al[5]	2017	8%
Present study	2020	18.17%

On analysing the discrepancies between paired FNA smears and cell blocks (table 4), the significant discrepancy occurred on an increment of malignant diagnosis from 32% (FNA smears) to 46.09% in cellblocks and reduction of negative for malignancy category from 43.7% (FNA smears) to 30.47% in a cell block. This finding could be attributed to increased cellular yield and better appreciation of cellular details and architecture in cell block preparation[5, 26, 27]. As shown in table 5, morphological and architectural preservation was significantly better for cell blocks than FNA smears.

Table 6 shows the level of agreement in diagnostic categories (malignant, suspicious for malignancy, benign and negative for malignancy), morphological and architectural preservation. Although the percent agreement for diagnostic categories is 68%, the k-statistic is 0.559 (p=0000). This k-statistic is statistically significant; however, it has a weak level of agreement. This observation is attributed to the fact that a cell block has less false negatives than smears. It is therefore not surprising that there is no agreement on cellularity (k-statistic 0.1156, P0.0279), morphological preservation (k-statistic -0.0488, P0.6924**)** andarchitectural preservation (K-statistic 0.1673, P0.279) between smears and cell blocks, and this lack of agreement is statistically significant. This finding agrees with a study by Khan S et al. [18] that found a poor agreement between similar diagnostic methods in cellularity, morphological and architectural preservation.

The immunocytochemical stains were done on cell blocks that were diagnosed with malignancy or suspicious for malignancy (table 8). In the 4 immunocytochemical stains performed, the staining intensity was satisfactory, with few cell blocks showing severe background staining. Generally, all the stained slides could be read with no difficulties. The role of immunocytochemistry in confirming, changing and/or modifying diagnoses has been shown in this study. This outcome supports Khan S et al. [18,] who confirmed the crucial role of fixative and optimal tissue processing in preserving the antigenicity of tumour cells for accurate immunocytochemical analyses. The antigen retrieval methods, dilution of the antibodies and enhancements used in the current study are by no means gold standard. Laboratories performing immunocytochemistry on cell blocks should optimise and validate their respective immunohistochemical assays to produce better staining characteristics[31].

Limitations of this study

One of the CAP recommendations is that for initial validation of every assay used clinically, laboratories should achieve at least 90% overall concordance between the new test and the comparative test[31]. Due to financial constraints, immunohistochemistry was not performed on all tissue (or resection) biopsies in this study. Therefore, the concordance level on immunohistochemistry between CRR-fixed cellblocks and paraffin-embedded tissue biopsies could not be evaluated. This challenge limited our validation of the immunocytochemistry done on the CRR-fixed cell blocks and in addition the FNAB passes were not assessed using rapid on-site

specimen evaluation.

Although vimentin staining suggests the mesenchymal origin of some tumours, there are numerous exceptions, making the stain non-specific. On the other hand, it may be the only positive stain in certain cases and thus confirms that the tissue is capable of staining. The issues of cost could not permit the addition of more mesenchymal markers.

The Covid-19 pandemic was the biggest challenge as the pandemic prevented us from getting more participants in the study. During the pandemic, the outpatient surgical department at KCH was closed,and only excision biopsies were being done. There was a suspension of diagnostic core needle biopsies and fine needle aspirations during the pandemic. We, therefore, did not get the required sample size of 184 participants. Airports were also closed, and logistically this posed challenges in moving the cell blocks on time from Malawi to South Africa. Molecular studies on the cellblocks could not be done. It was impossible to complete all components (including DNA assessment) of this study for the degree, but it may still be feasible as part of an ongoing study.

CONCLUSION

This study has shown improved sensitivity, cytomorphological and architectural preservation when using CRR-fixed cell blocks in conjunction with the routine FNA smears. Furthermore, this study confirmed that CRR-fixed cell blocks are adequate for performing immunocytochemical staining, thereby significantly improving the sensitivity for establishing important clinical diagnoses on FNA specimens. It is envisioned that CRR-fixed cell blocks will be a source of extractable, stable and usable DNA, enhancing their value as a source for biobanking and future research.

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