TITLE PAGE

Comparison of Fine Needle Aspiration Cytology and Cell Block to Formal Histology of Small Subcutaneous Mass Lesions in Children.

A Prospective Study at Red Cross War Memorial Children`s Hospital

By

ERNEST NAANWIN-IB KUNFAA JNR.

KNFERN001

Dissertation submitted for the completion of Master of Medicine in Paediatric Surgery

DIVISION OF PAEDIATRIC SURGERY

RED CROSS WAR MEMORIAL CHILDREN`S HOSPITAL

UNIVERSITY OF CAPE TOWN

2023

Supervisor:

Professor Sharon Cox

DECLARATION

FORM D19

FORM D19

Plagiarism Declaration

"This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor."

Name: ERNEST NAANWIN-IB KUNFAA

Student number: KNFERN001

Signature:

Signed by candidate

Date:
11/02/2024

COPYRIGHT INFORMATION

DECLARATION

I, Ernest Naanwin-Ib Kunfaa, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signed by candidate

Signature:

Date: 11/02/2024

ACKNOWLEDGEMENTS

With sincere gratitude to:

- Professor Sharon Cox, Charles FM Saint Chair and Head of Division of Paediatric Surgery, Red Cross War Memorial Children's Hospital and University of Cape Town, supervisor for this dissertation.

- Prof Alp Numanoglu, Emeritus Professor of Paediatric Surgery, Red Cross War Memorial Children's Hospital and University of Cape Town.

- Prof Komala Pillay and Shivani Singh for their guidance, time and timely reportage of reports.

- My colleagues at Red Cross Hospital who allowed me time and supported my research in diverse ways.

- Naseerah, the ward clerk for ward D2 at RCWMH, your support is very much appreciated.

- The staff of D2, Theatre, and laboratory (Histopathology unit at RCWMH and GSH), Statistical unit.

- To my wonderful wife (absolutely outstanding), kids, siblings and friends for their support all these years of study, you kept me going to the end.

CONTENTS

LIST OF TABLES

ABBREVIATIONS:

RCWMCH:	Red Cross War Memorial Children's Hospital
UCT:	University of Cape Town
WHO:	World Health Organisation
NHLS:	National Health Laboratory service
FNAC:	Fine needle aspiration Cytology
FNAB:	Fine needle aspiration Biopsy
SOPD:	Surgical Outpatient Department
FRC:	Faculty Research Committee
ICH GCP:	International Convention on Harmonisation Good Clinical Practice
TB:	Tuberculosis

ABSTRACT

Background

Fine-needle aspiration cytology (FNAC) has been widely accepted as a safe, cost-effective and accurate tool for the preoperative diagnosis of masses or lesions in adults with over 90% accuracy. The use of FNAC with adjunct Cell block in children is still not widely accepted.

Cell block technique refers to the processing of sediments, blood clots, or grossly visible tissue fragments from FNAB cytological specimens into paraffin blocks cut and stained as for formal histopathology. Use of Cell blocks as an adjunct to routine cytology smears can increase the sensitivity to a considerable extent leading to increased diagnostic yield.

Excision, incision or core biopsy and formal histology is the gold standard to diagnose pathological causes of small, palpable masses in children. Problems related to this approach include the need for preoperative admission, starvation, a general anaesthetic, an operative procedure, postoperative pain, higher costs, as well as time taken for results, while FNAC can be performed as an outpatient procedure with local anaesthetic if needed.

Objective

This study aims to primarily determine the accuracy of the combination of FNAC and Cell block in comparison to gold standard histopathological diagnosis for mass lesions in children.

Methods

This is a prospective single center study involving children who had both FNAC and excision or incision biopsy taken at the same operative time for histological diagnosis at Red Cross War Memorial Children's Hospital (RCWMCH). Different pathologists assessed the FNAC/Cell block samples and the formal histological samples in different laboratory areas, thus ensuring blinding of the results.

Results:

Fifty sets of samples were acquired from 49 nine participants, 29(59%) males and 20(41%) females. The age range was 17 days to 192 months, Pain 20(40%) was the most common presenting symptom, followed by fever 11(22%). The average duration of the lesions was 5 months (range 1 to 12 months). The average length of stay was 1 day (range 0 to 15 days). The head and neck 35(70%) were the most common site, followed by the axilla 5(10%). On formal histology there were 40(80%) benign lesions and 10(20%) malignant lesions. Nine patients had either bloody or inadequate FNAC samples, the Cell block was also bloody in 6 of these but gave the correct diagnosis in the other 3. The most common benign lesions were reactive lymph nodes 15(30%) and active mycobacterial infection 10(20%) whilst Hodgkin's Lymphoma 4(8%) was the most diagnosed malignancy. Five FNAC/Cell block combinations were falsely reported as reactive nodes, 3 of these represented Hodgkin's lymphoma on formal histology.

When analysing the ability of FNAC and Cell block combination to differentiate between benign and malignant lesions (as opposed to achieving the full histological diagnosis), 30 out of 35 sample sets correctly diagnosed benign conditions giving an 85.7% concordance. The sensitivity and specificity of FNAC and Cell block combination to correctly diagnose benign lesions is 97.1% and 66.7% respectively, with a positive and negative predictive value of 91.9% and 85.6% respectively.

For Malignant lesions 6 out of 9 sample sets correlated with the correct malignant diagnosis a concordance of 67%. The sensitivity and specificity of the FNAC Cell block combination to diagnose a malignant lesion was calculated at 66.6% and 97.1% respectively with a positive and negative predictive value of 85.6 and 91.9% respectively.

The combination of FNAC and Cell block gave the correct histological diagnosis in 36 (72%) of patients.

Limitations

Single-center study and a small sample size.

Conclusions:

FNAB is gradually being accepted as a diagnostic tool in the paediatric population. Cell block processing adds to the diagnostic potential of the FNAB. In this study, the FNAC Cell block combination showed an 85.7% concordance for benign conditions and 67% for malignant conditions when compared with formal histology.

In the absence of a bloody tap, inadequate sample or necrosis, and excluding samples with a diagnosis of a reactive node which would prompt further assessment, 98% of patients undergoing FNAC and Cell block were correctly diagnosed with pathology that was confirmed on formal histology.

The combination of FNAC and Cell block is a reliable method of diagnosing the histopathological diagnosis and in this series would have halved the number of patients needing a general anaesthetic to acquire a sample for formal histology. This represents a large saving in costs and theatre time, with the added advantage of patient safety.

CHAPTER 1: INTRODUCTION, LITERATURE REVIEW

Background:

Excision, incision or core biopsy and formal histology is the gold standard to diagnose pathological causes of small, palpable masses in children. Fine-needle aspiration biopsy (FNAB) has become increasingly popular for evaluating palpable masses in the adult population. Small palpable lesions - predominantly enlarged lymph nodes or other soft tissue tumours are a common presentation in the paediatric population. These masses generally have remained exempt from FNAB cytopathology, with open biopsy being the conventional and time-honoured approach to pathologic diagnosis(1). Problems related to this approach include the need for a preoperative admission and starvation, a general anaesthetic, an operative procedure creating a surgical wound, postoperative pain, as well as the high costs related to such a procedure.

Literature review

There are multiple recent papers that discuss FNAB in the adult population. Fine needle aspiration cytology (FNAC) remains an excellent diagnostic method for soft tissue tumours, with a sensitivity described as over 95% in detecting benign tumours, and a specificity of up to 99% when diagnosing malignancy (2-5). In addition, concordance between cytologic and histologic grading of soft tissue tumours can be as high as 75% (2).

FNAC has gradually become more accepted in the paediatric population but remains underused, possibly because of diagnostic difficulty related to some childhood tumours, many of which would fall in the ‘small round blue cell’ category (Neuroblastoma, non-Hodgkin’s lymphoma, Rhabdomyosarcoma, Primitive Neuroectodermal tumour and the blastemal component of a Wilms tumour) where the ultrastructural aspects of the tissue sample are important for definitive diagnosis as well as the perceived lower diagnostic accuracy in the paediatric population(6, 7).

The first studies examining paediatric FNAC were published in the 1980s (3, 7-12). In 1985, Akhtar et al described the concurrent use of light and electron microscopy to increase the

diagnostic accuracy of FNAB in paediatric patients and using these modalities together note that a precise diagnosis was possible in 94% of FNAB's yielding sufficient material, but also point out that this cannot be perceived as the accuracy rate because follow up biopsy specimens were not available in all cases(7). In 1989 Cohen et al reported a sensitivity of 97% and specificity of 95% of FNAB in determining the exact pathology in paediatric patients, however, this is after exclusion of those with insufficient material to analyze and only includes a small percentage of patients who had histological or clinical follow up (9). In 1984 Taylor and Nunez reported 64 FNAB's from 56 pediatric patients in which there were 19 malignant diagnoses, a sensitivity of 76%, and a specificity of 100% (13).

FNAB is, however, emerging as a preferred outpatient procedure for the diagnosis of soft tissue tumours in the paediatric population due to low cost of the procedure, fewer complications, feasibility, same day discharge and quick results. The procedure, being minimally invasive, can be done under local anaesthesia or sedation in children who would not tolerate local anaesthetic. Results from a FNAB could be known the same day with earlier initiation of further investigations or treatment (14). Thus, before resorting to surgical intervention, FNAB is a helpful procedure in the diagnosis of both neoplastic and non-neoplastic lesions (15).

In an inter-institutional study, review of FNAB in Paediatric oncology in South Africa, found sensitivity of the procedure for neoplastic lesions was 96.6%, the specificity 97.0%, positive predictive value 99.0%, and negative predictive value 90.1%, with a diagnostic accuracy of 96.7%. However, the ability of FNAB to enable a specific tumour diagnosis to be made with correct and accurate sub typing of the tumour on which chemotherapy or radiotherapy could be commenced was 75.5% (6). This would thus imply that up to 25% of paediatric malignancies undergoing FNAB would still need formal histology in order to determine the correct tumour subtype and hence guide the chemotherapy choice. Initially in this study, however, almost 10% of patients eligible for the study were excluded prior to calculation of these specificities due to inadequate smears (6).

Despite the increased use of fine-needle aspiration cytology there is therefore a set of patients who will need further ancillary testing in the form of immunohistochemistry, flow cytometry or formal histology, and in whom more tissue will be required.

There is limited information available to assess the contribution of Cell blocks, although the value of Cell blocks has been acknowledged (16, 17). Acquisition of tissue for cell block relies on a simple fine needle aspiration biopsy and is thus just as non-invasive as a simple FNAB test. Mathew et al in their work on the role of Cell block in cytopathologic evaluation, obtained satisfactory material on Cell block in 32 out of 46 cases (69.56%)(18). This was comparable to the study done by Nathan et al in which Cell block material was obtained in 300 out of 409 lesions (73.3%)(16). The Mathew et al study revealed that Cell blocks were superior to conventional FNAC smears with a sensitivity of 71.1%. An overall improvement in the final diagnosis was noted when smears were complemented by Cell block, with an increase in sensitivity of 84.4%. Even though diagnostic accuracy of Cell block was superior to FNAC in this study, there was no significant difference between conventional smears and Cell block (18).

There is very little literature comparing FNAC and Cell block to excision biopsy at the same time and of the same lesion to determine their efficacy or concordance. This study seeks to find out or to assess the accuracy, sensitivity and specificity of FNAC together with Cell block, as compared to formal histology in a paediatric population.

Motivation for the study:

Excision biopsy for histopathological diagnosis of small masses remains the gold standard of assessment and diagnosis in our institution. With this comes the problems of costs and time related to an admission and a surgical procedure. There is not much evidence comparing the accuracy and sensitivity and specificity of less invasive forms of diagnosis such as FNAC and Cell block combination compared to formal histology in the paediatric population. This study aims to compare FNAC with Cell block to formal histology in order to assess their correlation.

When performed simultaneously for this study, fine needle aspiration biopsy is a minor procedure in addition to the standard incision or excision biopsy for histopathology that patients would normally undergo at Red Cross War Memorial Children`s Hospital. As such, it should not result in any additional inconvenience or pain for the patient undergoing incisional or

excisional biopsy, but the outcome of this study has potential for having a great effect on procedures for future patients requiring characterization of their solid lesions.

This study aims to:

1. Primarily determine the accuracy of the combination of FNA cytology and Cell block in comparison to gold standard histopathological diagnosis.
2. Secondary aims are to identify the demographics of the involved patients and the distribution of cases according to histopathological diagnosis.

Ethics

Approval of the study by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee was obtained, REF. 747/2020. (Appendix 1). The Red Cross War Memorial Children's Hospital scientific committee granted permission for the study.

CHAPTER 2: THE STUDY

Methods

As a single centre study candidates who were considered included:

1. All patients who presented to the Division of Paediatric Surgery at Red Cross War Memorial Children's Hospital with a history of a palpable mass. These after clinical assessment with or without radiological investigation in the form of Ultrasonography or Magnetic Resonance imaging (MRI).
2. Those who required incisional biopsy or excision of the mass to obtain formal histology as the current local standard of care.

There was full explanation verbally and via an information sheet (Appendix 2) and signing of informed consent forms (Appendix 3) if the parent or legal guardian agreed. The assent of the child was also sought if the child was of an appropriate age and intellectual maturity normally around 8 years of age (Appendix 4). Consent and patient assent to participate in the study was separate to the standard procedure consent and assent required to perform a procedure under general anaesthetic (Appendix 5). A date for surgery was booked and parent or legal guardian informed, a telephonic call was made to remind them a day prior to surgery.

All participants consenting to the study were then identified on a Study Participant Log Sheet (Appendix 6) and were assigned a participant number. Each participant had a data sheet allocated to them where all relevant information was captured (Appendix 7). These data sheets were and remain separate to and filed apart from the clinical records. This data was entered to a Microsoft Excel spreadsheet for interrogation and comparison. Anonymised data was and remains stored in a password protected file.

Procedure:

The investigators were responsible for the study consent and assent as well as the organizational aspects of the study and related procedures for all patients admitted to the study.

The general anaesthetic was performed by the anaesthetist on duty and in accordance with the child's age, size clinical condition and co-morbid pathology. As far as was logistically possible,

one of the investigators were either the operative surgeon or assistant in the operation/ FNAB procedure. Should an investigator not be available due to other commitments, the surgeon on call performed the procedure. This was an infrequent occurrence and was further mitigated by discussion of patient orders on operative lists in the morning theatre 'Huddle' where theatre logistics were discussed. Only those staff members willing to participate and knowledgeable of the study procedures did so.

Prior to commencement of the study, all Paediatric Surgical registrars and consultants who were able to perform such procedures were briefed on the study. In addition, they underwent training on the FNAB procedure in the on-site Surgical Skills Training Centre so that the procedure was standardized.

Standard World Health Organization (WHO) checklist, induction, anaesthetic, skin preparation and drape occurred as per normal processes in theatre. Prior to any skin incision, a fine needle aspiration of the mass was performed in order to secure FNAC smears as well as a Cell block sample. If the mass was located close to major vasculature an ultrasound guided sample procurement was carried out, to reduce risk of injury to surrounding vasculature or organs.

Standard technique of FNAB:

Two samples were taken with the following fine needle aspiration technique. Using a 22-gauge needle, and a 10ml syringe, an initial 'non-aspirate' sample was taken. One 1ml of air was aspirated into the syringe, the needle was passed in and out of the mass 3 times in a fan shape, without removing the needle from the skin on each pass and without any negative pressure on the syringe. The material in the needle was then smeared on one or more cytology slides and fixed with cytological fixative by the National Health Laboratory Service (NHLS) assistant.

A second 'aspirate' sample was taken in the exact same manner, this time with negative pressure applied to the syringe, and this was smeared on a separate set of cytology slides and fixed accordingly (also for cytology). The same syringe was then flushed with saline and all contents injected into a test tube containing normal saline. This was the sample for Cell block analysis. Both cytology and Cell block specimens were labelled with the participant number and not patient details. The Cell block specimen is then spun down and the sediment processed

as for formal histology. Standard NHLS protocol for spinning the sample, staining of the cytological specimens and processing was performed in the NHLS laboratory. The process of FNAB added on average 3 minutes to the entire procedure and added no or negligible morbidity to the standard open excisional biopsy procedure.

Acquisition of samples for FNAC and Cell block was then followed by excision or incision biopsy of the mass as per standard protocol. Histology specimens were transported to the laboratory in formalin, and formal histology was performed according to standard NHLS protocol. These specimens were labelled with the patient's name and hospital record number. A total of two sets of cytology slides, one Cell block sample and the removed tissue was sent to 2 separate NHLS laboratory settings.

Blinding:

Both cytology and Cell block specimens were labelled with participant numbers and not the patient's details. Formal histology specimens were labelled with the patient's name and medical record number. Cytological examination and histological examination were performed in different venues and by different pathologists, thus blinding the pathologists performing formal histological evaluation to the cytology and Cell block results.

Postoperative care

Postoperative care was as per standard protocol. All patients continued to receive standard treatment which was be related to the cytology or histological findings and consisted of pain medications and consultation with Oncology unit or Infectious Disease unit if required.

Data collected was analysed to determine the accuracy and correlation of the various tests as well as look at the demographics and various pathological diagnoses in participants.

It is important to note that management of the participant was exactly as per normal standard patient management; the only difference was the addition of the fine needle aspiration that would add approximately 2 to 3 minutes to the procedure and would add no or negligible additional morbidity peculiar to fine needle aspiration which will be different from morbidity of the open excision of mass.

Population:

Patients aged less than 13 years undergoing incision or excision biopsy and meeting all inclusion criteria:

Inclusion Criteria

- All children less than 13 years old who presented to RCWMCH with a small palpable lesion suspicious for a pathological process requiring excision or incision biopsy and formal histology for diagnosis.
- Appropriate consent and assent granted.

Exclusion Criteria

- Patients who, deemed by clinical findings and hospital protocols required a FNAB only without need for excision biopsy.
- Masses that were out of reach for a FNAB
- Denied consent or assent.

Statistical Methods:

It was determined that between 50 and 60 excision biopsies are performed annually on patients with palpable masses at Red Cross War Memorial Children's Hospital. The biostatistician determined a sample size as per below would serve as a guide to the final sample size for the study.

In terms of sample size to determine non-inferiority of FNAC plus Cell block verses histology, the Biostatistician determined a number of scenarios:

1. Assuming histology has a diagnostic accuracy of 99.9% and FNAC/Cell block has a diagnostic accuracy of 97%, power = 90%, alpha = 5% (one sided): sample size = 88
2. Assuming histology has a diagnostic accuracy of 99.9% and FNAC/Cell block has a diagnostic accuracy of 97%, power = 80%, alpha = 5% (one sided): sample size = 46
3. Assuming histology has a diagnostic accuracy of 99.9% and FNAC/Cell block has a diagnostic accuracy of 95%, power = 90%, alpha = 5% (one sided): sample size = 46

4. Assuming histology has a diagnostic accuracy of 99.5% and FNAC/Cell block has a diagnostic accuracy of 97%, power = 80%, alpha = 5% (one sided): sample size = 108
5. Assuming histology has a diagnostic accuracy of 99.9% and FNAC/Cell block has a diagnostic accuracy of 97%, power = 90%, alpha = 1% (one sided): sample size = 102
6. Assuming histology has a diagnostic accuracy of 99.5% and FNAC/Cell block has a diagnostic accuracy of 95%, power = 90%, alpha = 1% (one sided): sample size = 98
7. [Sample size 0.995 0.90, one sample one sided alpha (0.01)]

Number of patients:

The authors would have liked to secure 100 participants over a 2-year period however this was limited to 50 observations for the following reasons:

1. Limited funding from a small research award for this protocol during the duration of this MMed
2. The timing of the study as relates to the need for write-up and graduation.
3. Patient accrual was decreased with COVID decreasing the number of theatre lists as well as the numbers of patients presenting.
4. Increased standalone FNAB's being performed during COVID. At the time, due to restraints imposed by COVID, the hospital protocol was altered to allow for FNAB as a safe preliminary investigation – resulting in fewer patients needing formal histology.

However, using researched figures of diagnostic accuracy of formal histology and Cell block, the sample size of 50 was sufficient. The study will, however, continue within the unit after completion of the MMed to acquire more data and possibly validate the procedure further.

Anticipated outcomes:

It was anticipated that the results of the Fine Needle Aspiration Cytology together with the added investigation of Cell block histology would be sufficient to determine the etiological diagnosis of palpable masses in the majority of patients, thus negating the need for formal histology in most patients.

Consent requirements, ethics and data storage:

The parent or legal guardian of each study participant signed an informed consent (Appendix 3) in a language of their choice prior to being admitted to the study. If the child was of an age

and maturity level that is appropriate, the child's assent was also sought (Appendix 4). Parents and guardians were informed that management of the child was standard and as per normal protocol (Appendix 5 standard hospital form) pertaining to the clinical problem, whether or not they agreed to being included to the study. They were informed of their right to refuse entry to the study or withdraw their consent at any time with no prejudicial treatment or alterations in the care of their child. They also gave consent for the taking of non-identifying clinical photographs, which were then stored electronically on the password protected theatre data system and could be used in the publication of the resulting paper.

The need for confidentiality was essential. Patient data was not kept together with identifying patient factors other than in the clinical notes and on the theatre record keeping system as per normal patient management. In order to achieve this; two data bases were established on separate computers. The first is a Participant Log sheet with patient name and number and a corresponding participant number which was and remains password protected (Appendix 6). The second data base has all clinical data from the data collection sheet (Appendix 7) with a corresponding participant number as previously allocated. All stored data is delinked from identifying details to preserve patient anonymity. Only study personnel have access to study data. All the research was done in accordance with the International Convention on Harmonisation Good Clinical Practice (ICH GCP) and declaration of Helsinki Guidelines (19).

Financial implications

The study was performed by the investigators who did not draw any salary. Stationary costs were drawn from Division of Paediatric Surgery research fund 411493 PDS 512 and a Post Graduate Research Training Grant (FRC Award 2020). Statistical support was through the statistician assigned for MMed support.

The management of study patients deviated from existing management protocols by the addition of FNAB while under anaesthetic. As the assessment of the FNAC and Cell block specimens are not standard of care, all costs incurred by stains for these procedures were borne by the study as elaborated in the budget (Appendix 8). All costs pertaining to hospital fees, scans, theatre and anaesthetic as well as the formal histology costs were covered by the state and patient as per the norms of the hospital billing system.

Participants and their families did not receive any remuneration for participation in the study as there is no deviation from normal management in these patients and no extra outpatient visits required.

Results

49 participants were entered into the study, one on 2 separate occasions, giving 50 sets of samples, each set including a FNAB that was processed for FNAC and Cell block assessment as well as a core, incision or excision biopsy which was sent for formal histopathology assessment. There were 29 (59.1%) males and 20 (39.9%) females.

The average age was 5 months (Range 17 days to 192 months). The average patient weight was 17kg (Range 3.2kg – 58.9kg).

Presenting Symptom	**Number of patients**
Asymptomatic mass	18
Pain	21
Fever	10
Weight loss	9
Skin change	4
Anaemia	3
Cough	3
Night sweats	2
Vomiting	1
Lower limb weakness	1
Urine retention	1
Constipation	1
Pus discharge	1
Headache	1
Pruritis	1

Table 1: Presenting symptoms.

An asymptomatic mass was present in 18(36%). The most common symptoms were pain in 21(42%) followed by fever in 10(20%), and weight loss in 9(18%) patients. Table 1 shows the presenting symptoms and their frequency. The head and neck 35(70%) were the most common site followed by lesions in the axilla 5(10%) and the inguinal area 3(6%). Nineteen (38%) patients stayed in hospital for 1 night, 16(32%) of patients did not stay overnight, and the rest

of the patients stayed between 3 and 15 days with longer stays due to urgent management needs, such as other oncological work-up, tunnelled line insertion, initiation of chemotherapy as examples.

Formal histopathology, the gold standard to which the rest of the results were compared, revealed 40(80%) patients had benign infective or non-infective pathology and 10(20%) had malignant conditions. Of the benign conditions, reactive lymph nodes 15(30%) were the most common finding, followed by active mycobacterial infection in 10(20%) and lipoma in 4(8%) cases. Hodgkin lymphoma, 4(8%), was the most common malignant condition. Table 2 delineates the confirmed diagnosis on formal histology.

Diagnosis		**Number of patients N (%)**
Benign	Reactive Lymph node	15 (30)
	Active mycobacterial infection	10 (20)
	Lipoma /Fibroadipose tissue	6 (12)
	Dermoid / epidermoid Cyst	2 (4)
	Non-necrotising granulomatous inflammation	1 (2)
	Sinus Histiocytosis (Rosai-Dorfman Disease)	1 (2)
	Lymphatic malformation	1 (2)
	Abscess Wall	1 (2)
	Pilomatrixoma	1 (2)
	Fat necrosis	1 (2)
	Nodular fasciitis	1 (2)
Malignant	Hodgkin Lymphoma	4 (8)
	Anaplastic Lymphoma	1 (2)
	Nephroblastoma	1 (2)
	Neuroblastoma	1 (2)
	Rhabdomyosarcoma	1 (2)
	Undifferentiated small round cell sarcoma of soft tissue	1 (2)
	Kaposiform Haemangioendothelioma	1 (2)

Table 2: Diagnosis based on formal histology.

Table 3 compares results of FNAC, Cell block and formal histology in all 50 sample sets. 29(58%) of the patients had concordance of all three investigations. A bloody or inadequate sample was obtained on FNAC in 9(18%) cases. Three of these 9 patients had a diagnosis

confirmed with Cell block analysis. In 2 patients FNAC diagnosed a reactive node while the cell block correctly diagnosed active mycobacterial disease in one and Hodgkins's lymphoma in the other. The Cell block thus confirmed the diagnosis in 5 additional patients as compared to the FNAC alone.

In two patients FNAC made the correct diagnosis with no added information obtained by the Cell Block: one of which was too bloody and the other showing myxoid fragments and reactive fibroblasts when the diagnosis was nodular fasciitis.

The combination of FNAC and Cell block thus gave the correct histological diagnosis in 36 (72%) of patients. When excluding patients with bloody samples on FNAC and Cell block, the FNAC and Cell block combination gave a correct diagnosis in 36 out of 44 sample sets an 82% concordance with formal histology.

There were 8(16%) patients where FNAC and Cell block were concordant but did not confirm the correct diagnosis. Two of these patients FNAC and Cell block showed necrosis, both revealing active mycobacterial infection on formal histology and 5(10%) had concordant FNAC and Cell block indicating reactive lymph nodes, while the true diagnosis was non-necrotising granulomatous inflammation, Sinus Histiocytosis (Rosai-Dorfman Disease) and Hodgkin lymphoma (1, 3). The other false positive was that of neuroblastoma on both FNAC and Cell block, while the true diagnosis on histology was that of reactive lymph node hyperplasia. Thus, when excluding patients with FNAC and Cell block bloody samples, 8/44(18%) of FNAB Cell block sample sets gave the incorrect diagnosis.

When analysing the ability of FNAC and Cell block combination ability to differentiate between benign and malignant lesions (as opposed to achieving the full histological diagnosis), 30 out of 35 sample sets correctly diagnosed benign conditions giving an 85.7% concordance. The sensitivity and specificity of FNAC and Cell block combination to correctly diagnose benign lesions is 97.1% and 66.7% respectively, with a positive and negative predictive value of 91.9% and 85.6% respectively.

For malignant lesions 6 out of 9 sample sets correlated with the correct malignant diagnosis – a concordance of 67%. The sensitivity and specificity of the FNAC Cell block combination to

diagnose a malignant lesion was calculated at 66.6% and 97.1% respectively with a positive and negative predictive value of 85.6 and 91.9% respectively.

FNAC	Cell Block	Formal Histology	FNAC/CB correlation to histology	Benign or Malignant
Bloody sample	Bloody sample	Fibroadipose tissue	Inconclusive	Benign
Bloody sample	Bloody sample	Reactive lymph node	Inconclusive	Benign
Bloody sample	Bloody sample	Kaposiform haemangio-endothelioma	Inconclusive	Malignant
Bloody sample	Bloody sample	lymphangioma	Inconclusive	Benign
Bloody sample	Bloody sample	AMB	Inconclusive	Benign
Bloody sample	Bloody sample	Reactive lymph node	Inconclusive	Benign
Inadequate/ Bloody sample	Lipoma	Lipoma	Positive	Benign
Inadequate sample	Lipoma	Lipoma	Positive	Benign
Inadequate sample	Abscess	Abscess wall	Positive	Benign
AMB	AMB	AMB	Positive	Benign
AMB	AMB	AMB	Positive	Benign
AMB	AMB	AMB	Positive	Benign
AMB	AMB	AMB	Positive	Benign
AMB	AMB	AMB	Positive	Benign
AMB	AMB	AMB	Positive	Benign
Necrosis	Necrosis	AMB	negative	Benign
Necrosis	Necrosis	AMB	negative	Benign
Anaplastic lymphoma	Anaplastic lymphoma	Anaplastic lymphoma	Positive	Malignant
calcification in fat necrosis	calcification in fat necrosis	fat necrosis	Positive	Benign
Cyst	Bloody sample	Dermoid cyst	Positive	Benign
Cyst: Keratinizing squamous epithelium	Cyst: Keratinizing squamous epithelium	Epidermoid inclusion cyst (Cyst: Keratinizing squamous epithelium)	Positive	Benign
Nodular fasciitis	Myxoid fragments, Reactive Fibroblasts	Nodular fasciitis	Positive	Benign
Fibroadipose tissue	Fibroadipose tissue	Lipoma	Positive	Benign
Fibroadipose tissue	Fibroadipose tissue	Fibroadipose tissue	Positive	Benign
Fibroadipose tissue	Fibroadipose tissue	Lipoma	Positive	Benign
Neuroblastoma	Neuroblastoma	Neuroblastoma	Positive	Malignant
Neuroblastoma	Neuroblastoma	Reactive lymph node	Negative	Benign
Pilomatrixoma	Pilomatrixoma	Pilomatrixoma	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign

Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Reactive lymph node	Positive	Benign
Reactive lymph node	Reactive lymph node	Non-necrotising granulomatous inflammation	Negative	Benign
Reactive lymph node	AMB	AMB	Positive	Benign
Reactive lymph node	Hodgkin lymphoma	Hodgkin lymphoma	Positive	Malignant
Reactive lymph node	Reactive lymph node	Sinus Histiocytosis (Rosai-Dorfman Disease)	Negative	Benign
Reactive lymph node	Reactive lymph node	Hodgkin lymphoma	Negative	Malignant
Reactive lymph node	Reactive lymph node	Hodgkin lymphoma	Negative	Malignant
Reactive lymph node	Reactive lymph node	Hodgkin lymphoma	Negative	Malignant
Rhabdomyosarcoma	Rhabdomyosarcoma	Rhabdomyosarcoma	Positive	Malignant
Triphasic nephroblastoma	Triphasic nephroblastoma	Triphasic nephroblastoma	Positive	Malignant
Undifferentiated small round cell sarcoma	Undifferentiated small round cell sarcoma	Undifferentiated small round cell sarcoma of soft tissue	Positive	Malignant

Table 3: Table comparing FNAC, Cell block and formal histology results of 50 sample sets. Correlation refers to whether the FNAC/Cell block combination differentiated benign and malignant lesions (AMB: Active Mycobacterial Infection, CB: Cell Block)

Discussion

This study aimed to primarily determine the accuracy of the combination of FNAC and Cell block in comparison to gold standard histopathological diagnosis for mass lesions in children. There were 29(59%) males and 20(41%) females within the age range of 17 days to 192 months. Pain 20(40%) was the most common presenting symptom, followed by fever 11(22%). The average duration of the lesions was 5 months (range 1 to 12 months) while the head and neck 35(70%) were the most common site, followed by the axilla 5(10%). Formal histology revealed 40(80%) benign lesions and 10(20%) malignant lesions. Reactive lymph nodes 15(30%) and active mycobacterial infection 10(20%) were the most common benign lesions respectively, whilst Hodgkin's Lymphoma 4(8%) was the most diagnosed malignant lesion.

The combination of FNAC and Cell block gave the correct histological diagnosis in 36 (72%) cases. FNAC Cell block combination showed an 85.7% concordance for benign conditions and 67% for malignant conditions when compared with formal histology. When those with a bloody tap, inadequate sample or necrosis and reactive nodes are excluded, 98% of patients undergoing FNAC and Cell block were correctly diagnosed, as corroborated by formal histology.

Palpable and non-palpable subcutaneous lesions is a presentation that is encountered by the Paediatric surgeon with the common practice being the excision or incision of the lesion for a histopathological diagnosis. However, this technique generally requires admission to hospital, a general anaesthetic, surgery results in a surgical wound and post-operative pain, and is both time and resource intensive.

Whilst still not the preferred biopsy technique, FNAB is gradually being accepted in the paediatric population, especially in the low-income countries, due to the advantages of using less resources, being less invasive and less time consuming. Due to the fact that this study included formal histology, 68% of patients required an overnight stay or longer, while the addition of a FNAB to the surgical procedure added just 3 minutes supporting the above comments on resource use for formal histological assessment compared to FNAB, which can be performed in an outpatient clinic.

With regards to accuracy in low and low middle income countries, Razak et al, in a study from South Africa, cited an overall sensitivity of cytology for detecting malignancy in a paediatric population was 96.6% with an accurate diagnosis allowing subtyping on which management could be based in 75.7% of malignant lesions undergoing FNAB while in another South African study, FNAB was found to be an acceptable diagnostic procedure for triage of paediatric lymphadenopathy(6, 20). In addition, FNAB may be favoured in patients that are not stable enough to undergo surgical excision (6, 20-23).

Cell blocks are prepared from residual cells within the FNAB needle and syringe. The addition of Cell block examination to routine smear analysis has greatly improved on the accurate diagnosis of both benign and malignant lesions via FNAC with subtyping of the malignant

lesions being possible as Cell blocks provide more tissue enabling a more definitive cytopathologic diagnosis (16, 22, 24). In a study relating to analysis of aspirated serous effusions, Cell block analysis was superior to the conventional smear as it provided both architectural information as well as diagnostic material for further studies such as immunohistochemistry to supplement the diagnosis (20).

This single center study sought to compare the results of FNAC/Cell block combination to the standard histology, seeking to evaluate the concordance of this combination investigation with formal histological evaluation the current gold standard. Several peer reviewed publications have suggested FNAC to be used as first line of investigation so as to avoid the constraints and complications that go with general surgery (6, 20, 23, 25). Material required for Cell block analysis is collected at the same time, in the same manner as that collected for the cytological smear, and hence adds no time or morbidity to the procedure. In addition, analysis of Cell blocks is generally fast and inexpensive in comparison to formal histology.

In our study 50 sample sets were collected. FNAB resulted in 7 bloody and 2 inadequate samples. These bloody or inadequate samples should prompt further investigation either as a repeat FNAB, or as formal histology. To avoid this, the clinician can perform further passes through a different area of the mass in an attempt to get less bloody slides at the time of initial biopsy should the sample appear overtly blood like. Also, one can still submit bloody samples for assessment as in 3/9 cases reported as bloody/ inadequate sample on FNACs, the Cell block still provided an answer. The 9(18%) bloody or inadequate sample that were obtained on FNAC is in keeping with the 20.6 % inadequacy rate which was said to be acceptable for this procedure performed on children in Sher-Locketz et al work (20). There were no recorded complications with any of the patients during the taking of FNAC samples, which goes to support the fact that FNAC has very minimal morbidity and mortality rate.

The majority (70%) of lesions in this study were in the head and neck area followed by the axilla, a similar distribution to other studies (20, 25, 26).

In this study reactive lymph nodes were the most common etiology followed by active mycobacterial infection for benign lesions, supporting findings in the literature that benign and

reactive lesions constitute a significant proportion of findings in children with small palpable lesions (25).

Of the patients with reactive lymph nodes diagnosed on FNAC, 7 out of 19 patients had false negative results, 2 of which had diagnoses confirmed on Cell block. FNAC and Cell block were thus correct in 14/19 reactive nodes with an accuracy of 74%. While this correlation is high, it is a concern that 5 patients had concordant results of reactive lymph nodes in both FNAC and Cell block, but formal histology revealed different diagnoses – 3 of them being Hodgkin's lymphoma. The low pick-up of Hodgkin lymphoma with FNAC in our study was also noticed by Razak et al who reported that the ability of FNAC to make specific diagnosis had less impressive rates for Hodgkin's lymphoma (68.2%) and rhabdomyosarcoma (9.1%) (5). The cytological diagnosis of Hodgkin's lymphoma in the absence of classic Reed Sternberg cells has been acknowledged and still remains a challenge (3, 5). This indicates that caution should be taken when clinical symptoms and signs do not correlate with a diagnosis of reactive lymph node, specifically if there are B-symptoms which would indicate TB or lymphoma. The authors feel due to the severity of the consequences of missing a malignancy or other differential diagnosis, and in the absence of any differentiating clinical features, or adjunct investigation for TB, a result indicating reactive lymph node may indeed warrant further investigation and or formal histological assessment.

Mycobacterial infection was the second most common benign condition occurring in 20% of this cohort. These children were investigated for TB using prior to FNAC. In other literature FNABs have a greater diagnostic yield than routine and more established diagnostic procedures including gastric aspirates and sputum sample (20). Our findings agree with Sher-Locketz and other writers that that mycobacterial infection can be accurately diagnosed with a high sensitivity and specificity on FNAB specimens (20). The 2 FNAC and Cell block sets diagnosed as necrotic tissue were confirmed to be mycobacterial infection on formal histology. Necrotic tissue on FNAC or Cell block should prompt repeat FNAC or formal histology as there is a possibility of necrotizing inflammation.

While the FNAC /Cell block combination missed the diagnosis of Hodgkin's lymphoma in 3 out of 4 patients and sampling was inadequate in one patient with Kaposiform

hemangioendothelioma, the combination had a 100% (5/5) pick rate for the small round blue cell tumours in this study.

When determining accurate histological diagnosis, excluding those with both FNAC and Cell block showing a bloody tap, Cell block analysis confirmed the diagnosis in 5/13 cases where FNAC was incorrect. In this study, FNAC / Cell block combination thus improved on the diagnostic accuracy of benign and malignant lesions as compared to FNAC or Cell block alone when each was compared to the standard histology. As Cell block creates no further morbidity, but does add value and improve diagnosis, it is suggested that all FNAB's be accompanied by a Cell block preparation and assessment. This is in line with Sharma et al. work where Cell block proved superior to smears for giving a definitive diagnosis and categorization of lesion (27). Thus, with a combination of FNAC and Cell block, 36/50 patients had the correct diagnosis. If excluding those whose Cell block was bloody, or necrotic, (which would both prompt further investigation), 36/42 (86%) patients had the correct diagnosis on non-invasive assessment methods alone. When excluding all patients with a reactive node – as already discussed these would most likely need further investigation by means of formal histology, 24/25 patients were diagnosed correctly - the only incorrect diagnosis being one FNAC and Cell block diagnosis of neuroblastoma was NOT confirmed on formal histology.

Thus, no matter which way the results are analysed, it is shown that addition of the Cell block technique increases the ability of FNAB to differentiate between benign and malignant lesions, and also increases the rate of correct histological diagnosis while providing sufficient sample for immunohistochemistry as well allowing appropriate treatment to be commenced.

Limitations

The limitations of this study include the single centre nature as well as the relatively small numbers due to both funding issues as well as the timing of the study that took place in the Covid 19 pandemic – where there were reduced numbers of patients undergoing both FNAB and formal histology. This could be addressed by further funded multicentre trials.

Conclusion

FNAB is gradually being accepted as a diagnostic tool in the paediatric population. Cell block processing adds to the diagnostic potential of the FNAB. In this study, the FNAC Cell block combination showed an 85.7% concordance for benign conditions and 67% for malignant conditions when compared with formal histology.

In the absence of a bloody tap, inadequate sample or necrosis, and excluding samples with a diagnosis of a reactive node which would prompt further histological assessment, 98% of patients undergoing FNAC and Cell block were correctly diagnosed with pathology that was confirmed on formal histology. It is advised to further investigate if FNAC and Cell block reveal an inadequate sample, necrosis or a bloody tap. While the sensitivity of the combination to diagnose malignancies is fairly high, at 66.7%, the authors feel this is too low to be safe, as the consequences of missing a malignancy specifically lymphoma, are dire. For this reason, the authors recommend that patients diagnosed with a reactive node on these 2 modalities need further investigation.

The combination of FNAC and Cell block is a reliable method of diagnosing the histopathological diagnosis, and in this series would have halved the number of patients needing a general anaesthetic to acquire a sample for formal histology. This represents a large saving in costs and theatre time, with the added advantage of patient safety. We strongly recommended that FNAC should be the first line of investigation and Cell block should be carried out in all FNAC investigations of all palpable lesions in children at RCWMH.

Disclosure of conflict of interest

None

References

1. Wakely Jr PE, Kneisl JSJCCIIJotACS. Soft tissue aspiration cytopathology: diagnostic accuracy and limitations. 2000;90(5):292-8.
2. Gulzar J, Yasin SB, Khan SP, Bashir N. Role of fine needle aspiration cytology (FNAC) in the diagnosis of soft tissue tumors. International Journal of Research in Medical Sciences. 2017;5(10).
3. Kocjan G. Fine needle aspiration cytology. Cytopathology. 2003;14(6):307-8.
4. Liu CC, Jethwa AR, Khariwala SS, Johnson J, Shin JJ. Sensitivity, Specificity, and Posttest Probability of Parotid Fine-Needle Aspiration: A Systematic Review and Meta-analysis. Otolaryngol Head Neck Surg. 2016;154(1):9-23.
5. Eslami A, Can NT, Ng DL. Infectious disease diagnosed by fine needle aspiration biopsy. Journal of the American Society of Cytopathology. 2020;9(3):152-8.
6. Razack R, Michelow P, Leiman G, Harnekar A, Poole J, Wessels G, et al. An interinstitutional review of the value of FNAB in pediatric oncology in resource-limited countries. Diagn Cytopathol. 2012;40(9):770-6.
7. Akhtar M, Ali MA, Sabbah R, Bakry M, Nash JE. Fine-needle aspiration biopsy diagnosis of round cell malignant tumors of childhood. A combined light and electron microscopic approach. Cancer. 1985;55(8):1805-17.
8. Wakely Jr PE, Kardos TF, Frable WJ. Application of fine needle aspiration biopsy to pediatrics. Human pathology. 1988;19(12):1383-6.
9. <The use of fine-needle aspiration biopsy in children.pdf>.
10. Betsill JR WL, Hajdu SI. Percutaneous aspiration biopsy of lymph nodes. American journal of clinical pathology. 1980;73(4):471-9.
11. Burt A, Smillie D, Cowan M, Adams F. Fine needle aspiration cytology: experience with a cell block technique. Journal of clinical pathology. 1986;39(1):114.
12. Fleisher G, Grosflam J, Selbst S, Ludwig S. Management of lymphadenitis in childhood: the role of percutaneous needle aspiration. Ann Emerg Med. 1984;13(10):908-11.
13. Taylor SR, Nunez C. Fine-needle aspiration biopsy in a pediatric population. Report of 64 consecutive cases. Cancer. 1984;54(7):1449-53.
14. Hossain T, Amin MR, Siddiqui M, Huq MAU, Rahman MMJJoPSoB. Correlation Between FNAC and Open Biopsy in Cervical Lymhadenopathy in Children. 2010;1(2):118-26.
15. Haque M, Talukder SJMmjM. Evaluation of fine needle aspiration cytology (FNAC) of lymph node in Mymensingh. 2003;12(1):33-5.
16. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology: improved preparation and its efficacy in diagnostic cytology. American journal of clinical pathology. 2000;114(4):599-606.
17. Zito FA, Gadaleta CD, Salvatore C, Filotico R, Labriola A, Marzullo A, et al. A modified cell block technique for fine needle aspiration cytology. Acta cytologica. 1995;39(1):93-9.
18. Mathew EP, Nair V. Role of cell block in cytopathologic evaluation of image-guided fine needle aspiration cytology. J Cytol. 2017;34(3):133-8.
19. Bioethique JI. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. 2004.
20. Sher-Locketz C, Schubert PT, Moore SW, Wright CA. Successful Introduction of Fine Needle Aspiration Biopsy for Diagnosis of Pediatric Lymphadenopathy. Pediatr Infect Dis J. 2017;36(8):811-4.
21. Wakely PE, Jr., Kneisl JS. Soft tissue aspiration cytopathology. Cancer. 2000;90(5):292-8.
22. Agrawal P, Srinivasan R, Rajwanshi A, Gupta N, Dey P, Kakkar N, et al. Fine needle aspiration cytology of paediatric soft tissue tumours highlighting challenges in diagnosis of benign lesions and unusual malignant tumours. Cytopathology. 2019;30(3):301-8.
23. van de Schoot L, Aronson DC, Behrendt H, Bras J. The role of fine-needle aspiration cytology in children with persistent or suspicious lymphadenopathy. J Pediatr Surg. 2001;36(1):7-11.
24. Suri J, Gandotra V, Abrol D, Bhardwaj S. Analysis of Cell Block Vs . Conventional Smear in Fluid Cytology. Journal of Evidence Based Medicine and Healthcare. 2015;2(39):6464-71.

25. Gupta S, Khatri S, Garewal J, Vijay S, Singh S, Kumar M. A Study to Correlate between FNAC and Histopathological Diagnosis of Enlarged Lymph Node. Annals of International Medical and Dental Research. 2016;2(5).
26. Jyoti D, Suman S, Kumar Bhattacharya B, Deo Narain C, Kumar Agrawal P. Clinicopathological Correlation of Fine Needle Aspiration Cytology with Histopathology in Peripheral Lymphadenopathy in Patients Attending a Tertiary Care Hospital in Eastern Bihar. Journal of Evolution of Medical and Dental Sciences. 2016;5(104):7654-6.
27. Sharma R. Role of Cell Block in Diagnostics-A New Paradigm in Cancer Diagnosis. International Clinical Pathology Journal. 2015;1(5).

CHAPTER 3: APPENDICES

Appendix 1: Ethics approval

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submisisons@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

03 May 2021

HREC REF: 747/2020

Prof S Cox
Division of Paediatric Surgery
6th Floor, ICH Building
Red Cross Children's Hospital
Email: sharon.cox@uct.ac.za
Student: naanwin-ib@yahoo.com

Dear Prof Cox

PROJECT TITLE: COMPARISON OF FINE NEEDLE ASPIRATION CYTOLOGY AND CELL BLOCK TO GOLD STANDARD HISTOPATHOLOGICAL DIAGNOSIS-MMED CANDIDATE-DR ERNEST KUNFAA

Thank you for your response letter, addressing the issues raised by the Faculty of Health Sciences Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 May 2022.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

The HREC acknowledge that the student: - Dr Ernest Kunfaa will also be involved in this study.

Please quote the HREC REF 747/2020 in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF 747/2020sa

Appendix 2: Information sheet

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

RCWMCH ROHVK
Children First

Informed Consent Form for parents/caregivers

Study number: __ __ __ __

PARTICIPANT INFORMATION LEAFLET AND INFORMED CONSENT - CASES

Each participant must receive, read and understand this document before any study-related procedure is performed.

STUDY TITLE: Comparison of fine needle aspiration cytology and Cell block to formal histology of small subcutaneous mass lesions in children.

INSTITUTION: Red Cross War Memorial Children's Hospital, University of Cape Town

Dear Parent / Legal Guardian

Hello. My name is _____ (name of study doctor) and I would like to ask if you are interested in allowing your child to take part in a research study. Research is the process in which we look for answers to questions that can help us improve the treatment of patients. With this specific research study we would like to learn more about the different ways in which we can find the cause of lumps and bumps in children. The different ways that we will investigate includes Fine Needle Aspiration Cytology, Cell block and formal Histology. The first two test needs only a needle and syringe to get the sample but the last one (the reason your child is here) requires removal of the part or the whole lump or bump in theatre to carry out the test.

Why is your child being asked to participate?

You are being asked for permission for your child to participate because he/she is being admitted to our ward so that we, as your child's doctors, can find a cause for and treat his/her lump/bump.

- This information leaflet is there to help you to decide if you would like your child to participate in this study. If you have any questions, do not hesitate to ask me.

- Before agreeing to participate, it is important that you read and understand the purpose of the study, the study procedures, benefits, risks, and discomforts.
- Taking part in this study is voluntary. You can choose not to take part and if you join, you may withdraw at any time.
- If you decide not to take part in this study, or have your child removed from the study, your child will still receive the usual care that he/she would have received otherwise.
- You should only agree to allow your child to take part if you are satisfied with all of the procedures involved.
- If you decide to allow your child to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will be given a copy of this form to keep.

What is the purpose of the study?

Like your child, there are many children who have some sort of small lump or bump. These lumps or bumps are then usually removed during a small surgery so that we can look at it under a microscope, called Formal Histology, to see what the cause is.

This means that your child will have to be admitted to the hospital in order for the surgery to be performed, which is an inconvenience for you and your child. The surgery may also leave a scar and there may be some pain after the operation. We are exploring other ways to find the cause of the lump or bump, instead of doing an operation, such as using a small needle and syringe to stick into the lump or bump to take samples for testing after giving medicine to prevent pain. This small needle test, (called a Fine Needle Aspiration cytology and Cell block) does not require admission to the hospital or an operation.

This study aims to compare the two small tests taken with fine needle to the formal histology which requires an operation. We hope to show that we can, in future, only do the small needle test which is quicker, easier and less painful, which will help ensure that fewer children will need an operation under general anesthesia and hopefully we will have fewer complications.

Length of the study and number of participants

The study will take place at Red Cross War Memorial Children's Hospital in Cape Town, South Africa. Approximately 100 children, with lumps and bumps, will be enrolled into the study

over an 18 month period. Should you agree to participate in the study, it will involve a maximum of 1 hour of your time.

What will happen in this study?

Your child will be treated like we have been treating all children with lumps and bumps thus far. He/she will go for a planned operation. When your child is asleep (under a general anesthesia), and before we make the cut on the skin, the surgeons will use a small needle and syringe to remove a few cells from the lump. This is the only extra part of the process, and is what we are asking permission for. After the needle test, we will proceed with the operation as we normally would do. The samples we collect will help us learn more about Fine needle aspiration cytology and Cell block test and how these compare to the operation results.

We will contact you by telephone or give you a follow up at the surgical outpatient clinic to inform you about the result/diagnosis of the lump/bump.

Will any of these study procedures result in discomfort or inconvenience?

Your child may experience some pain, slight bleeding from the point of needle injection and rarely minor haematoma (blood collection under the skin) after Fine needle injection. They will be given adequate pain medication to stop the pain. There are other risks, but these are from the standard open procedure of cutting and removing the lump (Excision biopsy) and these include bleeding, bruising, infection at the operation site, problems of wound healing and scarring.

What are the benefits?

There are no direct benefits to the participants other than the lump/bump being removed. However, the results of this study may help us to formulate a policy where other children in South Africa will not have to undergo surgery as the first step to diagnose their lump/bump.

What are your rights?

Your child's participation in this study is completely voluntary and you can decline his/her participation, without stating any reason. Your child's medical care will not be affected if you do not participate in this study.

Will I be compensated for my child to take part in the study?

You will not be paid for your child to take part in this study. You will not have to pay any additional costs if your child takes part in the study. Although injuries are very unlikely in this study, if any research related injuries your child will be seen at the Surgical emergency or at the surgical outpatient department where the both the research persons (Dr Ernest Kunfaa or Professor Sharon Cox) or the surgeon involved in the procedure. After assessments and intervention, you may be referred to a healthcare facility or specific doctor (specialist) for further management if need be.

How will my child's information be protected?

All of the research records will be kept confidential and stored securely. Your child will be identified by a unique study number and not his/her name. The doctors, members of the research team, the study monitors and members of the ethics committees that oversee the study, will need to see your child's health information, and will keep all personal information confidential.

What will happen to the samples collected from my child?

Your child's name will be removed from all needle samples and replaced with numbers, to ensure that samples can only be used by people closely involved in the research. Most of the samples collected will be stored and tested at a local laboratory.

What happens if I get hurt taking part in this study?

This research study is covered by an insurance policy taken out by the University of Cape Town. In the case that your child suffers a bodily injury because he/she is taking part in the study the insurer will pay for all reasonable medical costs required to treat his/her bodily injury. This will be according to the SA Good Clinical Practice Guidelines 2006 (or latest version), which are based on the Association of the British Pharmaceutical Industry Guidelines. The

insurer will pay without you having to prove that the research was responsible for your bodily injury. You may ask the study doctor for a copy of these guidelines.

The insurer will not pay for harm if, during the study, you:

- Use medicines or other substances that are not allowed.
- Do not follow the study doctor's instructions.
- Do not tell the study doctor that you have a bad side effect from the study medicine.
- Do not take reasonable care of yourself and your study medicine.

If you are harmed and the insurer pays for the necessary medical costs, usually you will be asked to accept that insurance payment as full settlement of the claim for medical costs. However, accepting this offer of insurance cover does not mean you give up your right to make a separate claim for other losses based on negligence, in a South African court.

It is important to follow the study doctor's instructions and to report straightaway if you have a side effect from the study medicine.

Who do I contact if I change my mind or if I want to withdraw from the study?

Researcher: Dr Ernest N. Kunfaa 06068368024

Principal Investigator: Dr Ernest N. Kunfaa 06068368024

Co-investigator: Professor Sharon Cox 021-658 5012

UCT Ethics Office: Mrs. Lamees Emjedi 021- 406 6338

Appendix 3: Consent form

INFORMED CONSENT FORM

Study number: __ __ __ __

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I asked have been answered to my satisfaction.

I consent voluntarily for my child to be a participant in the study to compare fine needle aspiration cytology, Cell block and formal histology of small lumps/bumps (subcutaneous mass lesions) in children.

Print Name of **Parent/Legal guardian**_____
Signature of **Parent/Legal guardian** _____
Date _____

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of **witness**_____
Signature of **witness** _____
Date _____

Statement by the researcher/person taking consent.

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Fine Aspiration of mass prior to incision/ excision biopsy under same General anesthesia
- Surgery will be done as same standard procedure.
- All samples to be taken will be sent to the laboratory for various testing at no additional cost to the patient.
- We may take non-identifying clinical pictures of lesion or microscopic staining.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of **researcher/ person taking consent** _____

Signature of **Researcher/Person taking the consent**_____

Date _____

Appendix 4: Assent Form

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

RCWMCH RQHVK
Children First

Assent to Participate in Research - Form

Study number: __ __ __ __

PARTICIPANT INFORMATION LEAFLET AND ASSENT FORM

INSTITUTION: Red Cross War Memorial Children's Hospital, University of Cape Town

STUDY TITLE: Comparison of fine needle aspiration cytology and Cell block to formal histology of small subcutaneous mass lesions in children. (FNA study)

RESEARCHERS NAME(S): Dr Ernest Kunfaa, Prof Sharon Cox, Prof Alp Numanoglu, Prof Pillay Komala, Dr Shivani Singh

ADDRESS:
Division of Paediatric surgery
Red Cross War Memorial Children Hospital
7700 Rondebosch

CONTACT NUMBER: 021 658 5054 or 021 658 5012

RESEARCH

We are asking you to be part of a research study. We as doctors do research to learn more about certain problems and diseases and to find new and better ways to treat these problems and disease.

WHY is the research study being done?

This study is being done to compare two different ways of finding out what the cause of lump/bump are in children like you. Currently the normal way of finding out is by doing an operation and taking part of or the whole lump out and sending it to the laboratory where it will be looked at under a special microscope, called histology. For the research we are doing, we will take a few pieces from the lump/bump using a small needle, called Fine Needle Aspiration, and then send these pieces to the laboratory to be looked at in two special tests. We want to find out if the test with the small needle, which does not need to be done as an operation can find the cause of the lump/bump just as good as the operation, so that we can use only this small needle test for other children in future.

Why have I been invited to take part in this research study?

You have been invited because you have a lump or bump that the doctors will remove to find the cause. Knowing what causes the lump or bump will help decide how to treat it.

Who is doing the research?

The surgeons working at Red Cross War Memorial Children Hospital are doing the research. Their names are at the top of this form.

What will happen to me in this study?

If you decide that you would like to take part in this study, we would like to keep the information about your lump/bump. When you go to the theatre for your operation, we will make you sleep so that you cannot feel any pain and we will then use the small needle to take some pieces of your lump before the surgeon takes it out.

Your information will be kept secret and only used by the doctors and other members involved in the research. Your name will be kept separately from your information, so that your personal details are kept as secrete as possible. We may take non-identifying clinical pictures of the lump or microscopic staining. We will contact you by telephone or a follow up at the surgical outpatient clinic with results or diagnosis of the lump or bump.

Can anything bad happen to me?

You will not experience any pain when the samples are taken but you may experience a little pain after the operation, but you will be given enough pain medicine to stop the pain. You may experience some pain, slight bleeding from point of needle injection and rarely minor haematoma (blood collection under the skin) after Fine needle injection. There are other risks but these are from the standard open procedure of cutting and removing the lump (Excision biopsy) and these include bleeding, bruising, infection at the operation site, problems of wound healing and scarring.

All of these are possible bad things that can happen from the operation for which you are here. Adding the fine needle test for which we are as your permission, will not add other bad things to the operation. We will be very careful and make sure to give clear instructions to you and your parents/guardian to help prevent these bad things from happening.

Can anything good happen to me?

The lump or bump will be removed from your body but this study will not directly affect you. The findings of this study might help other children with lumps and bumps in future.

Will anyone know I am in the study?

Your name will be replaced with a study number. Apart from the researchers, no one else will know your name or any other of your personal details.

Who can I ask questions about the study?

You can ask questions if do you do not understand any part of the study. If you have questions later that you do not think of now, you can talk to me or any of the researchers or ask your parent or Guardian or nurse to call the number provided above.

What if I do not want to do this?

It is your choice to be part of this research. You are allowed to say no. This will not affect the surgery for which you are here for neither will it affect the good care that you will receive from the facility. Also, you can withdraw from the research at any time.

Participant's Statement

The researcher told me about the research study and I understand.	YES	NO
I had a chance to ask questions.	YES	NO
I know I can ask questions at any time.	YES	NO
I know that I can stop taking part in the study at any time.	YES	NO
I want to be in the research study.	YES	NO

_____

Printed Name of Research Participant

_____

Signature of Research Participant **Date**

_____

Printed Name of Parent or Legal Guardian

_____

Signature of Parent or Legal Guardian

_____ _____

Date **Time**

Witness Statement

I have been present during the oral presentation of this research study.

_____

Printed Name of Witness

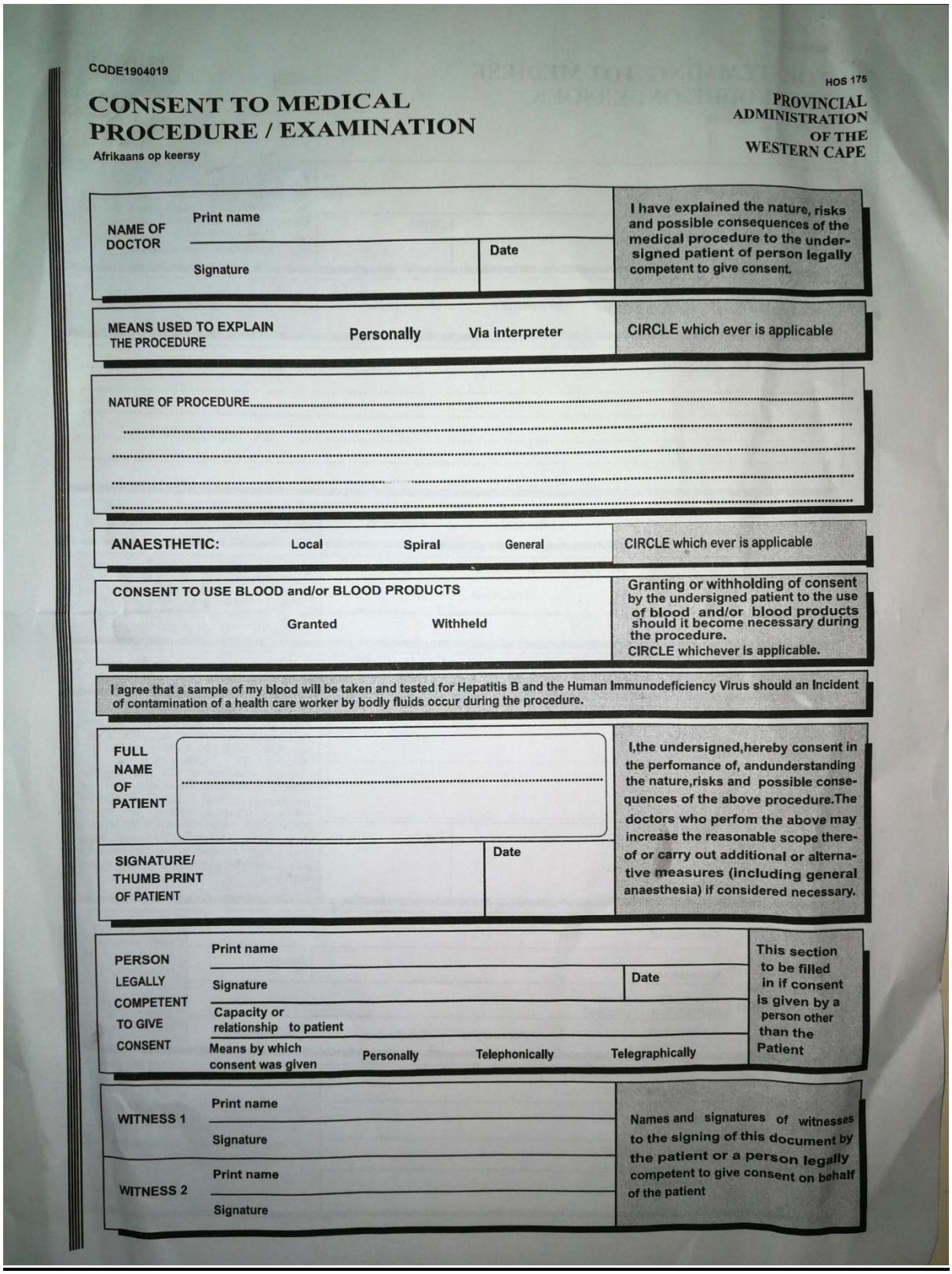
_____

Signature of Witness

_____

Date **Time**

Appendix 5: Consent to Medical examination



CODE1904019

HOS 175

CONSENT TO MEDICAL PROCEDURE / EXAMINATION

Afrikaans op keersy

PROVINCIAL ADMINISTRATION OF THE WESTERN CAPE

NAME OF DOCTOR	Print name Signature Date	I have explained the nature, risks and possible consequences of the medical procedure to the undersigned patient of person legally competent to give consent.
MEANS USED TO EXPLAIN THE PROCEDURE	Personally Via interpreter	CIRCLE which ever is applicable
NATURE OF PROCEDURE.....		
ANAESTHETIC:	Local Spiral General	CIRCLE which ever is applicable
CONSENT TO USE BLOOD and/or BLOOD PRODUCTS	Granted Withheld	Granting or withholding of consent by the undersigned patient to the use of blood and/or blood products should it become necessary during the procedure. CIRCLE whichever is applicable.
I agree that a sample of my blood will be taken and tested for Hepatitis B and the Human Immunodeficiency Virus should an Incident of contamination of a health care worker by bodly fluids occur during the procedure.		
FULL NAME OF PATIENT	.....	I,the undersigned,hereby consent in the perfomance of, andunderstanding the nature,risks and possible consequences of the above procedure.The doctors who perfom the above may increase the reasonable scope thereof or carry out additional or alternative measures (including general anaesthesia) if considered necessary.
SIGNATURE/ THUMB PRINT OF PATIENT	Date	
PERSON LEGALLY COMPETENT TO GIVE CONSENT	Print name Signature Date Capacity or relationship to patient Means by which consent was given Personally Telephonically Telegraphically	This section to be filled in if consent is given by a person other than the Patient
WITNESS 1	Print name Signature	Names and signatures of witnesses to the signing of this document by the patient or a person legally competent to give consent on behalf of the patient
WITNESS 2	Print name Signature	

Appendix 6: Study Participant log

Study Participant Log

Comparison of fine needle aspiration cytology and Cell block to formal histology of small subcutaneous mass lesions in children.

Date Enrolled	**Patient Name**	**Patient folder number**	**Participant Number**
			FNA 001
			FNA 002
			FNA 003
			FNA 004
			FNA 005
			FNA 006
			FNA 007
			FNA 008
			FNA 009
			FNA 010
			FNA 011
			FNA 012
			FNA 013
			FNA 014

Appendix 7: Data Collection Form

DATA COLLECTION FORM

Comparison of fine needle aspiration cytology and Cell block to formal histology of small subcutaneous mass lesions in children.

Study ID Number:

DEMOGRAPHICS

Date of birth (d/m/y) :

Age in months:

Weight (kg): _____

Gender: ☐ Male ☐ Female

Race: African☐ White☐ Coloured☐ Indian/ Asian☐

First language ☐ English ☐ Afrikaans ☐ Xhosa ☐ Other …

PATIENT INFORMATION

Date of admission:

Date of operation:

Date of Discharge:

Folder Number:

Duration of mass :

Associated symptoms : Pain☐ fever Weight loss☐ Vomiting☐ Skin change☐

Night sweats, cough,

ANATOMIC SITE OF BIOPSY

site	Number			Complications
	FNAC	Cell block	Incision/Excision biopsy	
Extremities				
Head and neck				
Abdomen				
Orbit				
Salivary gland				
Thorax				
Back				
Central nervous system				
Skin				
Pharynx				
Thyroid				

DISTRIBUTION OF BENIGN LESIONS

Lesion / Diagnosis	FNAC / Cell block diagnosis	Histopathological diagnosis	Number of Concordance	Number of Non-Concordance

DISTRIBUTION OF MALIGNANT LESIONS

Mass	FNAC / Cell block diagnosis	Histopathological diagnosis	Number of concordance	Number of non-Concordance

Completed by_____ Date_ _ /_ _ / 20_

Appendix 8: Budget

Funding for this study is from Department of Paediatric Surgery research fund 411493 PDS 512 (Stationary costs) and Post Graduate Research Training Grant (FRC Award 2020) for the statistician meetings and cost of stains.

			P950 FNA STUDY	
			Budget	
	No. of Patients		Test cost (Rands)-cytology and Cell block	Total (Rands)
year 1	50		460	23000
year 2	50		510	25500
	0			0
Total	**100**			**48500**

Table 4. Cytology and Cell block stains/processing

Statistical expect meetings	Cost per hour (Rands)	Actual expenditure
1	349.01	0 (Free)
2	349.01	349.01
3	349.01	349.01
4	349.01	349.01
5	349.01	349.01
Total		1396.04

Table 5: Statistical support service

No	Item	Unit cost (VAT incl.)	Total Cost (VAT incl.)
1	Stationary		R1000
2	Printing		R500
3	Miscellaneous		R1000
	Total		R2500

Table 6: FNAC research budget

No	Item	Unit cost (VAT incl.)	Total cost (VAT incl.)
1	Laboratory agents	R970	R48,500.00
2	Statistical service	R349.01	R1396.04
3	Stationary and printing	R	R1500
4	Miscellaneous	R	R1000
5	Total		R52396.04

Table 7: Total research Budget