The diagnostic pathway to a surgical lymph node excision biopsy service in a HIV and TB endemic region in a Western Cape Tertiary Institution

by

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Declaration

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Format

This is a Publication-ready format manuscript. We are currently in the process of submitting it to Southern African Journal of HIV Medicine.

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Acronyms and Abbreviations

AFB:	Acid-fast bacilli
ART:	Antiretroviral therapy
EPTB:	Extrapulmonary tuberculosis
FNA :	Fine needle aspiration
FNAC:	Fine needle aspiration for cytology
GSH:	Groote Schuur hospital
GXP:	GeneXpert MTB/RIF assay
HL :	Hodgkin lymphoma
HREC:	Human Research Ethics Committee
IQR:	Interquartile range
KS:	Kaposi Sarcoma
LAD :	Lymphadenopathy
LTFU:	Lost to follow up
MCD:	Multicentric Castleman Disease
NHLS:	National Health Laboratory Service
PWH:	People living with HIV
RADLAC:	Rapid access diagnostic lymphadenopathy clinic
TB:	Tuberculosis
WHO:	World Health Organization
Xpert:	GeneXpert MTB/RIF assay

Publication ready manuscript

Title

The diagnostic pathway to a surgical lymph node excision biopsy service in a HIV and tuberculosis endemic region in a Western Cape Tertiary Institution

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Abstract

In the HIV/TB endemic public care setting of the Western Cape, diagnostic consideration of patients with persistent lymphadenopathy is focused towards extra-pulmonary tuberculosis (EPTB), more than other infectious or malignant causes of lymphadenopathy. We investigated patients consecutively referred for lymph node excision at Groote Schuur Hospital for selection of, and results of, laboratory tests performed during the diagnostic pathway and possible impact on diagnostic delay. Eighty-six patients were included, 61 patients (71%) had no previous diagnosis to explain the lymphadenopathy, while 25 patients had a previous diagnosis of a haematological malignancy, cancer or tuberculosis. In the new patient group, EPTB was the commonest diagnostic outcome (24.6%, 15/61), followed by lymphoma (21.3%, 13/61) and cancer (14.8%, 9/61). HIV positive patients constituted 41% (n=25). Median time from presentation with lymphadenopathy to first excision biopsy was 55 days (IQR 22-106). Fine needle aspiration (FNA) cytology of lymphadenopathy was performed in 30/61 (49%) of patients and repeated in a third of these, while smear for AFBs and culture for *M. tuberculosis* were infrequently performed and GeneXpert MTB/RIF assay on FNA never performed. Furthermore, in the seven patients with a final diagnosis of lymphoma in whom FNA cytology was performed, cytology was not diagnostic of lymphoma. In patients with persistent lymphadenopathy, this study demonstrates how poorly structured diagnostic pathways contribute to unnecessary health care utilisation and diagnostic delay in readily treatable conditions. We need to implement accurate diagnostic pathways for patients with lymphadenopathy in South Africa's healthcare system, thus improving early diagnosis of both EPTB and lymphoma, potentially improving patient outcomes.

Introduction

South Africa is the epicentre of the dual tuberculosis (TB) and HIV epidemics. Patients presenting with persistent lymphadenopathy and constitutional symptoms in the public health care system are deemed in the first place to suffer from extra-pulmonary tuberculosis (EPTB) regardless of age or HIV status(1). Less diagnostic consideration is given for malignant causes of lymphadenopathy, such as haematological malignancies, predominantly lymphoma, and solid cancer metastases to the lymph nodes(2, 3).

In people living with HIV (PWH), EPTB accounts for up to 50% of TB cases, with TB adenitis the most common site of infection (1, 3, 4). The diagnosis of EPTB requires specific and sensitive tests to account for the pauci-bacillary nature of the disease in extrapulmonary sites. Enhanced diagnostic accuracy for detecting active TB in lymph nodes is readily available with the GeneXpert MTB/RIF assay (Xpert). Furthermore, Xpert has been recommended for first-line assessment in the World Health Organization (WHO) guidelines since 2013. In spite of this strong recommendation, Xpert has not been widely implemented in the investigation of lymphadenopathy in our region(5, 6). In common practice, it is found that lymph node examination to rule out TB adenitis or lymphoma, is focused on fine needle aspirate (FNA) sent for either cytology or TB microscopy, or for both (7). The unacceptably low diagnostic accuracy of these tests leads to failed detection of not only of TB, but also of lymphoma(8, 9). With both conditions inaccurately 'ruled out', a presumptive diagnosis of EPTB often follows, with empiric TB therapy started without definitive proof of mycobacterial infection, and without clinical follow-up to ensure response(1, 3).

It is imperative that the clinician is not only inclined towards EPTB as a differential diagnosis in patients with lymphadenopathy, but also consider lymphoma and solid cancer metastases. Considering these serious conditions as top differential diagnoses ensures that the clinician appropriately chooses the best tests to arrive at the correct diagnosis. Fine needle aspiration for cytology (FNAC) is a good first-line test for cancer metastasis. EPTB and lymphoma, however, are the more common, and more actionable, differential diagnoses of lymphadenopathy. The FNAC is not an ideal test to diagnose either lymphoma or EPTB, due to its unacceptably low

sensitivity for both lymphoma and EPTB(2, 8). We have previously found that the use of FNAC (especially repeated use) significantly delays the diagnosis of lymphoma by increasing the interval from presentation to diagnosis, *the healthcare practitioner interval*.(1)

We hypothesised that the choice of tests performed in the diagnostic pathway of lymphadenopathy have an impact on diagnostic delay and increase the *healthcare practitioner interval* in patients referred for lymph node excision biopsies. We further wanted to analyse the tests related to investigation of lymphadenopathy before excision biopsy including TB investigations and tests related to aspiration of the lymph node. To investigate this, we performed a retrospective review of all patients admitted during 2016 to the surgical biopsy clinic at Groote Schuur Hospital. These patients underwent a diagnostic excision biopsy of the lymph node, with the focus on obtaining a histological diagnosis.

Research methods and design

Study design and participant selection

Groote Schuur Hospital (GSH) is a tertiary referral institution in Cape Town, Western Province, South Africa. Patients that are 13 years and older are referred from within GSH or peripheral hospitals/health centres to the local anaesthetic day surgery list at GSH to be evaluated for excision of a palpable mass. Main presentations seen in this clinic, are lymphadenopathy or soft tissue swelling (like lipomas or sebaceous cysts). Patients are assessed clinically, and the need for an excisional biopsy are determined. The procedure is then performed under local anaesthesia and histology results are communicated to patients during a follow-up visit or with a phone call. In this retrospective analysis, we included all patients who had a lymph node excision biopsy between 01 January 2016 and 31 December 2016. Patients who underwent soft tissue biopsies and those who did not qualify for a biopsy after clinical examination were excluded.

Ethical considerations

Ethical approval was obtained from the University of Cape Town Human Research

Ethics Committee (HREC: 829/2020) and GSH. A waiver of informed consent was granted as this was a retrospective study using anonymized data.

Data Collection

Data was sourced from the clinic booking register, hospital electronic and paper medical records and the National Health Laboratory Service (NHLS) laboratory information system called TrakCare (InterSystems, Cambridge, Massachusetts, United States)

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Patient demographics. Age and sex were recorded for all participants.

Clinical characteristics. A medical history was recorded for all participants, including a history of solid cancer or haematological malignancy previously treated, as well as previously treated tuberculosis. History included HIV and ART status. The dates of first presentation with lymphadenopathy, and of the excisional biopsy (and if needed, a second biopsy) were recorded and this period was defined as the diagnostic period (or *health care practitioner interval* as defined previously). The site of excisional biopsy was recorded (neck, axillary, inguinal and other).

Investigations preceding excision biopsy. All laboratory tests relevant to lymphadenopathy carried out during the diagnostic period/healthcare practitioner interval preceding the excision biopsy were recorded. These tests were conducted as part of routine clinical practice and were analysed by pathologists employed by the NHLS at GSH. All sputum and FNA results were recorded along with dates of sample collection. Investigations to detect the presence of TB infection performed on sputum and FNA, included GeneXpert MTB/RIF (Xpert), Ziehl-Neelsen stain for acid-fast bacilli (AFBs), and mycobacterial culture, with their respective results recorded. In addition, all cytology results from FNAs were documented. For HIV-positive patients, the most recent CD4 counts (cells/mm3) and HIV viral load to categorise patients as virally suppressed (< 50 copies/mL) or unsuppressed were recorded.

Lymph node excision biopsy reports*.* The lymph node biopsy specimens were examined and reported by pathologists employed by the NHLS at GSH. All samples underwent routine histological review (including where indicated, the Ziehl-Neelsen stain for AFBs) with additional immunohistochemical and special stains requested by the anatomical pathologist. Xpert and mycobacterial culture were performed if requested by the surgeon. We used the final histological diagnoses to categorise

diagnostic outcomes as lymphoma (haematological malignancy), cancer, tuberculosis, other, or non-diagnostic. Lymphoma and cancer diagnoses were made according to the relevant WHO classification. According to the latest WHO classification(10), multicentric Castleman's disease was included under lymphoma (new diagnostic category of 'lymphoproliferations'). The patients categorised as tuberculosis included patients with both definite tuberculosis (culture positive on FNA/tissue for *M. tuberculosis* and/or AFBs identified on FNA/tissue) and probable tuberculosis (no other diagnosis that would account for lymphadenopathy with one or more of either macroscopic caseation on FNA/tissue, or, granulomas on FNA/tissue). All other benign lymph node pathologies were included under the diagnostic category of 'other'. An outcome was classified as non-diagnostic if a sample didn't have enough material for diagnosis or the material was not representative of a lymph node.

Biopsy Procedure

Informed consent for the procedure was obtained. The region around the planned lymph node excision was prepared and draped in the usual sterile fashion. Local anaesthesia: 1% lignocaine with or without adrenaline (or 50:50 mixture of 1% lignocaine and 0.5% bupivacaine) was used to infiltrate the region of the proposed incision. The node itself was not injected. An incision was made in the orientation of the skin line over the palpable lymph node. This incision was deepened to access the node using a combination of sharp and blunt dissection in combination with electrocautery for haemostasis. The node was dissected from surrounding tissues using electrocautery. Electrocautery was avoided on the specimen. Haemostasis achieved with electrocautery before closure of the wound. The skin was closed with 3-0 vicryl deep dermal sutures and a subcuticular suture of running 4/0 Monocryl.

Data Analysis

Data analysis was performed using STATA Version 18 (StataCorp LP, Texas; www.stata.com). Patients were analysed in two groups: "new diagnosis" - those with no previous diagnosis to explain the lymphadenopathy; "previous diagnosis" - those with a previous diagnosis of a haematological malignancy, cancer or tuberculosis that required an excision biopsy for further characterisation, response assessments

or for suspected relapse. In the “new diagnosis” group, patients were divided into five diagnostic outcome categories: lymphoma (haematological malignancy), cancer, tuberculosis, other, and non-diagnostic. Patients with a “previous diagnosis” were divided into four categories: known lymphoma/leukaemia, TB, cancer or cancer and TB. Categorical variables were described by frequencies and percentages and compared across groups using Pearson’s Chi-squared or Fisher’s exact tests, as appropriate. Numerical variables were described by medians and interquartile ranges as data were non-parametric. Numerical variables were compared across groups using Kruskal-Wallis tests.

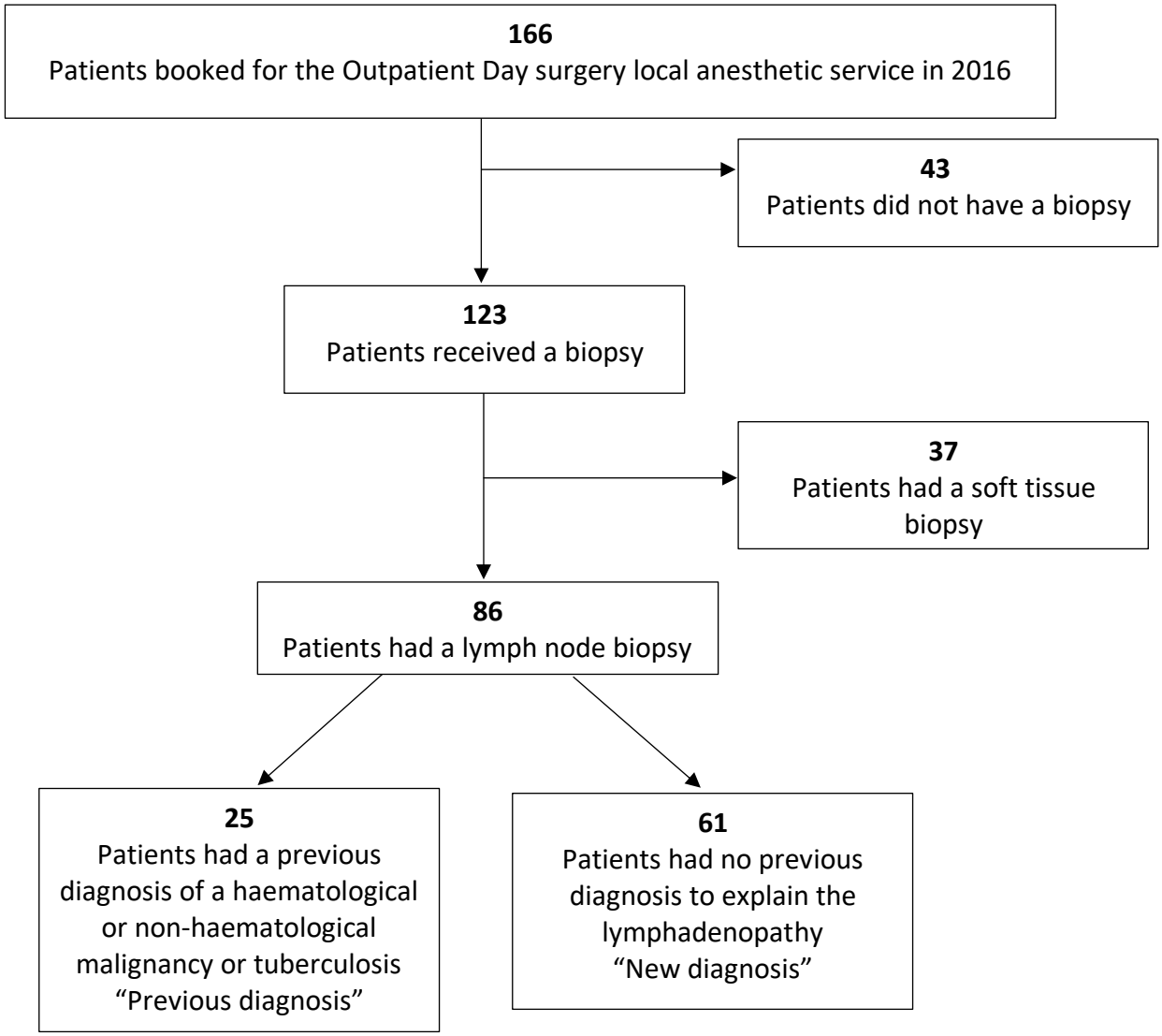

Figure 1: Flowchart showing participants included and excluded in this study.

Results

In 2016, 166 patients were evaluated in the surgery clinic for ‘lumps and bumps’ (Figure 1). Of these patients, 86 were included in the study as they had a histological confirmation of a lymph node excision; 59% of patients (n=51) were female with a median age of 41 years (IQR 30-53). The HIV status was known for 90.7% of patients (78/86), of whom 39.7% (31/78) were HIV positive. The most common site of biopsy was the neck (53.5%, 46/86), followed by axilla and inguinal (both 17.4%, 15/86) and other/unknown sites (11.6%, 10/86). The majority of patients (71%, 61/86) had no previous diagnosis to explain the lymphadenopathy and were classified as “New diagnosis”. See Table 1 and 2, and supplementary Table 1. The remaining 25 patients, classified as “Previous diagnosis”, were undergoing repeat assessment for a previously confirmed haematological or non-haematological malignancy or tuberculosis. The demographic and clinical characteristics as well as diagnostic tests performed in “Previous diagnosis” patients are in supplementary Tables 2 and 3.

New Diagnosis Cohort: Demographics and diagnoses

Demographics, clinical characteristics and time to diagnostic biopsy

Of the new patients, the majority (59.0%, 36/61) were female, and the median age was 41 years (IQR 29-51) (Table 1). People living with HIV (PWH) constituted 41.0% (25/61) of the group). Most PWH were on ART (68.0%, 17/25) and 45.8% (11/24) were virally supressed at the time of excision biopsy. The median time from presentation with lymphadenopathy to first excision biopsy was 55 days (IQR 22-106). The lymphoma group had the shortest median time from presentation, sputum analysis and FNA to first excision biopsy compared to the other groups. The median time from sputum Xpert/TB culture to first excision biopsy was longer than the time from FNA to biopsy (49 days - IQR 14-80 and 30 days - IQR 6-86, respectively).

Diagnostic outcomes, age and HIV status

Diagnostic outcomes are represented in Table 1. TB was the most common diagnostic outcome (24.6%, 15/61), followed by lymphoma (21.3%, 13/61) and cancer (14.8%, 9/61). Additionally, one patient with lymphoma had an isolated positive TB culture on sputum and one cancer patient had an isolated positive Xpert on sputum. The remainder of the biopsies were benign lesions, classified as “other”, or were non-diagnostic. The median age was highest in those with cancer, and was

significantly lower and comparable in patients with lymphoma and TB. Patients with lymphoma had the highest rate of HIV positivity 61.5% (8/15) and almost all were on ART and virally supressed with a median CD4 count of 278 cells/mm^3 (IQR 104-726). In contrast, patients with tuberculosis had the lowest median CD4 count of 83 cells/mm^3 (IQR 59-85) and none were virally supressed although 66.7% (4/6) were on ART.

New Diagnosis Cohort:

Diagnostic tests performed during the healthcare practitioner interval

The diagnostic tests performed during the diagnostic, or *health care practitioner* interval leading up to excisional biopsy, are presented in Table 2.

Sputum prior to excision biopsy

Prior to excision biopsy, more than half of all patients, and two thirds of those with a final diagnosis of TB, had at least one sputum analysis. Xpert was the most performed test and was positive in 8.6% of cases (3/35). Culture for *M. tuberculosis* was seldom performed and positive in 2/8 cases. Smear for AFBs was seldom performed and non-diagnostic.

FNA prior to excision biopsy

Half of the patients had at least one FNA, and 9.8% (6/61) had a second FNA. Cytology on FNA (FNAC) was almost always sent away (96.7%, 29/30 of FNA1was sent for cytology, and 83.3%, 5/6 of FNA2 sent for cytology). Staining for AFBs and culture for *M. tuberculosis* were less frequently performed and Xpert was never performed on the FNAC. FNAC was not diagnostic in the lymphoma cases. In one lymphoma case, which also had Kaposi sarcoma (KS), the cytology showed undifferentiated neoplasm but missed the lymphoma diagnosis. Staining for AFBs did not detect any cases of TB, and there was only one positive TB culture on a second FNA. Cytological examination of two definite TB cases showed one case with necrosis, and another with necrotising granulomatous inflammation. In one more case with granulomatous inflammation, the final diagnosis was necrotizing granulomatous inflammation diagnosed as *probable TB*.

Results of excision biopsy

Tuberculosis. Most patients (95.1%, 58/61) did not have a Xpert performed on the tissue, with none of the TB patients having a Xpert performed. In seven cases AFBs were found, six of whom had a final diagnosis of TB, and one a final diagnosis of TB and lymphoma. TB culture was only performed on 39.4% (24/61) of biopsies. Of the five patients in the TB group that had a culture, only two had a positive result. Of the six patients with positive AFBs on histology, only one had a TB culture performed, which was positive. *Lymphoma*. Among the 13 lymphoma cases, there were seven classical HL, one Burkitt Lymphoma and five multicentric Castleman disease (MCD). The nine cancer cases consisted of three adenocarcinomas, one seminoma, two KS, two neuroendocrine carcinomas and one renal cell carcinoma. There were 2 patients classified under lymphoma that had additional diagnoses on histology, in one case MCD and *M. Kansasii* and the other additionally had KS. Of the 15 tuberculosis cases, 10 were definite tuberculosis and five were probable tuberculosis. *Other*. The "Other" subgroup included 10 dermatopathic lymphadenitis, five reactive lymphoid hyperplasia, two reactive lymph nodes, and one case each of follicular hyperplasia, HIV-related follicular hyperplasia, and fibrosis. The majority of second biopsies were diagnostic (88.9%, 8/9). Of the four patients who had a non-diagnostic first biopsy, only one patient had a second biopsy which was also non-diagnostic.

Previous diagnosis cohort: Demographics and diagnoses

The 25 patients with a prior diagnosis included 14 patients with a previous haematological malignancy, eight with cancer, two with TB and one with both cancer and TB. The majority (60.0%, 15/25) were female, and the median age was 43 years (IQR 35-53). A quarter (6/24) of the group were PWH. In the patients known with a haematological malignancy, the excision biopsy was congruent with their original diagnosis in 71.4% (10/14) of cases. In the remaining cases, one patient known with myelofibrosis had a new diagnosis of Acute Leukaemia, one patient known with HL had a reactive lymph node and two patients had non-diagnostic biopsies (both previously known with lymphoma). The biopsy result remained unchanged in 62.5% (5/8) of cancer cases. The other three biopsy results showed dermatopathic lymphadenitis. In the patients with known tuberculosis or tuberculosis and cancer, the biopsy results were also consistent with the original diagnosis.

Table 1: The demographic and clinical characteristics of patients who had an excision biopsy for an unknown cause of lymphadenopathy: New Diagnosis Cohort

		Outcome of excision biopsy					
	Total (N=61)	Lymphoma (n=13)*	Cancer (n=9)	TB (n=15)	Other (n=20)	Non-diagnostic (n=4)	P-value
Median age at biopsy	41 (29-51)	35 (29-41)	58 (43-64)	31 (23-45)	43.5 (34.5-53)	39 (21-60.5)	**0.016**
Sex							
Male	25/61	7/13	6/9	6/15	5/20	1/4	0.216
Female	36/61	6/13	3/9	9/15	15/20	3/4	
HIV status							
HIV positive	25/61	8/13	3/9	6/15	6/20	2/4	0.136
HIV negative	30/61	4/13	5/9	8/15	13/20	0/4	
Unknown	6/61	1/13	1/9	1/15	1/20	2/4	
Median CD4 count, cells/µL (n=22)	181 (85-400)	278 (104-726)	264 (105-456)	83 (59-85)	400 (337-527)	129 (75-183)	0.121
ART status							
On ART	17/25	7/8	1/3	4/6	4/6	1/2	0.104
Not on ART	5/25	1/8	2/3	2/6	0/6	0/2	
Unknown	3/25	0/8	0/3	0/6	2/6	1/2	
Virally suppressed	11/24	7/8	0/3	0/6	3/6	1/2	**0.002**
Median days from presentation with lymphadenopathy to biopsy	55 (22-106)	32 (14-44)	74 (49-107)	74 (35-92)	79 (27-156)	18.5 (11-171)	0.066
Median days from sputum to biopsy (n=31)	49 (14-80)	10.5 (4-42)	49 (35-107)	46 (16-122)	70 (52-80)	130 (6-254)	0.143
Median days from FNA to biopsy (n=30)	30 (6-86)	10 (5.5-44)	30 (6-34)	74 (24-86)	91 (0-235)	17 (2-94)	0.385

P-values calculated using Pearson's Chi-squared/Fisher's exact tests for categorical variables and Kruskal-Wallis tests for numerical variables

ART: anteritroviral therapy; FNA: fine needle aspiration; TB: tuberculosis

*Includes 2 patients with Lymphoma and Kaposi Sarcoma

Table 2: Diagnostic tests performed in patients who had an excision biopsy for an unknown cause of lymphadenopathy: New Diagnosis Cohort

		Total (N=61)	Lymphoma (n=13)*	Cancer (n=9)	TB (n=15)‡	Other (n=20)	Non-diagnostic (n=4)†
Sputum 1	GXP positive	2/26	0/4	0/7	2 /8	0/5	0/2
N=31	AFB positive	0/2	0/0	0/0	0/2	0/0	0/0
	TB Culture positive	1 /7	1 /2	0/1	0/1	0/2	0/1
Sputum 2	GXP positive	1/9	0/2	1 /2	0/3	0/1	0/1
N=10	TB culture positive	1/1	0/0	0/0	1/1	0/0	0/0
	AFB positive	0/12	0/3	0/3	0/5	0/1	0/0
	TB culture positive	0/4	0/1	0/1	0/2	0/0	0/0
	Cytology†						
	Atypical	6/29	1/7	1/5	2/9	0/5	2/3
FNA 1	Negative for malignancy	9 /29	4 /7	2/5	2/9	1/5	0/3
N=30	Cancer	3/29	1/7	2/5	0/9	0/5	0/3
	Necrosis	1/29	0/7	0/5	1/9	0/5	0/3
	Necrotising granulomatous inflammation	1/29	0/7	0/5	1/9	0/5	0/3
	Granulomatous inflammation	1/29	0/7	0/5	0/9	1/5	0/3
	Non-diagnostic (inadequate material for diagnosis)	8/29	1/7	0/5	3/9	3/5	1/3
	AFB positive	0/3	0/1	0/1	0/1	0/0	0/0
FNA 2	TB culture positive	1/1	0/0	0/0	1/1	0/0	0/0
N=6	Cytology						
	Negative for malignancy	3/5	0/1	1/1	0/1	1/1	1/1
	Necrotising suppurative lymphadenitis	1/5	1/1	0/1	0/1	0/1	0/1
	Non-diagnostic	1/5	0/1	0/1	1/1	0/1	0/1
	AFBs/GXP						
Histology	No AFBs found/ No GXP performed	51/61	11/13	8/9	9/15	20/20	3/4
N=61	No AFBs found/ GXP neg	3/61	1/13	1/9	0/15	0/20	1/4
	AFBs found/ No GXP performed	7/61	1/13	0/9	6/15	0/20	0/4
	GXP positive	0/61	1/13	0/9	0/15	0/20	0/4
	TB Culture positive	2/24	0/9	0/4	2/5	0/5	0/1

AFB, acid-fast bacilli; FNA, fine-needle aspiration ; GXP, GeneXpert MTB/RIF assay; TB, tuberculosis

P-values calculated using Pearson's Chi-squared/Fisher's exact tests for categorical variables and Kruskal-Wallis tests for numerical variables

*Includes 2 patients with Lymphoma and Kaposi Sarcoma

† Non-diagnostic cytology includes not representative, suboptimal and reactive.

‡ In the tuberculosis category we include five patients who had probable tuberculosis (defined in the methods)

Discussion

Lymphadenopathy and its most anticipated aetiology, EPTB, are important diagnostic dilemmas in the Western Cape region. We describe the performance of individual tests used prior to excisional biopsy and how inappropriate test selection may contribute to diagnostic delay. Our retrospective analysis of diagnostic laboratory tests reveals that FNA was frequently performed but mostly utilised for cytology, a test with poor sensitivity for the main differential of TB and lymphoma. The most obvious rule-out TB test on FNA, Xpert, WHO recommended since 2013, was never performed. In this cohort, histological assessment of excisional biopsies showed that over 60% of patients had one of three notable diagnoses: namely TB, lymphoma, and metastatic cancer. The first two conditions respond well to prompt treatment, but poorly to late treatment, underscoring the urgency of improving pathways to timely and accurate diagnosis of these lymphadenopathy disease differentials.

Gold standard histological diagnosis of lymphadenopathy is obtained through a surgical excision biopsy, and in this cohort, it took a median of 55 days (IQR 22-106) from presentation with persistent lymphadenopathy to a diagnostic biopsy. In the UK NICE guidelines, 28 days was defined as an acceptable target from presentation to diagnosis when a patient presents with suspected haematological malignancy(1, 11). The UK NICE guidelines further specify that ideally the specialist centre to whom a patient is referred should perform a biopsy within 2-3 weeks. The recommended diagnostic interval was not achieved in this cohort. In a previous study, we showed that investigations and interventions performed during the *healthcare practitioner interval* contributed significantly to diagnostic delay in lymphoma, with a median delay of 48 days from first healthcare contact to diagnostic biopsy(1).. In this study we confirm that repeat procedures contributed to an increase in the *healthcare practitioner interval.* A total of 10 patients had repeat sputum tests for Xpert, where an Xpert on FNA would have been more appropriate. FNA for cytology was repeatedly performed and seldom gave a *rule-in* diagnosis, while poor sensitivity of FNA for either TB or lymphoma, made a confident *rule-out* diagnosis impossible. Performing a test that is sensitive and specific from the very beginning, reduces healthcare utilization and improves timely diagnosis(12).

Our analysis shows that the diagnostic tests aimed at a diagnosing TB adenitis in this patient cohort prior to biopsy, were poorly chosen, with sub-optimal sensitivity and specificity to identify or exclude common causes of lymphadenopathy. Approximately half of patients underwent FNA cytology and, in a smaller proportion, a smear for AFBs was requested. Both these tests lack sensitivity for TB diagnosis due to paucibacillary setting of lymph node infection(13, 14). The use of Xpert was solely confined to sputum and, in a third of these patients, the sputum Xpert was even repeated. This in a patient with readily accessible pathologic lymphadenopathy. This practice contradicts the 2013 WHO Policy Update on Xpert which clearly recommends Xpert testing of non-respiratory specimens, including lymph node tissue, as the first-line diagnostic test for EPTB(15, 16). From the authors' continuing observations in clinical service in the Western Cape, Xpert is still not widely utilised for ruling out EPTB in enlarged lymph nodes. In two diagnostic comparison studies of TB adenitis, the sensitivity of Xpert (49.3% and 60.1%) was significantly higher than the sensitivity of smear microscopy (14.5% and 27.8%)(13, 17). More recently we reported a 100% specificity and a 70% sensitivity for tuberculosis using an Xpert assay in 43 patients with TB adenitis(5). Even if patients present with pulmonary symptoms in addition to significant lymphadenopathy, it should be recognised that not only the lung, but also the lymph node, can reveal the diagnosis. In such a patient, concurrent Xpert of the LN and sputum should be considered, and a lymph node biopsy performed if Xpert is either negative, or if there is a poor response to TB therapy.

Lymphoma accounted for 21% of the cases and most clinicians referring to this clinic rightly noted it as a differential. However, the use of FNAC in lymphoma diagnosis - which was performed in more than half of cases - is strongly discouraged due to extremely low test sensitivity unless auxiliary tests are available to enhance accuracy(18, 19). A meta-analysis of 42 studies (1989-2012) that reviewed the sensitivity of FNAC to detect lymphoma, reported only 12% diagnostic accuracy for lymphoma(3). Furthermore, Antel et al reported a similar sensitivity of 11%(63/90) of FNACs that were suggestive, but not yet diagnostic of lymphoma(1). In other words, even in these 11% of patients the suspicion of a lymphoma diagnosis had to be confirmed on histology. This poor performance is confirmed by the results in this

cohort where no patient was diagnosed to have lymphoma with a FNAC, and only one patient was noted to have “atypical” findings – a finding also noted in other conditions. Lymphoma is an important differential in the investigation of significant lymphadenopathy in patients of all ages. Our research highlights that patients of any age and HIV status can have TB adenitis or lymphoma(5).

In contrast to the lack of utility of FNAC for lymphoma, the use of FNAC in the solid cancer diagnostic pathway is well established. In patients of an older age, as the importance of metastatic cancer rises, FNAC has an acceptable sensitivity for a first-line investigation(20). This sensitivity was not evident in the 15% with solid cancer in our cohort, likely as they were a highly selected group.

This study highlights the importance of a structured pathway in managing lymphadenopathy to optimize resource use(18). Furthermore, this analysis of patients undergoing surgical excision of lymphadenopathy in 2016, provided the proof of concept for the implementation of the Rapid Access Diagnostic Lymphadenopathy Clinic (RADLAC) at GSH at the end of 2017 - a physician-led biopsy clinic where safe, feasible and effective tests are performed to assess lymphadenopathy(1-3). The key concept around which the RADLAC is built, is the performance of a point-of-care core biopsy sent away for both histology and Xpert. Subsequent implementation of the clinic showed a reduction of the *health care practitioner interval* to 27 days(3).

Our study has limitations. The sample size was small, the study was limited to one year, the procurement of reports retrospective and we had to rely on second-hand information from doctors’ notes and request forms that could lead to interpretation bias Although we tried by way of records of visits, of tests performed, and of history given, to ascertain the first date on which healthcare intervention was sought by patients, the retrospective review with missing data most likely led to an underestimation of the duration of the *healthcare practitioner interval*. All participants had an accessible peripheral lymph node; therefore, our findings may not be generalisable to patients with tuberculosis or lymphoma without peripheral nodes.

Conclusion

This retrospective analysis of patients with persistent lymphadenopathy demonstrates how poorly structured diagnostic pathways increase healthcare utilisation and contribute to diagnostic delay in urgently treatable conditions. The median diagnostic interval of 55 days, more than double the UK NICE target of 28 days, results from inappropriate test selection, reliance on FNAC, repeated testing without due consideration of differential diagnoses, and perceived or real barriers to referral for lymph node excision biopsy.

Our data confirms the requirement for the focused investigations of Xpert and histological examination when making at least two of the three key diagnoses in patients with lymphadenopathy — TB and lymphoma. Implementing improved clinical pathways to diagnosis for patients with lymphadenopathy in South Africa's public healthcare system will improve timely diagnosis of both TB and lymphoma, potentially leading to better patient outcomes.

Acknowledgements

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Author contributions

Dr Tennis Tomo Camagu reviewed the patient's medical details and reports, analysed data and wrote the article. Dr Potelwa and Dr Sheree Gray collected the data for the study. Dr Estelle Verburgh conceptualised the study, edited and reviewed the manuscript. Drs Francois Malherbe and Christo Kloppers led the surgical teams performing procedures, as well as reviewing the manuscript. Jenna Oosthuizen and Karryn Brown assisted with data management and performed the statistical analysis. David Richardson assisted with reviewing the manuscript. All authors discussed the results and contributed to the final manuscript.

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Data availability

Raw data were generated at Groote Schuur Hospital. Derived data supporting the findings of this study are available from the corresponding author Dr Tennis Tomo Camagu Potelwa.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agenda of the authors.

References

1. Antel K, Levetan C, Mohamed Z, Louw VJ, Oosthuizen J, Maartens G, Verburgh E. The determinants and impact of diagnostic delay in lymphoma in a TB and HIV endemic setting. BMC Cancer. 2019;19(1):384.
2. Antel K, Louw VJ, Maartens G, Oosthuizen J, Verburgh E. Diagnosing lymphoma in the shadow of an epidemic: lessons learned from the diagnostic challenges posed by the dual tuberculosis and HIV epidemics. Leuk Lymphoma. 2020;61(14):3417-21.
3. Antel K, Oosthuizen J, Brown K, Malherbe F, Loebenberg P, Seaton C, et al. Focused investigations to expedite cancer diagnosis among patients with lymphadenopathy in a tuberculosis and HIV-endemic region. Aids. 2023;37(4):587-94.
4. Sharma SK, Mohan A, Kohli M. Extrapulmonary tuberculosis. Expert Rev Respir Med. 2021;15(7):931-48.
5. Antel K, Oosthuizen J, Malherbe F, Louw VJ, Nicol MP, Maartens G, Verburgh E. Diagnostic accuracy of the Xpert MTB/Rif Ultra for tuberculosis adenitis. BMC Infect Dis. 2020;20(1):33.
6. Vogt SL, Maloma L, Xian RR, Ambinder RF, Philip V, Patel M, et al. Significance of lymph node fine needle aspiration for the diagnosis of HIV-associated lymphoma in a low-resource setting. Aids. 2022;36(10):1393-8.
7. Antel K, Verburgh E. Lymphadenopathy in a tuberculosis-endemic area: Diagnostic pitfalls and suggested approach. SAMJ: South African Medical Journal. 2019;109:712-4.
8. Hehn ST, Grogan TM, Miller TP. Utility of Fine-Needle Aspiration As a Diagnostic Technique in Lymphoma. Journal of Clinical Oncology. 2004;22(15):3046-52.
9. Frederiksen JK, Sharma M, Casulo C, Burack WR. Systematic Review of the Effectiveness of Fine-Needle Aspiration and/or Core Needle Biopsy for Subclassifying Lymphoma. 2015;139(2):245-51.
10. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Araujo IBO, Berti E, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. Leukemia. 2022;36(7):1720-48.
11. Guideline N. Suspected cancer: recognition and referral. 2023:26.
12. Six KA, Aban IB, Daniels GM, Wolfson J, Beierle EA, Kutny MA, et al. Outcomes of Referrals in Pediatric Patients With Peripheral Lymphadenopathy. J Pediatr Hematol Oncol. 2024;46(6):322-6.
13. Tadesse M, Abebe G, Abdissa K, Aragaw D, Abdella K, Bekele A, et al. GeneXpert MTB/RIF Assay for the Diagnosis of Tuberculous Lymphadenitis on Concentrated Fine Needle Aspirates in High Tuberculosis Burden Settings. PLoS One. 2015;10(9):e0137471.
14. Hafez NH, Tahoun NS. Reliability of fine needle aspiration cytology (FNAC) as a diagnostic tool in cases of cervical lymphadenopathy. J Egypt Natl Canc Inst. 2011;23(3):105-14.
15. Committee WWGAbtGR. Automated Real-Time Nucleic Acid Ampli- fication Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children: Policy Update.Geneva, Switzerland: World Health Organization. 2013.
16. WHO. Xpert MTB/RIF Implementation Manual: Technical and Operational 'How-To'; Practical Considerations. Geneva: World Health Organization; 2014 Available from: https://wwwncbinlmnihgov/books/NBK254323/. 2014.

17. Fantahun M, Kebede A, Yenew B, Gemechu T, Mamuye Y, Tadesse M, et al. Diagnostic accuracy of Xpert MTB/RIF assay and non-molecular methods for the diagnosis of tuberculosis lymphadenitis. PLoS One. 2019;14(9):e0222402.
18. Chau I, Kelleher MT, Cunningham D, Norman AR, Wotherspoon A, Trott P, et al. Rapid access multidisciplinary lymph node diagnostic clinic: analysis of 550 patients. Br J Cancer. 2003;88(3):354-61.
19. Kroft SH, Sever CE, Bagg A, Billman B, Diefenbach C, Dorfman DM, et al. Laboratory Workup of Lymphoma in Adults: Guideline From the American Society for Clinical Pathology and the College of American Pathologists. Arch Pathol Lab Med. 2021;145(3):269-90.
20. Ha HJ, Lee J, Kim DY, Kim JS, Shin MS, Noh I, et al. Utility and Limitations of Fine-Needle Aspiration Cytology in the Diagnosis of Lymphadenopathy. Diagnostics (Basel). 2023;13(4).

Supplementary Tables

Supplementary Table 1: Outcomes of first and second excision biopsy and survival of patients who had no previous diagnosis

		Outcome of excision biopsy				
	Total (N=61)	Lymphoma (n=13)	Cancer (n=9)	TB (n=15)	Other (n=20)	Non-diagnostic (n=4)
Outcome 1st excision biopsy						
Cancer	5/61	0/13	5/9	0/15	0/20	0/4
Kaposi Sarcoma	2/61	0/13	2/9	0/15	0/20	0/4
Lymphoma	10/61	10/13	0/9	0/15	0/20	0/4
Kaposi Sarcoma and lymphoma	2/61	2/13	0/9	0/15	0/20	0/4
TB	9/61	0/13	0/9	9/15	0/20	0/4
Query TB	6/61	0/13	0/9	6/15	0/20	0/4
Other	18/61	0/13	0/9	0/15	18/20	0/4
Non-diagnostic	9/61	1/13	2/9	0/15	2/20	4/4
Outcome 2nd biopsy						
Cancer	3/9	0/1	3/3	0/1	0/3	0/1
Lymphoma	1/9	1/1	0/3	0/1	0/3	0/1
TB	1/9	0/1	0/3	1/1	0/3	0/1
Other	3/9	0/1	0/3	0/1	3/3	0/1
Non-diagnostic	1/9	0/1	0/3	0/1	0/3	1/1
Patient survival						
Alive 3-5 years after diagnosis	3/61	1/13	0/9	1/15	1/20	0/4
Alive >5 years after diagnosis	32/61	7/13	1/9	9/15	13/20	2/4
LTFU	13/61	2/13	3/9	5/15	2/20	1/4
Dead	13/61	3/13	5/9	0/15	4/20	1/4

P-values calculated using Pearson's Chi-squared/Fisher's exact tests for categorical variables and Kruskal-Wallis tests for numerical variables

TB, tuberculosis; LTFU,lost to follow up

Supplementary Table 2: The demographic and survival characteristics of patients who had a previous diagnosis

		Previous diagnosis			
	Total (N=25)	Lymphoma/Leukaemia (n=14)	Cancer (n=8)	TB (n=2)	TB & Cancer (n=1)
Median age at biopsy	43 (35-53)	40 (31-53)	50.5 (45-57)	34.5 (27-42)	35
Sex					
Male	10/25	5/14	2/8	2/2	1/1
Female	15/25	9/14	6/8	0/2	0
HIV status					
HIV +	6/25	3/14	0/8	2/2	1/1
HIV-	17/25	10/14	7/8	0/2	0
Unknown	2/25	1/14	1/8	0/2	0
Patient survival					
Alive 3-5 years after diagnosis	1/25	1/14	0/8	0/2	0
Alive >5 years after diagnosis	8/25	3/14	4/8	0/2	1/1
LTFU	5/25	2/14	1/8	2/2	0
Dead	11/25	8/14	3/8	0/2	0

TB, tuberculosis; LTFU,lost to follow up

P-values calculated using Pearson's Chi-squared/Fisher's exact tests for categorical variables and Kruskal-Wallis tests for numerical variables

Supplementary Table 3: Clinical characteristics and diagnostic tests performed in patients who had a previous diagnosis

			Previous diagnosis			
		Total (N=25)	Lymphoma/Leukaemia (n=14)	Cancer (n=8)	TB (n=2)	TB & Cancer (n=1)
Sputum 1 N=3	GXP positive	3/3	0/0	0/0	2/2	1/1
Sputum 2	GXP positive	2/2	0/0	0/0	1/1	1/1
N=3	TB culture positive	0/1	0/0	0/0	0/1	0/0
FNA 1	GXP positive	0/0	0/0	0/0	1/1	0/0
N=10	AFB positive	1/1	0/0	0/0	1/1	0/0
	TB culture positive	1/1	0/0	0/0	1/1	0/0
	Cytology					
	Atypical	4/10	1/2	3/8	0/0	0/0
	Negative for malignancy	1/10	0/2	1/8	0/0	0/0
	Cancer	2/10	0/2	2/8	0/0	0/0
	Non-diagnostic	2/10	0/0	2/8	0/0	0/0
FNA 2	Granulomatous inflammation	1/2	0	0/1	1/1	0/0
N=2	Non-diagnostic	1/2	0	1/1	0/1	0/0
Histology	AFBs/GXP					
N=25	No AFBs found/ No GXP performed	25/25	14/14	8/8	2/2	1/1
	TB culture positive	0/2	0/1	0/1	0/0	0/0
Biopsy 1	Acute leukaemia	2 (8.0)	2/14	0/8	0/2	0/1
outcome	Cancer	5 (20.0)	0/14	5/8	0/2	0/1
N=25	Kaposi Sarcoma	1 (4.0)	0/14	0/8	0/2	1/1
	Lymphoma	9 (36.0)	9 (64.3)	0/8	0/2	0/1
	TB	2 (8.0)	0	0/8	2/2	0/1
	Other	3 (12.0)	0	3/8	0/2	0/1
	Non-diagnostic	3 (12.0)	3	0/8	0/2	0/1
Biopsy 2 outcome N=1	Cancer	1/1	0	1/1	0	0
Final	Solid cancer	6/25	0/14	5/8	0/2	1/1
Diagnosis	Lymphoma/leukaemia	11/25	11/14	0/8	0/2	0/1
	TB	2/25	0/14	0/8	2/2	0/1
	Dermatopathic lymphadenitis	3/25	0/14	3/8	0/2	0/1
	Non-diagnostic	3/25	3/14	0/8	0/2	0/1

AFB, acid-fast bacilli; FNA, fine-needle aspiration ; GXP, GeneXpert MTB/RIF assay; TB, tuberculosis

P-values calculated using Pearson's Chi-squared/Fisher's exact tests for categorical variables and Kruskal-Wallis tests for numerical variables

UNIVERSITY OF CAPE TOWN
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Human Research Ethics Committee

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14 December 2020

HREC REF: 829/2020

A/Prof E Verburgh
Division of Clinical Haematology
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Dear A/Prof Verburgh

PROJECT TITLE: THE DIAGNOSTIC OUTCOMES OF A LYMPH NODE EXCISION BIOPSY SERVICE IN AN HIV AND TB ENDEMIC REGION-MMED CANDIDATE- DR TENNIS POTELWA

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

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 December 2021.

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 Tennis Potelwa will also be involved in this study.

Please quote the HREC REF 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, FHS 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.

Western Cape Government
Health

GROOTE SCHUUR HOSPITAL
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Professor Estelle Verburgh
MEDICINE - HAEMATOLOGY

E-mail: Estelle.Verburgh@uct.ac.za

Dear Professor Verburgh,

RESEARCH PROJECT: The Diagnostic Outcomes Of A Lymph Node Excision Biopsy Service in An HIV And TB Endemic Region (MMed. Dr Tennis Potelwa)

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until 30 December 2021.

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) Confidentiality must always be maintained.
d) No additional costs to the hospital should be incurred as indicated in your Annexure 2 i.e. Lab, consumables or stationery. **If access to TRACK Care/NHLS is required, kindly attach our letter of approval to the application form and approach Information Management to assist with data.**
e) **No patient folders may be removed from the premises or be inaccessible.**
f) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
g) Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) Please contact Michelle Riley (Patient Fees) at ext. 2276 to ascertain if there will be charges for conducting the Research and to obtain a quote or to discuss charges
m) Kindly submit a copy of the publication or report to this office on completion of the research.
n) At no time should any posters encouraging patients to partake in research, be displayed within a clinical area.
o) Please adhere to ALL COVID-19 regulations and Groote Schuur Hospital policies.

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 9 March 2021

C.C. Mr. L. Naidoo / Professor N. Ntusi / Professor V. Louw

Southern African Journal of HIV Medicine – Instructions for Authors

Original Research Article

Original research articles should be focused on HIV treatment, prevention and related topics relevant to clinical and public health practice.

Submission status	open
Word limit	5000 words (excluding the abstract, tables, figures, graphs, and references)
Abstract	maximum: 250 words Structural headings: Background, Objectives, Method, Results and Conclusion
What this study adds	maximum: 50 words
Main text	Structural headings: Introduction, Methods, Results, Discussion, Conclusion 'Ethical considerations' is a sub-section in the methods and must include: • Name of the ethical review committee • Study approval number • Manner of consent (written, oral) for human participants • Description of measures taken to maintain the confidentiality of data • If the study was determined to be non-human subjects research or exempt, the authors must provide a statement with those details in this section.
References	maximum 60, adhere to the Vancouver referencing style
Tables, figures and graphs	maximum 7, adhere to the Illustrations requirements found in the AOSIS House style guide
Formatting requirements	apply the guidelines located on the Formatting requirements page and the AOSIS house style guide
Compulsory supplementary file(s)	the Authorship, disclosure statements, copyright, and license agreement form, Ethical Clearance/Waiver Documentationand any other relevant form applicable to your submission
Ethical clearance/waiver documentation	evidence of ethical clearance for the study, such as the study approval letter or certificate from the Institutional Review Board (IRB), a waiver from the IRB et cetera

Original Research Article full structure

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of six paragraphs labelled Background, Objectives, Method, Results and Conclusion.

- Background: Why do we care about the problem? State the context and purpose of the study. (What practical, scientific or theoretical gap is your research filling?)
- Objectives: What problem are you trying to solve? What is the scope of your work (e.g. is it a generalised approach or for a specific situation)? Be careful not to use too much jargon.
- Method: How did you go about solving or making progress on the problem? State how the study was performed and which statistical tests were used. (What did you actually do to get the results?) Clearly express the basic design of the study; name or briefly describe the basic methodology used without going into excessive detail. Be sure to indicate the key techniques used.
- Results: What is the answer? Present the main findings (that is, as a result of completing the procedure or study, state what you have learnt, invented or created). Identify trends, relative changes or differences on answers to questions.
- Conclusion: What are the implications of your answer? Briefly summarise any potential implications. (What are the larger implications of your findings, especially for the problem or gap identified in your motivation?)

Do not cite references and do not use abbreviations excessively in the abstract.

What this study adds: What key insights into the research results and its future function are revealed? How do these insights link to the focus and scope of the journal? It should be a concise statement of the primary contribution of the manuscript; and how it fits within the scope of the journal.

Introduction: The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:

- Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by the use of evidence from the literature.
- Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic and should clarify the knowledge gap that this study will address. Your argument should be supported by the use of evidence from the literature.
- Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.

- Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.

Research methods and design: This must address the following:

- Study design: An outline of the type of study design.
- Setting: A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.
- Study population and sampling strategy: Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.
- Intervention (if appropriate): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.
- Data collection: Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.
- Data analysis: Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used or steps followed in qualitative data analysis.
- Ethical considerations: Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution's name and permit numbers should be stated here.

Results: Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data. All units should conform to the SI convention and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion: The discussion section should address the following four elements:

- Key findings: Summarise the key findings without reiterating details of the results.
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- Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.

Conclusion: Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

Acknowledgements: Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named. Refer to the acknowledgement structure guide on our Formatting Requirements page.

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