UNIVERSITY of RWANDA

GENEXPERT MTB/RIF ASSAY COMPARED WITH NON-MOLECULAR METHODS ON FINE NEEDLE ASPIRATION SAMPLES FOR DIAGNOSIS OF TUBERCULOUS LYMPHADENITIS AT UNIVERSITY TEACHING HOSPITAL OF KIGALI (CHUK)

Louise Munezero, MD

Master of Medicine (Anatomical Pathology) Dissertation
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A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Medicine in Anatomical Pathology of the University of Rwanda.

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December 2021

CERTIFICATION FOR AWARD

The under-designed certify that they have read and hereby recommend for acceptance by the University of Rwanda, College of Medicine and Health Sciences a dissertation entitled **"GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK)"**, in partial fulfillment of the requirements for the degree of Master of Medicine in Anatomical Pathology of the University of Rwanda.

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DECLARATION AND COPYRIGHT

I Louise Munezero, declare that this dissertation entitled "**GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK)**" is the result of my own work and it has not been submitted for other degree at the University of Rwanda or any other institution.

Louise Munezero

ACKNOWLEDGEMENT

DEDICATION

I dedicate my dissertation to the Almighty God for his mercy.

I also dedicate my dissertation to my lovely husband Dr. Japhet Ntezamizero for his everyday encouragement and helpful discussions about the research. To our daughter Ineza Brie Chrissa for tolerating my inadequate attention during my busy time. Then to my father Célestin Ndekezi, my mother Félicité Nyiramana, my brother Célestin Ntirabishaka and my sisters Angelique, Lydia, Lea and Rachel, for their love and moral support.

ABSTRACT

Background

Tuberculosis remains one of the major public health problems with a considerable number of new cases of rifampicin resistance. Extra pulmonary tuberculosis (EPTB) accounts for a significant proportion of tuberculosis cases, and tuberculous lymphadenitis (TBL) is one of the most common form of EPTB whose diagnosis still faces many challenges. Conventional methods like auramine stain which is mostly used in our settings, generally has a low sensitivity and specificity. The gold standard culture takes long time to grow, delaying the treatment. Recently, WHO recommended GeneXpert to be used as the initial diagnostic test of Extra-pulmonary tuberculosis and there are limited data about GeneXpert test on fine needle aspiration samples. GeneXpert is a rapid molecular test which gives results within 2 hours as well as the status of rifampicin resistance. The aim of this study was to determine the diagnostic performance of GeneXpert and compare GeneXpert with auramine and combined auramine/GeneXpert tests on fine needle aspiration samples.

Method

The study was prospective cross-sectional of 10 months period from June 2020 to March 2021. We have enrolled patients with palpable lymph node suspected to be TBL at the University Teaching Hospital of Kigali (CHUK). The total of 98 patients were received and lymph node specimen were collected for FNAC (Fine Needle Aspiration Cytology), auramine-O, GeneXpert MTB/RIF assay and culture on Lowenstein-Jensen medium for each patient. Analysis was done by Statistical Product and Service Solutions (SPSS) version 27.0 (IBM Corporation, Armonk, NY), GraphPad Prism (GraphPad Software, Inc., CA 92037 USA) version 9 and MedCalc statistical software (MedCalc Software Ltd, Ostend, Belgium) version 20.010. Descriptive statistics, chi-square, Fisher's exact, odd ratio, Cohen's kappa coefficient, positive/negative likelihood ratio and receiver operator characteristic (ROC) with area under the curve (AUC) were used accordingly.

Results

In our study the sensitivity, specificity, PPV and NPV of GeneXpert taking culture as gold standard were 100%, 88.4%, 54.5% and 100%, respectively. Considering composite reference standard (positive auramine, positive culture or both) specificity and PPV were improved significantly and the values of sensitivity, specificity, PPV as well as NPV were as follow: 94.7%, 94.9%, 81.8% and 98.7%, respectively. A moderate level of agreement between

GeneXpert and auramine was observed with k=0.74 and the almost perfect level of agreement between GeneXpert and combined auramine/GeneXpert was noted with k=0.97. Taking culture as gold standard, GeneXpert was compared with auramine and combined auramine/GeneXpert. The GeneXpert showed the excellent accuracy with AUC of 0.942, followed by combined auramine/GeneXpert (AUC: 0.936, excellent accuracy) and auramine with AUC of 0.787 (fair accuracy), $p < 0.001$. This has been supported by the value of likelihood ratios where GeneXpert showed a higher LR+ of 8.620 than combined auramine/GeneXpert (8.196) and auramine (7.172). The values of LR- are 0, 0.367 and 0 for GeneXpert, auramine and combined auramine/GeneXpert respectively. During this study there was no rifampicin resistance detected by the GeneXpert MTB/RIF assay.

Conclusion

The study highlighted that GeneXpert MTB/RIF assay is more accurate than both combined auramine/GeneXpert and auramine in diagnosis of TBL on fine needle aspiration samples. The implementation of GeneXpert may improve early and accurate diagnosis of TBL and guide for proper treatment.

Key words

Tuberculous lymphadenitis; fine needle aspiration; GeneXpert; Auramine-O; accuracy

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LIST OF ABREVIATIONS

AFB: Acid Fast Bacilli

AIDS: Acute Immunodeficiency Syndrome

AUC: Area under the Curve

CHUB: Centre Hospitalier Universitaire de Butare (University Teaching Hospital of Butare)

CHUK: Centre Hospitalier Universitaire de Kigali (University Teaching Hospital of Kigali)

CMHS: College of Medicine and Health sciences

CRS: Composite Reference Standard

EPTB: Extra Pulmonary Tuberculosis

FFP: Formalin Fixed Paraffin Embedded tissue

FIND: Foundation for Innovative New Diagnostics

FM: Fluorescent Microscopy

FNA: Fine Needle Aspiration

FNAC: Fine Needle Aspiration Cytology

HIV: Human Immunodeficiency Virus

IBM: International Business Machine

IDC: Infectious Diseases Society of America

IRB: Institutional Review Board

IUATLD: International Union against Tuberculosis and Lung Disease

LJ: Löwenstein-Jensen

LN: Lymph node

MDR-TB: Multidrug-resistant TB

MTB: Mycobacterium Tuberculosis

NPV: Negative Predictive Value

PCR: Polymerase Chain Reaction

PPV: Positive Predictive Value

PTB: Pulmonary tuberculosis

RIF: Rifampicin Resistance

ROC: Receiver Operating Characteristic

RNL: Rwanda National Laboratory

SPSS: Statistical Package for Social Science/Statistical Product and service Solution

TB: Tuberculosis

TBL: Tuberculous lymphadenitis

UR: University of Rwanda

WHO: World Health Organization

CHAPTER I.   INTRODUCTION

1.1 Background

Tuberculosis (TB) continues to be among health problems in developing countries with enormous social and economic implications (1). As a consequence of increased human immunodeficiency virus (HIV) prevalence and increasing immigration rate, TB also is re-emerging as a health care problem in developed countries (2,3). Tuberculous lymphadenitis (TBL) is one of the most common forms of extra pulmonary tuberculosis (EPTB) whose diagnosis still faces many challenges (4).

In 2017, the World Health Organization (WHO) reported that TB is the ninth leading cause of death worldwide with 6.3 million new cases and estimated 1.3 million TB deaths among HIV-negative people with an additional 374,000 deaths among HIV-positive people (5). EPTB counted for 15% of the 6.3 million incident cases (5). There were 600,000 new cases with resistance to rifampicin and an estimated 170,000 deaths from multidrug resistance tuberculosis (MDR-TB) worldwide in 2016 (6).

European Union countries showed a marked decrease in rate of pulmonary tuberculosis (PTB) and increase of EPTB rate in 2013 (7). The African region proportion of new TB cases was 25% in 2016 (8) and the rate of EPTB among patient with PTB in Sub-Saharan Africa was 26.6% in 2013 (9). In India, TBL was seen in nearly 35% of EPTB which constituted about 20 % of all cases of TB (10).

In 2017, the incidence of TB for Rwanda was 57 per 100,000 population with estimated 1.5% of new TB cases with MDR-TB and 5.1% of MDR-TB in previously treated TB cases (5). There were 61% of rifampicin resistance in new TB cases and 69% of rifampicin resistance in previously treated TB cases (5).

The diagnosis of EPTB poses a particular challenge because of different ways in which the disease presents and pauci-bacillary nature of the specimen. Patients with EPTB are more likely to have negative sputum smear results as many EPTB cases do not have concomitant lung involvement (11). Diagnosis of EPTB requires high clinical suspicion and special diagnostic procedures like fine needle aspiration cytology (FNAC) for TBL. Diagnosis of TBL on histology or culture are also done but takes long time and delay treatment (12).

TBL does not have pathognomonic cytomorphological patterns (11), hence adjunct stain or culture are required for an accurate diagnosis (3). Most of studies done for diagnosis of TBL on concentrated fine needle aspirates (FNA), showed that molecular tests have high specificity than direct smear microscopy. In 2015 a study done in high tuberculosis burden settings revealed that GeneXpert is more specific than auramine with a specificity of 91.1% and 57.8% respectively (11). In march 2020, we have published a case of tuberculous lymphadenitis from Kigali University Teaching Hospital with cytomorphology in favor of TBL, auramine negative and the GeneXpert positive (13). WHO has recommended the use of GeneXpert as a rapid method in the confirmation of TB in other than respiratory samples (14) and that was a conditional recommendation that needs further research to support its feasibility. Despite this background, the diagnosis of TBL in Rwanda is still done using morphology and auramine stain alone, because GeneXpert is practically used for the cases of PTB. Moreover, there is no study done in Rwanda to evaluate the sensitivity and specificity of auramine stain nor GeneXpert for the diagnosis of TBL.

The present study was to demonstrate the feasibility of GeneXpert on lymph node aspirates and evaluate the sensitivity and specificity for this test as well for auramine stain, in comparison to culture. This study can serve as evidence-based tool in the review of the current protocols of diagnosis of EPTB in Rwanda.

1.2 Problem statement

The diagnosis of lymphadenopathies depends mainly on excision of lymph nodes and histological examination. For this, general anesthesia and hospitalization are required (15). Nowadays, FNAC is being used worldwide as a first approach to lymphadenopathies because it causes minimal discomfort to the patient, gives a rapid report (4,16) and on this FNA samples, different stains and molecular tests can be done (17). *Mycobacterium tuberculosis* detection techniques, based on microscopic examination of Ziehl–Neelsen or auramine-stained specimens are used for diagnostic confirmation, but they fail to provide an optimal sensitivity and specificity (18). Recently, WHO recommended GeneXpert to be used as the initial diagnostic test for EPTB (11) as a rapid molecular test with high specificity.

In our settings, the diagnosis of tuberculous lymphadenitis is mainly based on cytomorphological features and sometimes auramine O Fluorescent Microscopy (FM), which generally has a low sensitivity and specificity as it stains a lot of microorganisms (19). The

gold standard *Mycobacterium* culture is also rarely done because it is expensive (compared with GeneXpert which is free of charge) and takes a long time to grow (20,21).

This study aimed at determining the accuracy of the morphology-auramine that is currently used; demonstrate the feasibility of GeneXpert on FNA material, and assess its sensitivity and specificity in the diagnosis of TBL in order to provide an evidence-based recommendation to incorporate this test in the protocol of diagnosis of EPTB in Rwanda, especially for the diagnosis of TBL.

1.3 Study justification

There are some cases clinically suspected to be TBL and auramine stain negative which are reported as chronic granulomatous lymphadenitis. Causes of granulomatous lymphadenitis are many including *mycobacterium* tuberculosis, atypical mycobacterium, non-tuberculous mycobacteria, sarcoidosis, fungi, neoplasms etc (2).

If there is high index of TBL suspicion, lymph node excision biopsy or culture are done and their results take more than 2 weeks (18), delaying treatment. Once auramine is still negative after excision, nothing else can be done to prove its real negativity as there is no way of performing molecular tests on formalin-fixed paraffin-embedded tissue (FFPET) in our settings. Even if auramine stains positive, it is not specific because it also stains other non-tuberculous mycobacteria and other different microorganisms such as *Cyclospora, Isospora, Actinomyces, Nocardiae* and *Cryptosporidium parvum* (22).

GeneXpert can be used by operators with minimal technical expertise, enabling the diagnosis of TB and simultaneous detection of rifampicin resistance within 2 hours (11). The aim of this study was to evaluate the performance of GeneXpert for the diagnosis of TBL on FNA samples and compare its sensitivity and specificity with that of auramine and combined auramine/GeneXpert. The results of this study would help to improve diagnostic accuracy of TBL and early treatment.

1.4 Research question

Does auramine stain combined with GeneXpert improve diagnostic accuracy than either alone for diagnosis of tuberculous lymphadenitis?

1.5 Objectives

1.5.1 General Objective

To determine the diagnostic performance of GeneXpert, auramine and combined auramine/GeneXpert on fine needle aspiration samples.

1.5.2 Specific objectives

1. To describe AFB positivity in TBL according to cytomorphological patterns.
2. To determine the sensitivity and specificity of auramine, GeneXpert and combined auramine/GeneXpert in the diagnosis of tuberculous lymphadenitis
3. To compare sensitivity and specificity of auramine, GeneXpert, and combined auramine/GeneXpert in the diagnosis of tuberculous lymphadenitis.
4. To determine the number of rifampicin resistant cases of TBL at CHUK.

CHAPTER II. LITERATURE REVIEW

2.1 Epidemiology

Globally, tuberculosis is one of top 10 causes of death worldwide and leading cause of death from a single infectious agent ranking above HIV/AIDS (20). There were an estimated 1.2 million TB deaths among HIV-negative people in 2019 and additional 208 000 deaths among HIV-positive people (20). Number of people infected by TB is increasing every year where in 2019, 7.1 million people with TB were reported to have been newly diagnosed from 7 million in 2018 and from 6.4 million in 2017 (20). The incidence rate of tuberculosis was 1.9% per year in 2015-2016 (23). The estimates of TB prevalence for Rwanda was 91 per 100 000 population in 2012 and estimated HIV-positive incident TB cases was 2.5 per 100 000 population (14).

Tuberculosis typically affects lungs (pulmonary tuberculosis) but can affects other organs and tissue(extra pulmonary tuberculosis) (24). About 20% of all TB cases present an EPTB form (25) and approximately 30% of all EPTB cases are TBL (26). There are many forms of EPTB including bone, brain, pleural, genital urinary tract, gastrointestinal tract, skin and lymph node. Commonest form of EPTB are lymph node, pleural and disseminated TB (25). TBL is the most common form in countries where TB is endemic and should be suspected in any patient with enlarged lymph node that are firm, asymmetric and more than 2cm in diameter. They can also be fluctuant or become fistulized depending on stage (24).

2.2 Spread of tuberculosis

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis.* *Mycobacterium tuberculosis* is one of more than 190 species of mycobacterium genus and mycobacterial species other than *Mycobacterium tuberculosis* and *Mycobacterium leprae* are classified as atypical mycobacteria (ATM), nontuberculous mycobacteria (NTM) or environmental mycobacteria. Tuberculosis is a communicable disease from person to person by inhalation. Individual who is affected by PTB can transmit the infectious droplets when coughing, talking or sneezing into the air but for NTM, they are transmitted from environment by ingestion, inhalation or inoculation and the portal of entry may be oral mucosa or gingiva (27,28). The infection may result in in active disease (primary disease), may be cleared by the host immune system or suppressed into inactive form (latent tuberculosis infection). The EPTB

can occur as part of primary disease, latent infection, and reactivation or generalized infection via lymphatic and hematogenous spread (29).

2.3 The clinical presentation of tuberculosis

There are classical clinical features associated with active PTB like cough, fever, weight loss, night sweats, hemoptysis, chest pain and fatigue. Signs and symptoms for EPTB may be nonspecific and depend on organ involved and weather there is an association with PTB (25). For TBL, up to 57% present with lymph node swelling for weeks to months without associated systemic symptoms. It may present as single or multiple lymph node and the most lymph node group involved by TB is the cervical followed by mediastinum, axillar, mesenteric, hepatic, per-hepatic and inguinal (30). Clinical features of TBL depend upon the stage of the disease as described by Jones and Campbell who classified peripheral tuberculosis lymph node into following five stages (31):

1. Stage of lymphadenitis: It is characterized by non-tender, firm, mobile and discrete palpable lymph nodes showing non-specific reactive hyperplasia.
2. Stage of peri-adenitis or matting: Lymph nodes are large, rubbery and fixed to the surrounding tissue and to each other. This stage typically shows epithelioid granulomas, lymphocytes and caseating necrosis.
3. Stage of cold abscess: This is due to extensive caseation of the lymph node. The caseated node may liquefy, breaks down and gives rise to variable consistency with firm lymph node, soft areas of cold abscess or soft cystic and fluctuant. There is no local rise in temperature, no tenderness and no redness.
4. Stage of collar-stud abscess: The abscess burst out of the lymph node mass and extend into the subcutaneous tissue.
5. Stage of sinus formation: The abscess burst and give rise to a persistent, discharging sinus. The discharge from sinuses may infect the surrounding skin and cause extensive tuberculous ulcer.

2.4 Diagnosis of TBL

2.4.1 Final needle aspiration cytology (FNAC)

Fine needle aspiration was first described by Kun in 1847 and has since become a widely used diagnostic technique of tumor pathologies including different causes of lymphadenopathies as alternative to invasive procedures (20,32,33). FNA is applicable to lesions that are easily

palpable but new radiological techniques for internal imaging of organs and lesions in sites not easily accessible, helped for FNA of deeper impalpable structures (34). The low risk of complications is an additional advantage that allows FNA cytology to be performed as an office procedure and in outpatient departments. The FNA technique involves passing a fine bore needle through a presenting lesion to obtain cytological material that can be analyzed often immediately (33). The commonly used needles are 23 gauge or smaller with a 5 or 10ml syringe (32). The aspirated specimen is used in different ways such as immediate smearing and staining for light microscopy analysis or preparation for ancillary tests. The cytological criteria for the diagnosis of possible tuberculous lymphadenitis have been defined as epithelioid cell granulomas with or without multinucleated giant cells and necrosis (35). The specificity and sensitivity of FNAC in the diagnosis of TBL are ranging from 60 to 75% in different studies (36).

The above-described cytological criteria are not specific for TBL, addition stain to demonstrate Bacilli like acid fast bacilli stains, culture which is the gold standard or molecular methods including GeneXpert are needed and all can be performed on fine needle aspiration samples.

2.4.2 Auramine-O Fluorescent Microscope (FM)

Smear microscopy is the simplest currently available procedure to detect Acid Fast Bacilli (AFB) and auramine-O are nonspecific fluorochrome dyes that have an affinity for acid fast organisms (37,38). Dyes bind to the mycolic acid contained in the cell wall of the mycobacterium, allowing the penetration of the stain. This complex resist decolorization by acid-alcohol solution and the counterstain potassium permanganate helps to prevent non-specific fluorescence. Under the microscope with UV illumination, acid fast cells are yellow or bright orange against dark background (38). Conventional method for acid fast bacilli (AFB) like auramine-O plays a role in the diagnosis of TB. Its major disadvantages are low sensitivity, low specificity and time consuming (21).

2.4.3 GeneXpert MTB/RIF Assay

The GeneXpert MTB/RIF Assay is an automated test, that uses nested real time polymerase chain reaction (PCR) for quantitative detection of Mycobacterium tuberculosis as well as the rifampicin-resistance associated mutations of the rpoB gene (39). In addition to the detection of MTB and resistance to treatment, the GeneXpert MTB/RIF offers quantitative estimation of mycobacterial load in form of cycle threshold (Ct) value, inversely correlate with the concentration of mycobacterial burden (40). The GeneXpert MTB/RIF assay has been

validated and optimized for sputum samples to diagnose TB and multidrug-resistant TB in 2010 and in 2014, WHO has recommended GeneXpert MTB/RIF to be used not only for respiratory samples but also for other non-respiratory specimens (lymph nodes and other tissues) for diagnosis of EPTB but this was a conditional recommendation (8). GeneXpert is a molecular test that plays an important role in detection *Mycobacterium tuberculosis* and resistant tuberculosis strains within 2 hours (11).

2.5 Culture

Since 1882 after Koch's description of Human tubercle bacillus, *Mycobacterium tuberculosis* and other species of mycobacteria have been isolated and characterized (41). Culture test is one way of studying bacteria by growing that bacteria on different substances. These substances are either solid substances on culture plates or bottles of liquid known as culture broth.

The Löwenstein-Jensen (LJ) medium is more commonly used solid medium especially for mycobacterium species. When grown on LJ medium, M. tuberculosis appears as brown, granular colonies called "buff, rough and tough") (42,43). All specimens are digested, decontaminated and concentrated before inoculation. The processed specimen sediment are inoculated in the Löwenstein-Jensen (LJ) and then incubated at 37°C up to 8 weeks (43). Liquid culture is more rapid than LJ culture media and give results in about 15 days (20).

2.6 Treatment

The treatment of TB is based on poly-chemotherapy and the standardized regimens recommended by the WHO are administered in two phases (initial and continuation phases) (25). Chemotherapy consist of rifampicin, isoniazid, pyrazinamide and ethambutol for 2 months followed by rifampicin and isoniazid for 4 months (25). The basic principles for the treatment of PTB apply to EPT as well. Corticoids are added in some cases like in milliary TB and airway compression by TB adenopathy (25). In 2019, the overall response to the treatment was 94% (20). The Infectious Diseases Society of America (IDS) guidelines recommended surgical excision only in unusual circumstances but these circumstances were not Cleary defined (44). Some studies has recommended to consider surgical lymph node excision as an adjunct to anti-TB treatment for disease caused by drug resistant organisms or atypical mycobacterium, lymphadenopathies of ≥ 3cm or in case of abscess and fistula formation (44,45).

CHAPTER III. RESEARCH METHODS

3.1 Study design

This was a cross-sectional study of 10-months period from June 2020 to March 2021, determining and comparing sensitivity as well as specificity of GeneXpert, auramine and combined auramine/GeneXpert in diagnosis of tuberculous lymphadenitis.

3.2 Settings

The study was conducted at University Teaching Hospitals of Kigali (CHUK), a national referral hospital in Rwanda. Pathology department has many units, the concerned ones were Anatomical Pathology and Mycobacteriology. All patients with suspected TBL, sent for FNAC, meting inclusion criteria during the time period of the study were enrolled.

3.3 Study population

All patients with palpable lymphadenopathies and suspected TBL, meting inclusion criteria during the time period of the study were enrolled.

3.4 Selection of study population
3.4.1 Inclusion criteria

Patients with palpable lymph node, suspected to be TBL who come for FNAC at CHUK.

3.4.2 Exclusion criteria

Patients who were taking anti-tuberculous therapy during the study period**.**

3.5 Main outcome to be measured

The main outcomes to be measured in this study were:
1. Demographic data (age, sex and province of origin) of the patients with suspected TBL.
2. Clinical data (site and size of LN, presence of B-symptoms and previous history of TB)
3. AFB positivity in TBL by different diagnostic tests (auramine and GeneXpert) according to cytomorphological patterns.
4. Diagnostic performance of GeneXpert against culture as gold standard and against CRS (auramine positive or culture positive or both).

5. Comparison of GeneXpert, auramine and combined auramine/GeneXpert taking culture as gold standard.

3.6 Sample size calculation

The minimum representative sample size was calculated using logical reasoning of statistical methods for calculating an appropriate sample size for cross-section study (46) as follows:

$$n = \frac{(Z_{1-\left(\frac{\alpha}{2}\right)})^2 \times P \times (1-P)}{d^2}$$

n= the sample size $Z_{1-\alpha/2}$ = the standard normal variate   P= the expected proportion
d= Absolute error

$Z_{1-\alpha/2}$: The standard normal variate of 1.96 was considered at 5% type 1 error and P value less than 0.05 to be considered significant.

P: The expected proportion (P) of 6.8% of tuberculous lymphadenitis was considered based on a one-year (2018) period pilot study at CHUK.

d: Absolute error of 5% was used (d=0.05).

$$n = \frac{(Z_{1-\left(\frac{\alpha}{2}\right)})^2 \times P \times (1-P)}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.068 \times (1-0.068)}{(0.05)^2} = 97.38 \approx 98$$

The sample size is calculated to be at least 98 cases.

3.7 Sample collection and processing

For each patient with palpable lymph node, FNA sample was collected in the pathology unit at CHUK. Aspiration was done using 23-gauge needle with attached 10 ml disposable syringe. The first few drops of the aspirate were smeared, air dried and stained with Diff-Quick for cytomorphological diagnosis. One air dried slide was reserved for auramine stain and the remaining sample was processed for culture and GeneXpert in mycobacteriology unit after rinsing the needle within normal saline to have at least 4ml of the sample.

For FNAC diagnosis and cytomorphological pattern classification, two blinded observers were considered and for discrepancies in findings, a consensus were made. The smears suggestive of TBL on FNAC were grouped into 3 categories-(I) necrosis only, (II) epithelioid granulomas with necrosis with or without Langhans type multinucleated giant cells; (III) epithelioid granulomas without necrosis with or without Langhans type multinucleated giant cells.

The auramine-stained smears were examined for the presence of AFB using florescent microscopy and AFB smear positive slides were graded based on WHO and International Union against Tuberculosis and Lung Disease (IUATLD) scale as shown in Table 1.

Table 1. Acid-fast bacilli grading by WHO/IUATLD

N^o of AFB (Objective 20)	N^o of AFB (Objective 40)	Report
No AFB/Length	No AFB/Length	Negative
1-29 AFB/ Length	1-19 AFB/ Length	Record exact number
30-299 AFB/ Length	20-199 AFB/ Length	1+
10-100 AFB/ Field	5-50 AFB/ Field	2+
>100AFB/ Field	>50AFB/ Field	3+

AFB: acid-fast bacilli; IUATLD: International Union against Tuberculosis and Lung Disease; WHO: World Health Organization.

For GeneXpert assay, 1.5ml of the sample reagent supplied with the test were added to 2ml of the sample. The mixture was vortexed and incubated at room temperature for 15minutes. 2ml of the reagent-sample mix were taken and transferred to an Xpert cartridge and cartridge was loaded onto GeneXpert machine (Cepheid, Version 5.1). The results were automatically generated within 2 hours and read as positive or negative for *M. Tuberculosis* as well as quantitative estimation of mycobacterial load in form of cycle threshold (Ct) values as very low, low, medium and high. Rifampicin resistance results was recorded as susceptible, resistant or indeterminate. Anti-TB treatment was initiated after auramine and/or GeneXpert positive results.

The remaining 2 ml were used for mycobacterial culture on egg-based Lowenstein-Jensen (LJ) medium provided by Rwanda National Laboratory (RNL). The sample was processed using the standard N-acetyl L-cysteine-sodium hydroxide (NALC-NaOH) method. The samples inoculated on LJ medium were incubated at 37^{0}C for 4 to 8 weeks. Tubes without specimen

and tubes with a known cultured *M. tuberculosis* were used as negative and positive controls respectively. Culture positive were confirmed by GeneXpert MTB/RIF assay.

3.8 Data processing analysis

Data were collected using a pre-established questionnaire. Data entry and analysis were done using Statistical Product and Service Solutions (SPSS) version 27.0 (IBM Corporation, Armonk, NY), GraphPad Prism (GraphPad Software, Inc., CA 92037 USA) version 9 and MedCalc statistical software (MedCalc Software Ltd, Ostend, Belgium) version 20.010. Demographic and clinical presentation data were analyzed statistically in terms of frequency and percentages. Relationship between clinical variables and TBL were assessed with Chi-square or Fisher's exact test and odd ratio. The level of agreement among different diagnostic tests was determined using Cohen's kappa coefficient.

Diagnostic test performance characteristics including sensitivity, specificity, predictive values, positive and negative likelihood ratio were evaluated with 95% confidence interval. The plot of sensitivity *versus* 1-specificity known as receiver operating characteristic (ROC) curve with the area under the curve (AUC) was used to compare GeneXpert, auramine and combined auramine/GeneXpert as well as a measurement of accuracy. *P* value < 5% was considered statistically significant.

3.9 Ethical consideration

The research proposal was presented at the Institutional Review Board (IRB) of the University of Rwanda (UR) College of Medicine and Health sciences (CMHS) and the approval n umber 079/CMHS IRB/2020 was obtained. The permission to conduct the study was provided by Research Ethics Committee of study hosting hospital (CHUK) and approval number was EC/CHUK/045/2020. Before collecting data, informed consent was signed by adult patients; however, for children and young adults <18-year-old, an assent form was signed by their parents/ guardians. For every patient, a corresponding code number was given and all patients' identifier information was stored on a personal password protected computer. Participation in the study was voluntary, and the information obtained was treated confidentially, and only used for this research purposes.

3.10 Strength of the study

To the best of our knowledge, this study is the first to be done in our country providing data for GeneXpert on fine needle aspiration samples. It also showed that some cases are possibly missed by the commonly used tests for diagnosis of TBL in our settings.

CHAPTER IV. RESULTS

4.1 Demographic and clinical characteristics of study participants

A total of 98 patients meeting inclusion criteria were enrolled in the study. The age of participants ranged between 0–67 years the majority were male 58(59.2%) as shown by Table 2. The high proportion of participants were between 25–34 years which account 25 (25.5%). Most study participants (61.2%) were living in Kigali city, and small proportion of participants (5.1%) were from Western province.

The predominant lymph node group was neck (n=82, 83.7%), followed by axillar (n=9, 9.2%) and inguinal (n=7, 7.1%). Lymph node size was categorized into three groups a as follow: <2cm (n=24, 24.5%), 2-4cm (n=71, 72.4%) and >4cm (n=3, 3.1%).

Presumptive TBL patients showed associated symptoms like fever in 64 cases (65.3%), night sweats (40 cases, 40.8%), weight loss (20 cases, 20.4%) and cough (13 cases, 13.3%). The above-described symptoms were present for more than two weeks in 79 (80.6%) cases and two weeks and less in 19 (19.4%) cases. Most of patients (n=79 or 80.6%) were not previously treated for any form of tuberculosis while only 3 (1%) were previously treated of TBL and showed persistent lymph node swelling.

Table 2. Demographic and clinical characteristics of enrolled patients.

Characteristics	Number (%)
Sex	
Male	58 (59.2)
Female	40 (40.8)
Age (Mean=30.02; Median=30; Mode=25) years	
0-5	11 (11.2)
6-15	10 (10.2)
16-24	14 (14.3)
25-34	25 (25.5)
35-44	17 (17.3)
45-54	11 (11.2)
55-64	8 (8.2)
≥65	2 (2)
Residence	
Kigali city	60 (61.2)
North	18 (18.4)
South	8 (8.2)
East	7 (7.1)
West	5 (5.1)
Lymph node site	
Neck	82 (83.7)
Axillar	9 (9.2)
Inguinal	7 (7.1)
Lymph node size	
<2cm	24 (24.5)
2-4cm	71 (72.4)
>4cm	3 (3.1)
Associated symptoms	
Fever	64 (65.3)
Cough	13 (13.3)
Night sweats	40 (40.8)
Weight loss	20 (20.4)
Duration of symptoms	
>2weeks	79 (80.6)
≤2weeks	19 (19.4)
Previous TB treatment	
New	95 (96.9)
Previously treated	3 (1)
HIV status	
Positive	8(8.2)
Negative	90(91.8)
Specimen appearance	
Purulent	15 (15.3)
Caseous	21 (21.4)
Bloody stained	62 (63.3)

HIV: human immunodeficiency virus

4.2 Cytomorphological findings in favor of tuberculous lymphadenitis

For all 98 cases included in the study, four diagnostic methods were done including FNAC for morphological diagnosis, auramine, GeneXpert and culture. On FNAC, most of cases (n=62 or 63.3%) were not showing cytomorphological features of TBL while only 36 (36.7%) cases showed features consistent with TBL (included in one of three cytomorphological patterns) as shown on Figure 1. Of these 36 cases with cytomorphological patterns of TBL, the first pattern which is made of necrosis only was the frequent one, seen in 20 (55.6%) cases, followed by a pattern made of necrosis with epithelioid granuloma with or without multinucleated giant cells (n=12 or 33.3%) and the third pattern made of epithelioid granuloma without necrosis with or without multinucleated giant cells (n=4 or 11.1%).
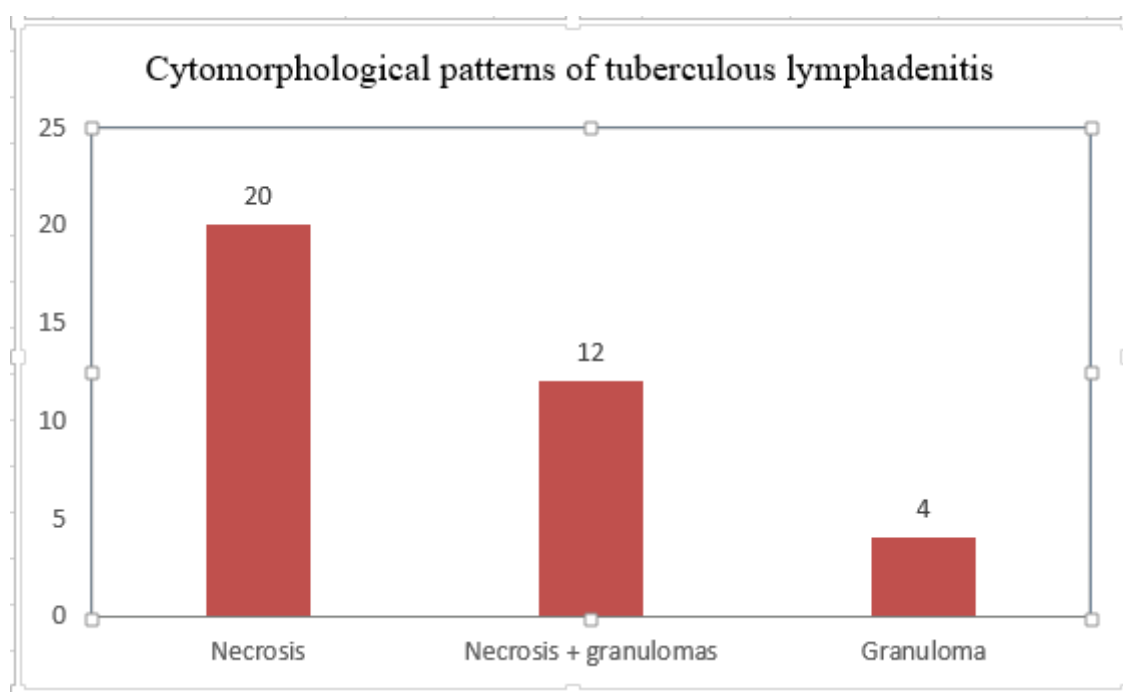
Figure 1. Cytomorphological patterns of Tuberculous Lymphadenitis
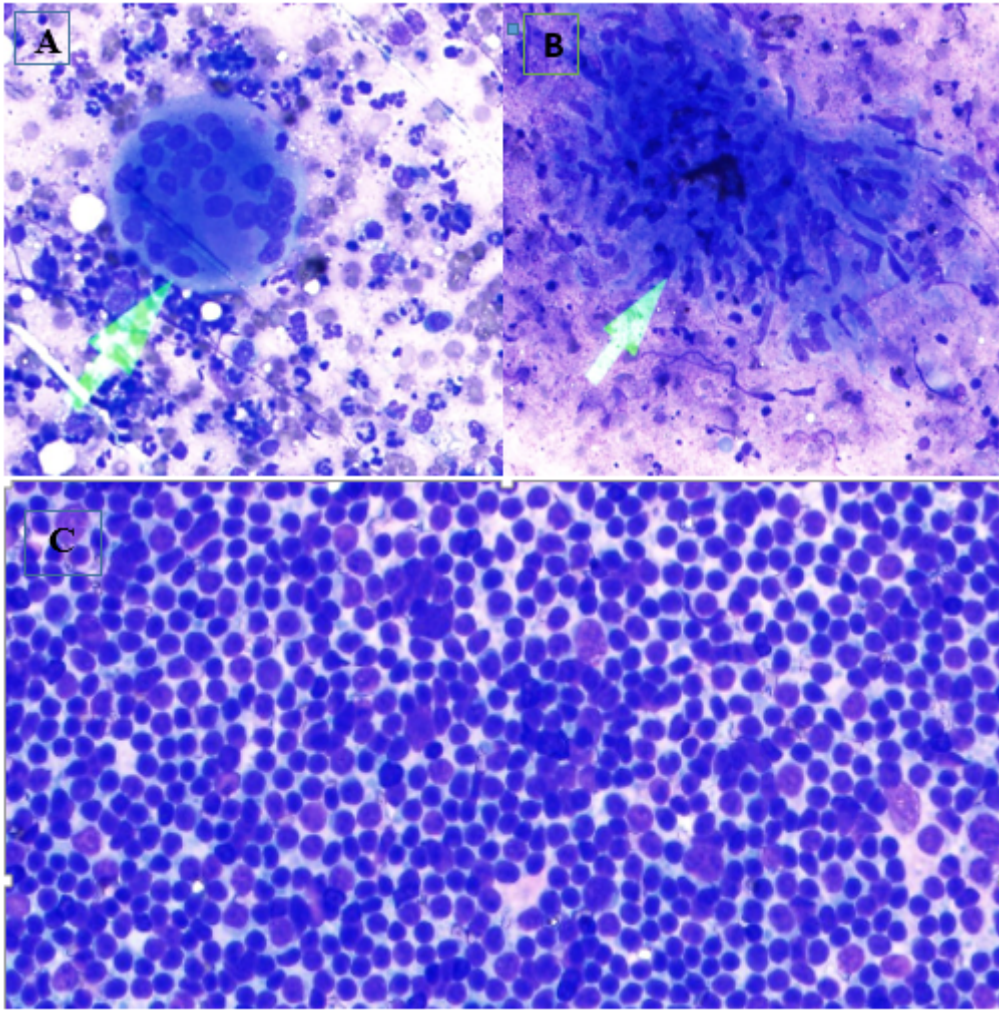
Figure 2. Figure illustrating DiffQuick-stained cytology findings.

Micro-photograph A: Multinucleated giant cell (DiffQuick stain, 400X). B: Aggregate of Epithelioid histiocytes (DiffQuick stain, 200X). C: Polymorphous population of lymphocytes (DiffQuick stain, 200X).

4.3 Fine needle aspiration cytology diagnosis

Majority cases (n=58 or 59.2%) showed features of reactive lymphadenitis as shown by Figure 3. The cytological features consistent with TBL were observed in 14 (14.3%). Chronic granulomatous inflammation and suppurative lymphadenitis were equally diagnosed with 8(8.2%). Other diagnosis including lymphoma, carcinoma, other neoplasms and abscess were diagnosed in 10(10.1%).
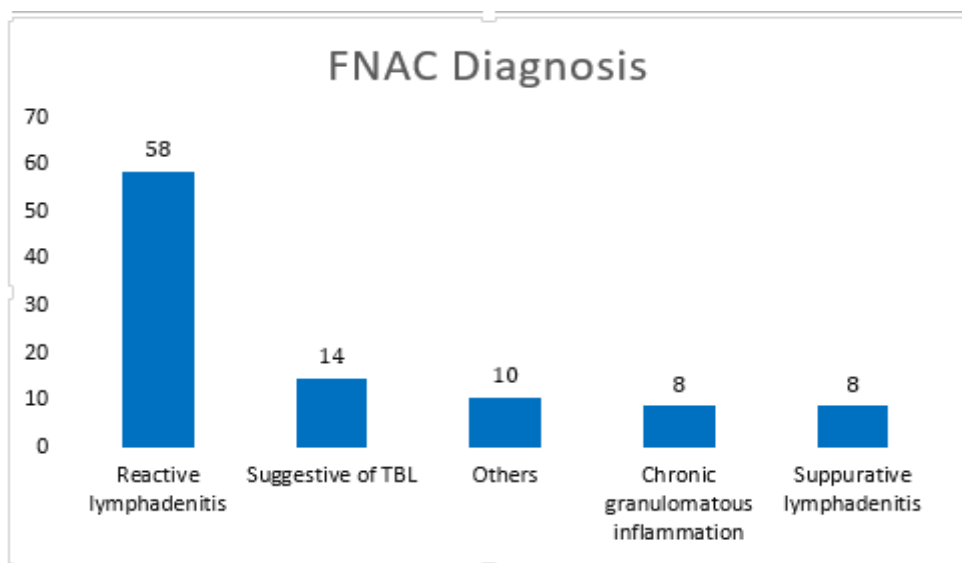
Figure 3. Diagnosis on Fine needle aspiration cytology (FNAC)

FNAC: Fine Needle Aspiration Cytology; TBL: Tuberculous lymphadenitis

4.4 Mycobacteriological findings

4.4.1 Tuberculous lymphadenitis positivity by different diagnostic methods

Table 3 shows TBL positivity by cytology, auramine, GeneXpert, culture, combined auramine/GeneXpert and combined auramine/culture.

Table 3. Tuberculous lymphadenitis positivity by different diagnostic methods.

Methods	Positive	Negative
Cytology, n (%)	14 (14.3%)	84 (85.7%)
GeneXpert, n (%)	22 (22.4%)	76 (77.6%)
Auramine, n (%)	16 (16.3%)	82 (83.7%)
Culture, n (%)	12 (12.2%)	86 (87.8%)
Combined auramine/GeneXpert, n (%)	23 (23.5%)	75 (76.5%)
Combined auramine/culture, n (%)	19 (19.4%)	79 (80.6%)

Twenty-three cases were positive by at least one test and, of these 23 cases of confirmed TBL, 8 (34.7%) cases were positive in all three tests, 7 (30.4%) were positive auramine and GeneXpert (Figure 4), whereas 3 (13.1%) and 1 (0.4%) were positive for GeneXpert alone and for auramine alone, respectively. There was no positive case for culture only. GeneXpert was positive in 7 (30.4%) cases missed by auramine test.
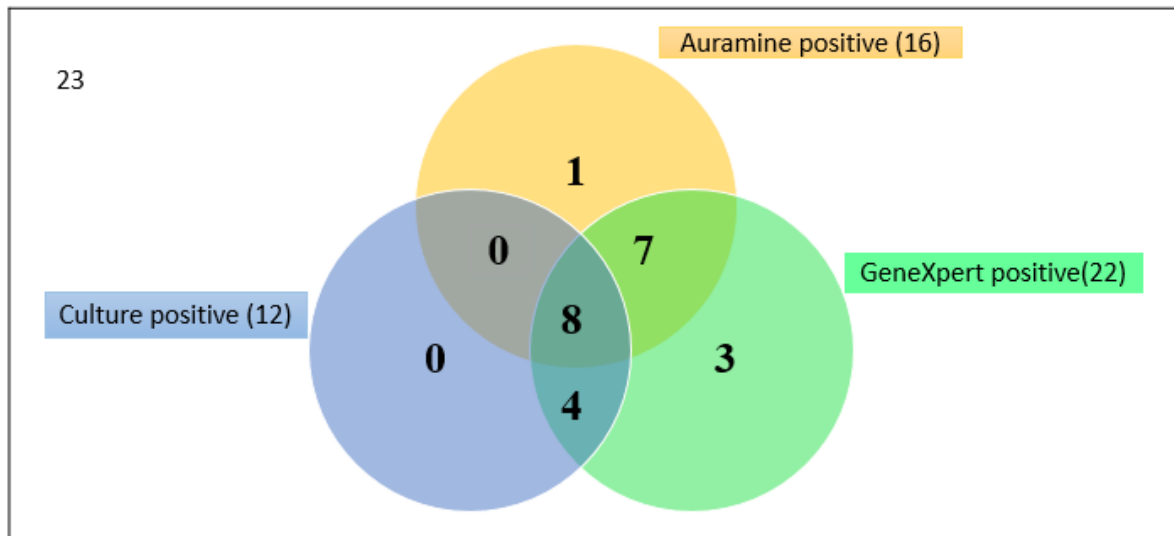
Figure 4. Venn diagram of positive culture, Auramine O and GeneXpert

4.4.2 Tuberculous lymphadenitis positivity by Auramine O fluorescent microscopy and AFB grading

Auramine stained slides were read on fluorescent microscopy and AFB grading was made based on WHO and IUATLD scale for grading bacillary load. The results for 16 auramine positive cases showed predominance of first grade (record exact number of AFB) with 74.9% distributed as follow; 2AFB (25%), 3AFB (12.5%), 4AFB (18.8%), 5AFB (6.3%), 7AFB (6.3%) and 7AFB (6.3%) as shown by Figure 5. Other grades were 1+ 6.3% and 2+ 18.8%.
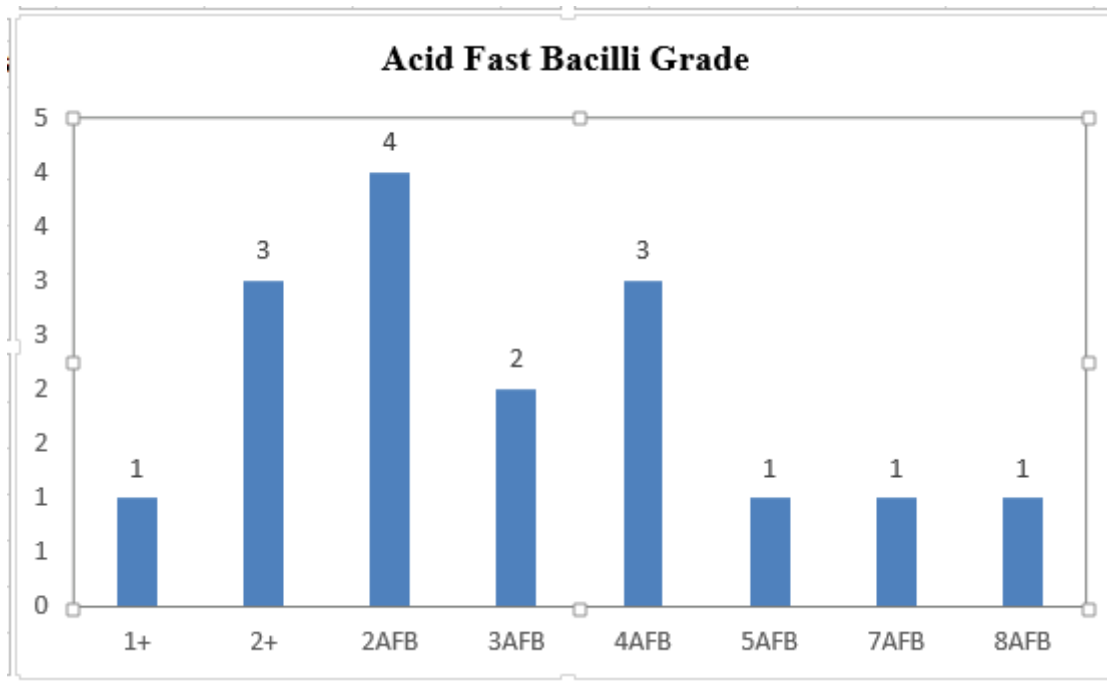
Figure 5. Grade of acid-fast bacilli (AFB) positive cases

AFB: Acid-fast bacilli

4.4.3 Tuberculous lymphadenitis positivity by GeneXpert MTB/RIF assay

GeneXpert positive (22/98) cases did not show rifampicin resistance and there was no indeterminate case identified. As the GeneXpert provides a quantitative estimation of mycobacterial load in form of cycle threshold (Ct) values as very low, low, medium and high, these values were also recorded and most of cases showed very low bacillary load accounting for 14/22(63.6%), followed by low (n=6 or 27.3%) and the medium load (n=2 or 9.1%) as presented in Figure 6.
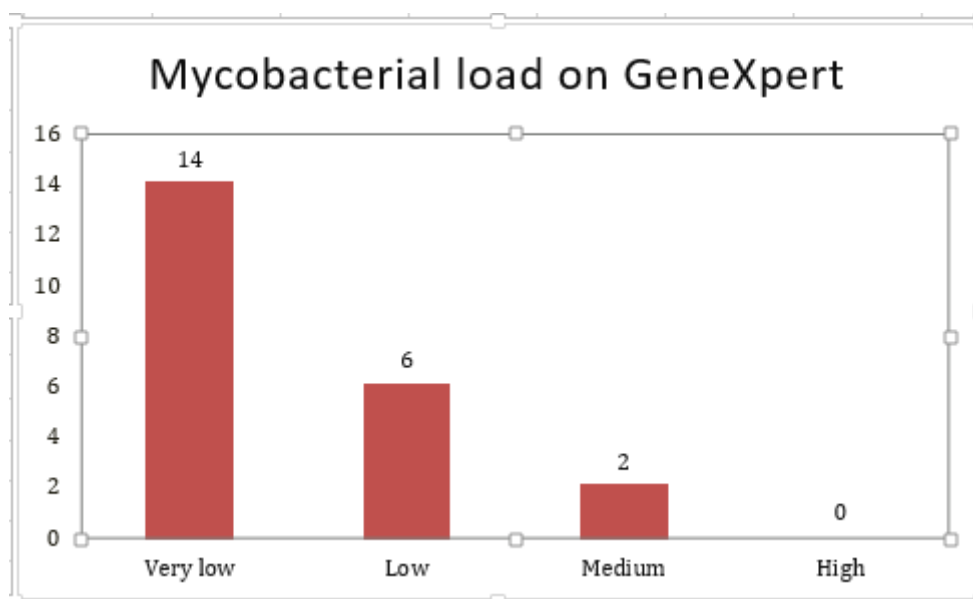
Figure 6. Bacillary load by GeneXpert MTB/RIF assay

MTB: Mycobacterium Tuberculosis; RIF: Rifampicin Resistance

4.4.4 Tuberculous lymphadenitis positivity by MTB Culture

In a total of 98 cases for which culture was done in LJ culture media, 12 (12.2%) were positive for Mycobacterium tuberculosis and 86 (87.8%) were negative. There is no contamination noted. The time period between sampling and culture was put into 3 categories and findings show that culture done within 7 days account for 37 (37.8%) cases, within 8 to 14 days for 27 (27.6%) cases and after 14 days for 34(34.7%) cases. As shown in Table 4, the positivity rate was not statistically affected by the time period between sampling and culture, in that positive cases were 2/37 (16.7%) cases of the first category, 4/27 (33.3%) cases of the second category and 6/34 (50%) cases of third category (Chi-square P=0.259).

Table 4. Culture positivity and time period between sampling and inoculation in culture media

Number of days between sampling and culture	Positive	Negative	*P* value
0–7	2	35	0.259
8–14	4	23	
>14	6	28	

P value by Chi-square

4.5 Relationship between clinical data and detection of tuberculous lymphadenitis.

In this study we have found no statistical significance in TBL detection (by either auramine or GeneXpert) *versus* gender, age group and lymph node size as shown by Table 5. A statistical significance was noted in TBL detection *versus* lymph node site, HIV status and presence of fever but presence of B symptoms did not show a statistical significance. The statistical significance for two tests (Fisher' exact test and odds ratio) was considered.

Table 5. Clinical data and detection of tuberculous lymphadenitis

Category	Positive	Negative	OR (95% CI)	*P**
Sex				
Male	11	44	1.0960	>0.999
Female	9	31	(0.42-2.84)	
Age group (years)				
0-14	1	18	0.1439	0.038
≥15	22	57	(0.02-1.14)	
Lymph node site				
Neck	16	66	0.3117	0.052
Axillar and inguinal	7	9	(0.10-0.96)	
Lymph node size				
<2cm	6	18	1.1176	>0.999
≥2cm	17	57	(0.38-3.26)	
Associated symptoms				
Fever				
Yes	20	44	4.6970	0.013
No	3	31	(1.28-17.19)	
Cough				
Yes	4	9	1.5439	0.496
No	19	66	(0.42-5.57)	
Night sweats				
Yes	15	25	3.7500	0.008
No	8	50	(1.40-10.02)	
Weight loss				
Yes	5	15	1.1111	>0.999
No	18	60	(0.35-3.47)	
B symptoms				
Yes	4	12	1.4872	0.506
No	13	58	(0.41-5.35)	
Duration of symptoms				
>2weeks	21	58	3.0776	0.227
≤2weeks	2	17	(0.65-14.46)	
HIV status				
Positive	5	3	6.6667	0.016
Negative	18	72	(1.45-30.53)	

* P value by Fisher's exact test. OR: odds ratio. CI: Confidence interval.

The positivity rate for GeneXpert, auramine and culture was highest in purulent aspirate followed by caseous aspirate and bloody aspirate (Figure 7). These findings were statistically significant with *P*< 0.05 by either Chi-square test or Chi-square test for trend.
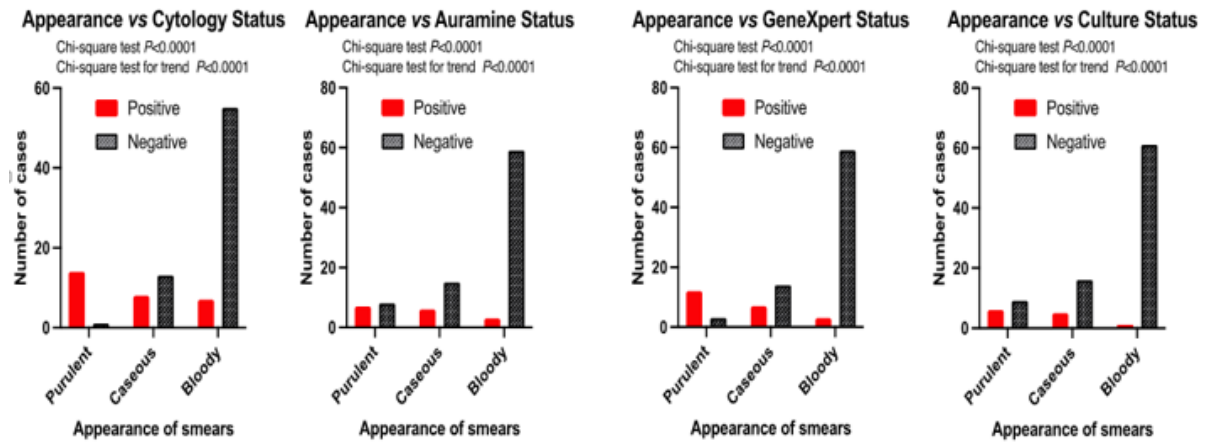
Figure 7. Type of aspirate and detection of tuberculous lymphadenitis

4.6 Relationship between cytomorphology patterns of TBL and confirmatory mycobacteriological tests (Auramine, GeneXpert and Culture)

The positivity rate of GeneXpert and Auramine was highest in first pattern made of necrosis only followed by the second pattern made of necrosis with epithelioid granuloma with or without multinucleated giant cells and the third pattern of epithelioid granuloma without necrosis with or without multinucleated giant cells (Figure 8). The positivity rate of culture was highest in the second pattern made of necrosis with epithelioid granuloma with or without multinucleated giant cells, followed by the first pattern made of necrosis only and the third pattern which is made of epithelioid granuloma without necrosis with or without multinucleated giant cells. There was a statistical significance difference between cytomophological pattern and detection of TBL by either GeneXpert, Auramine or Culture with *P*< 0.05 by Chi-square test. As the *P* value by chi-square for tend > 0.05 for all three tests (GeneXpert, auramine and culture), there was no evidence of trend for positivity rate from first pattern with necrosis only followed by second pattern of necrosis with epithelioid granuloma and the third pattern which is made of epithelioid granuloma without necrosis with or without multinucleated giant cells.
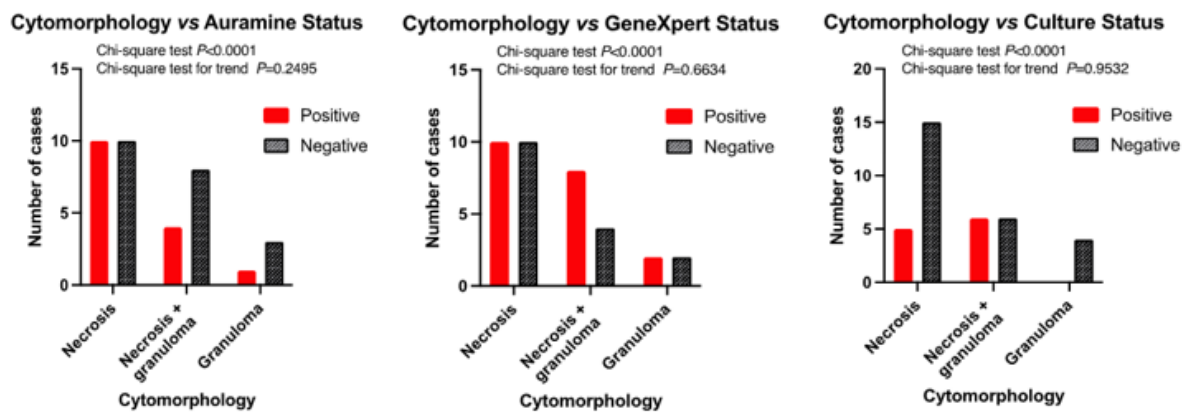
Figure 8. Tuberculous lymphadenitis detection according to cytomorphological patterns

4.7 Correlation of cytology diagnosis and confirmatory mycobacteriological tests (Auramine, GeneXpert and Culture)

During our study we found a moderate level of agreement between cytology diagnosis and auramine as well as for culture (Cohen k coefficient for inter-test reliability k: 0.48 and 0.55 respectively). There was an almost perfect level of agreement between cytology diagnosis and GeneXpert with k: 0.94. A statistical significance difference was noted between cytology diagnosis and auramine, cytology diagnosis and GeneXpert as well as in cytology diagnosis and culture as shown in Table 6. Cases that showed features suggestive of TBL, suppurative lymphadenitis and reactive lymphadenitis were mostly confirmed by GeneXpert. Auramine and culture showed variable positivity across different diagnosis made on FNAC. All cases diagnosed as lymphoma or other neoplasm were negative for TBL by auramine, GeneXpert and culture.

Table 6. Cytology diagnosis and positivity of mycobacteriological tests

Diagnosis on FNAC	Mycobacteriological tests								
	Auramine			GeneXpert			Culture		
	Pos	Neg	*P**	Pos	Neg	*P**	Pos	Neg	*P**
Suggestive of TBL	11	13	<0.0001	16	8	<0.0001	9	15	<0.0001
Suppurative lymphadenitis	4	3		4	3		2	5	
Reactive lymphadenitis	1	56		2	55		1	56	
Others	0	10		0	10		0	10	

P value by Chi-square; Pos: Positive; Neg: Negative; TBL: Tuberculous Lymphadenitis

4.8 Performance of GeneXpert MTB/RIF against culture as gold standard and against CRS

The sensitivity, specificity, PPV and NPV of GeneXpert with culture as gold standard are 100%, 88.4%, 54.5% and 100% respectively as shown by Table 7. Considering CRS (Composite Reference Standard), the sensitivity, specificity, PPV and NPV of GeneXpert are 94.7%, 94.9%, 81.8% and 98.7%, respectively. This study found the increase of specificity and PPV when CRS is considered.

Table 7. Diagnostic performance of GeneXpert with culture as gold standard and with reference to composite reference standard (CRS)

Gold standard: Culture								
	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
GeneXpert	12	76	10	0	100% (75.8-100)	88.4% (79.9– 93.6)	54.5% (34.7-73.1)	100% (95.2-100)
Gold standard: CRS								
GeneXpert	18	75	4	1	94.7% (75.4-99.7)	94.9% (87.7-98)	81.8% (61.5-92.7)	98.7% (92.9-99.9)

TP: true positive; TN: true negative; FP: false positive; FN: false negative; PPV: positive predictive value   NPV: negative predictive value; CRS: Composite reference standard

4.9 Comparison of GeneXpert MTB/RIF with non-molecular methods for diagnosis of tuberculous lymphadenitis

A moderate level of agreement between GeneXpert and auramine was observed with k=0.74 and the almost perfect level of agreement between GeneXpert and combined auramine/GeneXpert was noted with k=0.97. Taking culture as gold standard, GeneXpert was compared with auramine and combined auramine/GeneXpert. The accuracy measurements used were sensitivity, specificity, PPV, NPV, Likelihood ratios and AUC. GeneXpert showed the highest sensitivity, specificity, PPV and NPV as follow (100%, 87.8%, 54.5% and 100%) as shown in Table 8. Combining sensitivity and specificity for each diagnostic test, GeneXpert has also shown the highest positive likelihood ratio (8.620) and excellent AUC (0.942) as well

as statistically significant P value. The Receiver operator characteristics (ROC) curve of four diagnostic tests is shown on Figure 9.

Table 8. Comparison of GeneXpert, auramine and combined auramine/GeneXpert taking culture as gold standard

Operational characteristics	Diagnostic methods		
	GeneXpert	Auramine	Combined auramine/GeneXpert
Sensitivity	100%	66.7%	100%
(95% CI)	(75.8-100)	(39.1- 86.2)	(75.7-100)
Specificity	88.4%	90.7%	87.8%
(95% CI)	(79.9– 93.6)	(82.7-95.2)	(78.5-92.7)
PPV	54.5%	50%	52.2%
(95% CI)	(34.7-73.1)	(28-72)	(33-70.8)
NPV	100%	95.1%	100%
(95% CI)	(95.2-100)	(88.1-98.1)	(95.1-100)
LR+	8.620	7.172	8.196
LR-	0	0.367	0
AUC	0.942	0.787	0.936
(95% CI)	(98.7%-95.1)	(6.3%-95.1)	(88.9%-98.3)
P value	<0.0001	<0.001	<0.0001

PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; AUC: area under the curve; CI: confidence interval

GeneXpert showed a big area under the curve followed by combined auramine/GeneXpert and auramine alone (Figure 9).
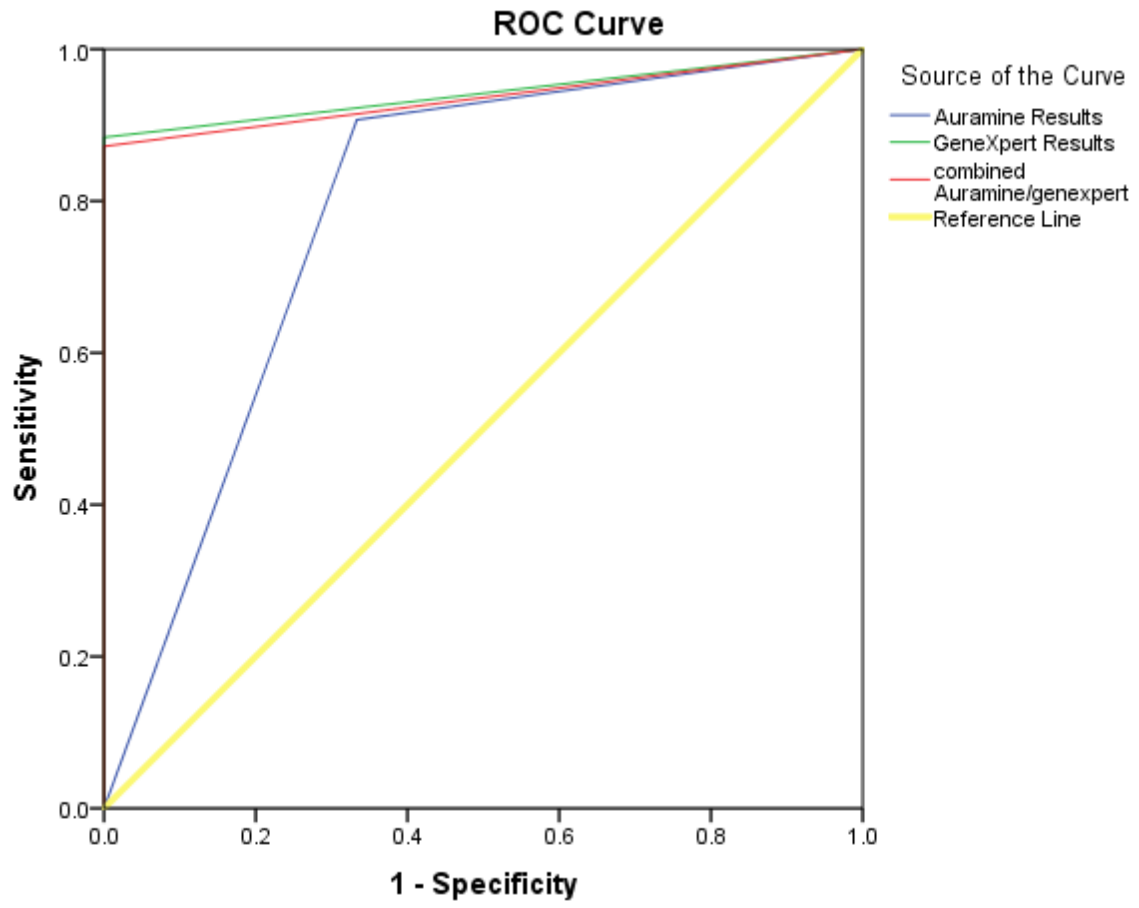
Figure 9. ROC curve of GeneXpert, auramine and combined auramine/GeneXpert tests with culture as gold standard.

ROC: Receiver operator characteristics

CHAPTER V. DISCUSSION

Diagnosis of EPTB pose challenges due to the way the disease present and pauci-bacillary nature of the specimens (6). TBL is one of the most common forms of EPTB which can be diagnosed on FNA samples as alternative method to the lymph node excision which is an invasive procedure (47). On FNA samples, diagnostic tests of tuberculosis can be performed including conventional methods (auramine-O, auramine-rhodamine and Ziehl-Neelsen stains), molecular tests and culture on liquid or solid medium.

Recently the WHO has recommended the GeneXpert to be used for the diagnosis of EPTB but this was a conditional recommendation that encouraging further research (5). The present study aimed at determining the feasibility of GeneXpert test on fine needle aspiration samples and to compare the GeneXpert test with other non-molecular tests for the diagnosis of TBL on fine needle aspiration samples considering culture as gold standard and against composite reference standard (auramine or culture positive or both).

5.1 Characteristics of study participants and association with TBL detection

The study population were mostly from Kigali city (61.2%) and this can be explained by the location of the study area which is CHUK, a referral hospital located is in Kigali city with high density of population compared with other areas so that it was easy to access the hospital. The second reason can be the rarity of pathology services in rural areas.

The present study showed male predominance (59.2%) and the similar findings were observed by Amer *et al*. (48) who found the male predominance of 53.2%. The contradictory observation was demonstrated by Mengistu *et al*. (49) with female predominance of 55.5%. The Predominant age group of patients with lymphadenopathies suspected to be TBL was young group of 25–34-year-old (25.5%) followed by a group of 34–44-year-old accounting for 17.3%. These age groups were different from those described by Mengistu *et al*. (49), where they found the age group of 15–24 year-old as the predominant one with 28.9% followed by the age group of 24–34 years old accounting for 27%. Pediatric age group showed a certain protection to TBL compared with adult but it is not statistically significant (p=0.038, OR=0.1439 CI=0.02-1.14). HIV positive was noted in 8.2% and, furthermore, 62.5 % of HIV positive patients were confirmed to have TBL. Bakesiima *et al.* (40) found that 96/385(24.9%) cases of suspected TBL were HIV positive (40). Patients with HIV are 6.6 times tended to be diagnosed with TBL (OR: 6.6667; 95%CI: (1.45-30.53), *p* value<0.05).

The most affected lymph node group was neck (83.7%) followed by axillar (9.2%) and inguinal (7.1%). The similar observation was noted by Ruma *et al*. (2) with neck lymph node group accounting for 89%) followed by axillar (9%) and inguinal (2%). During this study neck lymph node group were less likely to be TBL compared with axillar combined with inguinal ones (OR: 0.3117; 95% CI: 0.10-0.96; *p* value 0.052). To the best of our knowledge there is no study done which have calculated OR for lymph node sites to compare with. As we have a small number of inguinal and axillar nodes, further research with a big sample size can be done to investigate more. Most of lymph nodes were having medium size of 2–4cm, followed by <2 cm and few of them were > 4cm. These findings were supported by Moncef *et al*. (50). Systemic symptoms are not common in TBL and if present the most frequent symptoms are fever, night sweats and weight loss. Cough is the less common associated symptom as supported by Raghab *et al*. (51).The duration of symptoms is variable ranging from weeks to months as found by different authors (35,38,40).

Three patients were previously treated for TBL lymphadenitis and had persistent lymph node swelling. On cytology two of them showed epithelioid granuloma with necrosis and the third one showed epithelioid granuloma without necrosis. On mycobacteriological tests, two of them were auramine positive and one of the auramine positive cases was positive on GeneXpert. All three cases were culture negative. Raghab *et al*. (9) and Fordham *et al*. (44) found that some cases of TBL lymphadenitis show persistent lymph node swelling after medical treatment then require surgical therapy especially when TBL is due to nontuberculous mycobacterium.

5.2 Cytology findings and acid-fast bacilli positivity according to cytomorphological patterns in favor of tuberculous lymphadenitis

The predominant percentage of aspirated specimen was bloody stained followed by purulent and caseous but the positivity rate for GeneXpert, auramine and culture was highest in purulent followed by caseous and bloody stained aspirate. The above findings were also observed by Mengistu *et al*. (49). The highest bacilli positivity in purulent aspirate is explained by the stage of disease progression whereby AFB concentration is more in aspirate showing purulent or necrotic material (49).

On direct microscopy, most of cytological findings suggestive of TBL were confirmed by either auramine or GeneXpert with or without culture positive. A high proportion of suppurative lymphadenitis were diagnosed as TBL by one or more tests. These findings were

also demonstrated by Mengistu *et al*. (49) . This study showed a considerable percentage (3.4%) of reactive lymphadenitis cases confirmed TBL. Tadesse *et al* found 9% of reactive lymphadenitis confirmed to be TBL (11).

Among three cytomorphological patterns in favor of TBL, AFB positivity rate by auramine and mycobacterial tuberculosis positivity rate by GeneXpert were highest in first pattern made of necrosis only followed by the second pattern made of necrosis with epithelioid granuloma with or without multinucleated giant cells and the third pattern of epithelioid granuloma without necrosis with or without multinucleated giant cells. Similar findings were noted in several reports (15,47,52). This trend of *Mycobacterium tuberculosis* positivity rate is explained by the disease progression as stated above (11). *Mycobacterial tuberculosis* positivity rate by culture was highest in the second pattern made of necrosis with epithelioid granuloma with or without multinucleated giant cells, followed by the first pattern made of necrosis only and the third pattern which is made of epithelioid granuloma without necrosis with or without multinucleated giant cells. The highest positivity rate of *mycobacterium tuberculosis* is justified by the stage of the disease (11). This trend is not statistically significant and most of studies found that pattern with granulomas and necrosis has a high positivity rate (a combination of two first patterns) (15,47). To the best of our knowledge there is no study done which have calculated chi-square for trend of positivity rate by culture and cytomorphological patterns to compare with.

In general, fine needle aspiration samples show low bacillary load either on auramine or GeneXpert where the two first grades (record the exact number and 1+) are the predominant ones and have been found in more than 50% in our study. The low bacillary load nature of fine needle aspiration samples were also observed by Tadesse *et al*. (53).

The positivity of culture was low in our study 12.2% compared with 22.4% of GeneXpert. The slight decrease in positivity of culture of 44.7% versus 49.3% of GeneXpert was seen by Mengistu *et al*. (49) and their samples were immediately inoculated in culture media. Even though the WHO recommends to inoculate samples in culture media within 7 days because the probability of successful culturing bacilli decreases with time (54), some of our samples were delayed up to 28 days due to financial issues. Approximately 16% of samples processed within 7 days were positive, 33% of samples processed within 8–14 days were positive and 50% of positive cases were samples processed within more than 14 days but not later than 28 days. Before inoculation in culture media samples were stored in refrigerator at 2–8°C. There was no

statistical significance difference between mycobacterial tuberculosis growth and delayed sample inoculation in culture media (p value >0.005).

During our study, one case was auramine positive but GeneXpert and culture were negative and the patient was not on anti-TB treatments. The sample for this case was processed for culture after 14days in a refrigerator. This can be the cause of false culture negative or it can be tuberculosis caused by nontuberculous mycobacterium (NTM*) which are not detected by GeneXpert MTB/RIF assay. Other causes of false negative GeneXpert including very low mycobacterial load and CPR inhibitors (55). Quality of sample and antibiotics such as fluoroquinolones use preceding sampling are additional factors of false negative culture results (55,56). Arora *et al,* (57) had similar findings of some auramine positive cases with negative GeneXpert and culture positive or negative. In such cases, non-tuberculous mycobacterium is highly suspected hence the importance of culture in conjunction with smear microscopy.

Two cases were positive by GeneXpert and negative by auramine and culture. For both cases the GeneXpert showed very low mycobacterial load and this can support one of the factors of false negative auramine especially in low bacillary load specimens. The auramine requires at least 10 000 colony forming unit/ml to be positive (57,58). These cases need clinical correlation as they can be dead bacilli (57).

In this study we have found 7 cases with positive auramine and GeneXpert but with negative culture. If conventional smear is positive for AFB, the GeneXpert confirms that it is mycobacterium tuberculosis at 99% and there are many reasons of false negative culture as it have been found in other studies (49,56,58).

5.3 Diagnostic performance of GeneXpert MTB/RIF assay taking culture as gold standard and CRS

Although the GeneXpert has shown a good sensitivity and specificity in many studies as well as in our findings, when the composite reference standard is set, its sensitivity, specificity, positive and negative predictive values improve (49,59,60), as shown in Table 9.

Table 9: Other studies findings on the diagnostic performance of GeneXpert MTB/RIF assay taking culture as gold standard and composite Reference standard (CRS)

Gold standard: Culture				
Authors	Sensitivity	Specificity	PPV	NPV
Our study	100%	88.4%	54.5%	100%
Mengistu *et al.* (49)	78%	74%	71%	81%
Biadglegne *et al.* (59)	93.5%	69.1%	34.1%	98.4%
Bankar *et al.* (60)	84.9%	86.7%	33%	89.6%
Gold standard: CRS				
	Sensitivity	Specificity	PPV	NPV
Our study	94.7%	94.9%	81.8%	98.7%
Mengistu *et al.* (49)	92%	98.7%	96.9%	89.6%

PPV: Positive predictive value; NPV: Negative predictive value

5.4 Performance of GeneXpert assay compared with auramine and combined auramine/GeneXpert taking culture as gold standard

The evaluation of GeneXpert diagnostic performance over auramine and combined auramine/GeneXpert on fine needle aspiration samples of suspected TBL against culture as gold standard was made and showed that the GeneXpert is the best diagnostic test followed by combined auramine/GeneXpert and auramine. This conclusion was made based on values of positive and negative likelihood ratios as well as AUC as shown by Table 8. To the best of our knowledge no study done on fine needle aspiration samples comparing auramine and GeneXpert against culture as old standard which has calculated positive likelihood ratio, negative likelihood ratio and area under the curve to compare with our findings. Studies that have been conducted on pulmonary samples showed the excellent accuracy for GeneXpert with area under the curve of 0.94 and 0.95 (61,62).

Mangistu *et al*. (49) and Louis *et al*. (63) have compared auramine and GeneXpert against culture as gold standard and they have used sensitivity, specificity, PPV and NPV alone. Their observations were concordant to ours. Combining all above mentioned three studies comparing auramine and GeneXpert, the GeneXpert showed high sensitivity and auramine showed a high specificity. PPV and NPV were variable for both auramine and GeneXpert (Table 10). As those four parameters are not consistent, that is why we have supplemented them with LR+, LR- and

AUC that combine sensitivity and specificity, to demonstrate the excellent performance of GeneXpert MTB/RIF assay on fine needle aspiration samples.

Table 10: Other studies findings on comparison of auramine and GeneXpert MTB/RIF assay

Study	Tests	Sensitivity	Specificity	PPV	NPV
Our study	Auramine	66.7%	90.7%	50%	95.1%
	GeneXpert	100%	88.4%	54.5%	100%
Mengistu *et al.* (49)	Auramine	47%	94%	86.5%	68.7%
	GeneXpert	78%	74%	71%	81%
Louis *et al.* (63)	Auramine	75.9%	94.7%	95.7%	94.1%
	GeneXpert	86.1%	84.2%	90.3%	72%

PPV: Positive predictive value; NPV: Negative predictive

CHAPTER VI. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

To the best of our knowledge, this study is the first done in Rwanda providing data of GeneXpert MTB/RIF assay on fine needle aspiration samples and comparing the GeneXpert with auramine. Taking culture as gold standard and composite reference standard (auramine positive or culture positive or both), GeneXpert showed a considerable sensitivity and specificity so that it can be used to diagnose TBL on fine needle aspiration samples.

Based on calculated positive and negative likelihood ratios and area under the curve which has been used as a measurement of accuracy, the evaluation of GeneXpert diagnostic performance over auramine and combined auramine/GeneXpert showed that the GeneXpert is better than combined auramine/GeneXpert and auramine in the diagnosis of TBL on FNA samples.

We have found that cytomorphological pattern with necrosis is more favoring TBL than other patterns without necrosis and the positivity rate of GeneXpert and auramine was highest in pattern made of necrosis only followed by the pattern made of necrosis with epithelioid granuloma with or without multinucleated giant cells and the pattern of epithelioid granuloma without necrosis with or without multinucleated giant cells. This is justified by stage of the disease progression.

The positivity rate for GeneXpert, auramine and culture was highest in purulent aspirate followed by caseous aspirate and bloody aspirate. The highest bacilli positivity in purulent aspirate is explained by the stage of disease progression whereby AFB concentration is more in aspirate showing purulent or necrotic material (advanced stage) as it has been even found by other studies.

During the study period there was no rifampicin resistant case found by GeneXpert MTB/RIF assay.

6.2 Recommendations

Based on findings of this study, the following recommendations are made:

- The GeneXpert MTB/RIF assay is rapid, more accurate than auramine and provide the status rifampicin resistance. The implementation of GeneXpert MTB/RIF assay may improve early and accurate diagnosis of TBL and guide for proper treatment that is why we recommend GeneXpert to be used as the first approach for TBL diagnosis.

- Policymakers should consider including GeneXpert MTB/RIF assay in the diagnosis of TBL by training personnel and providing GeneXpert machines. The implementation of GeneXpert especially at the peripheral health care level may help in early diagnosis, correct treatment and good outcome.

- Pathologists should consider GeneXpert especially in auramine negative cases with cytomorphology in favor of TBL or reactive lymphadenitis. If TBL is highly suspected and the GeneXpert is negative auramine can be considered as it can be tuberculosis other than mycobacterium.

- Laboratory services with functioning mycobacteriology unit have to avail culture media in order to avoid delaying in sample inoculation especially for previously treated cases of tuberculosis to whom the decision making mostly relays on culture results.

- Further research with ability to perform culture immediately after sampling and with long period of time to assess the response to treatment, especially for these cases with AFB positive by auramine but with negative GeneXpert and culture are needed.

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APPENDICES

1. Data collection sheet

GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK).

1. Patient's code: ▭   Date: ▭

2. Age: ▭

3. Sex: Male ▭   Female ▭

4. Residency: a. Kigali city ▭ b. North ▭ c. South ▭ d. East ▭ e. West ▭

5. Lymph node sites: a. Head and Neck ▭   b. Axillar ▭

           c. Inguinal ▭   d. More than 1 site ▭

6. Lymph node size in cm:

7. Associated symptoms: a. Fever ▭   b. Cough ▭

           c. Night sweats ▭   d. Weight loss ▭

8. Duration of symptoms: a. More than 2weeks ▭   b. Less than 2 weeks ▭

9. History of TB contact: a. Yes ▭   b. No ▭

10. Previous TB treatment: a. New ▭   b. Previously treated ▭

11. Specimen appearance: a. Purulent ▭ b. Caseous ▭ c. Bloody stained ▭

12. Cytomorphological appearance:

    a. Granulomas with necrosis with/without Giant cells ▭

    b. Granulomas without necrosis with/without Giant cells ▭

    c. Necrosis only ▭

13. FNA cytology results:

a. Suggestive of tuberculous lymphadenitis [　　]

b. Chronic granulomatous inflammation [　　]

c. Suppurative lymphadenitis [　　]

d. Reactive lymphadenitis [　　]

e. Lymphoma [　　]

f. Carcinoma [　　]

g. Others [　　　　　　　]

14. Auramine result: a. AFB Positive [　　]

b. AFB Negative [　　]

15. AFB Grade: a. No AFB [　　]   b.1+ [　　]   c.2+ [　　]   d. 3+ [　　]

16. Gene Xpert result: a. Positive [　　]   b. Negative [　　]   c. Invalid or error [　　]

17. GeneXpert Cycle threshold range: a. Very low [　　]   b. Low [　　]

c. Medium [　　]   d. High [　　]

18. Rifampicin resistance: a. susceptible [　　]   b. Resistant [　　]   c. Indeterminate [　　]

19. MTB Culture result: a. Positive   b. Negative [　　]   c. Other growth [　　]

20. Days between sampling and culture:

21. HIV status: a. Positive [　　]   b. Negative [　　]

2. CONSENT FORM

CONSENT FORM IN ENGLISH (Adults)

GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK).

We are doing a study on different diagnostic method of tuberculous lymphadenitis at CHUK, Rwanda. The purpose of this study is to prove the most effective method to be used for early diagnosis of tuberculous lymphadenitis, in order to make recommendations after the study. It will help in early diagnosis and patient's management.

I …………………………………… agree to participate in the study "GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK). I am aware that participation in the study is voluntary and I will not be paid for the participation.

 In addition, all information provided will be treated with confidentiality and that my anonymity will be maintained. I am aware that the result of this study may be published but I will not be identified as an Individual. I reserve the right to withdraw from the study at any time if I so wish, without any consequence to the care provided to me. …………………………………….. …………………… …………………….. …………………

Name of participant   Signature of participant   Date

……………………………………. ……………………….. …………………………………

Name of researcher   Signature of researcher   Date

Contacts for further information:

Principle researcher contacts: Dr Louise Munezero

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Supervisor: Dr Belson Rugwizangoga, Pathologist

Email: belson77@gmail.com, Tel: 0788546597

CMHS IRB Chairperson, Tel: 0788 490 522

AMASEZERANO YO KWEMERA KUJYA MU BUSHAKASHATSI (ABAKURU)

Turi gukora ubushakashatsi bufite umutwe: GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK).

Integoy"ubu bushakashatsi ni ukugereranya uburyo butandukanye bukoreshwa mu gupima igituntu kitari icyo mu bihaha, bikababigamije gukomeza gushyiraho uburyo buboneye kandi bwihuse bwo gupima igituntu kitari icyo mu bihaha.

Jyewe,…………………………………………………...nemeye kujya mu ubushakashatsi bwitwa "GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK).

Nasobanuriwe ko kujya muri ubu bushakashatsi ari ubushake bwanjye, ko nta gihembo ntegereje guhabwa, kandi ko nzagirirwa ibanga kugiti cyanjye ndetse n"amakuru yose nzatanga.

Nasobanuriwe ko ibizava muri ubu bushakashatsi bizatangazwa ariko ko ntazerekanwa nk"umuntu kugiticye. Mfite uburenganzira bwo kuva muri ubu bushakashatsi igihe cyose nabishakira, kandi nijejwe ko ntangaruka byatera kubuvuzi mpabwa.

Amazinan"umukono by"uwasobanuriwe …………………………………………………..

Italiki…………………………………………………………………………………..
Umukonon'amazina y'umushakashatsi……………………………………………….

Italiki...

Ukeneye ibindi bisobanuro wahamagara:

Uyoboye ubushakashatsi: Dr Louise Munezero

Email: louisemunezero113@gmail.com , Tel: 0783133925

Umujyanama: Dr Belson Rugwizangoga, Pathologist

Email: belson77@gmail.com, Tel: 0788546597

CMHS IRB Chairperson, Tel: 0788 490 522

3. ASSENT FORM

ASSENT FORM (for children)

Project title: GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK).

We are doing a research study on different diagnostic method of tuberculous lymphadenitis at CHUK, Rwanda.

The purpose of this study is to prove the most effective method to be used for early diagnosis of tuberculous lymphadenitis, in order to make recommendations after the study. It will help in early diagnosis and patient's management.

If you decide to be part of this study, you will be asked to answer questions related to the study. You can ask questions any time, now or later. If you do not want to be in this study, no one will be mad at you. We will also ask your parents or guardians if they would like you to be in the study. Even if you say yes now, you can change your mind later. When we will finish this study, we will write a report about what was learnt. This report will not include your name or that you were in the study.

ASSENT

I want to take part in this study. I know I can change my mind at any time, without any consequence to the health care provided to me.

Name of the child:

………………………………………………………………………………………..

Name and signature of the next of kin

…………………………………………………………………………………………

Verbal assent given: Yes   Date: … /……/……

Name   of   participant   Signature   of   participant   Date

…………………………………….. …………………….. …………………………

Name of researcher   Signature of researcher   Date

Contacts for further information:

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Supervisor: Dr Belson Rugwizangoga, Pathologist

Email: belson77@gmail.com, Tel: 0788546597

CMHS IRB Chairperson, Tel: 0788 490 522

ICYEMEZO CYUBURENGANZIRA BWO KWINJIRA MUBUSHAKASHATSI (ABANA)

UMUTWE W'IBYIGWA: GeneXpert MTB/RIF assay compared with non-molecular methods on Fine Needle Aspiration samples for diagnosis of tuberculous lymphadenitis at University Teaching hospital of Kigali (CHUK).

Integoy"ubu bushakashatsi ni ukugereranya uburyo butandukanye bukoreshwa mu gupima igituntu kitari icyo mu bihaha, bikaba bigamije gukomeza gushyiraho uburyo buboneye kandi bwihuse bwo gupima igituntu kitari icyo mu bihaha.

Niwemera kwitabira ubu bushakashatsi, umuganga azagira ibibazo akubaza bijyanye nubushakashatsi. Ushobora kubaza ikibazo igihe icyo aricyo cyose. Ntabwo ari itegeko kwitabira ubu bushakashatsi. Ntawe uzakurakarira nuba utitabiriye. Tuzabaza n'ababyeyi bawe niba bemera ko witabira ubu bushakashatsi. Nubwo wakwemera ubu wemerewe kuva muri ubu bushakashatsi igihe cyose ushakiye kandi ntangaruka byagira kubuvuzi uhabwa. Niturangiza ubu bushakashatsi, tuzandika amakuru y' ibyo twabonye ariko izina ryawe ntaho rizagaragara. Nemeye kwitabira ubu bushakashatsi

Izina ry" umwana………………………………………………………………………..

Izina ry" uhagarariye umwana……………………………………………………………….

Itariki………………………………………………………….

Amazina y'usobanuriye uwitabiriye……………………………………………….

Itariki…………………………………………………

Ukeneye ibindi bisobanuro wahamagara:

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UNIVERSITY of RWANDA

COLLEGE OF MEDICINE AND HEALTH SCIENCES

DIRECTORATE OF RESEARCH & INNOVATION

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 20th May 2020

Dr. Louise MUNEZERO
School of Medicine and Pharmacy, CMHS, UR

Approval Notice: No 079/CMHS IRB/2020

Your Project Title *"Genexpert MTB/RIF Assay On Fine Needle Aspiration Samples For Diagnosis Of Tuberculous Lymphadenitis At University Teaching Hospital Of Kigali (CHUK) And Butare (CHUB) "* has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
			No (Reason)	
		Yes	Absent	Withdrawn from the proceeding
Prof Kato J. Njunwa	UR-CMHS		X	
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS		X	
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS	X		
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 20th May 2020, **Approval has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 20th May 2020

Expiration date: The 20th May 2021

Professor GAHUTU Jean Bosco
Chairperson Institutional Review Board,
University of Rwanda College of Medicine and Health Sciences

Cc:
- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Ethics Committee / Comité d'éthique

16,Jun,2020

Ref.:EC/CHUK/045/2020

Review Approval Notice

Dear Louise Munezero,

Your research project: *"GeneXpert Mtb/Rif assay on Fine Needle Aspiration Samples for diagnosis of Tuberculous Lymphadenitis At University Teaching Hospital of Kigali (CHUK) and Butare (CHUB)."*

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 16,Jun,2020 to evaluate your request for ethical approval of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your research project.

You are required to present the results of your study to CHUK Ethics Committee before publication by using this link:www.chuk.rw/research/fullreport/?appid=120&&chuk.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

Dr Emmanuel Rusingiza Kamanzi
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

ETHICS COMMITTEE
CHUK

Scan code to verify.

~ University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations "

B.P. :655 Kigali- RWANDA www.chuk.rw Tél. Fax : 00 (250) 576638 E-mail :chuk.hospital@chukigali.rw