Xpert MTB/RIF Ultra and mycobacterial culture in routine clinical practice at a Tertiary Paediatric Hospital

By

ANTHONY KWAME ENIMIL
ENMANT001

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Faculty of Health Sciences

University of Cape Town

Supervisors: Professor Brian Eley and Dr James Nuttall

Department of Paediatrics and Child Health, University of Cape Town

Faculty of Health Sciences, University of Cape Town

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DECLARATION

I, Anthony Kwame Enimil, hereby declare that the work on which this dissertation is based is my original work and that acknowledgements have been indicated in situations where another person's work has been referenced or quoted.

To the best of my knowledge, neither part nor whole of this work has been, or is being submitted for another degree in this University or another University.

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ABSTRACT

Introduction

World Health Organization approved the use of Xpert MTB/RIF Ultra (Ultra) in children due to quick turn-around time, improved yield over smear microscopy, and ability to detect rifampicin resistance despite culture being the “gold standard”.
This study reviewed published literature on current childhood tuberculosis diagnostic modalities. It also retrospectively compared demographic, clinical, and radiological features of children with confirmed and unconfirmed PTB, reviewed criteria for microbiologically unconfirmed PTB, and assessed incremental microbiological yield on second and third Ultra and/or mycobacterial culture results in routine clinical care at a tertiary paediatric hospital.

Method

For the review on childhood TB diagnostic modalities, PubMed was searched using Boolean terms OR/AND between childhood tuberculosis and words such as diagnosis, polymerase chain reaction, molecular, histology, imaging, and cultures. All abstracts were read after which selected articles that met the objectives of the thesis were fully reviewed and referenced appropriately.

The retrospective study was conducted in children (0 to 13 years) treated for Pulmonary TB (PTB) between 1 February 2018 and 31 January 2019 and who had at least one respiratory specimen investigated by Ultra and/or mycobacterial culture before TB treatment was commenced.

Relevant demographic, clinical information, tuberculin skin test results and laboratory results were abstracted from paper-based medical records and electronic database. Baseline chest radiographic findings were obtained from the radiology digital imaging database.

All data was entered anonymously into a Microsoft Excel spreadsheet and exported to R-statistical software for statistical analysis. Descriptive and inferential statistics were used in the analysis. Incremental yield of Ultra and/or mycobacterial cultures on sequential respiratory specimens was determined.

Results

Ultra is an important diagnostic method for confirming TB in children even though mycobacterial culture, molecular, and histology tests are also available. Other modalities such as imaging and immunologic tests support the diagnosis of microbiologically unconfirmed TB.

174 children with PTB ± EPTB were included in the retrospective study. The median age was 2.5 years. Tuberculosis was microbiologically confirmed in 93 (53.4%). Yield on Ultra in first respiratory specimens was 39.1%. When the results of Ultra and mycobacterial culture on first respiratory specimens were combined, 47.1% (82/174) had microbiologically confirmed TB.

Microcytic anaemia and pulmonary pathology were more common in confirmed TB.

Of 81 children with microbiologically unconfirmed TB, 31 (38.3%) met a consensus definition of unconfirmed intrathoracic TB formulated by an international expert committee.

In the subset of children (n=70) who were screened by Ultra on two sequential respiratory specimens, the incremental yield was 30.3%. When the results of Ultra and mycobacterial culture were combined the incremental yield in children who had 2 sequential respiratory specimens tested was 24.4% and 3.1% on Ultra and mycobacterial culture, respectively.

Conclusion

Ultra and/or mycobacterial culture on single respiratory specimens resulted in high microbiological yield. Ultra on second sequential respiratory specimens increased microbiological confirmation. The value of additional Ultra and/or mycobacterial culture testing in routine clinical practice requires further study.

Keywords: Diagnosis, confirmed, unconfirmed, childhood, tuberculosis

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ABBREVIATIONS

ADA- adenosine deaminase

BAL- bronchoalveolar lavage

BCG- Bacillus Calmette-Guerin

CGI- chronic granulomatous inflammation

CI- confidence interval

CN- caseating necrosis

CNS- central nervous system

CT- computerised tomography

cTB- confirmed TB

CXR-chest radiography

DNA- deoxyribonucleic acid

EPTB- extrapulmonary TB

Hb- haemoglobin

HIV- human immunodeficiency virus

IGRA- interferon gamma-release assay

ICU- intensive care unit

inhA- inhibin subunit alpha

IY- incremental yield

LAM- lipoarabinomannan

LAMP- loop-mediated isothermal amplification assay

LED- light-emitting diodes

LJ- Lowenstein-Jensen

LPA- line probe assay

MC- mycobacterial culture

MCV- mean corpuscular volume

MDR- multidrug-resistant

MGIT- mycobacteria growth indicator tube

MODS- microscopic-observation drug-susceptibility

MRI- magnetic resonance imaging

MTB- *Mycobacterium tuberculosis*

MTBc- *Mycobacterium tuberculosis* complex

MUWFA- moderate underweight-for-age

NAAT- nucleic acid amplification test

NGT- nasogastric tube

NHLS- National Health Laboratory Service

PANTA- Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim and Azlocillin

PCR- polymerase chain reaction

POCT- point-of-care test

PPD- purified protein derivative

PTB- pulmonary tuberculosis

RCWMCH- Red Cross War Memorial Children's Hospital

RNA- ribonucleic acid

SD- standard deviation

TB- tuberculosis

TST- tuberculin skin test

TU- tuberculin units

USG- Ultrasonography

uTB- unconfirmed TB

UWFA- underweight for age

WHO- World Health Organization

XDR- extensively drug-resistant

Xpert- Xpert MTB/RIF assay

Ultra- Xpert MTB/RIF Ultra assay

ZN- Ziehl-Neelsen

CHAPTER 1: INTRODUCTION

1.1 Context

Globally, in 2020 there were approximately 10 million new cases of tuberculosis (TB), 12% of which were children. The global TB incidence rate was 130 new cases per 100,000 population per annum for all ages.[1] However, the actual burden of TB in children is likely to be higher given the challenges in diagnosing childhood TB.[2] With the scale-up of antiretroviral therapy (ART) and improved TB diagnostics and management, the incidence of TB in South Africa has declined significantly over a 10 year period across all age groups.[3]

Microbiological confirmation of childhood tuberculosis is challenging as a result of both the pathophysiology of childhood TB disease and for logistical reasons and, as a result, is uncommon.[4] Respiratory specimens are difficult to collect in young children, and the reported bacteriologic yield is low.[5] Nicol et al., in a study evaluating 452 children with a median age of 19.4 months admitted to hospital with suspected PTB in 2009-2010 in Cape Town, found 6% had a positive smear result, 16% had a positive culture result, and 13% had a positive Xpert MTB/RIF test (Xpert) result.[6] Microbiology laboratory capacity is lacking in many African countries and diagnosis frequently relies on a combination of symptoms, signs, radiological findings, a tuberculosis contact history and tuberculin skin testing.[7]

Common investigations used in the diagnosis of childhood tuberculosis

Tuberculin skin testing (Mantoux test)

Short of demonstrating viable organisms in body tissues and fluids, the tuberculin skin test (TST) was the only method of detecting *Mycobacterium tuberculosis* (MTB) infection in an individual until the introduction of the interferon gamma-release assay (IGRA). Both are used in the diagnosis of TB infection in individual patients, as well as in epidemiological settings to measure the prevalence of tuberculous infection in populations.[8]

The Mantoux test is affordable, but it cannot distinguish true TB infection from tuberculosis or the effect of BCG vaccination which is widely used in developing

economies. The result of a Mantoux test is read in millimetres of induration 48-72 hours after injection and the interpretation is dependent on the immune status of the child. In an immunodeficient child (e.g., HIV-infected not on ART or malnourished), a Mantoux test is considered positive when the transverse diameter of the skin induration reaction is ≥5 mm. In an immunocompetent child, a reaction ≥10mm is considered positive.[9]

Imaging modalities

Chest radiographic imaging is one of the oldest imaging techniques used for diagnosing respiratory conditions including suspected TB.

On chest radiography (CXR), intrathoracic TB in infants and young children is typically characterised by enlarged lymph nodes and parenchymal opacities (lympho-bronchial TB). Pleural effusion develops mainly in children older than 5 years following recent primary infection.[10] Adolescents and young adults with TB more commonly present with apical consolidation and cavitation, fibrosis, and atelectasis.[11] Tuberculosis of the spine (Pott's disease) commonly involves the thoracic vertebrae and may be detected on CXR.[12]

However, CXR is limited by its two-dimensional orientation as well as high inter-interpreter and intra-interpreter variability in identifying lymphadenopathy.[13], [14] Poor image quality and co-infections in HIV positive children reduces the specificity of CXR as a diagnostic tool. In some studies specificity was < 50%.[15], [16]

Ultrasonography (USG) of the abdomen may identify micro-abscesses in the liver and spleen and USG of the chest helps identify lymph nodes and effusions. With additional history, USG findings could suggest TB, especially in HIV-infected patients.[17] Using the supra- sternal window, USG can detect lymph node abnormalities more frequently than radiography.[18]

Computerised tomography scan (CT scan) and magnetic resonance imaging (MRI) are imaging modalities that provide cross-sectional as well as three-dimensional spatial information to visualise multiple coexisting lesions, thus enhancing sensitivity and providing non-invasive monitoring for individuals.[19] CT is superior for detecting lymphadenopathy, better at visualisation of airway compression, pneumonia, lymph node necrosis, and lung necrosis than CXR.[20], [21]

CT scanning is limited by exposure to radiation and the need for intravenous contrast to enhance visualisation.[20]

Microbiological confirmation

Sputum smear microscopy

Sputum smear microscopy is widely used to detect TB. Light-emitting diodes (LED) have been developed to offer the benefits of fluorescence microscopy over conventional Ziehl-Neelsen (ZN) microscopy.[22] It has a number of limitations, including low sensitivity especially in HIV-positive individuals and children, and the inability to detect drug-resistance.[23] With mycobacterial culture as the reference standard, Xpert identified twice as many cases (75·9%) as did smear microscopy (37·9%) in one study [6] whilst in a systematic review and meta-analysis, the sensitivity of Xpert was 36-44% more than microscopy.[24]

Xpert MTB/RIF Ultra assay

World Health Organization (WHO) in December 2010 approved the use of Xpert as a replacement for sputum smear microscopy, especially in settings with high rates of HIV-associated TB and multidrug-resistant TB (MDR-TB).[25] It is capable of detecting MTBc and simultaneously screens the β subunit of the mycobacterial ribonucleic acid (RNA) polymerase gene for the presence of mutations conferring rifampicin resistance.

More recently, the Xpert MTB/RIF Ultra (Ultra) assay was developed to overcome the limited sensitivity of Xpert in the detection of PTB particularly in patients with

paucibacillary disease or HIV infection.[26] Dorman et al. in a study among adults with suspected TB who produced at least three sputum specimens in two days, the yield on Xpert and Ultra was 83% and 88% respectively on all culture positive specimens. Among smear negative, culture positive specimens, the yield on Xpert and Ultra was 46% and 63% respectively. Among HIV-infected patients with a culture positive specimen, Xpert and Ultra yielded a positive result on 77% and 90% respectively.[26]

A South African study published in 2018 investigated the comparative accuracy of Xpert and Ultra on induced sputum for diagnosing PTB in children. Among 76 children with a positive Xpert, Ultra or mycobacterial culture, Xpert detected 63.2%, Ultra 73.7% and culture 82.9%, (P = 0.117 for comparison of Xpert and Ultra).[27]

Mycobacterial culture

Lowenstein-Jensen (LJ) as a solid culture medium had been the gold standard for the diagnosis of TB for over a century. Its median time to positivity is four to six weeks. Commercial automated liquid culture methods including the mycobacterial growth indicator tube (BACTEC MGIT 960 - Becton Dickinson USA) are widely used for routine TB diagnosis.[28]

The BACTEC MGIT liquid culture system has a shorter turnaround time than the conventional LJ method for both smear positive and negative clinical specimens with a median detection time of 2 weeks.[28] In a study in which smear positive specimens were also cultured, the yield of positive results was 66.7% and 87.4% on LJ and MGIT methods respectively. On smear negative specimens the yield was 13.4% and 17.4% on LJ and MGIT methods respectively [29]

Mycobacterial culture is superior to Ultra for diagnosing PTB in children because childhood TB is often paucibacillary.[27] Thus, Ultra is used in combination with mycobacterial culture when investigating children for PTB at hospitals in South Africa.

The microscopic-observation drug-susceptibility (MODS) assay is a liquid culture-based method for detecting living mycobacteria premised on two well-known characteristics of MTB namely the growth in liquid medium is faster than that on solid medium, and the microscopic visualisation of the unique cording of MTB in liquid culture.[30] It is a low-cost, low-technology tool for high-performance detection of MTB and MDR TB.[31] Compared to smear microscopy (28.2%), MODS (39.7%) was found to be more sensitive at detecting MTB in children.[32]

Fine needle aspiration biopsy

Lymph nodes may be sampled by fine needle aspiration biopsy (FNAB), providing diagnostic material for mycobacterial culture and drug susceptibility testing, cytology, as well as nucleic acid amplification testing (NAAT).[33]

About 30% of children with PTB also have extrapulmonary disease, with tuberculous lymphadenitis as the commonest manifestation.[34] Tuberculous lymphadenitis is considered to be the local manifestation of the systemic disease, whereas lymphadenitis due to nontuberculous mycobacteria is truly a localised disease.[35]

A prospective study in Cape Town, South Africa showed that in high-risk populations, FNAB using a combination of cytomorphology, autofluorescence, and ZN staining provided a rapid and definitive diagnosis of mycobacterial infection, allowing initiation of therapy pending culture and sensitivity testing.[36] In a prospective diagnostic study in adults using fine-needle aspirates (FNA) at Groote Schuur Hospital, Cape Town, Ultra sensitivity was 75% using mycobacterial culture on FNA as reference.[37]

Tissue histology

A histological finding of chronic granulomatous inflammation (CGI) concomitant with caseating necrosis (CN) from a peripheral lymph node excision biopsy can be strong evidence of active TB even though not confirmatory.[38] Other infectious (e.g. cat scratch disease) and non-infectious (e.g. sarcoidosis) conditions produce CGI.[39]

Other investigations

Table 1 shows other tests less frequently used in the diagnostic workup of children with suspected TB.

Table 1 Other tests for TB diagnosis in children

Test	Technique	Advantages	Disadvantages
Molecular/Antigen test			
LAMP[40]	A nucleic acid amplification method designed to amplify a specific DNA region under isothermal conditions	User friendly Less infrastructure needed More sensitive than sputum smear microscopy	Less sensitive than Xpert MTB/RIF Ultra
Urine-LAM[41]	Detects cell wall lipopolysaccharide lipoarabinomannan in urine	Commercially available as a POCT	More applicable in adult HIV patients with advanced disease
Immune response test			
ADA[42]	Enzyme involved in T-cell proliferation	Supportive for TB pleural effusion	Variable cut-off values indicating a significant result
IGRA[43]	Measures interferon (IFN)-gamma release in response to antigens present in *Mycobacterium tuberculosis*	Differentiates *Mycobacterium tuberculosis* infection from BCG	Test is performed using blood (invasive procedure) Expensive

LAMP-Loop-mediated isothermal amplification assay; LAM- Lipoarabinomannan; ADA- Adenosine Deaminase; IGRA-Interferon Gamma release assay; POCT-Point-of-care test; BCG- Bacillus Calmette-Guerin vaccine.

Conclusion

All diagnostic modalities have limitations in the diagnosis of TB in children and clinical judgement remains important in the decision to start TB treatment.

The revised classification of intrathoracic tuberculosis defines confirmed intrathoracic TB as detection of *Mycobacterium tuberculosis* complex (MTBc) by either culture or Ultra on at least 1 respiratory specimen. Unconfirmed TB is defined as (1) bacteriological confirmation NOT obtained AND at least 2 of the following: the presence of suggestive symptoms or signs of TB, chest radiography consistent with TB, close TB exposure or immunologic evidence of MTB infection, a positive response to TB treatment or (2) immunological evidence of MTB (either a positive

TST or a positive IGRA result) plus at least 1 of TB clinical diagnosis criteria OR CXR consistent with TB OR positive clinical response to anti-TB therapy.[44]

The consensus statement helps classify unconfirmed intrathoracic TB for both clinical and research purposes, but was primarily designed to standardise the diagnosis of childhood intrathoracic TB in research studies.[44]

This study reviewed the routine use of Ultra and mycobacterial culture at RCWMCH and compared unconfirmed TB with the consensus statement on a revised classification of intrathoracic TB. It also reviewed the incremental yield from second and third sequential respiratory specimens for Ultra and/or mycobacterial culture in a subset of patients.

1.2 Ethical considerations

The study was submitted for approval to the Departmental Research Committee, Department of Paediatrics and Child Health, University of Cape Town; Human Research Ethics Committee (HREC), Faculty of Health Sciences, University of Cape Town (Appendix 1 and Appendix 2); and the Research Committee at Red Cross War Memorial Children's Hospital (Appendix 3). The study was done in accordance with the Declaration of Helsinki. The HREC approval number is 049/2019.
The data was collected retrospectively, thus consent was not obtained from parents/legal guardians.

The data sheets included the names and hospital folder numbers of study subjects which enabled the researchers to check information from the hospital folders after data collection had been completed. Each name and hospital folder number were linked to a study number. Study numbers but not names or hospital folder numbers were entered into an electronic database for anonymous analysis and reporting.

Risks to study participants

There were no risks to study participants. Data was collected retrospectively and analysed anonymously.

Benefits to study participants

There were no direct benefits to study participants.

1.3 Author instructions for the International Journal of Infectious Diseases

International Journal of Infectious Diseases (IJID) is the official Publication of the International Society for Infectious Diseases. The journal is peer-reviewed and deals with the epidemiology, clinical diagnosis, treatment, and control of infectious diseases with particular emphasis placed on those diseases that are most common in under-resourced countries. IJID had an impact factor of 3.2 in 2019. Original research articles do not exceed 3500 words, refer to appendix 4 for the complete author guidelines.

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CHAPTER 2: PUBLICATION-READY MANUSCRIPT

TITLE PAGE

Xpert MTB/RIF Ultra and mycobacterial culture in routine clinical practice at a Tertiary Paediatric Hospital

Anthony Enimil[1], James Nuttall[1], Chad M. Centner[2,3], Natalie Beylis[2,3], Brian Eley[1]

[1]Paediatric Infectious Diseases Unit, Red Cross War Memorial Children's Hospital and Department of Paediatrics and Child Health, University of Cape Town, Cape Town, South Africa
[2]Division of Medical Microbiology, University of Cape Town, South Africa
[3]National Health Laboratory Service, Groote Schuur Hospital and Green Point, Cape Town, South Africa

Authors email addresses

Anthony Kwame Enimil - tonash@gmail.com, enmant001@myuct.ac.za
James Nuttall - james.nuttall@uct.ac.za
Chad Centner - chadc@nis.za
Natalie Beylis - natalie.beylis@yahoo.co.uk
Brian Eley - brian.eley@uct.ac.za

The present address for correspondence: Anthony K Enimil, Department of Child Health, Komfo Anokye Teaching Hospital and Kwame Nkrumah University of Science and Technology, Kumasi-Ghana
Email address: tonash@gmail.com, enmant001@myuct.ac.za

HIGHLIGHTS

- Children with microbiologically confirmed TB had more airway compression, cavitary disease, and miliary TB.
- Microcytic anaemia was more frequent in microbiologically confirmed TB.
- Microbiological yield increased on combining Xpert Ultra and mycobacterial culture results.
- A second sequential Xpert Ultra test increased microbiological yield.

ABSTRACT

Objective

This study described the demographic, clinical, and radiological characteristics of children treated for pulmonary tuberculosis and evaluated Xpert MTB/RIF Ultra (Ultra) and mycobacterial cultures in routine clinical care at a tertiary paediatric hospital.

Methods

The findings of this retrospective study were summarised using descriptive and inferential statistics.

Results

174 children were included. The median age was 2.5 years. Tuberculosis was microbiologically confirmed in 93 (53.4%). Yield on Ultra in first respiratory specimens was 39.1% (68/174). When the results of Ultra and mycobacterial culture on the first respiratory specimens were combined, 47.1% (82/174) had microbiologically confirmed TB. Microcytic anaemia was more common in children with confirmed TB.

In the subset of children (n=70) screened by Ultra on two sequential respiratory specimens, the incremental yield was 30.3%. In the subset of children screened by Ultra on three sequential respiratory specimens (n=16), the incremental yield on the second and third specimens was 16.7% and 0% respectively. When Ultra and mycobacterial culture results were combined the incremental yield in children who had 2 sequential respiratory specimens tested was 24.4% and 3.1% on Ultra and mycobacterial culture, respectively.

Conclusion

Xpert Ultra and mycobacterial culture on a single respiratory specimen resulted in high microbiological yield. Incremental yield of Ultra on a second respiratory specimens increased microbiological confirmation. Additional diagnostic testing may require further study.

Keywords: Diagnosis, confirmed, unconfirmed, childhood, tuberculosis

Globally, in 2020 there were approximately 10 million new cases of tuberculosis (TB), 12% of which were children. The global TB incidence rate was 130 new cases per 100,000 population per annum for all ages. South Africa is acknowledged as a high-burden country for TB, HIV-TB coinfection, and multidrug-resistant TB (MDR-TB). In 2019 it had one of the highest incidence rates at 615 new TB cases per 100,000 population per annum with 360,000 incident cases.[1]

Sputum smear microscopy is widely used to detect TB. It has a number of limitations, including low sensitivity especially in HIV-infected individuals and children, and the inability to detect drug-resistant TB.[2] The Xpert MTB/RIF assay (Xpert) was endorsed by the World Health Organization (WHO) in December 2010 as a replacement for sputum smear microscopy, particularly in settings with high rates of HIV-associated TB and multidrug-resistant TB.[3] It is capable of detecting *Mycobacterium tuberculosis* complex (MTBc) and simultaneously screens the β subunit of bacterial ribonucleic acid (RNA) polymerase gene for the presence of mutations conferring rifampicin resistance.

South Africa introduced Xpert to clinical practice in March 2011.[4] Prior to this intervention, confirmation of pulmonary TB (PTB) in children was done by smear microscopy and mycobacterial culture. The sensitivity of Xpert is superior to that of microscopy in children under investigation for TB.[5]–[7] However, because childhood TB is paucibacillary, mycobacterial culture is superior to Xpert for diagnosing PTB in children.[8] Thus, Xpert is used in combination with mycobacterial culture when investigating children for PTB at hospitals in South Africa.

An important paediatric TB diagnostic challenge is that most children are unable to produce an expectorated sputum specimen. Instead, alternative specimen types notably induced sputum and gastric lavage aspirates are used in the investigation of children with suspected PTB. Diagnostic performance of Xpert is comparable in induced sputum and gastric lavage specimens.[9]

The sensitivity of Xpert may be increased by screening two or more sequential respiratory specimens from children under investigation for PTB.[5], [10] More recently, the Xpert MTB/RIF Ultra (Ultra) assay was developed to overcome the

limited sensitivity of Xpert in the detection of PTB particularly in patients with paucibacillary disease or HIV infection.[11] The diagnostic performance of Ultra for confirming PTB in children was evaluated using banked sputum specimens obtained from children previously investigated for PTB. Compared to mycobacterial culture, the sensitivity and specificity of Ultra were 75.3% and 95% respectively. In a subset of children, the sensitivity of Ultra was superior to that of Xpert, but mycobacterial culture outperformed both Xpert and Ultra. Furthermore, Ultra was unable to detect MTBc in specimens of 25% of children with culture-confirmed TB and hence cannot be used as a replacement test for mycobacterial culture, especially in settings where mycobacterial culture is part of the routine diagnostic work up.[12]

In February 2018, Ultra replaced Xpert as a screening tool for TB in children at Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town. In this study we compared demographic, clinical, and radiological features of children in routine care with confirmed and unconfirmed PTB, reviewed criteria for microbiologically unconfirmed PTB, and assessed incremental microbiological yield on second and third Ultra and/or mycobacterial culture results at a tertiary paediatric hospital during the first year after the introduction of Ultra.

METHODS

Study design and Setting

This retrospective study was conducted at RCWMCH in children treated for PTB and who had at least one respiratory specimen investigated by Ultra and/or mycobacterial culture before TB treatment was commenced. Red Cross War Memorial Children's Hospital in Cape Town, South Africa is a 282-bed teaching hospital of the University of Cape Town. It serves as a tertiary-level paediatric referral hospital for sick children aged 0 to 13 years from the Western Cape province as well as surrounding provinces.

Study population

Children aged 0 to 13 years who were investigated for TB and initiated on treatment for microbiologically confirmed or microbiologically unconfirmed PTB between 1 February 2018 and 31 January 2019. These children were identified from the RCWMCH pharmacy and National Health Laboratory Service (NHLS) microbiology databases.

Inclusion Criteria:

- Children between 0 and13 years who were treated for PTB including those with concomitant extra-pulmonary tuberculosis (EPTB) at RCWMCH from 1 February 2018 to 31 January 2019 AND
- Children who had results for Ultra ± mycobacterial culture for at least one respiratory specimen recorded in the NHLS microbiology database.

Exclusion criteria:

- Children with EPTB without evidence of PTB.
- Children who were initially commenced on TB treatment during the study period but then had their TB treatment stopped as they had an alternative diagnosis.
- Children with PTB who started TB treatment before 1 February 2018.

The proportion of children with microbiologically unconfirmed TB who met a consensus case definition of unconfirmed TB as defined by an international expert committee was determined.[13]

Data collection

Relevant demographic, clinical information, and tuberculin skin test results were abstracted from paper-based medical records. Baseline chest radiographic findings were obtained from the radiology digital imaging database. Chest radiographs were viewed in both anterior posterior or posterior anterior (age-dependent) and lateral views. Relevant pathology was determined based on the integration of the radiologist's report and researcher's interpretation, and recorded on a standardised radiology results sheet.[14] Ultra and mycobacterial culture results, as well as selective haematology and HIV results were abstracted from the NHLS electronic databases. All information was entered on study-specific data collection forms.

Tuberculin skin testing

Tuberculin skin testing was performed by the Mantoux method. Briefly, 0.1 mL of 2 tuberculin units of purified protein derivative (Tuberculin PPD RT 23, 2 TU, AJ Vaccines, Copenhagen, Denmark) was administered intradermally with a short bevel needle. The extent of induration was measured after 48-72 hours. The result was interpreted according to WHO guidelines.[15]

Respiratory specimen collection

Respiratory specimens were collected according to standardized methods summarized in the supplementary file.

Microbiological procedures

All microbiology testing was conducted at the NHLS microbiology laboratory, Groote Schuur Hospital, Cape Town, South Africa. Respiratory specimens were processed and analysed according to the laboratory's standard operating procedures and/or relevant manufacturers' instructions.

In brief, specimens were decontaminated by the addition of N-Acetyl-L-Cysteine-NaOH solution to achieve a final NaOH concentration of 1.5%. After 20 minutes, an

equal volume of phosphate buffer was added, followed by centrifugation at 3000xg for 15 minutes. The supernatant was then decanted to leave 1 ml of sediment, which was split; 500 µl was inoculated into an Ultra (Cepheid, Sunnyvale, CA) cartridge. Molecular semi-quantitation of MTBc load by Ultra was categorized as trace, very low, low, medium, high, and very high. The remaining 500 µl of sediment was resuspended in 500 µl phosphate buffer and inoculated into BD BBL Mycobacteria Growth Indicator Tubes (MGIT). The MGITs had been prepared with 800 µl PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) antibiotic mix (Becton Dickinson, Franklin Lakes, NJ). MGITs were incubated at 37ºC in the BD BACTEC MGIT 960 Mycobacteria Culture System (Becton Dickinson) and continuously monitored for 42 days or until positive for growth of acid-fast bacilli that was identified using the Ziehl Neelsen stain. The presence of MTBc was confirmed by GenoType MTBDRplus line probe assay (Hain Life science GmbH, Nehren, Germany), which also detects rifampicin and isoniazid-resistance associated mutations in MTBc. Species identification of non-tuberculous mycobacteria was not routinely performed.

Study definitions

Confirmed TB (cTB) was defined as microbiological confirmation of MTBc by either culture or Ultra on at least 1 respiratory specimen.[13] Unconfirmed TB (uTB) was diagnosed if the attending clinician treated a child for TB based on the presence of suggestive symptoms or signs of TB, chest radiograph consistent with TB, a close TB contact and/or a positive tuberculin skin test but without microbiological confirmation.

Pulmonary tuberculosis (PTB) is any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or tracheobronchial tree.

Extrapulmonary TB (EPTB) is any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs. Tuberculous intrathoracic lymphadenopathy (mediastinal and/or hilar) or tuberculous pleural effusion, without radiographic abnormalities in the lungs, constituted EPTB[15]

HIV infection:

- for a child < 18 months old: a positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test on an independent blood sample.
- for a child >18 months old: two positive serological test results (HIV rapid test or HIV ELISA) or a positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test.[16]

Moderate or severe underweight for age (UWFA) were defined as weight-for-age z score (WAZ) <−2 standard deviations (SD) below the median WHO growth reference standards.[17]

Haemoglobin (Hb) cutoff of < 11 g/dl was defined as anaemia and mean corpuscular volume (MCV) < 70 fL was defined as microcytosis.[18] Microcytic anaemia was defined as Hb <11 g/dl and MCV < 70 fL.[19]

Tuberculosis treatment regimens for uncomplicated, complicated, and central nervous system (CNS) TB as described in the South African childhood Tuberculosis guidelines were used to treat the patients included in this study.[20]

Liver friendly regimen: An alternative TB treatment regimen containing non-hepatotoxic drugs such as ethambutol, amikacin, levofloxacin and/or linezolid.[21]

Tuberculosis contact history refers to household or close exposure of a child to an individual with PTB.[15]

Statistical analysis

All data was entered anonymously into a Microsoft Excel spreadsheet and exported to R statistical software version 3.5.1 for statistical analysis.[22]

Demographic, clinical, and radiological categorical variables were presented as proportions and percentages of total. Chi-squared test or Fisher's exact test was used to assess association of categorical variables between confirmed PTB and

unconfirmed PTB. When categorical variables were small values (5 and below) Fisher's exact test was used.

Normally distributed continuous variables were summarised by mean and standard deviation (SD). Student's t-test was used to compare mean values of normally distributed variables in children with cTB and uTB. Skewed continuous variables were summarised by median and interquartile range (IQR). The Wilcoxon rank-sum test was used to compare medians of non-normally distributed continuous variables in children with cTB and uTB. Statistical significance was set at $p<0.05$.

All participants had at least one respiratory specimen for Ultra and/or mycobacterial culture. The microbiological yield was reported as number (%). For children with two or three specimens processed by Ultra and/or mycobacterial culture the incremental yield between the first and second specimens and between the second and third specimens was calculated according to the method of Rachow et al.[10]

RESULTS

Study participants and respiratory specimens

During the study period 308 children at RCWMCH received TB treatment, of whom 174 (56.5%) initiated TB treatment for PTB ± EPTB and were included in the analysis, figure 1.

A total of 260 respiratory specimens were submitted for Ultra and/or mycobacterial culture in the 174 children. Specimen types were induced sputum, 176 (67.7%); expectorated sputum, 42 (16.1%); tracheal aspirate, 25 (9.6%); gastric lavage, 14 (5.4%) and bronchoalveolar lavage, 3 (1.2%).

TB classification

Tuberculosis was microbiologically confirmed in 93 (53.4%) of the participants. The prevalence of clinical features, tuberculin skin test reactivity and PTB exposure history in the 81 children with uTB are summarised in Table 1. Of these 81 children, 37 (45.7%) had documentation of Mantoux testing, of which 31 (83.8%) were positive. Of the 31 with a positive Mantoux reaction, 8 (25.8%) met the consensus case definition of uTB. The six with Mantoux negative results all had at least two of TB clinical diagnostic criteria, chest radiographs suggestive of PTB, or exposure to PTB i.e., all six met the consensus definition of uTB. Of the 44 (54.3%) patients without Mantoux results, 17 (38.6%) had at least two of TB clinical diagnostic criteria, chest radiographs suggestive of PTB, or exposure to PTB i.e., consistent with the consensus definition of uTB.

Characteristics of confirmed and unconfirmed TB cases

The characteristics of the children with cTB and uTB are summarised in Table 2. Most of the children were less than 5 years of age. Among the 93 cases of cTB, 55 (59.1%) were diagnosed by both Ultra and mycobacterial culture, 23 (24.7%) by Ultra alone and 15 (16.2%) by mycobacterial culture alone. A significantly higher proportion of children with cTB experienced EPTB, p=0.001. Among the 45 cTB cases with EPTB, 23 (51.1%) had central nervous system (CNS) TB including 11 with miliary TB, 13 (28.9%) abdominal TB, and 9 (20.0%) had EPTB involving other organ systems. Among the 15 uTB cases with EPTB, 9 (60.0%) had CNS TB

including 5 with miliary TB, 3(20.0%) had abdominal TB, and 3 (20.0%) had EPTB involving other organ systems. Significantly higher proportions of children with cTB were treated for complicated or CNS TB, p=0.001.

The mean (SD) of haemoglobin, white cell count and platelet count were 9.47(1.95) g/dL, 14.7 (7.33) x 10^9/L and 494(211) x 10^9/L, respectively. There were no statistically significant differences when comparing these parameters in children with cTB and uTB. However, a greater proportion of children with cTB, 33/92 (35.9%), [95% CI (26.1%-46.5%)]) compared with uTB, 15/74(20.3%), [95% CI (11.8%-31.2%)] had microcytic anaemia, p=0.03.

Radiological features

Table 3 summarises the chest radiographic findings of children with cTB and uTB. Higher proportions of children with cTB experienced airway compression, cavitary disease and miliary TB.

Of 168 (96.5%) participants with chest radiograph reports, 72(42.8%) were 0-2 years old, 50(29.8%) were between 2 and 5 years of age, and 46 (27.4%) were above 5 years of age. Nodal disease was found in 35 (48.6%), 20 (40%), and 20 (43.5%) of the participants aged 0-2 years, 2 to 5 years, and above 5 years, respectively. Airspace opacification was found in 43 (59.7%), 24 (48%), and 25 (54.3%) of participants aged 0-2 years, 2 to 5 years, and above 5 years, respectively.

Microbiologically confirmed TB.

Table 4 summarises the results of the respiratory specimens of the children processed by Ultra. The yield from Ultra on the first respiratory specimens was 39.1% (68/174). Seventy (40%) of children had a second respiratory specimen screened by Ultra. The first respiratory specimens of these children yielded 23 positive Ultra results. The second specimens of these 70 children yielded a further 10 positive Ultra results, an incremental yield of 30.3%. Sixteen (9.2%) had three respiratory specimens screened by Ultra. There was no incremental yield from the third Ultra respiratory specimen.

Table 5 summarises the results of the respiratory specimens of the children processed by both Ultra and mycobacterial culture. The yield from Ultra and/or mycobacterial culture on the first respiratory specimens was 47.1% (82/174). For children with two respiratory specimens, first respiratory specimens yielded 31 positive Ultra and/or mycobacterial culture results. Testing by Ultra of the second respiratory specimens added 10 positive results, an incremental yield of 24.4%. Mycobacterial culture testing of second respiratory specimens added 1 positive result, an incremental yield of 3.1%. For children with three respiratory specimens, there was no incremental yield from the third respiratory specimen.

DISCUSSION

This study retrospectively compared demographic, clinical, and radiological features of children with confirmed and unconfirmed PTB at RCWMCH during the first year after the introduction of Xpert MTB/RIF Ultra. It also reviewed criteria for microbiologically unconfirmed PTB at RCWMCH in relation to a consensus case definition of unconfirmed TB defined by an international expert committee.[13] Incremental microbiological yield was assessed on second and third Ultra and/or mycobacterial culture results.

The median age among study participants was 2.5 years. This was similar to previous paediatric PTB studies at RCWMCH.[23], [24] This was an expected finding because children under 5 years have less developed immune systems and therefore more easily progress from primary infection to TB in high burden TB countries than older children.[25] HIV prevalence was 12.1% among children with PTB in this study. Two earlier prospective studies at RCWMCH that enrolled 452 and 195 participants reported HIV prevalence of 24% and 16.4% respectively among PTB patients.[5], [24] The lower prevalence in our study is likely attributable to the impact of the prevention of mother-to-child transmission of HIV interventions implemented in South Africa.

The prevalence of moderate or severe underweight-for-age among participants was 31.6% in this study. Zar et al.[24] and Nicol et al.[5] reported weight for age Z-scores <-2 prevalence of 24.6% and 35.2% respectively among their participants. In this study, the prevalence of a positive Mantoux reaction in HIV uninfected participants with uTB was 80%. Zar et al.[24] and Nicol et al.[5] recorded positive Mantoux reaction prevalence of 65% and 52% respectively among HIV uninfected participants with uTB. The higher Mantoux positive prevalence estimate of 80% in the current study may be in part due to the small sample size inflating this prevalence estimate. The prevalence of radiological changes suggestive of PTB among study participants with uTB was 70.5% whilst Nicol et al. reported a similar prevalence of 68%.[5]

In this study, the prevalence of microcytic anaemia in microbiologically confirmed TB (cTB) and uTB participants were 35.9% and 20.3% respectively. In a prospective, cross-sectional study conducted at another tertiary referral hospital in Cape Town in

1999, the prevalence of microcytic anaemia in cTB and probable TB was 26% and 29% respectively.[26] Relatively high prevalence in both studies suggests that microcytic anaemia is endemic among children with TB in Cape Town, and should be routinely screened for in these children.

This study found 54.8% airspace opacification, 42.5% nodal disease, and 14.9% airway compression among all children treated for TB. In a paediatric study completed in Mozambique airspace opacification was also the predominant feature of PTB.[27] In the current study, airspace disease was more frequent than nodal disease in all age categories, whereas in a retrospective case record review in British Columbia, nodal disease was more prevalent than airspace opacification in young and older children.[28] The reason for these differences is not clear although HIV infection could have influenced the radiological findings; in the current study 12% of participants had HIV infection whilst in the British Columbia study none of the participants had HIV infection.

Of the 174 participants with at least one respiratory specimen, the overall sensitivity of Xpert Ultra (Ultra) was 44.8% (78/174) on any respiratory specimen. In a prospective study that consecutively recruited 195 hospitalised patients with at least one nasopharyngeal aspirate (NPA) and at least one induced sputum (IS), the sensitivity of Ultra was 10.3% and 15.9% on NPA and IS respectively.[24] Whilst Zar et al.[24] obtained NPA and IS specimens from each study participant, the current study retrospectively analysed a mixture of respiratory specimens types (expectorated sputum, gastric lavage, induced sputum, and bronchoalveolar lavage) collected during routine clinical care.

In this study, of 70 participants with two Ultra results on any respiratory specimen, the incremental yield from the second Ultra test result was 30.3% and in the 16 participants with three Ultra results the incremental yield from the third Ultra test result was 0%. Furthermore, when we combined Ultra and/or mycobacterial culture results, the incremental yield from the second respiratory specimen tested by Ultra and mycobacterial culture was 24.4% and 3.1% respectively. Rachow et al. prospectively enrolled 164 patients with suspected TB. Each participant provided up to three sputum specimens for smear microscopy, Xpert and mycobacterial culture.

An incremental yield on second specimens and most third specimens was obtained when smear microscopy, Xpert and mycobacterial culture results were analysed separately and when Xpert and mycobacterial culture results were combined.[10] In our study, the absence of microbial yield from the third respiratory specimen was probably due to the small number enrolled in this study. Thus, larger studies are probably needed to determine whether there are advantages to testing more than two respiratory specimens in routine clinical practice, particularly in settings where Ultra and mycobacterial culture are routinely performed.

This study had limitations. Some clinical information was not documented or available at the time of reviewing the participants information. Sample size for participants with at least one respiratory specimen was small. Numbers of participants with second and third respiratory specimens were also limited. Respiratory specimen types were requested during routine clinical care and determined by the attending clinicians. Thus, whilst the results suggest that processing two specimens by Ultra and/or mycobacterial culture improved results, larger studies are needed to confirm this observation and to determine whether additional specimens should be tested in routine clinical practice.

CONCLUSION

In the first year of introduction of Ultra at RCWMCH, the median age of children investigated and treated for TB was 2.5 years. Children with microbiologically confirmed TB were more likely to have microcytic anaemia than those with unconfirmed TB. Cavitary disease, airway compression, and miliary TB disease on chest radiograph were more common in children with Ultra and/or mycobacterial culture positive results than in children with negative results. Combining Ultra and mycobacterial cultures on a single respiratory specimen improved the prevalence of confirmed PTB in routine clinical care at RCWMCH. Testing second respiratory specimens increased the microbiological yield.

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ETHICAL CONSIDERATIONS

Ethics approval was obtained from the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, reference number: HREC REF 049/2019 and the RCWMCH Research Committee approved the study, reference number: RCC 177/2019. The study was conducted in accordance with the principles of the Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

Anthony Enimil extracted the data from the RCWMCH patient files, radiology digital imaging, and the NHLS results databases for the investigated cases and wrote the manuscript. Brian Eley developed the concept and provided guidance on the title and objectives of the study as well as the study literature review, data analysis and

manuscript development. James Nuttall assisted with the study protocol and manuscript development. Natalie Beylis supported protocol development and data retrieval from NHLS database. Chad Centner supported manuscript writing particularly for the description of microbiology methods and assisted with retrieval of data from NHLS database. All authors reviewed and approved the final draft.

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Figures and Tables

Figure 1 Selection of children with confirmed and unconfirmed PTB for data analysis

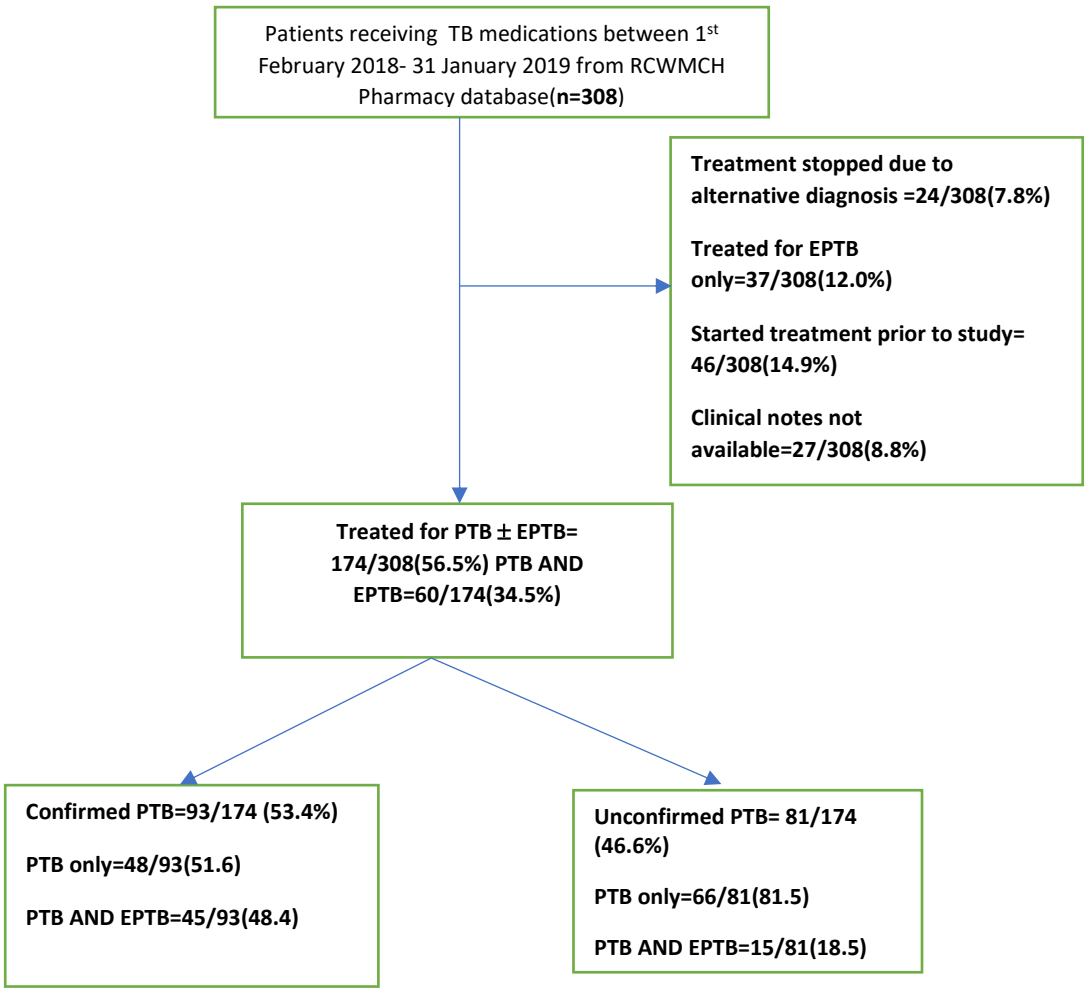

Table 1 Prevalence of manifestations in children with unconfirmed PTB

Variable	Total Number=81(100%)	95% Confidence Interval
Clinical signs/symptoms suggestive of TB		
Persistent cough > 2 weeks	20(24.7)	15.8-35.5
Weight-for-age z score of ≤−2	24(29.6)	20.0-40.8
Persistent (>1 week) and unexplained fever (>38°C)	4(4.9)	1.4-12.1
Persistent, unexplained lethargy/reduced playfulness, n/N (%)	3/78(3.8)	0.80-10.8
Chest radiograph findings suggestive of PTB, n/N (%)	55/78(70.5)	58.2-79.5
A positive Mantoux test		
≥10 mm if HIV uninfected children, n/N (%)	24/30(80.0)	72.3-90.4
≥5 mm if HIV-infected or weight-for-age Z-score <-2, n/N (%)	7/7(100)	64.5-100
Exposure to an individual with PTB	40(49.4)	37.4-60.2
Unconfirmed cases fulfilling clinical, radiological, positive Mantoux and/or PTB exposure history criteria		
Paediatric cases fulfilling all four criteria	7(8.6)	3.5-17.0
Paediatric cases fulfilling three criteria	31(38.3)	27.7-49.7
Paediatric cases fulfilling two criteria	34(42.0)	31.1-53.5
Paediatric cases fulfilling one criterion	9(11.1)	5.2-20.0

N=total number; n=number of events within a group; %= percentage

Table 2 Demographic and clinical characteristics of confirmed and unconfirmed PTB

Variables	All Subjects N=174 n (%)	Confirmed PTB N=93 n (%)	Unconfirmed PTB N=81 n (%)	p-value
Median age (IQR), years	2.5(1.1-5.3)	2.5(0.92-6.50)	2.4(1.42-4.75)	0.9
Gender				
Female	93(53.4)	49(52.7)	44(54.3)	0.83
Males	81(46.6)	44(47.3)	37(45.7)	
Ages in years				
<5 years	126(72.4)	65(69.9)	61(75.3)	0.43
≥5 and <14 years	48(27.6)	28(30.1)	20(24.7)	
Childhood immunization status				
Completed for age	135(77.6)	71(76.3)	64(79.0)	0.67
Incomplete for age	39(22.4)	22(23.7)	17(21.0)	
Moderate or severe underweight for age (Z-score < -2)	55(31.6)	31(33.3)	24(29.6)	0.6
HIV infected	21(12.1)	11(11.8)	10(12.3)	0.91
History of TB contact	73(41.9)	33(35.4)	40(49.4)	0.06
Contacts with positive GeneXpert, n/N (%)	51/73(69.9)	25/33(75.8)	26/40(65.0)	0.32
Disseminated Disease (PTB + EPTB)	60(34.5)	45(48.4)	15(18.5)	***0.001***
Types of TB treatment Regimen				
Uncomplicated	56(32.2)	17(18.3)	39(48.2)	ref
Complicated	81(46.5)	48(51.6)	33(40.7)	***0.001***
Central Nervous system	32(18.4)	23(24.7)	9(11.1)	***0.001***
Individualised	1(0.6)	1(1.1)	0(0)	0.99
Liver-friendly	4(2.3)	4(4.3)	0(0)	0.98

N=total number; n=number of events in a group; %= percentage

Table 3 Radiological features of confirmed and unconfirmed PTB

Intrathoracic lesions	**Total N=168* n (%)**	**Confirmed PTB N=90 n (%)**	**Unconfirmed PTB N=78 n (%)**	**p-value**
Airspace opacification	92(54.8)	45(50.0)	47(60.2)	0.24
Cavitary disease	5(3.0)	5(5.6)	0(0)	***0.035***
Lymph nodal disease	74(42.5)	41(45.6)	33(42.3)	0.67
Airway compression	25(14.9)	18(20.0)	7(9.0)	***0.04***
Pleural effusions	20(11.9)	8(8.9)	12(15.4)	0.19
Miliary disease	16(9.5)	11(12.2)	5(6.4)	***0.014***

N=total number; n=number of events in a group; %=percentage; *6 participant did not have chest radiographs

Table 4 Microbiological yield when respiratory specimens were evaluated by Xpert MTB/RIF Ultra but not mycobacterial culture.

Patients grouped according to number of respiratory specimens submitted	**Number (%) of Children N= 174**	**Number (%) of positive Ultra results on the 1st respiratory specimens**	**Additional number (%) of positive Ultra results on the 2nd respiratory specimens**	**Additional number (%) of positive Ultra results on the 3rd respiratory specimens**
1 specimen	174 (100)	68 (39.1)	-	-
2 specimens	70 (40)	23 (32.9)	10 (14.3)	-
3 specimens	16 (9.2)	4 (25.0)	1 (6.3)	0 (0)

N=total number; %= percentage; Ultra=Xpert MTB/RIF Ultra

Table 5 Microbiological yield when respiratory specimens were evaluated by both Xpert MTB/RIF Ultra and mycobacterial culture.

Patients grouped according to number of respiratory specimens submitted	**Number (%) of Children N= 174**	**Number (%) of positive Ultra and/or MC results on the 1st specimens**	**Additional number (%) of positive Ultra results on the 2nd specimens**	**Additional number (%) of positive MC results on the 2nd specimens**	**Additional number (%) of positive Ultra results on the 3rd specimens**	**Additional number (%) of positive MC results on the 3rd specimens**
1 specimen	174(100)	82(47.1)	-	-	-	-
2 specimens	70(40)	31(44.3)	10(14.3)	1(1.4)	-	-
3 specimens	16(9.2)	5(31.2)	1(6.3)	0(0)	0(0)	0(0)

N=total number; %= percentage; Ultra=Xpert MTB/RIF Ultra; MC= mycobacterial culture

Supplementary file

Respiratory specimen collection

Respiratory specimens were collected by standardised induced sputum, gastric lavage, expectoration, tracheal aspirate and bronchoalveolar lavage (BAL) methods.

Induced sputum specimen collection: After a 2-3-hour fast, the patient was pretreated with inhaled salbutamol, followed by sputum induction with 5mls of 5% sterile saline. A sterile mucus extractor of catheter size 6 or 7 was used to collect a sputum specimen.[1]

Gastric lavage specimen collection: The child underwent early morning gastric lavage after an overnight fast of at least 4 hours. Before the child arose, a nasogastric tube (NGT) was passed, and the gastric contents aspirated. If the aspirate volume was less than 20 mL, 20 mL of normal saline was inserted down the NGT, left for 2–3 minutes, then aspirated. Additional 5–10 mL volumes of normal saline were inserted and aspirated until a minimum of 20 mL of aspirate was obtained.[2]

Expectorated sputum specimen collection: For an older child who was able to expectorate, a sputum specimen was collected after deep, spontaneous coughing.[3]

Tracheal aspirate collection: Before the procedure, the patient was preoxygenated for 2 minutes, then disconnected from the ventilator. The tracheal aspirate was obtained by inserting an appropriately sized suction catheter through the endotracheal tube and suctioning for a maximum of 5 seconds. The tracheal aspirate was collected into a mucus trap. After specimen collection, the patient was reconnected to the ventilator.[4]

Bronchoalveolar lavage (BAL) collection: This was done either as a completely blind procedure or under direct vision. Blind BAL was done in the intensive care unit (ICU) by inserting a 10 ml aliquot of warm, sterile saline through the endotracheal tube, followed by suctioning with an appropriately sized suction catheter into a mucus trap. Bronchoalveolar lavage under direct vision was done by a pulmonologist or a

pulmonology trainee in theatre under general anaesthetic during bronchoscopy. Once the area of the lung was located, 10 ml of warm saline was inserted through the flexible bronchoscope channel. The saline was then suctioned into a specimen container using the suction channel of the flexible scope.[5]

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APPENDICES

1. FACULTY OF HEALTH SCIENCES ETHICS APPROVAL LETTER

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

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

25 January 2019

HREC REF: 049/2019

Prof B Eley
Division of Paediatric and Child Health
Room 520, 5th Floor
ICH Building
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Prof Eley

PROJECT TITLE: XPERT ULTRA MTB/RIF AND MYCOBACTERIUM CULTURE IN ROUTINE CLINICAL PRACTICE AT RED CROSS WAR MEMORIAL CHILDRENS HOSPITAL (MPhil: DR A ENIMIL)

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.

Approval is granted for one year until the 30 January 2020.

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)

We acknowledge that the student: Dr Anthony Enimil 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

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

2. 2021 ANNUAL PROGRESS REPORT/ ETHICS RENEWAL

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

HUMAN RESEARCH ETHICS COMMITTEE
19 JAN 2021
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

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This serves as notification of annual approval, including any documentation described below.			
☐ Approved	Annual progress report	Approved until/next renewal date	30.01.22
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	20/1/22

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

Please clarify your plan for research-related activities during COVID-19 lockdown

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	19 January 2021		
HREC REF Number	049/2019	Current Ethics Approval was granted until	30 January 2021
Protocol title	Xpert ultra MTB/RIF and Mycobacterium culture in routine clinical practice at Red Cross War Memorial Children's Hospital		
Principal Investigator	Brian Eley		
Department / Office Internal Mail Address	Room 520, 5th floor ICH building, Red Cross War Memorial Children's Hospital, Klipfontein Road, Rondebosch, 7700		
1.1 Does this protocol receive US Federal funding?		☐ Yes	✓ No

2. Protocol status (tick ✓)

☐	Research-related activities are ongoing
✓	Data collection is complete, data analysis only

Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository.

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		174
Total number of records or specimens collected, reviewed or stored since last progress report		64
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	✓ Yes	☐ No

4. Signature

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Signature of PI		Date	19 January 2021

Signatures Removed

3. APPROVAL LETTER FROM HOSPITAL RESEARCH COMMITTEE

Western Cape Government
Health

DR AN PARBHOO
Manager: Medical Services
Red Cross War Memorial Children's Hospital
Email: Anita.Parbhoo@westerncape.gov.za
Tel: +27 21 658 5430 Fax: +27 21 658 5006/5166

11 March 2019

Prof B Eley
Dr A Enimil

Dear Prof Eley and Dr Enimil,

RESEARCH: RXH: RCC 177

PROJECT TITLE: Xpert ultra MTB/RIF and Mycobacterium culture in routine clinical practice at Red Cross War Memorial Children's Hospital

It is a pleasure to inform you that the hospital Research Review Committee has approved your application to conduct above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely,

Signature Removed

DR AN PARBHOO
MANAGER: MEDICAL SERVICES

4. AUTHORS INSTRUCTIONS for the INTERNATIONAL JOURNAL OF INFECTIOUS DISEASES

The International Journal of Infectious Diseases

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Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance, or proofreading the article, etc.).

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[2] Van der Geer J, Hanraads JAJ, Lupton RA. The art of writing a scientific article. Heliyon. 2018;19: e00205. https://doi.org/10.1016/j.heliyon.2018.e00205

Reference to a book:
[3] Strunk Jr W, White EB. The elements of style. 4th ed. New York: Longman; 2000.

Reference to a chapter in an edited book:

[4] Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

Reference to a website:

[5] Cancer Research UK. Cancer statistics reports for the UK, http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/; 2003 [accessed 13 March 2003].

Reference to a dataset:

[dataset] [6] Oguro M, Imahiro S, Saito S, Nakashizuka T. Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1; 2015. https://doi.org/10.17632/ xwj98nb39r.1.

Reference to software:

[7] Coon E, Berndt M, Jan A, Svyatsky D, Atchley A, Kikinzon E, Harp D, Manzini G, Shelef E, Lipnikov K, Garimella R, Ultra C, Moulton D, Karra S, Painter S, Jafarov E, Molins, S. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo; 2020 (March 25). https://doi.org/10.5281/zenodo.3727209.

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5. DATA COLLECTION SHEET

SECTION A: PATIENT DEMOGRAPHY

Review Date	Folder no	Study number
Age in years	Date of birth	Gender
Weight in KG	WAZ score	Residence
Immunization status Up to date Complete incomplete	Date of admission/First review	Date of discharge/Transfer

SECTION B: HISTORY /CLINICAL INFORMATION

HISTORY (Yes-Y; No-N; Not stated-NS)				
SYMPTOMS	CHECK			DURATION (days)
All children	Y	N	NS	
Cough	Y	N	NS	
Weight loss/poor gain	Y	N	NS	
Fever	Y	N	NS	
Persistent/unexplained Fever	Y	N	NS	
Loss of appetite	Y	N	NS	
Persistent Lethargy	Y	N	NS	
Seizures/convulsions	Y	N	NS	
Headaches	Y	N	NS	
Vomiting	Y	N	NS	
Others	Y	N	NS	

SECTION C: EXAMINATION FINDINGS

EXAMINATION (Yes-Y; No-N; Not stated-NS)				
General Examination	CHECK			Comments
Temperature	Y	N	NS	Value:
Jaundice	Y	N	NS	
Pallor	Y	N	NS	
cyanosis	Y	N	NS	
Lymphadenopathy	Y	N	NS	
oedema	Y	N	NS	
clubbing	Y	N	NS	
Others-	Y	N	NS	
Respiratory Examination	Y	N	NS	L-left; R-Right; B-Bilateral
Respiratory rate	Y	N	NS	Value
Dullness to percussion	Y	N	NS	
Crackles	Y	N	NS	

Wheeze	Y	N	NS	
Reduced breath sounds	Y	N	NS	
Cardiovascular	Y	N	NS	Comments
Heart rate	Y	N	NS	Value
Apex beat displaced	Y	N	NS	
Pericardial rub	Y	N	NS	
Distant heart sounds	Y	N	NS	
Abdominal	Y	N	NS	
Distended	Y	N	NS	
Ascites	Y	N	NS	
Splenomegaly	Y	N	NS	
Hepatomegaly	Y	N	NS	
Central Nervous System	Y	N	NS	
Glasgow coma score	Y	N	NS	Value:
Neck stiffness/Kernicks +ve	Y	N	NS	
Altered consciousness?	Y	N	NS	
Focal neurology?	Y	N	NS	

SECTION D TB CONTACT HISTORY

Documentation of anyone in the household or close family, including those who have died, EVER been treated for TB?	Yes……. No…… Not stated……
If yes relationship was stated	Parent Grandparent Sibling Other relatives Others:
Type of TB	Confirmed AFB Xpert MTB/RIF TB Culture Unconfirmed: Radiological/clinical Not stated
Xpert sensitivity	Sensitive/ Resistant/not stated
Mycobacterium culture	List sensitivity if available……. Not stated
Treatment started	Yes……. No…… Not stated (NS)

SECTION E: LABORATORY INVESTIGATION -
FBC/CSF/LFT/RFT/INFLAMMATORY MARKERS

Type	Done	Date	Results	Value
FBC	Y N		White Cell Count Haemoglobin MCV Platelet Count	
Smear/comments: Microcytic Normochromic macrocytic				

CSF	Y N	Date	Polymorphs		AFB microscopy	

			Lymphocytes		TB culture	
			Red Blood Cells		India Ink	
			Glucose mmol/L		CLAT	
			Protein g/L		Bacterial culture	
LFT			ALT AST			
Inflammatory Markers			CRP PCT ESR			

SECTION F: HIV STATUS FN

Item	Done			Type
Is there screening test	Y	N	NS	Rapid/PCR
Is there confirmation test	Y	N	NS	Rapid/Eliza/PCR/VL
Test results	Positive Negative NS			
If Positive, is there evidence of receiving ART	Y	N	NS	
Is CD4 documented (Closest to review)	Y	N	NS	Value
Is Viral Load documented (if more than one state latest)	Y	N	NS	Value Log:
What is the duration of treatment	……. years ……. months ……. days			
List types of ARVs	□ stavudine □ lamivudine □ zidovudine □ didanosine □ abacavir □ efavirenz □ nevirapine □ kaletra			
Sequence of ARV meds:	□ TB meds started before ARVs □ TB meds started with ARVs □ ARVs started before TB meds □ Not stated			

SECTION G: MICROBIOLOGY TESTS/ CULTURES

Item	Done			Date	Organism Isolated
Blood culture	Y	N	NS		
CSF	Y	N	NS		
Urine	Y	N	NS		
Mycobacterium Culture	Y	N	NS		

SECTION H: SUPPORTIVE TUBERCULOSIS DIAGNOSTICS

Mantoux test	Taken	Date of reading	Induration - transverse diameter (mm)

Tuberculin Skin Test	Y N NS		…….mm

SECTION I REVISED CONSENSUS CASE DEFINITION

Status	Date	Positive=P; Negative=N, Not stated=NS		
Confirmed Tuberculosis				
Microscopy AFBs				
Xpert Ultra				
Mycobacterium culture				
Unconfirmed Tuberculosis	Date	Yes –Y; No-N; Not stated=NS		
Symptomatic		Y	N	NS
Chest Xray		Y	N	NS
Immunologic (TST)		Y	N	NS
Contact History		Y	N	NS
Unlikely Tuberculosis				
Bacteriologically not confirmed		Y	N	NS
TST positive		Y	N	NS

SECTION J: COMORBID CONDITIONS FN

SECTION K: TUBERCULOSIS TREATMENT PLAN AND OUTCOME

Item	Description	Yes –Y; No-N; Not stated=NS
Type of Tuberculosis	Intrathoracic (IT) only	Y N NS
	IT Plus EPTB	Y N NS
Treatment initiated	Y N NS	
Choice treatment	R H Z E Et steroid Pyridoxine	
	2nd line list…….	
Factors influencing medication choice	ARVs-1	Y N NS
	Uncomplicated-2 Complicated-3 Miliary/Disseminated-4 TBM-5	Y N NS
	Liver friendly meds 6	Y N NS
	Others 7	Y N NS
Outcome	Discharged to a TB clinic, Transferred to an intermediate facility (St Joseph's) Transferred to a TB hospital	Y N NS
	Died	Y N NS
Death	Date of death	

	Cause of death

SECTION L: INTRATHORACIC SAMPLING AND OUTCOMES ON XPERT Ultra

Results Ultra	Sample 1	Sample 2	Sample 3	Sample 4		Sa
Negative=0 Positive/Trace/ Indeterminate = 1						
Sample type: ES=1 GL=2 IS=3 TA=4 Others=5						

APPENDIX B CHEST XRAY DATA EXTRACTION FORM FN:

Date CXR performed: ____ / _____ / _____

Section 1: Parenchymal (Ghon) focus?

1. Right upper zone Yes □ No □
2. Right middle zone Yes □ No □
3. Right lower zone Yes □ No □
4. Left upper zone Yes □ No □
5. Left middle zone Yes □ No □
6. Left lower zone Yes □ No □
7. Upper zone Yes □ No □
8. Middle zone Yes □ No □
9. Lower zone Yes □ No □

Section 2: Cavity?

1. Right upper zone Yes □ No □
2. Right middle zone Yes □ No □
3. Right lower zone Yes □ No □
4. Left upper zone Yes □ No □
5. Left middle zone Yes □ No □
6. Left lower zone Yes □ No □
7. Upper zone Yes □ No □
8. Middle zone Yes □ No □
9. Lower zone Yes □ No □

Section 3: Soft tissue density suggestive of lymphadenopathy?

1. Right(peri-hilar) Yes □ No □
2. Right(para-tracheal) Yes □ No □
3. Right(calcified) Yes □ No □
4. Left(peri-hilar) Yes □ No □
5. Left(para-tracheal) Yes □ No □
6. Left(calcified) Yes □ No □

Section 4: Airspace opacification?

1. Right upper zone Yes □ No □
2. Right middle zone Yes □ No □
3. Right lower zone Yes □ No □

4. Left upper zone Yes □ No □
5. Left middle zone Yes □ No □
6. Left lower zone Yes □ No □
7. Upper zone Yes □ No □
8. Middle zone Yes □ No □
9. Lower zone Yes □ No □
10. Bilateral Miliary picture? Yes □ No □

Section 5: Airway compression and/or tracheal displacement?

1. Right paratracheal Yes □ No □
2. Right main bronchus Yes □ No □
3. Right bronchus intermedius Yes □ No □
4. Left main bronchus Yes □ No □

Section 6: Pleural effusion?

1. Right Yes □ No □
2. Left Yes □ No □