Evaluation of Diagnostic Advances in Musculoskeletal Tuberculosis; The Automated Xpert MTB/RIF Assay.

Thesis presented for the degree of

DOCTOR OF PHILOSOPHY

IN ORTHOPAEDIC SURGERY

FACULTY OF HEALTH SCIENCES

UNIVERSITY OF CAPE TOWN

BY

MICHAEL HELD

MMed - University of Cape Town, South Africa

Dr. med. - Ludwig-Maximilian University of Munich, Germany

FC Orthopaedic Surgery (South Africa)

Specialist Orthopaedic and Trauma Surgery (Germany)

SUPERVISORS: Prof R Dunn, Prof H Zar

Declaration

I, Michael Held, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions from others as outlined in the acknowledgements. The assistance I received with study management, data collection, analysis and manuscript review from the co-authors of the publications that form part of this thesis is described.

Neither the substance nor any part of the above thesis has been submitted in the past, or is being, or is to be submitted for a degree at this University or at any other university.

I grant the University of Cape Town free licence to reproduce the above thesis in whole or in part, for the purpose of research.

I present this thesis for examination for the degree of PhD.

Signed

Signature   Date: February 12th 2016

Contents

Abstract

Background

Xpert MTB/RIF (Xpert) is a rapid, automated, onsite nucleic acid amplification test for tuberculosis (TB). It is effective for the diagnosis of pulmonary TB but there is limited evidence for its usefulness in extrapulmonary TB, particularly musculoskeletal TB.

Aims and hypothesis

The aim of this thesis was to investigate the diagnostic accuracy of Xpert for musculoskeletal TB and for rifampicin resistance against a gold standard of culture or histology.   Site of disease, HIV status, and age of patients, and accuracy in spinal compared to extraspinal TB were investigated as secondary objectives.

The overarching hypothesis was that Xpert is more accurate and would provide results faster than the gold standard for musculoskeletal TB, and that it would have a higher yield in HIV infected patients, adult patients, and patients with spinal disease.

Methods

Prospective studies of patients with suspected musculoskeletal TB, at the tertiary care hospitals Groote Schuur and Red Cross Children's Hospital in Cape Town, South Africa, were undertaken from June 2013 to March 2015. The diagnostic accuracy of Xpert was compared to culture or histopathology.

Findings

206 biopsies of 201 patients older than 13 years of age (23% HIV positive) were analysed.

The sensitivity and specificity of Xpert was 92.3% and 99.1% respectively. Xpert detected 8

cases more than culture (p = 0.069) and positive results were available 17 days earlier (<0.001). The sensitivity of Xpert in HIV positive patients was 96.9% (31/32) versus 89.6% (43/48) in HIV negative patients (p=0.225). The sensitivity of Xpert for spinal biopsies was 93.8% (95% CI 86.0-97.9) with specificity of 97.6% (95% CI 87.4 – 99.9), compared to extra-spinal biopsies with a sensitivity of 81.8% (95% CI 48.2 – 99.7, p=0.164) and specificity of 100% (95% CI 95.1 – 100%, p=0.186).

109 osteoarticular samples of children 12 years of age or younger, with a median age of 5.6 years (IQR 2.2 – 8.7) were analysed. Xpert provided a sensitivity of 73.9% (95% CI 51.6-89.8) with a specificity of 100% (95% CI 95.7 - 100) and was available at a mean of 0.8 days (0.46-1.4) compared to 21 days (19 – 30) for culture (p <0.001).

All rifampicin resistant cases were correctly diagnosed. A trend towards higher sensitivity in spinal tissue as well as HIV infected patients was observed. This study also provides evidence that Xpert has a lower sensitivity in children than in adults, yet, still detects more cases of paediatric musculoskeletal TB and is faster than culture. Histology was a useful test for the diagnosis of musculoskeletal TB, especially in children, and should be used alongside Xpert to provide the highest yield possible to detect TB.

Conclusion:

These first large studies on the accuracy of Xpert for musculoskeletal TB provide evidence for the usefulness of Xpert in the diagnosis of spinal TB, extraspinal TB, in HIV positive patients, and in childhood musculoskeletal TB. Based on these results, Xpert should be recommended as the initial test for diagnosis as it is more sensitive and faster than the gold standard of liquid culture.

Acknowledgments

Projects in Proteomics, Immunohistology and Genetics have been ignited from our meetings. **Prof Mayosi** from the Department of Medicine and Dean of the Medical Faculty at the University of Cape Town (as of 1st of September 2016) has given direction and advice beyond this thesis and has invested hours in leading me to decisions which will influence my career in a positive way. From these discussions sparked the establishment of our own Orthopaedic Research Unit with a special research group for musculoskeletal tuberculosis. The advice to become an IMPI ('worrier') has made me understand that one clear research question will create a simple but powerful answer, which can overcome major challenges we face and spark new questions along the way.

This was an extremely selfish project and anything I gained will never make up for the time lost with my family. The support received from my wife and family was the true sacrifice of this endeavour.

Abbreviations and Style

Style and format

Parts of the introduction, methods and conclusion of this thesis have been taken from the included manuscripts and publications with permission of the doctoral degree board. For the first publication we used the name 'GeneXpert' to describe the index test, thereafter 'Xpert' or 'Xpert MTB/RIF'; all names represent the same test. To increase the consistency in the terminology with have changed GeneXpert to Xpert MTB/RIF in this thesis wherever possible. The included manuscripts are displayed as submitted for publication and their references have been combined at the end of this thesis with the references of other chapters to ensure a uniform style (Vancouver referencing). Tables have been incorporated in the same style as for the publication to ensure the same organized display of the results.

Abbreviations

AIDS	Acquired immune deficiency syndrome
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
ESR	Erythrocyte Sedimentation Rate
GeneXpert	Xpert MTB/RIF, Xpert
HIV	Human Immunodeficiency Virus
LPA	Line Probe Assay
MDR	Multi-drug resistance
N	Number
PCR	Polymerase Chain Reaction
PTB	Pulmonary TB
TB	Tuberculosis
WHO	World Health Organization
Xpert	Xpert MTB/RIF, GeneXpert

Chapter 1

Introduction

This chapter has the following contents:

1. Context and literature review

    1.1. Review of the epidemiology of musculoskeletal TB

    1.2. Diagnostic tests for musculoskeletal TB

    1.3. Xpert MTB/RIF assay and its diagnostic accuracy for musculoskeletal TB

    1.4. Reporting on diagnostic accuracy

2. Summary and problem statement

3. Aims

4. Hypotheses

1.   Context and Literature review

1.1.   Epidemiology of musculoskeletal tuberculosis

Incidence of TB

Worldwide, 9 million new TB cases with 3.5% multidrug resistant TB (MDR-TB) and 1.5 million TB related deaths are reported annually. Of the new TB cases 25% are reported in Africa and more than 500 cases per 100.000 inhabitants in South Africa. Extrapulmonary disease accounts for 10% to 42% of TB cases, of which around 10-25% have musculoskeletal TB (Figure 1). [1-3]

In the Western Cape, the incidence of pulmonary TB is even higher with 1400 per 100 000

inhabitants.[4]
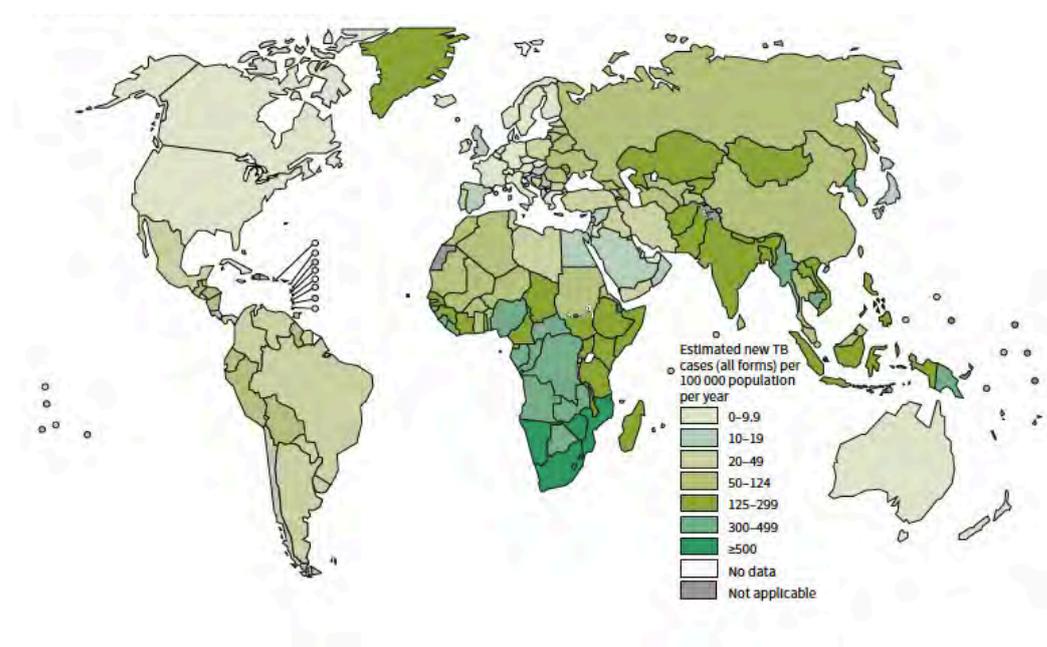
Figure 1. Estimated TB incidence rates in 2013.[2]

Epidemiology of extrapulmonary TB

In the 1960s extrapulmonary TB (EPTB) comprised 7.6% of all TB cases in developed

countries. This has since increased to 20–40 % as reported in recent studies[5-7] with about

20% in children. Although EPTB is usually not infectious it can cause death if undiagnosed or

untreated.[8, 9] Transmission risk of patients with EPTB is given when concurrent pulmonary TB

is present, which is the case in between 7% and 20% of EPTB.[7, 10, 11]

Epidemiologic studies in industrialized countries as well as resource-constrained areas found

that young patients, females, and persons of African or Asian origin had a higher risk for

EPTB.[10-12] HIV co-infection led to an increased rate of EPTB and in such cases also to a higher

mortality. [13, 14]

Epidemiology of musculoskeletal TB

Extrapulmonary TB accounts for 10% to 42% of TB cases, of which around 10-25% have

musculoskeletal TB. [1-3] The estimated global prevalence of joint and bone TB is

approximately 19 million to 38 million cases. [15] The most affected site of infection is the spine (50-69%), followed by hip, knee, and ankle/foot (10-13% each).[12]

Musculoskeletal TB in children

Childhood TB is estimated to account for 15-20% (pulmonary and extrapulmonary TB) of the total caseload in African countries[2]; 3% of these have musculoskeletal TB, but epidemiological data regarding musculoskeletal TB in children is very rare.[16, 17] At Red Cross Children's Hospital in Cape Town, the largest children's hospital in sub-Saharan Africa, approximately 20 children under the age of 12 years are treated for musculoskeletal TB each year (60% spinal TB, 20% in knee, 16% in hip and less then 5% TB of the ankle or upper limb).[18]

HIV/TB co-infection and multidrug resistant (MDR) TB

An estimated 1.1 million (13%) of the 9 million people who developed TB in 2013 were HIV-positive.[2] The prevalence of HIV co-infection among TB patients is highest in the African region.[2] Among the high TB/HIV burden countries, this ranged from 7% in Mali to 74% in Lesotho and Swaziland (Figure 2).[2]
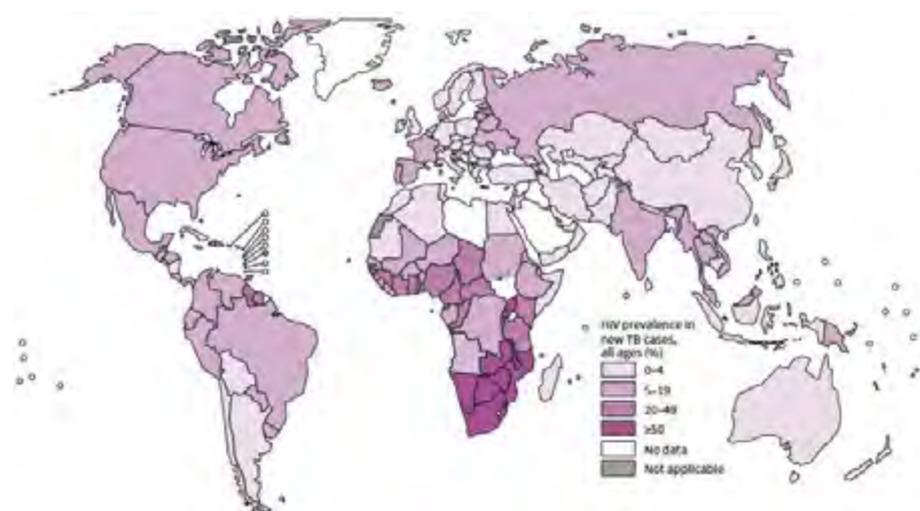
Figure 2. Estimated HIV prevalence in new and relapse TB cases in 2013.[2]

Similar to HIV/AIDS, MDR-TB remains an unsolved problem as the WHO estimates 3.5% of new cases and 20.5% of previously treated cases to be multidrug resistant. Although South Africa has one of the world's highest TB and MDR-TB rates[1], the epidemiology for EPTB and especially musculoskeletal TB remains largely unknown. We estimate that MDR-TB has a prevalence of 5% in musculoskeletal TB in our population[19] making a correct diagnosis on tissue biopsy imperative to identify resistance and initiate optimal treatment.

1.2. Diagnostic tests for musculoskeletal TB

Conventional Tests

For musculoskeletal TB, clinical and radiological diagnosis has poor accuracy, as it is a great mimicker of various other skeletal conditions.[20] But accurate and timely diagnosis is essential to prevent joint destruction, growth arrest, and contractures in large joints as well as deformity with neurological compromise in spinal disease, which may lead to lifelong morbidity and disability.[21-25] The current gold standard to test for osteoarticular TB is mycobacterial culture and histology.[26] For pulmonary samples, a meta-analysis reported a time to detection of 22 (smear positive) to 34 (smear negative) days in Loewenstein-Jensen medium and 12 (smear positive) to 18 days (smear negative) in radiometric Bactec 460 medium. The sensitivity and specificity in detecting pulmonary TB with BACTEC 460 systems is 87.7 and 89.7%, respectively.[27] However, joint biopsy samples usually contain only a small number of bacteria, making culture and staining for acid-fast bacilli challenging.[15, 28] The sensitivity of TB culture (Loewenstein-Jensen medium) compared to a composite reference standard for childhood musculoskeletal TB is approximately 73% (70% in hips, 71% in knees, 70% in ankles, 75% in feet and ankles).[18, 24, 29, 30] Similar sensitivities are reported for musculoskeletal TB in adults[31] with up to 90% for spinal TB.[32] These conventional tests require experienced microbiologists and extensive laboratory setup and their shortcomings demand a new, fast and accurate test, which is readily available and affordable.

Non-automated Nucleic Acid Amplification Tests (NAAT) in musculoskeletal TB

Most nucleic acid amplification tests for TB use polymerase chain reaction (PCR). This method uses high temperature to split double stranded DNA into single strands. DNA polymerase is then used to synthesize a complementary DNA strand and form double stranded DNA again with added oligonucleotides. This process can be repeated multiple times to amplify specific DNA sequences. For musculoskeletal TB, various studies have reported on the sensitivity of non-automated NAATs ranging from 61% to 83% in extra-spinal osteoarticular samples[33,34,35] and 94% in spinal samples.[36] One study also reported a higher sensitivity in spinal biopsies (90%) compared to extraspinal biopsies (63%) with culture as a reference standard.[37] In Children, non-automated NAATs of synovial joint biopsies have a reported sensitivity of only 40% when compared to culture or histology.[23]

1.3.   Xpert MTB/RIF (Xpert)

The Foundation for Innovative New Diagnostics (FIND) has collaborated with an industry partner (Cepheid, Sunnyvale, CA) to develop Xpert® MTB/RIF. Further funding was provided by the US National Institutes of Health. The laboratory of Professor David Alland at the University of Medicine and Dentistry in New Jersey provided technical support. Xpert MTB/RIF (Xpert) carries a registered trademark and is manufactured by Cepheid.

The Xpert assay is a cartridge based, automated, in vitro form of a NAAT (Figure 3). The system uses single-use cartridges to eliminate cross-contamination between samples. Its primers amplify part of the rpoB gene containing an 81 base pair core region. Mutations in this region, which have previously been isolated and conserved, are associated with rifampicin resistance and can be detected by means of five molecular beacons (A-E) each labelled with a different fluorophore.

Being automatic, It is therefore not only very fast but also obviates the need for a labour- and cost-intensive laboratory set-up necessary for non-automated NAATs. It is therefore an attractive test for the resource constrained environments of countries with a high TB burden.[38]
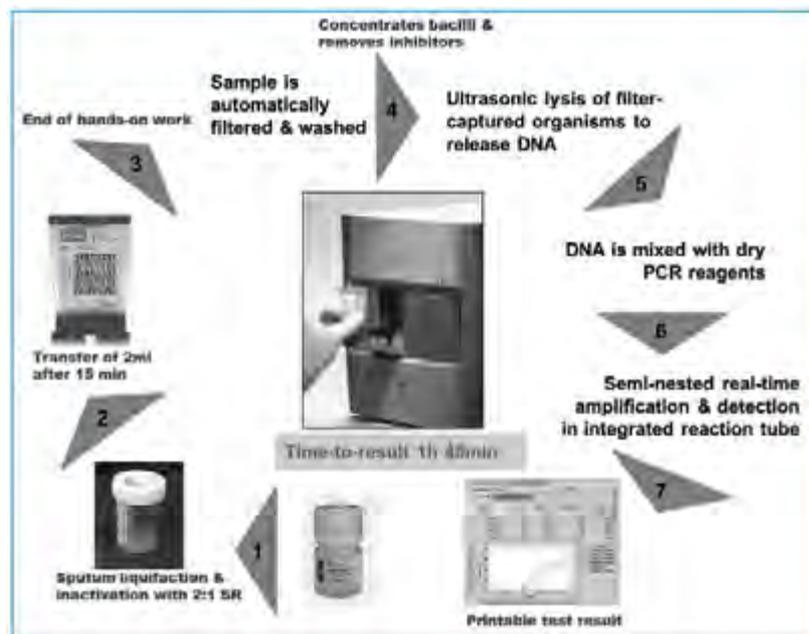
Figure 3. Steps using the Xpert assay.[39]

Advantages and Disadvantages of Xpert

Xpert has the advantage of requiring much smaller quantities of M. tuberculosis to demonstrate infection. Its detection limit of 130 mycobacteria per millilitre in various clinical samples increases the diagnostic predictability in extra-pulmonary tuberculosis even with samples with only a few bacteria present. [40,41] A disadvantage of Xpert is that it will lead to positive results even if the pathogens are non-viable and so remains positive for long periods after treatment.[42] In these cases active TB will have to be confirmed clinically and by means of various imaging modalities. The automated sample processing in Xpert has a step which removes DNA of non-intact cells to limit false positivity. Yet, in one of 7 patients with previous TB retreated for new onset pulmonary TB, Xpert is found to be false positive.[43] This

is a higher false positive rate than for new cases. Fewer years since TB treatment completion and a negative chest X-Ray are clinical features associated with false positivity. [43]

Costs for Xpert MTB/RIF

The concessional price for a four-module Xpert instrument is approximately 17 000 US dollars, with a cartridge price of 9.98 US dollars). The cartridge price for the private market varies widely between 40 and 155 US dollars.[44] Xpert also generates hidden costs as it needs a stable electricity supply, constant temperature and needs to be calibrated yearly. In comparison, the automated BACTEC MGIT 960 by Diagnostic Systems, Sparts MD (Franklin, Lakes, NJ, USA) with an annual capacity of 8300 tubes is 38 950 US Dollars. A fee of 5 000 US Dollars will cover 3 years of Instrument service. A test tube is 1.95 US Dollars. These prices have been negotiated for South African public sector hospitals by the Foundation for Innovative New Diagnostics (FIND). Additional costs which need to be considered are infrastructure, human resources, as well as delivery costs and pathways for reporting. Assessing the costs to prevent each disability-associated life-year (defined as number of years lost due to a disease, disability or early death), modelling studies have found that in countries of high disease burden, Xpert is cost effective, either in addition to or as a replacement test of conventional testing methods. [45-49] A high rate of empirical TB treatment practiced in low and middle income countries with a high disease burden could undermine this estimated effect of Xpert.[50-52] In a recent study cost per screened subject was 13.09 US Dollars and the cost per notified TB case was 90.7 US Dollars when using Xpert. [53] This cost could be reduced with automated chest radiography analysing for TB via computer software and screening cases prior to Xpert testing.

Accuracy of Xpert in pulmonary TB of adults and children

Xpert has been validated for pulmonary TB in adults and in children but not for musculoskeletal TB. For adult pulmonary TB, Xpert MTB/RIF (Xpert) has shown to have the

potential to become a faster alternative to the current reference standard, which is sputum microscopy and culture.[42, 54, 55] In a recent meta-analysis it had a sensitivity of 90%, and an accuracy to detect rifampicin resistance of 94% for pulmonary TB.[56] Another meta-analysis of studies on the diagnostic accuracy of Xpert in adults showed, that Xpert is more sensitive and specific compared with microscopy and culture).[57] When comparing respiratory samples of HIV-negative to HIV-positive patients a slight increase in sensitivity has also been shown for HIV-infected cases [86% (95% CI 76-92%) compared to 79% (95% CI 70-86%) HIV-uninfected]. For childhood PTB at Red Cross Children's Hospital, the sensitivity of Xpert on repeated induced sputum specimens compared to liquid culture is approximately 70%[58] and in a recent meta-analysis[59] the pooled sensitivity of a single Xpert was 62%. Another meta-analysis[60] reported that Xpert had moderate sensitivity and high specificity of 65% (95% CI: 61 - 69%) and 99% (95% CI: 98 - 99%), respectively for childhood pulmonary TB. Sensitivity and specificity for paediatric rifampicin resistance were 94.0% (95% CI: 80.0 - 93.0%) and 99.0% (95% CI: 95.0 - 98.0%), respectively.

Similar findings have recently been confirmed by a large multicentre trial[61] reporting a sensitivity of 68% (95% CI, 50%-82%) and specificity of 100% (95% CI, 97%-100%) in children with suspected pulmonary TB when compared with culture. Xpert detected 1.7 times more culture-confirmed cases than smear microscopy with a similar time to detection. Another recent multicentre trial assessed Xpert in alternative specimens in HIV-Infected children.[62] Sensitivities in all, standard, and alternative samples were 79.3% (95% CI 60.3-92.0), 72.4% (95% CI, 52.8-87.3), and 75.9% (95% CI, 56.5-89.7), respectively. Specificities were ≥97.5%. Xpert combined on nasopharyngeal aspirate and stool had sensitivities of 75.9% (95% CI, 56.5-89.7) and 75.0% (95% CI, 47.6-92.7), respectively.

Accuracy of Xpert in extrapulmonary tissue samples

The number of bacteria in extrapulmonary specimens is often lower than in pulmonary specimens, influencing the detection rate and making diagnosis more challenging. There are

few diagnostic accuracy studies of extrapulmonary samples since extrapulmonary TB is less common and invasive procedures have to be performed to obtain tissue. In one meta-analysis for adult tissue samples of various origins, the sensitivity varied widely between 42% and 100%.[39] Another meta-analysis on studies reporting diagnostic accuracy of Xpert in mostly extra-osseous extrapulmonary samples found a pooled sensitivity of 81.2% (95% CI, 67.7 – 89.0%) and specificity of 98.1% (95% CI, 87.0 – 99.8%).[63]

There are no studies on the diagnostic accuracy of Xpert of extrapulmonary TB in children.

Rifampicin resistance testing with Xpert

Rifampicin resistance occurs most commonly via mutation of the rpoB gene encoding a subunit of the bacterial RNA polymerase, which is the binding site for the antibiotic.[64] The Xpert does not detect resistance to any other antibiotics (multidrug resistance) used to treat TB and therefore culture is still recommended. A line probe assay (LPA), is a DNA strip test, such as GenoType® MTBDRplus or GenoType® Mycobacterium CM lineprobe assays (Hain Lifescience, Nehren, Germany). It is used to detect multidrug resistance. The WHO therefore recommends the use of molecular LPA for the rapid diagnosis of MDR-TB in high TB-burden, low-income settings.[64]

Current evidence supports the accuracy of Xpert to detect resistance to rifampicin. Its sensitivity and specificity in adults for pulmonary TB is high (95% and 98% respectively). [39] In a meta-analysis, data on resistance testing in tissue was available for 566 samples from 13 studies. Xpert missed 2 of 41 rifampicin resistant samples. The average prevalence of rifampicin resistance was 5.4%.[39]

Accuracy of Xpert in musculoskeletal TB

Data on Xpert of bone and joint tissue samples are very limited (Table 1). One study on 29 adults with spinal disease reported Xpert to have a sensitivity of 72% in HIV negative and 82% in HIV positive patients, but this was compared to a clinical reference standard.[65]

Another study[66] compared Xpert in 60 adult orthopaedic fluid samples with culture and found a sensitivity of 63.6% (14/22). Large studies of the accuracy of Xpert in children or adults are not available for extrapulmonary tissue samples, let alone musculoskeletal samples.

Study	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens RIF (%/N)	Reference Standard	Design	Age	N	Type
Monni 2012 [65]	72 (82 in HIV)	100	100	67	not done	Clinico-radiologic signs	prospective	adults	29	tissue
Gu 2015 [66]	63.6 (14/22)	not given	not given	not given	100 (6/6)	culture or histology	prospective	adults	60	pus

Table 1. Current evidence on diagnostic accuracy of Xpert in musculoskeletal samples.

Sens = Sensitivity, Spec = Specificity, PPV = positive predictive value, NPV = negative predictive value, Sens RIF = sensitivity of Xpert to detect Rifampcin resistance, N = number, Type = Type of specimen collected.

2. Summary and problem statement

There is a lack of data on an accurate, rapid diagnostic test in musculoskeletal TB. A new, rapid diagnostic method, the Xpert assay, is an automated sample preparation and real-time PCR instrument. For pulmonary TB, this test has shown to have high accuracy and perform faster than the current reference standard, which is sputum microscopy and culture. Currently no large studies have assessed the accuracy of Xpert in adult and childhood musculoskeletal TB or in HIV-infected compared to uninfected patients with musculoskeletal disease.

3. Aims

The overall aim was to assess the diagnostic accuracy of Xpert to detect musculoskeletal TB and rifampicin resistance in tissue of bone or joints. As the diagnostic yield of conventional

tests might be influenced by site of disease, HIV status and age of the patients, specific objectives included assessing the accuracy of Xpert in Children (0-12 years of age), HIV infected patients, as well as spinal and extraspinal TB.

4. Hypotheses

4.1 Xpert has been established as the first line test for pulmonary TB and has been proven to be faster and more accurate than culture. We therefore hypothesized, that for osteoarticular TB, Xpert will outperform the current gold standard (culture and histology) in accuracy and time to diagnosis and will have potential to become the first line test.

4.2 Xpert is accurate for detection of rifampicin resistance in pulmonary and extrapulmonary TB. We therefore hypothesized it would detect rifampicin resistance in musculoskeletal specimens with high accuracy.

4.3 Affected tissue in children and extraspinal TB contain paucibacillary disease compared to that in adults or spinal TB. We therefore hypothesized that Xpert would be more accurate in adults than in children, as well as in spinal tuberculosis compared to extraspinal tuberculosis.

4.4 Xpert has a higher accuracy in pulmonary samples of HIV-infected patients compared to HIV negative patients. We therefore hypothesized that Xpert would have a higher accuracy in HIV-infected patients with musculoskeletal TB compared to HIV negative patients.

Chapter 2

Study outline and methodology

Study Design

This was a prospective study on the diagnostic accuracy of Xpert® MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, CA) in patients with suspected musculoskeletal TB in a TB and HIV endemic setting. The diagnostic performance and time to availability of the results of the Xpert assay were compared to standard tissue microscopy and culture.

Recruitment

All patients presenting with features suspicious of musculoskeletal TB (Table 1) to Groote Schuur Hospital and Red Cross Children's Hospital in Cape Town from June 2013 to March 2015 were included. Biopsies were collected by a specialized orthopaedic service. Spinal TB was managed by a subspecialist spinal service. Paediatric patients were managed by a subspecialised paediatric orthopaedic team.

Inclusion Criteria

Patients were included in the study with the following criteria:

1. Patients presented with suspected musculoskeletal TB (Table 1).

2. A tissue biopsy was performed as part of the routine clinical workup

3. A valid Xpert and TB culture were obtained

4. Age of paediatric group: age 0-12 years, recruited at Red Cross Children's Hospital

5. Age of adult group: older than 12 years of age, recruited at Groote Schuur Hospital

Clinical Red Flags	Radiological Red Flags
• History of chronic pain for more than 3 months • Constitutional symptoms: low grade fever, night sweats, loss of appetite, weight loss • Chronic cough • Elevated ESR • History of Tuberculosis contact • Gibbus • Neurological deficit • Positive Mantoux test • Immune compromise/HIV	• Loss of anterior vertebral height • Paravertebral shadow on XRs • Shadow of a psoas abscess • Adjacent Vertebral endplate changes with preserved disc height • Changes on Chest X-Ray suspicious of TB • Paravertebral abscess on MRI

Table 1: Clinical and radiological red flags on which the suspected diagnosis of TB was made.

Exclusion Criteria

Patients were excluded if one of the following was present:

1. Invalid Xpert assay or TB culture

2. Tissue biopsy not indicated (prior diagnosis of TB)

3. No consent given.

Research Procedures

Index Test – Xpert MTB/RIF (Xpert)

Specimens were submitted in sterile saline in duplicate to the mycobacterial culture laboratory. The first specimen was processed for onsite Xpert® MTB/RIF testing (Cepheid, Sunnyvale, CA).

Xpert SR lysis buffer was added in a 1:3 ratio and the specimen was vortexed initially and again after 10 minutes. Two millilitres of the mixture were processed automatically according to Xpert protocols the result was read after approximately 90 min. A trained laboratory technician performed the test and was blinded to the results of the reference test.

Reference Standard

The reference standard was liquid culture or histology. The cultures were processed with the automated BACTEC MGIT 960 by Diagnostic Systems, Sparts MD (Franklin, Lakes, NJ, USA). A Ziehl-Nielson (ZN) stain and a haematoxylin and eosin (HE) stain were done in the microbiology and histopathology laboratory respectively.

Quantification of acid-fast bacilli was read according to the specifications from the Centres for Disease Control and Prevention. The isolate was assessed for multidrug resistant TB (MDR-TB) using the GenoType® MTBDRplus or GenoType® Mycobacterium CM lineprobe assays (Hain Lifescience, Nehren, Germany). The accuracy of Xpert to detect rifampicin resistance was assessed. "MDR-TB" was defined as resistance to isoniazid and rifampicin, with or without resistance to other first-line drugs.[2]

A trained pathologist experienced in diagnosing TB reviewed the histopathology slides. Histological features of TB were caseous necrosis, epitheloid cell granulomas or Langhans giant cells. Clinical data, imaging, as well as the Xpert test results were available to the pathologist.

Sample collection

The sample collection for the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA), culture and histopathology was done as part of the routine clinical management and according to local guidelines for the management of patients with suspected musculoskeletal TB. There was no additional surgery or surgical insult to the patient to obtain tissue or perform research related biopsies. Tissue was taken from the predetermined area of disease and then divided into equal parts to be processed for the index and reference tests. If indicated clinically, repeat biopsies were performed on the basis of an unclear diagnosis or as part of a protocol of multiple washouts to control sepsis. Musculoskeletal tissue samples were collected surgically in an operating room under sterile conditions from radiographically predetermined areas of disease. Tissue samples were taken from synovium in articular

biopsies and bone in extra-articular biopsies. Spinal tissue biopsies were performed by a subspecialised spine team. Extraspinal lesions were biopsied by a specialised orthopaedic team. Paediatric patients were managed and biopsied by a subspecialist paediatric orthopaedic team. All extraspinal biopsies were carried out as open procedure targeting the area of suspected disease. For spinal TB, percutaneous biopsies were carried out by a transpedicular approach in the thoracic and a paraspinal approach in the lumbar spine. An open biopsy was performed when debridement of granulomata and drainage of paravertebral abscesses or stabilization of the spinal column was found to be necessary. Indications included instability of the spinal column, acute deterioration of neurology, a large paravertebral abscess, and airway compromise in lesions of the cervical spine. Abscesses were drained through a costotransversectomy in the thoracic spine and through an anterolateral retroperitoneal approach in the lumbar spine. A thoracotomy was carried out when stabilization of the anterior column of the thoracic spine was necessary. The Smith-Robinson approach was used for lesions of the cervical spine.

Biopsy material included pus swabs and/or tissue biopsies from diseased bone or soft tissue. All samples were subjected to Xpert assay and histopathological examination as well as microbiological testing.

Definitions

For the first publication on spinal TB (chapter 3), 'definite TB' was defined as a positive *Mycobacterium Tuberculosis* culture. In patients with negative cultures, 'probable TB' was assumed if histology along with clinical and radiological findings (Table 1) were suggestive of TB and if patients improved on TB therapy. 'NOT TB' was assumed in cases who tested negative for culture and histology and who improved without TB treatment.

For the publications in chapter 4 and 5 "confirmed TB" was defined as a positive *M tuberculosis* culture or positive histology. A negative culture and histology with improvement of symptoms without TB treatment after at least a month of follow up was

considered 'NOT TB'. Improvement was assessed by means of signs of sclerosis on X-Ray, clinical improvement such as weight gain and diminished pain as well as decreasing Erythrocyte Sedimentation Rate (ESR). The diagnostic accuracy of Xpert was compared to liquid culture or histology (confirmed TB or probable TB).

Medical treatment was provided for confirmed or probable TB cases according to the protocol of the local infectious disease guidelines and was given for 9 months. Patients underwent follow-up evaluations every 3 months.

Statistical Analysis

The sensitivity, specificity and predictive values of Xpert with 95% confidence intervals (95% CI) were calculated using TB culture or histology as reference standard.

The data was analysed as per sample using STATA 13 statistical software (STATA Corporation, College Station, TX USA). Descriptive statistics were used to characterise the study population, normally distributed continuous data were summarized by mean and 95% confidence interval, non-normally distributed continuous data by median and interquartile range. Categorical data were summarized as proportions with 95% confidence intervals. Statistical tests included two-sample test of proportions, chi squared test, Kruskal Wallis test and Wilcoxon rank-sum test. All statistical tests were two-sided at $\alpha = 0.05$.

Ethics

The procedure was explained to patients and / or caregivers as well as its risks and benefits. A patient or caregiver information sheet was provided. Informed written consent was taken in all cases undergoing surgery. The study was approved by the human research ethics committee of the Faculty of Health Science, University of Cape Town (Appendix: HREC REF 206/2013).

Description of risks and benefits

Patients received usual treatment according to local and South African guidelines, which

conform to internationally accepted standards of care for musculoskeletal TB.

Data safety and Reimbursement

All patients' names and folder numbers were removed from the data stream. This study adhered to the Declaration of Helsinki 2013. There was no reimbursement.

Reporting

As part of the EQUATOR toolkit for authors, the Standards for the Reporting of Diagnostic accuracy studies (STARD) were used to give a structured guidance for the design and reporting of this diagnostic accuracy study.[67]

Chapter 3

Xpert MTB/RIF for spinal Tuberculosis - An accurate and rapid diagnostic test.

M Held[1], M Laubscher[1], HJ Zar[2], R Dunn[1]

Affiliation:

[1]Orthopaedic Research Unit, Department of Orthopaedic Surgery, Groote Schuur Hospital

University of Cape Town

Address: H49 Old Main Building, Groote Schuur Hospital, Observatory 7925, South Africa

[2]Department of Pediatrics and Child Health, Red Cross War Memorial Children's Hospital

University of Cape Town and MRC Unit on Child & Adolescent Health, University of Cape

Town, South Africa. Address: Klipfontein Road, Rondebosch 7700, South Africa

Abstract

The lack of an accurate, rapid diagnostic test for M. tuberculosis (TB) is a major challenge in spinal TB. Xpert MTB/RIF (Xpert), a new, rapid molecular diagnostic test is recommended as the first line investigation for suspected pulmonary TB in areas of high HIV prevalence or drug resistance, yet it has not been validated for the diagnosis of musculoskeletal TB. The aim of this study was to assess the accuracy of Xpert for spinal TB. A prospective clinical study of 69 consecutive adults hospitalised with suspected spinal TB was conducted at a tertiary hospital in an area with one of the highest TB incidence and prevalence worldwide. The Xpert was performed on tissue samples of the enrolled patients and the diagnostic accuracy was compared to a reference standard of tissue in liquid culture. Seventy-one spine samples

from 69 patients (2 re-biopsies) were included in the study. The Xpert test showed a high sensitivity of 95.6 % and specificity of 96.2% in spinal TB. The test results were available on day 2 for the Xpert test, compared to day 35 for cultures. All multi- drug resistant TB (MDR-TB) cases were diagnosed accurately with the Xpert test. The MDR-TB rate was 5.8%.

Introduction

Accurate diagnosis of musculoskeletal tuberculosis (TB) is essential to enable timely and effective treatment. Rapid availability of test results and initiation of treatment is important to halt disease progression and prevent further morbidity. In spinal TB, diagnostic delay can lead to devastating neurological compromise and irreversible disability.

Xpert® MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, CA), an automated Polymerase Chain Reaction (PCR) diagnostic test that simultaneously detects TB and Rifampicin resistance, has recently become available. The World Health Organization (WHO) has endorsed this for use in patients with suspected pulmonary TB in areas of high HIV or drug resistance. For pulmonary TB, this test method has often been quoted with similar sensitivity and specificity to conventional testing methods such as TB culture.[1-4]

A recent meta-analysis of the Cochrane Infectious Diseases Group has analysed 18 studies with 7816 patients. The Xpert was carried out on sputum in suspected pulmonary TB and a sensitivity of approximately 90% with a false negative rate of 2% was reported. The sensitivity for rifampicin resistance was 94%.[5]

According to the WHO Global Tuberculosis Report 2013 the Xpert test will be made available universally in future with massive roll out programs by the WHO.[6] However, there is no data on its accuracy for diagnosis of musculoskeletal TB.

The aim of this study was to investigate the accuracy of the Xpert assay compared to the gold standard of culture and microscopy.

Methods

The methods are stated in chapter 2.

Results

Seventy-one spine samples from 69 patients (2 re-biopsies) were included in the study, 31 were males (44.9%). Their median age was 40 years (IQR 27 - 60). Twenty-two (31.9%) of the 69 patients were HIV positive, 37 patients were HIV negative (53.6%), in 10 patients HIV testing was not done.

Overall 36 (52.2%) patients were classified as definite TB (culture positive), 8 (11.6%) as probable TB (histology positive but culture negative) and 25 (36.2%) as not TB (culture and histology negative) (Table 2). A total of 44 patients had a positive Xpert result. Xpert was positive in 97.2% of the patients in the "definite TB" group, 100.0% (8) in the "probable TB" group and 4.0% (N=1) in the "not TB" group. (Table 2)

Table 3 shows the per sample analysis of all cases with either culture positive ('definite TB') or histology positive ('probable TB') samples, the sensitivity of Xpert PCR test was 95.6% (95% CI [84.9 – 99.5]), specificity 96.2% ([80.4 – 99.9]), positive predictive value 97.7% (95% CI [88.0 – 99.9]) and negative predictive value 92.6% (95% CI [75.7 – 99.1]). There were 2 false negative and 1 false positive Xpert sample in this analysis.

A secondary analysis was done to compare Xpert to only culture positive samples (definite TB) as the reference standard (Table 3). Culture detected 37 TB cases and histology detected a further 8 cases. With Xpert the yield of TB detection compared to cultures alone increased by (8 of 37 cases) 21.6%. This demonstrates that the 8 cases which where only detected on histology (probable TB) could likely be false negative TB cultures. The sensitivity of Xpert for this secondary analysis was 92.6%, detecting 25 of 27 cases (95% CI [75.7 – 98.9] with a specificity of 79.55% (35/44); 95% CI [64.7 – 90.2], a positive predictive

value of 73.5% (25/34); 95% CI [55.6- 87.1] and negative predictive value of 94.6% (35/37); 95% CI [81.8 – 99.2].

In the Xpert test, the results were available at a maximum of 2 days compared to a median of 35 (IQR [15-43]) days for culture.

In 4 patients rifampicin-resistant TB was evident on the Xpert test (5.8%) and possible multi-drug resistant TB (MDR-TB) was therefore suspected. All 4 patients could be started on the correct MDR-TB treatment regimen one day after the biopsy. In 3 of the 4 patients MDR-TB was confirmed with sensitivity testing for Rifampicin and Isoniazid once the culture was available. In one of the 4 Xpert samples with Rifampicin resistance, the TB culture was negative and therefore MDR-TB may have been missed without the Xpert test.

Conversely, in another Xpert test sample, Isoniazid mono-resistance was evident only on culture as the Xpert tests solely for Rifampicin resistance

Discussion

Clinical and radiological diagnosis of musculoskeletal TB notoriously has poor accuracy, as TB is a great mimicker of various other skeletal conditions.[8]   Often the TB culture is time consuming (on average 6-8 weeks) and one often relies on histological evidence. This requires experienced microbiologists and close communication with the treating physician but most importantly demands a test, which is accurate, readily available, rapid and cheap.

With the advent of PCR methods to test for TB, various studies have reported on the sensitivity of non-automated PCR tests ranging from 61% to 83%.[9-14] The PCR method has the advantage of requiring much smaller quantities of M. tuberculosis to demonstrate infections and leads to a rapid diagnosis through amplification of nucleic acid of the pathogen. Its detection limit of 130 colony-forming units (CFU) per millilitre (versus 10.000 CFU/ml in cultures) in various clinical samples increased the diagnostic predictability in extra-pulmonary tuberculosis even with samples with only a few bacteria present.[15,16]

In this study, the sensitivity of the Xpert test was 95.6% (95% CI [84.9 – 99.5]), the specificity was 96.2% ([80.4 – 99.9]), the positive predictive value was 97.7% (95% CI [88.0 – 99.9]) and negative predictive value was 92.6% (95% CI [75.7 – 99.1]).

Four MDR-TB, 5.8% of all patients, were accurately diagnosed with the Xpert test. In one of these patients culture results were false negative, reported at 43 days post biopsy. These patients would have been discharged into the community without adequate treatment for 43 days, and in one case without treatment at all, highlighting the benefit of the Xpert test.

Another major benefit of the Xpert test is its rapid time for test results to be available. In our study, In the Xpert test, the results were available at a maximum of 2 days compared to median of 35 (IQR [15-43]) days for culture. The automated process of the Xpert device takes only about 90 minutes to completion and most of the time lost was due to the infrastructure required to run the test. Compared to other PCR testing methods, the automated, cartridge based test obviates extensive laboratory support. This makes it a tool which can be moved closer to smaller hospitals and clinics cutting down long transport times and complex logistics often present in countries with a high TB burden.

One group has found no difference in time compared to conventional testing methods when the Xpert device was not onsite. Time delays in transport made it similar to their reference standard of cultures and histology and they found no added benefit.[17]

One of the disadvantages of PCR testing is that in contrast to TB culture, PCR testing will lead to positive results even if the pathogens are non-viable.[1] In these cases active TB will have to be confirmed clinically and by means of various imaging modalities.

Another concern for the Xpert method is that it tests drug resistance only for Rifampicin therefore a mono-resistance for Isoniazid can only be detected with another PCR testing method (Hain). In our series, one case of Isoniazid mono-resistance was discovered. Though, according to current treatment guidelines Isoniazid mono-resistance does not

require any change to the conventional TB regimen for patients without Isoniazid mono-resistance. Thus there was no clinical impact.[18]

Conclusion

The Xpert shows a high sensitivity of 95.6 % and specificity of 96.2% in spinal TB. The yield of TB detection with Xpert compared to cultures alone increased by 21.6%. The test results were available on day 2 for the Xpert test, compared to day 35 for cultures. All MDR-TB cases were diagnosed accurately with the Xpert test. The MDR-TB rate was 5.8%. The index test was therefore more sensitive and lead to faster access to test results than the gold standard.

Tables

Table 1. Clinical and radiological red flags on which the suspected diagnosis of TB was made.

Clinical Red Flags	Radiological Red Flags
• History of chronic pain for more than 3 months • Constitutional symptoms: low grade fever, night sweats, loss of appetite, weight loss • Chronic cough • Elevated ESR • History of Tuberculosis contact • Gibbus • Neurological deficit • Positive Mantoux test • Immune compromise/HIV	• Loss of anterior vertebral height • Paravertebral shadow on XRs • Shadow of a psoas abscess • Adjacent Vertebral endplate changes with preserved disc height • Changes on Chest X-Ray suspicious of TB • Paravertebral abscess on MRI

Table 2. Baseline characteristics of the patient cohort.

	Overall	Definite TB	Probable TB	Not TB	P value
N	69	36	8	26	0.28
HIV +	22 (31.9%)	13 (36.1%)	4 (50.0%)	5 (20.0%)	0.28
Sex (male)	31 (44.9%)	14 (38.9%)	6 (75.0%)	11 (44.0%)	0.18
Age -Median (IQR)	40 (27 - 60)	31 (27 - 46)	37.5 (23 - 57.5%)	51 (40 - 63)	0.06
Xpert +	44/69 (63.8%)	35/36 (97.2%)	8/8 (100.0%)	1/25 (4.0%)	

Table 3. Per sample accuracy of Xpert MTB/RIF for detecting TB. PPV: positive predictive value. NPV: negative predictive value.

Reference Standard	All (N)	Sensitivity % (N)	Specificity % (N)	PPV % (N)	NPV % (N)
TB Culture **or** histology positive	71	95.6% (44/46)	96.2% (25/26)	97.7%(44/45)	92.6% (25/27)
TB Culture positive **only**	71	92.6% (25/27)	79.5% (35/44)	73.5% (25/34)	94.6% (35/37)

Chapter 4

Diagnostic accuracy of Xpert MTB/RIF in HIV positive and HIV negative patients with musculoskeletal tuberculosis

This has been submitted to the Bone & Joint Journal (BJJ), formerly known as The Journal of Bone & Joint Surgery (British Volume), published by The British Editorial Society of Bone & Joint Surgery.

M Held[1], M Laubscher[1], L Workman[2], HJ Zar[2], R Dunn[1]

Affiliation:

[1]Orthopaedic Research Unit, Department of Orthopaedic Surgery, Groote Schuur Hospital

University of Cape Town

Address: H49 Old Main Building, Groote Schuur Hospital, Observatory 7925, South Africa

[2]Department of Pediatrics and Child Health, Red Cross War Memorial Children's Hospital

University of Cape Town and MRC Unit on Child & Adolescent Health, University of Cape Town, South Africa

Address: Klipfontein Road, Rondebosch 7700, South Africa

Abstract

Aim

We aimed to investigate the diagnostic accuracy of Xpert MTB/RIF (Xpert) in musculoskeletal TB of HIV positive and negative patients.

Methods

A prospective study of patients older than 13 years of age with suspected TB of bone and joints, who presented to a large tertiary care hospital in South Africa was undertaken from June 2013 to March 2015. The diagnostic accuracy of Xpert was compared to culture or histopathology as the gold standard.

Results

206 biopsies of 201 patients (23% HIV positive) were evaluated. The sensitivity and specificity of Xpert was 92.3% and 99.1% respectively. Xpert detected 8 cases more than culture (p = 0.069) and positive results were available 17 days earlier (<0.001). Xpert detected all four multidrug resistant TB (MDR-TB) cases and an additional two rifampicin resistant cases in culture negative samples. The sensitivity of Xpert in HIV positive patients was 96.9% (31/32) versus 89.6% (43/48) in HIV negative patients (p=0.225).

Conclusion

Xpert is an accurate test for the diagnosis of musculoskeletal TB in HIV positive and HIV negative patients. A positive Xpert result should be regarded as microbiologic confirmation of osteoarticular TB.

Introduction

Musculoskeletal TB occurred in approximately 3% of the estimated 8.6 million people who developed tuberculosis (TB) in 2012.[2] Amongst these, spinal TB is the most common orthopaedic manifestation, and may lead to neurological deficits in 23-76% of cases. [21] Timely and accurate diagnosis of musculoskeletal TB with initiation of appropriate therapy is crucial to prevent such associated morbidity.

In a recent meta-analysis, Xpert® MTB/RIF (Cepheid, Sunnyvale, CA) was reported to have a sensitivity of 90% for *M tuberculosis* and of 94% for rifampicin resistance in pulmonary TB in adults.[56] In a second meta-analysis of the accuracy of Xpert on tissue samples (other than lymph nodes) using culture as a reference standard[39] the reported pooled sensitivity was 81.2% (95% CI, 67.7 – 89.0%) and specificity was 98.1% (95% CI, 87.0 – 99.8%). However, there are no large published studies on the accuracy of Xpert for musculoskeletal disease in either HIV-infected or uninfected adults. Non-automated nucleic acid amplification assays have reported sensitivities of 61% - 83% for musculoskeletal TB.[37, 68-71] A few small studies have investigated Xpert for musculoskeletal disease including a small pilot study we undertook,[19] a case report[72] and a study of 29 spinal tissue samples.[65] In this study the reference standard was based on clinical and radiological findings. Xpert had a sensitivity of 72% in HIV negative and 82% in HIV positive patients. However, Xpert was not done onsite and results were only available at approximately six days, compared to 27 days for culture.

This study aimed to assess the accuracy of Xpert, for musculoskeletal TB in HIV infected and uninfected adults. We also compared the time to availability of results for Xpert to that of culture and differences in accuracy in spinal and extra-spinal samples.

Methods

A prospective study of consecutive patients older then 13 years of age with clinico-radiological features of musculoskeletal TB was done. All patients underwent a

musculoskeletal tissue biopsy for suspected TB from June 2013 to March 2015 as part of the routine clinical workup. Included were patients who presented to our institution, a large a tertiary care hospital in South Africa, with symptoms suspicious of TB and the fact that the patients had received the Xpert assay. Excluded were samples in which Xpert or TB cultures were faulty, insufficient or contaminated.

Clinical features and red flags for suspected TB were defined as the presence of constitutional symptoms, chronic cough, elevated ESR, history of TB contact, spinal gibbus, neurological deficit in suspected spinal TB, history of pain for more than 3 months, and immune compromise or HIV. Radiological features for suspected TB were loss of anterior vertebral height, paravertebral shadow on radiographs, shadow of a psoas abscess, adjacent vertebral endplate changes with preserved disc height, changes on chest radiographs suspicious of TB and paravertebral abscess formation on MRI. Biopsies were performed by a specialized orthopaedic service at our hospital.

Musculoskeletal tissue samples were collected surgically in an operating room under sterile conditions from radiographically predetermined areas of disease. Tissue samples were taken from synovium in articular biopsies and bone in extra-articular biopsies. Spinal tissue biopsies were performed by a subspecialist surgical spinal team. Extraspinal lesions were biopsied by a specialised orthopaedic team. Extraspinal biopsies were done by open approach and dissection to the area of suspected disease. Indications for open spinal biopsies included instability, deterioration of neurology, a large abscess or airway compromise.

Specimens were submitted in sterile saline in duplicate to the microbiology laboratory to be processed simultaneously. The first specimen was processed for Xpert® MTB/RIF (Xpert) testing (Cepheid, Sunnyvale, CA). Xpert SR lysis buffer was added in a 1:3 ratio and the specimen was vortexed initially and again after ten minutes. Two millilitres of the mixture were processed automatically and the result was read after approximately 90 minutes. A

trained laboratory technician blinded to the results of the reference test performed Xpert testing.

The reference standard was liquid culture or histology. Culture was done on the second specimen using the automated liquid culture BACTEC MGIT system (960 by Diagnostic Systems, Sparts MD (Franklin, Lakes, NJ, USA). A Ziehl-Nielson (ZN) stain and a haematoxylin and eosin (HE) stain were done in the microbiology and histopathology laboratory respectively.

Quantification of acid-fast bacilli (AFB) was according to the specifications from the Centres for Disease Control and Prevention. "MDR-TB" was defined as resistance to isoniazid and rifampicin. The isolate was assessed for multidrug resistant TB (MDR-TB) using the GenoType® MTBDRplus or GenoType® Mycobacterium CM lineprobe assays (Hain Lifescience, Nehren, Germany). Lineprobe assays are PCR tests, which can detect TB and identify rifampicin resistant strains by detecting mutations of the rpoB gene. Mutations in the the *katG* gene or inhA gene can be detected and are used for testing isoniazid resistance. Lineprobe assays need at least regional level laboratories due to their complexity and bio safety requirements.

A trained pathologist experienced in diagnosing TB reviewed the histopathology slides. Clinical data, imaging, as well as the Xpert test results were available to the pathologist. Histological criteria for TB were caseous necrosis, epitheloid cell granulomas or Langerhans giant cells.

"Confirmed TB" was defined as a positive *M tuberculosis* culture or positive histology. A case was considered to be "NOT TB" if culture and histology were negative and where there was improvement on follow-up without TB treatment. The diagnostic accuracy of Xpert (Cepheid, Sunnyvale, CA, USA) was compared to liquid culture or histology.

The study was approved by the human research ethics committee of the Faculty of Health Sciences, University of Cape Town and was performed in accordance with the Helsinki

Declaration (1964, amended in 2008) of the World Medical Association (See Appendix). Written consent was obtained in all patients.

Statistical Analysis

The sensitivity, specificity and predictive values of Xpert with 95% confidence intervals (95% CI) were calculated using TB culture or histology as the reference standard. Data were analysed as per sample using STATA 13 statistical software (STATA Corporation, College Station, TX USA). Descriptive statistics were used to characterise the study population, normally distributed continuous data were summarized by mean and 95% confidence intervals and non-normally distributed continuous data by median and interquartile range. Categorical data were summarized as proportions with 95% confidence intervals. Statistical tests included two-sample test of proportions, chi squared test, Kruskal Wallis test and Wilcoxon rank-sum test. All statistical tests were two-sided at $\alpha = 0.05$.

Results

207 samples were collected from 202 patients with suspected musculoskeletal TB; 5 patients had repeat biopsies. One sample was excluded as the culture sample was sent in formalin and had to be discarded, 206 samples met the inclusion criteria. The biopsy sites are shown in Table 1; 122 of 206 biopsies (59.2%) were done for spinal TB. The remaining biopsies were for suspected extra-spinal TB of joint or bone. 196 (95.1%) were tissue samples and ten (4.8%) were pus samples. The median age of the patients was 40 years (IQR 27-54), 97 were male (48.3%). In the per sample analysis 76 (38.5%) were culture positive (definite TB) of which Xpert was positive in 85 samples (41.3%) (Table 2).

The sensitivity for Xpert in musculoskeletal samples was 92.3% (95% CI 84.8 – 96.9) with specificity of 99.1% (95% CI 95.2 – 99.8) (Table 2).

For culture confirmed TB only, the sensitivity of Xpert was 90.8% (95% CI 81.9 – 96.2, P=0.724) with specificity of 87.7% (95% CI 80.8 – 92.8, P<0·001). All except one of the culture negative but Xpert positive samples showed features of TB on histology.

In 203 of 206 samples (98.5%) a Ziehl-Nielson Stain was available for quantification of Acid Fast Bacilli (AFB), 32 (15.8%) were positive, and 171 (84.2%) were negative. The sensitivity when compared to our gold standard was 33.7% (95% CI 24.2% - 44.3%) with a specificity of 99.1% (95% CI 95.1% - 99.9%) (Table 2).

Xpert was positive at a median (IQR) of 1 day (1 – 1) compared to 18 days (12 – 26) for culture, p <0.001. Xpert detected more cases than culture (84/91; 92.3% compared to 76/91; 83.5% on culture p = 0.069).

Drug resistant TB: All four MDR-TB cases detected with the lineprobe assay were also correctly identified with Xpert. Xpert detected an additional two patients with rifampicin resistance, in which culture was negative (Table 3). Therefore 6 of 90 patients (6.7%) with TB disease had rifampicin resistance. In one case isoniazid (INH) mono-resistance was found on line probe assay.

Accuracy in samples of HIV infected and uninfected patients: Forty-six patients were HIV infected (22.9%), 102 patients were HIV uninfected (50.7%) and 53 (26.4%) were of unknown HIV status. Amongst HIV infected patients, 32 of 46 (69.5%) had TB compared to 47 of 102 HIV negative patients (46.1%) and 11 of the 55 patients with unknown HIV status (20.7%); p <0.001 (Table 1). The sensitivity of Xpert was 96.9% (95% CI 83.8-99.9) in HIV positive compared to 89.6% (95% CI 77.3-96.5, p=0.225) HIV negative patients. The specificity was 100% (95% CI 78.5-100.0) for HIV positive compared to 98.3% (95% CI 90.8-99.9, p=0.621) in HIV negative individuals. (Table 4).

Accuracy in spinal and extra-spinal samples: The sensitivity of Xpert for spinal biopsies was 93.8% (95% CI 86.0-97.9) with specificity of 97.6% (95% CI 87.4 – 99.9), compared to extra-

spinal biopsies with a sensitivity of 81.8% (95% CI 48.2 – 99.7, p=0.164) and specificity of 100% (95% CI 95.1-100%, p=0.186)(Table 4).

Discussion

This is the first large study to investigate the accuracy of Xpert for the diagnosis of musculoskeletal TB in both HIV positive and negative patients. We found that Xpert had high sensitivity and specificity for spinal and extra-spinal disease and provided additional diagnostic yield over culture. We noted a higher accuracy than reported in a meta-analysis evaluating Xpert in extrapulmonary TB.[39] In addition, Xpert was more sensitive than non-automated nucleic acid amplification assays in musculoskeletal TB reported in other studies with sensitivities of 61% - 83%.[37, 68-71] Reliance on culture may lead to delay in diagnosis and treatment, with resultant serious morbidity such as joint destruction or paralysis in spinal TB.[21] The results for Xpert, including drug resistance, were available much faster than liquid culture enabling timely diagnosis and initiation of therapy.

A further advantage of Xpert is the ability to rapidly detect resistant cases, which is especially important in our setting with one of the highest prevalence rates of drug resistant disease worldwide. [2] A meta-analysis of Xpert for resistance testing in tissue of 566 samples from 13 studies, reported the prevalence of Rifampicin resistance to be 5.4%. Xpert missed 2 of 41 rifampicin resistant samples.[39] We found 4 (4.4%) patients with MDR-TB and Xpert detected an additional 2 cases of rifampicin resistant TB who were culture negative. Therefore 6 patients (6.7%) had rifampicin resistant TB and Xpert detected all correctly. The use of Xpert in these cases enabled rapid initiation of appropriate therapy and detected another 2 cases, which would have been missed otherwise. A limitation of Xpert is its inability to detect INH mono-resistance, which was the case in one sample, yet the treatment is identical to that for drug-sensitive TB in South Africa. [73]

Xpert had a similar sensitivity in samples from HIV positive compared to HIV negative patients. This trend has also been reported in another small study.[65] HIV positive patients may be at particular risk for rapid progression of disease and morbidity,[1] therefore use of Xpert may be especially useful in such populations.

A limitation of the study is that it was conducted amongst adult patients with advanced disease requiring surgery at a referral hospital. The generalizability of these results to patients with less severe disease is therefore unclear; further studies of patients with milder forms of musculoskeletal disease and in peripheral hospitals should be done. A further limitation is that the histological diagnosis was made by a single pathologist. However, clear criteria for histopathological diagnosis were used, and the biopsies were consistently reviewed in a standardized way by an experienced pathologist. We also did not blind the pathologist to the Xpert results, which might have introduced bias. Though test results are usually not part of the information given or retrieved by the pathologists at the time of the analysis of the specimen. Different methods of sample collections were used, guided by the anatomic area as well as severity of the disease, which could have potentially resulted in suboptimal sample collection. More aggressive (open) approaches might have resulted in a standardized way of surgical sample collection but may have resulted in unacceptable morbidity. A further limitation was that in several patients the HIV status was unknown, thus reducing the sample size for comparison of HIV positive and negative adult patients.

Conclusions

Xpert is an accurate, rapid and effective test for the diagnosis of musculoskeletal TB in HIV positive and HIV negative individuals. It should be recommended as a first line investigation for musculoskeletal TB and a positive result should be regarded as microbiologic confirmation of musculoskeletal TB.

Funding

Tables

Table 1. Characteristics of patient cohort and biopsy sites.

	Overall	TB Culture positive only	TB Culture negative but histology positive	Not TB	P value
N (%) of patients	201	75 (37.3)	15 (7.5)	111 (55.2)	
HIV infected	46 (22.9)	24 (32.0)	8 (53.3)	14 (12.6)	
HIV uninfected	102 (50.7)	41 (54.7)	6 (40.0)	55 (49.6)	<0.001
HIV status unknown	53 (26.4)	10 (13.3)	1 (6.7)	42 (37.8)	
Sex (male)	97 (48.3)	33 (44.0)	9 (60.0)	55 (49.6)	0.485
Age -Median (IQR)	40 (27 – 54)	35 (25 – 51)	35 (24 – 52)	47 (28 – 57)	0.099

Biopsy Sites	N	%
Spine	122	59.2
Hip	10	4.8
Knee	30	14.6
Ankle and foot	9	4.4
Shoulder	6	2.9
Elbow	5	2.4
Wrist/Hand	3	1.5
Bone	20	9.7
Sacroiliac joint	1	0.5
Total	206	100

N=Number, IQR=Inter Quartile Range.

Table 2. Per-sample comparison of the accuracy of TB culture to Xpert with the reference standard set as TB culture or histology positive.

Reference Standard TB culture or histology positive (N = 84)	All (N) samples	Sensitivity % (N) CI 95%	Specificity % (N) CI 95%	PPV % (N) CI 95%	NPV % (N) CI 95%
TB Culture	76/206 (36.9%)	76/91 83.5 (74.3 – 90.5)	115/115 100 (96.8 – 100)	76/76 100.0 (95.2 – 100)	115/130 88.5 (81.7 – 93.4)
Xpert	85/206 (41.3%)	84/91 92.3 (84.8 – 96.9)	114/115 99.1 (95.2 – 99.8)	84/85 98.8 (93.6 – 99.8)	114/121 94.2 (88.4 – 97.6)
P-Value		0.069	0.316	0.343	0.107
AFB	203/206 (98.5%)	31/92 33.7 (24.2 – 44.3)	110/112 99.1 (95.8 – 99.9)	31/32 96.9 (83.8 – 99.9)	110/171 64.3 (56.7 – 71.5)

N = number of patients. PPV = positive predictive value. NPV = Negative predictive value. AFB = evidence of acid fast bacilli on Ziehl-Nielson stain.

Table 3. Concordance between Xpert and culture drug susceptibility testing for rifampicin resistance.

	Culture resistant	Culture sensitive	Culture inconclusive	Culture negative	Total
Xpert resistant	4	0	0	2	**6**
Xpert sensitive	0	64	1	13	**78**
Xpert Inconclusive	0	1	0	0	**1**
Xpert negative	0	7	0	114	**121**
	4	72	1	129	**206**

4. Accuracy of Xpert in samples comparing HIV positive to HIV negative patients as well as spinal to extra spinal samples.

Reference Standard TB Culture or histology positive	All N (%)	TP	FP	FN	TN	Sensitivity N% CI 95%	Specificity N% CI 95%	PPV N% CI 95%	NPV N% CI 95%
Over all (samples)	206	84	1	7	114	84/91 92.3 (84.8–96.9)	114/115 99.1 (95.3–100)	84/85 98.8 (93.6 –100)	114/121 94.2 (88.4–97.6)
SPINAL VERSUS EXTRASPINAL SAMPLES									
Spinal biopsies	122 (59.2)	75	1	5	41	75/80 93.8 (86.0 – 97.9)	41/42 97.6 (87.4 – 99.9)	75/76 98.7 (92.9–99.9)	41/46 89.1 (76.4–96.4)
Extraspinal biopsies	84 (40.8)	9	0	2	73	9/11 81.8 (48.2 – 97.7)	73/73 100 (95.1 – 100)	9/9 100 (70.1–100)	73/75 97.3 (90.7–99.7)
P-value						0.164	0.186	0.729	0.061
HIV NEGATIVE VERSUS HIV POSITIVE SAMPLES									
HIV negative	106 (51.5)	43	1	5	57	43/48 89.6 (77.3 – 96.5)	57/58 98.3 (90.8 – 99.9)	43/44 97.7 (88.0–99.9)	57/62 91.9 (82.2–97.3)
HIV positive	46 (22.3)	31	0	1	14	31/32 96.9 (83.8 – 99.9)	14/14 100 (78.5 – 100)	31/31 100 (90.0–100)	14/15 93.3 (68.1–99.8)
P-Value						0.225	0.621	0.398	0.856
HIV unknown	54 (26.2)	10	0	1	43	10/11 90.9 (58.7 – 99.8)	43/43 100 (91.8 – 100)	10/10 100 (72.2 –100)	43/44 97.7 (88.0–99.9)

Chapter 5

Diagnostic accuracy of Xpert MTB/RIF in tissue samples of children with suspected musculoskeletal TB

M Held[1], M Laubscher[1], S Mears[1], S Dix-Peek[1], L Workman[2], HJ Zar[2], R Dunn[1]

Affiliation:

[1]Orthopaedic Research Unit, Department of Orthopaedic Surgery, Groote Schuur Hospital

University of Cape Town

Address: H49 Old Main Building, Groote Schuur Hospital, Observatory 7925, South Africa

[2]Department of Pediatrics and Child Health, Red Cross War Memorial Children's Hospital

University of Cape Town and MRC Unit on Child & Adolescent Health, University of Cape

Town, South Africa

Address: Klipfontein Road, Rondebosch 7700, South Africa

Abstract

Aim

Xpert MTB/RIF (Xpert) is useful for the diagnosis of extrapulmonary TB in adults, but there is limited evidence on its usefulness in children. This study investigated the accuracy of Xpert for the diagnosis of childhood musculoskeletal TB.

Methods

The diagnostic accuracy of Xpert was compared to a reference standard of culture or histopathology in children hospitalized with suspected osteoarticular TB in Cape Town, South Africa from June 2013 to May 2015.

Results

109 samples of 102 patients (60 male; 58.8%) with a median age of 5.6 years (IQR 2.2 – 8.7) were included. There were 23 samples with confirmed TB by culture or histology (21.1%); histology was positive in all of these, while culture was positive in 14 samples (12.8%). Xpert was positive in 17 samples (15.6%), providing a sensitivity of 73.9% (95% CI 51.6-89.8) and specificity of 100% (95% CI 95.7 - 100). Xpert was positive at a mean of 0.8 days (0.46-1.4) compared to 21 days (19 – 30) for culture, p <0.001. Multidrug resistant TB was detected on culture in a single sample that was negative on Xpert testing.

Conclusion

Xpert confirmed bone and joint TB in children more accurately and faster than culture and should be used as a first line test. The sensitivity of Xpert is relatively low when compared to histology which is a useful test for musculoskeletal TB in children.

Introduction

The World Health Organization (WHO) estimates 9 million newly diagnosed tuberculosis cases in 2013, 25% in Africa and more than 500 cases per 100.000 inhabitants in South Africa; childhood TB is estimated to account for 15-20% of the total caseload in African countries.[2] Extrapulmonary disease accounts for 10% to 42% of TB cases, of which around 10-25% have musculoskeletal TB. [2,1,3]

At Red Cross Children's Hospital in Cape Town, the largest children's hospital in sub-Saharan Africa, approximately 20 children under the age of 12 years are treated for musculoskeletal TB each year (60% spinal TB, 20% in knee, 16% in hip and less then 5% TB of the ankle or upper limb).[18]

The sensitivity of TB culture compared to a composite reference standard for childhood musculoskeletal TB is approximately 73% (70% in hips, 71% in knees, 70% in ankles, 75% in feet and ankles).[18, 24, 29, 30] Non-automated nucleic acid amplification tests in synovial joint biopsies of children have a sensitivity of only 40% when compared to culture or histology,[23] highlighting the need for a more accurate test for musculoskeletal TB. Accurate and timely diagnosis of musculoskeletal TB is essential to prevent joint destruction, growth arrest, and contractures in large joints as well as deformity with neurological compromise in spinal disease, which may lead to lifelong morbidity and disability.[21-25] Additionally, MDR TB has a prevalence of 5% in musculoskeletal TB in our population[19] making a correct diagnosis on tissue biopsy imperative to identify resistance and initiate optimal treatment.

The Xpert® MTB/RIF (Xpert) assay (Cepheid, Sunnyvale, CA) is an automated nucleic acid amplification test, which has been validated for pulmonary TB in children but not for musculoskeletal TB. At Red Cross Hospital, the sensitivity of Xpert compared to liquid culture, on repeated induced sputum specimens for PTB is approximately 70%[58] and in a recent meta-analysis[59] the pooled sensitivity of a single Xpert was 62%.

Data on Xpert of bone and joint tissue samples are very limited. One study on 29 adults with spinal disease reported a sensitivity of 72% in HIV negative and 82% in HIV positive patients, but this was compared to a clinical reference standard.[65] Another study [66] compared Xpert in 60 adult orthopaedic fluid samples with a composite reference standard and found a sensitivity of 82% (41/50) versus culture with a sensitivity of 48% (24/50). Currently no large studies have assessed the accuracy of Xpert in childhood osteoarticular TB. We therefore aimed to assess the diagnostic accuracy of Xpert in bone and joint samples of children with suspected musculoskeletal TB.

Methods

A prospective study of Xpert for diagnosis of musculoskeletal TB was done in children admitted to Red Cross Children's Hospital in Cape Town, South Africa, from June 2013 to May 2015. Children under 13 years of age who presented with suspected musculoskeletal TB were enrolled. Symptoms or signs suspicious of musculoskeletal TB included joint or back pain with insidious onset, associated with elevated inflammatory markers, TB contact, constitutional symptoms, chronic cough, or HIV. Suspicious radiological signs were osteopenia and erosions involving both sides of the affected joint. In spinal imaging, anterior vertebral height loss, paravertebral or psoas shadow suggesting abscess formation, adjacent endplate changes with preserved disc height as well as local kyphosis were suggestive of TB. Children were excluded if samples were inadequate or incorrectly processed or tested. Informed consent was taken from a parent or legal guardian. After informed consent was taken from a parent or legal guardian, radiologically predetermined areas of disease were biopsied surgically as part of the routine clinical workup. This was done through an open approach, under sterile conditions and general anaesthesia. Spinal biopsies were performed by a subspecialist spinal unit. A subspecialist paediatric orthopaedic unit performed all extraspinal biopsies.

"Confirmed TB" was defined as a positive *M tuberculosis* culture or positive histology. A negative culture and histology with improvement of symptoms without TB treatment after at least a month of follow up was considered 'NOT TB'. Improvement was assessed by means of a decrease in ESR readings, signs of sclerosis on XR, as well as clinical improvement such as weight gain and diminished pain.

The accuracy of Xpert was compared to culture or to histology as a reference standard.

Tests: Specimens were collected in duplicate and sterile saline was added. One specimen was used for Xpert® MTB/RIF testing (Cepheid, Sunnyvale, CA). The specimen was mixed with Xpert SR lysis buffer at a ratio of 1:3. Two millilitres of this fluid were automatically processed adhering to Xpert protocols. The laboratory technician processing the Xpert test was blinded to the culture or histology results.

The primary reference standard was TB culture on BACTEC MGIT 960 [Diagnostic Systems, Sparts MD,Franklin, Lakes, NJ, USA) or histology. The microbiology and histopathology laboratories performed Ziehl-Nielson (ZN) stains and haematoxylin and eosin (HE) stains respectively. Specifications from the Centres for Disease Control and Prevention were used for quantification of acid-fast bacilli (AFB). The pathologist was blinded to the Xpert and culture results but not to the clinical history. TB was diagnosed if histology showed granulomatous necrosis with epitheloid cells or Langhans giant cells

The culture isolate was tested for drug resistance using the GenoType® MTBDRplus or GenoType® Mycobacterium CM lineprobe assay (Hain Lifescience, Nehren, Germany). "MDR-TB" was defined as resistance to isoniazid and rifampicin, with or without resistance to other first-line drugs.[7]

Statistical Analysis: The accuracy of Xpert (sensitivity, specificity and predictive values) with 95% confidence intervals (95% CI) was calculated using positive TB culture or histology for *M tuberculosis* as the reference standard. Per sample analysis was done using STATA 13 statistical software (STATA Corporation, College Station, TX USA). The study population was

analysed using descriptive statistics, mean and 95% confidence intervals were used in normally distributed data, median and interquartile range was used for non-normally distributed continuous data. Categorical data were reported as proportions with 95% confidence intervals. Statistical tests included two-sample test of proportions, chi squared test, Kruskal Wallis test and Wilcoxon rank-sum test. All statistical tests were two-sided at α = 0.05.

Results

We collected 120 samples from 113 patients; three patients had repeat biopsies and one patient had multiple biopsies of different sites. Of 120 samples, 11 were excluded (7 TB cultures were contaminated and four samples were incorrectly processed or tested). Therefore 109 samples from 102 patients (60 male; 58.8%) with a median age of 5.6 years (IQR 2.2 – 8.7) were included. In 30 patients (29.4%) the HIV status was known, 3/30 (10%) were HIV positive, 27/30 (90%) were HIV negative.

The biopsy sites are shown in Table 1. The main areas of biopsy were the knee (48 of 109 biopsies; 44.0%), hip (18 biopsies; 16.5%), spine (14 biopsies; 12.8%), and foot or ankle (11 biopsies; 10.1%).   One pus sample (0.9%) and 108 (99.1%) tissue samples were collected. In 23 samples (21.1%) of 22 patients (one repeat biopsy) the histology was positive. Culture was positive in 14 samples (12.8%), all of which also had histology indicating TB. A total of 23 samples (21.1%) were therefore histology or culture positive (confirmed TB). Xpert was positive in 17 (15.6%) samples and detected 3 samples more when compared to culture (Table 2). Of the 109 samples, 86 were TB negative, with 41 (37.6%) pyogenic joint infections, 35 (32.1%) acute or chronic synovitic joint pathology, five (4.6%) tumors, and five (4.6%) samples without a specific diagnosis or abnormal features.

When comparing Xpert to a gold standard of culture or histology positive for TB, the sensitivity was 73.9% (95% CI 51.6-89.8) and specificity 100% (95% CI 95.7 - 100) (Table 3).

The sensitivity of TB culture was 60.9% (14/23; 95% CI 38.5-80, P=0.345) with a specificity of 100% (86/86; 95% CI 95.7-100) when compared to histology confirmed TB. Of the 109 samples, 108 (99.1%) were tested for Acid Fast Bacilli (AFB), of which 15 (13.8%) were positive. The sensitivity and specificity of smear compared to culture or histology was 60.9% (95% CI 38.5 – 80.3%) and 98.8% (95% CI 93.6% - 99.9%) respectively (Table 3).

Xpert was positive at a mean (range) of 0.8 days (0.46-1.4) compared to 21 days (19 – 30) for culture, p <0.001 (Table 4). Two samples were drug resistant on testing with a line probe assay; the first was MDR but Xpert was negative, while the 2nd showed isoniazid (INH) mono-resistance, which cannot be detected with Xpert (Table 5).

Discussion

This is the first large report to show the accuracy of Xpert for the diagnosis of musculoskeletal TB in children. Xpert provided results much earlier than culture and detected more TB cases than culture. Although the sensitivity of Xpert was consistent with that reported on respiratory specimens in childhood pulmonary TB,[74] the sensitivity was lower than that published for musculoskeletal samples in adults.[19,65,70] The lower sensitivity in children may reflect paucibacillary disease or relatively early disease compared to that in adults. Culture provided additional information on drug resistance in two cases. Hence, our local policy is to use culture with Xpert and histology to test for musculoskeletal TB in children.

Histology was positive for all children who were culture positive, while some children were culture negative but positive on histology. Lack of viability of the organisms or technical issues in the laboratory or during transport of the specimen may explain culture negative histology positive cases. Although it cannot provide microbiologic confirmation or resistance testing, which is crucial in our patient population, histology is therefore still important to detect musculoskeletal TB. Xpert detected more cases of histology proven TB

than did culture and results were available much sooner than for culture, however, an advantage of culture was the ability to detect drug resistance beyond rifampicin resistance. A limitation of our study was that some TB culture samples were contaminated or were not processed adequately. The relatively small number of HIV positive children limited the power to investigate the impact of HIV on the accuracy of Xpert and further research is needed. The small number of HIV positive children might reflect the success of the mother to child HIV prevention program. The accuracy for histology might also not be generalizable to a setting with low TB burden, as our pathologist was experienced in and preconditioned for the histological diagnosis of TB given the high incidence of TB in South Africa. Nevertheless, the histological signs of TB are clearly delineated and should be recognizable by any pathologist.

In conclusion, Xpert confirmed musculoskeletal TB in children more accurately and faster than liquid culture, the current gold standard. It should therefore be used as a first line test. Yet, the sensitivity of Xpert is relatively low when compared to histology. Histology therefore remains a useful test for diagnosis of musculoskeletal TB in children.

Tables

Table 1. Sites of biopsies.

Site	Number (%)
Spine	14 (12.8)
Hip	18 (16.5)
Knee	48 (44.0)
Ankle and foot	11 (10.1)
Shoulder	3 (2.7)
Elbow	6 (5.5)
Wrist/Hand	1 (1.0)
Bone	8 (7.3)
Sacroiliac joint	0 (0.0)
Total	109 (100)

Table 2. Characteristics of children (per-patient).

Definite TB 22/102 (21.6%)	Overall	TB culture positive	histology positive (culture negative)	Not TB	P value
N (%) of patients	102	13 (12.8%)	9 (8.8%)	80 (78.4%)	
Sex (male)	60 (58.8%)	7 (53.9%)	3/9 (33.3)	50/80 (62.5)	0.224
Median age (months)	66.8	51.1	60.2	68.3	0.479
IQR	(26.1 – 104.0)	(40.7 – 82.9)	(48.6 – 121.1)	(22.1 – 109.7)	

N=Number, IQR=Inter Quartile Range.

Table 3. Per-sample comparison of the accuracy of TB culture to Xpert with the reference standard set as TB culture positive or histology positive (23/109 samples).

	All (N) samples	Sensitivity % (N) CI 95%	Specificity % (N) CI 95%	PPV % (N) CI 95%	NPV % (N) CI 95%
Xpert	17 (15.6%)	17/23 73.9 (51.6 – 89.8)	86/86 100.0 (95.7 – 100.0)	17/17 100.0 (81.6 – 100.0)	86/92 93.5 (86.3 – 97.6)
AFB only*	15 (13.8%)	15/23 65.2 (42.7 – 83.6)	85/85 100.0 (95.4 – 100.0)	15/15 100.0 (79.6 – 100.0)	85/93 91.4 (83.8 – 96.2)
Histology	23 (21.9%)	23/23 100.0 (85.7 – 100.0)	82/82 100.0 (95.5 – 100.0)	23/23 100.0 (85.7 – 100.0)	82/82 100.0 (95.5 – 100.0)

*one AFB not done N=108

 N = number of patients, PPV = positive predictive value, NPV = Negative predictive value. AFB = evidence of acid fast bacilli on Ziehl-Nielson stain.

Table 4. Time to availability of results (in days)

	Time to result (CI 95%)	Time to positive result (CI 95%)	Time to negative result (CI 95%)
Xpert	0.8 (0.5 – 1.4)	0.8 (0.3 – 1.9)	0.7 (0.5 – 1.4)
Culture	44 (43 – 45)	21.5 (19 – 30)	44 (43 – 46)
P-Value	<0.001	< 0.001	<0.001

Table 5. Concordance between Xpert and culture drug susceptibility testing for rifampicin resistance.

	Culture resistant	Culture sensitive	Culture inconclusive	Culture negative	Total
Xpert resistant	0	0	0	0	0
Xpert sensitive	0	9	1	7	17
Xpert inconclusive	0	0	0	0	0
Xpert negative	1	2	1	88	92
Total	1	11	2	95	109

Chapter 6

Discussion and Conclusion

Discussion

These are the first large studies on the accuracy of Xpert® MTB/RIF (Cepheid, Sunnyvale, CA) on musculoskeletal TB in adults and children as well as in HIV positive and HIV negative patients. It underlines that Xpert should be recommended as the initial test for suspected musculoskeletal TB as it is more sensitive and gives faster results than the current gold standard (culture and histology).

Xpert was found to have high sensitivity in adults which is higher than reported in a meta-analysis evaluating Xpert in extrapulmonary TB.[39] We also found that Xpert has a lower sensitivity in musculoskeletal samples of children than of adults,[19,65,70] however detecting more cases of TB than culture. The lower sensitivity in children may reflect paucibacillary disease or relatively early disease compared to adults. The sensitivity of Xpert in childhood musculoskeletal TB was also consistent with that reported on respiratory specimens in childhood pulmonary TB,[74] for which Xpert is the recommended first line test. A further finding was that histology remains a useful test for the diagnosis of musculoskeletal TB. It should be used in addition to Xpert to compensate for its lower sensitivity observed in children.

Xpert is also an accurate test to detect rifampcin resistance as all rifampcin resistant cases were correctly diagnosed. This is in line with current evidence supporting the accuracy of Xpert to detect resistance to rifampicin in pulmonary and extrapulmonary samples.[39]

We also noted a rate of rifampicin resistance of approximately 5%, which, due to the size of our cohort and prospective data collection, represents important evidence of drug resistant

musculoskeletal TB. This number is comparable to the rifampicin resistance in extrapulmonary TB reported by the WHO in a large metaanalysis.[39]

We found no significant difference in the subgroup analysis of spinal compared to extraspinal TB, as well as HIV positive compared to HIV negative patients. There was however, a trend to higher sensitivity in spinal tissue as well as HIV positive patients. HIV positive patients may have a greater bacillary load and therefore be more likely to be Xpert positive, as has been reported in a previous study and as has been reported for childhood pulmonary TB.[65, 74] HIV positive patients may be at particular risk for rapid progression of disease and morbidity,[1] therefore use of Xpert may be especially useful in such populations.

A major benefit of Xpert is its rapid time for test results to be available including Rifampicin resistance, as the automated process of Xpert takes only approximately 90 minutes to completion. This is crucial to ensure early and adequate treatment, which is especially important in our setting with one of the highest prevalence rates of drug resistant disease worldwide.[2] Without Xpert, affected patients may wait on average 40 days before culture results would detect drug resistance. Furthermore, reliance on culture with delay in diagnosis and treatment may also result in serious morbidity such as joint destruction or paralysis in spinal TB.[21] Early diagnosis with Xpert can therefore influence the progression of the disease and the implementation of appropriate drug therapy.

For these reasons, the Xpert assay should ideally be performed onsite. One study found no difference in time or added benefit of the Xpert compared to conventional testing methods when Xpert was not onsite.[17] The Xpert assay should therefore be placed in hospitals and clinics, to enable easy and rapid access. However, as our studies have shown, Xpert carried out in tertiary care hospitals with transport of specimens to a central testing facility was still effective for rapid diagnosis in suspected musculoskeletal TB.

Limitations of this study

One of the disadvantages of PCR testing is that in contrast to TB culture, PCR testing may be positive even if the pathogens are non-viable.[1] In these cases active TB will have to be confirmed clinically, by culture and by means of various imaging modalities. However, unlike pulmonary TB, this is not an important issue in musculoskeletal disease, where an initial biopsy is usually sufficient to make the diagnosis and where re-biopsy is unlikely.

A further limitation is that Xpert tests only rifampicin resistance therefore mono-resistance for isoniazid cannot be detected, nor can more extensive drug resistance be evaluated. However, current treatment guidelines recommend no change to the conventional TB regimen for isoniazid mono-resistance. Thus, in cases of isoniazid mono-resistance, there will be no impact in treatment regime.[18] Further, next generation Xpert tests are being developed that will include PCR targets for INH and for MDR TB.

Another limitation of the studies is that, especially for our spinal TB group, they were conducted in patients with advanced disease requiring surgery at referral hospitals. The generalizability of these results to patients with less severe disease is therefore unclear; further studies of patients with milder forms of musculoskeletal disease and in peripheral hospitals should be done.

A further limitation is that the histological diagnosis was made by a single pathologist. However, clear criteria for histopathological diagnosis were used, and the biopsies were consistently reviewed in a standardized way by an experienced pathologist. The use of a single pathologist may conversely strengthen reliability and consistency in histological reporting. The accuracy for histology, especially for our paediatric cohort, might also not be generalizable to a setting with low TB burden, as our pathologist was sensitised to the histological diagnosis of TB given the high incidence of TB in this geographical area.

Nevertheless, the histological signs of TB are clearly delineated and should be recognizable by any trained pathologist.

A further limitation is the use of different methods of sample collection, guided by the anatomic area as well as severity of the disease, which could have potentially resulted in suboptimal sample collection. More aggressive (open) approaches might have resulted in a standardized way of surgical sample collection but may have resulted in unacceptable morbidity.

Another limitation was that in several patients the HIV status was unknown, thus reducing the sample size for comparison of HIV positive and HIV negative adults. However, these numbers are similar to reports published by the WHO on the African region, in which 76% of TB patients knew their HIV status in 2013. The relatively small number of HIV positive children limited the power to investigate the impact of HIV on the accuracy of Xpert, but this reflects a strong HIV preventative program in this geographical area, reducing the incidence of paediatric HIV.

We also did not record previous or current TB treatment. This might influence the accuracy of Xpert or increase the number of false positive culture samples. Furthermore, a recent study[43] has shown a higher false positive rate in these cases compared with new cases.

Another limitation of our paediatric cohort was that some TB culture samples were contaminated or were not processed adequately; the exclusion of these samples might have potentially influenced the calculated accuracy of Xpert. However, this only occurred in a very small number of specimens; laboratory contamination of around 5% is internationally regarded as acceptable practice.

Future research

The higher rate of spinal disease amongst our adult patients with confirmed TB (80%) compared to international reports (approximately 60%) is an area that requires further research. Study of patients with less severe forms of musculoskeletal disease should be undertaken to enable greater generalizability of these results.

The question why Xpert has lower accuracy in children, HIV negative patients and extraspinal disease remains unanswered and larger studies, especially for childhood musculoskeletal TB are needed.

Investigation and comparison of the yield of Xpert from less invasive samples with those from musculoskeletal biopsies, such as superficial lymph nodes or samples from respiratory tract are needed. Future research should therefore assess the sensitivity of Xpert in other samples compared to musculoskeletal samples.

As improved next generation Xpert tests are developed, it will be important to undertake further research to evaluate these for diagnosis of musculoskeletal disease.

Conclusions and Recommendations

These are the first large studies on the accuracy of Xpert® MTB/RIF (Cepheid, Sunnyvale, CA) for musculoskeletal TB. We have produced evidence, which covers a large research gap for diagnostic accuracy studies in musculoskeletal TB, especially in extraspinal TB, in TB/HIV co-infections, and in childhood musculoskeletal TB. Based on these results, Xpert should be recommended as the initial test as it is more sensitive and faster than culture. The National Health Laboratory System in the Western Cape has already implemented this. All rifampcin resistant cases were correctly diagnosed. We also noted a rate of rifampicin resistance of approximately 5%, which, due to the size of our cohort and prospective data collection,

represents rare evidence for musculoskeletal TB. A trend towards higher sensitivity in spinal tissue as well as HIV positive patients was observed. This study also provides evidence that Xpert has a lower sensitivity in children than in adults, yet, still detects more cases of paediatric musculoskeletal TB and is faster than culture. As an additional finding histology was found to remain a useful test for the diagnosis of musculoskeletal TB, especially in children, and should be used alongside Xpert to provide the highest yield possible to detect TB.

Dissemination and Publications included

The dissemination of this project was realized by means of podium presentations at local,

national, and international meetings and congresses, publications in international

orthopaedic journals with high impact factors, and by influencing regional policy changes.

Influencing Changes in Policy of testing musculoskeletal TB

This study, amongst others has proven the benefit of Xpert® MTB/RIF (Cepheid, Sunnyvale,

CA) for extrapulmonary tissue.   Therefore, after being implemented nationally for selected

extrapulmonary specimens, the National Health Laboratory System (NHLS) altered the

policies regarding testing for musculoskeletal TB: Xpert will now be the first test for

musculoskeletal TB in the Western Cape. The algorithm includes a reflex culture if result

shows rifampicin resistance or is Xpert is negative, but NOT if result indicates rifampicin

susceptible TB.

Dissemination in meetings and congresses in form of podium presentations:

1. Surgical Research Day, 2013, University of Cape Town (**Research Prize**)

2. National Meeting of South African Orthopaedic Congress, Sun City, 2013 (**GT Du Toit
    Prize for best presentation**)

3. National Meeting of South African Orthopaedic Congress, Cape Town, South Africa,
    2014[75]

4. 21st International Meeting on Advanced Spine Techniques (IMAST) Valencia, Spain
    July 16-19, 2014[76]

5. 13th International Meeting of the Combined Orthopaedic Associations (COMOC -
    England, Australia, New Zealand, USA, Canada and South Africa), Cape Town 2016.

6. SICOT Orthopaedic World Congress. Rome. 2016. Accepted.

Publications and manuscripts

Manuscript 1 (published)

Xpert MTB/RIF for spinal tuberculosis: an accurate and rapid diagnostic test. Bone Joint J. 2014 Oct;96-B(10):1366-9. doi: 10.1302/0301-620X.96B10.34048.

Held M, Laubscher M, Zar HJ, Dunn RN

Manuscript 2 (submitted to Bone & Joint Journal)

Diagnostic accuracy of Xpert MTB/RIF in HIV positive and HIV negative patients with musculoskeletal tuberculosis

M Held, M Laubscher, L Workman, HJ Zar, R Dunn

Manuscript 3 (accepted for publication in the Pediatric Infectious Disease Journal)

Diagnostic accuracy of Xpert MTB/RIF in tissue samples of children with suspected musculoskeletal TB

M Held, M Laubscher, S Mears, S Dix-Peek, L Workman, HJ Zar, R Dunn

About the Journals

The Bone & Joint Journal (BJJ), formerly known as The Journal of Bone & Joint Surgery (British Volume), is published by The British Editorial Society of Bone & Joint Surgery. It ranks amongst the worlds top 10 orthopaedic journals with an impact factor of 2.801 (August 2015).

The Pediatric Infectious Disease Journal is published by the European Society for Paediatric Infectious Diseases and has an impact factor of 2.7.

Contribution of Authors

Introduction, Methods and Conclusion

I have written the introduction and conclusion, which was revised by Prof Dunn and Prof Zar. Parts of the methods section were taken out of the included manuscripts. The co-authors of the included publications reviewed the methods sections of the respective publications. Lesley Workman reviewed the section on statistical tests. Dr Bamford and N. Beylis provided insight into laboratory tests and the Xpert assay and reviewed this section of our methods.

Chapter 3 and 4

Authors: M Held, M Laubscher, L Workman, HJ Zar, R Dunn

Chapter 3: Xpert Polymerase Chain Reaction for spinal Tuberculosis - An accurate and rapid diagnostic test.
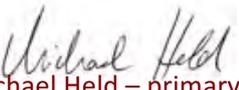
Chapter 4: Diagnostic accuracy of Xpert MTB/RIF in HIV positive and HIV negative patients with musculoskeletal tuberculosis

For these publications I developed the research idea, the research design and protocol. Dr Laubscher advised on the research design and protocol and assisted with the setup of the study sites. I collected the data and analyzed the data under the supervision of Lesley Workman and Prof Zar. Dr Bamford and N. Beylis provided insight into laboratory tests and the Xpert assay and reviewed this section of our methods. Patients were recruited and operated on by Prof Dunn at his Orthopaedic and Spinal Unit. Prof Dunn is also the principle investigator of this study. I wrote the manuscript, which was reviewed by Dr Laubscher, Prof Dunn and Prof Zar. Journal submission and revision of this publication was done by me and I am the corresponding author.
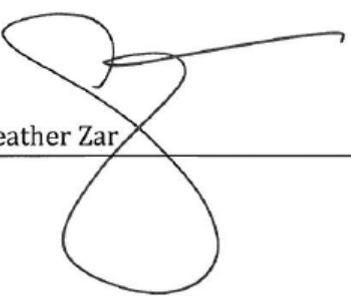
Chapter 5: Diagnostic accuracy of Xpert MTB/RIF in tissue samples of children with suspected musculoskeletal TB

For these publications I developed the research idea, the research design and protocol. Dr Laubscher advised on the research design and protocol and assisted with the setup of the study sites. Pediatric patients with suspected spinal TB were operated on by Prof Dunn at his spinal unit at Red Cross Hospital. Prof Dunn is also the Principle Investigator of this study. Dr Dix-Peek and Dr Mears were in charge of the recruitment and clinical management of pediatric patients at Red Cross Children's Hospital. I collected the data and analyzed the data under the supervision of Lesley Workman and Prof Zar. Dr Bamford and N. Beylis provided insight into laboratory tests and the Xpert assay and reviewed this section of our methods. I wrote the manuscript, which was reviewed by Dr Laubscher, Dr Dix-Peek, Dr Mears, Prof Dunn and Prof Zar. Journal submission and revision of this publication was done by me and I am the corresponding author.

We confirm the above:

12.2.16

Dr Michael Held – primary author   Date

14.2.16

Prof Robert Dunn – Supervisor   Date

14.2.16

Heather Zar   Date

Tables

Table 5. Concordance between Xpert and culture drug susceptibility testing for rifampicin

resistance.

Figures

Chapter 1

Figure 1. Estimated TB incidence rates in 2013.

Figure 2. Estimated HIV prevalence in new and relapse TB cases in 2013.

Figure 3. Steps using the Xpert assay.

References

1.   Anley CM, Brandt AD, Dunn R. Magnetic resonance imaging findings in spinal tuberculosis: Comparison of HIV positive and negative patients. Indian journal of orthopaedics 2012;46(2):186.

2.   WHO. Global Tuberculosis report 2014. World Health Organisation 2014.

3.   Trecarichi E, Di Meco E, Mazzotta V, Fantoni M. Tuberculous spondylodiscitis: epidemiology, clinical features, treatment, and outcome. Eur Rev Med Pharmacol Sci 2012;16(Suppl 2):58-72.

4.   Lawn SD, Bekker LG, Middelkoop K, Myer L, Wood R. Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: the need for age-specific interventions. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2006;42(7):1040-7.

5.   Gunal S, Yang Z, Agarwal M, Koroglu M, Arici ZK, Durmaz R. Demographic and microbial characteristics of extrapulmonary tuberculosis cases diagnosed in Malatya, Turkey, 2001-2007. BMC Public Health 2011;11:154.

6.   Jutte PC, van Loenhout-Rooyackers JH, Borgdorff MW, van Horn JR. Increase of bone and joint tuberculosis in The Netherlands. The Journal of bone and joint surgery British volume 2004;86(6):901-4.

7.   Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2009;49(9):1350-7.

8.   Rockwood RR. Extrapulmonary TB: what you need to know. Nurse Pract 2007;32(8):44-9.

9.   Friesen J. The other TB. How to treat extrapulmonary tuberculosis & protect yourself. JEMS : a journal of emergency medical services 2009;34(7):36, 8.

10. Wang X, Yang Z, Fu Y, et al. Insight to the Epidemiology and Risk Factors of Extrapulmonary Tuberculosis in Tianjin, China during 2006-2011. PloS one 2014;9(12):e112213.

11. Gomes T, Reis-Santos B, Bertolde A, Johnson JL, Riley LW, Maciel EL. Epidemiology of extrapulmonary tuberculosis in Brazil: a hierarchical model. BMC Infect Dis 2014;14:9.

12. Johansen IS, Nielsen SL, Hove M, et al. Characteristics and Clinical Outcome of Bone and Joint Tuberculosis From 1994 to 2011: A Retrospective Register-based Study in Denmark. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2015;61(4):554-62.

13. Moszynski P. Coinfection of tuberculosis and HIV poses global threat. Bmj 2008;336(7657):1330-1.

14. Bruchfeld J, Correia-Neves M, Kallenius G. Tuberculosis and HIV Coinfection. Cold Spring Harb Perspect Med 2015;5(7):a017871.

15. Malaviya A, Kotwal P. Arthritis associated with tuberculosis. Best Practice & Research Clinical Rheumatology 2003;17(2):319-43.

16. Holland TS, Sangster MJ, Paton RW, Ormerod LP. Bone and joint tuberculosis in children in the Blackburn area since 2006: a case series. Journal of children's orthopaedics 2010;4(1):67-71.

17. Schaaf HS, Marais BJ, Whitelaw A, et al. Culture-confirmed childhood tuberculosis in Cape Town, South Africa: a review of 596 cases. BMC Infect Dis 2007;7:140.

18. Maqungo S, Oleksak M, Dix-Peek S, Hoffman E. Tuberculosis of the foot and ankle in children. SA Orthopaedic Journal 2012;11(2):23-8.

19. Held M, Laubscher M, Zar HJ, Dunn RN. GeneXpert polymerase chain reaction for spinal tuberculosis: an accurate and rapid diagnostic test. The bone & joint journal 2014;96-B(10):1366-9.

20.   De Vuyst D, Vanhoenacker F, Gielen J, Bernaerts A, De Schepper AM. Imaging features of musculoskeletal tuberculosis. European radiology 2003;13(8):1809-19.

21.   Watts HG, Lifeso RM. Tuberculosis of bones and joints. The Journal of bone and joint surgery American volume 1996;78(2):288-98.

22.   Rajasekaran S, Shanmugasundaram T. Prediction of the angle of gibbus deformity in tuberculosis of the spine. The Journal of Bone & Joint Surgery 1987;69(4):503-9.

23.   Hoffman EB, Allin J, Campbell JAB, Leisegang FM. Tuberculosis of the Knee. Clinical orthopaedics and related research 2002;398:100-6.

24.   Campbell J, Hoffman E. Tuberculosis of the hip in children. Journal of Bone & Joint Surgery, British Volume 1995;77(2):319-26.

25.   Mohideen M, Rasool M. Tuberculosis of the hip joint region in children. SA Orthopaedic Journal 2013;12:38-43.

26.   Tenover FC, Crawford JT, Huebner RE, Geiter LJ, Horsburgh Jr C, Good R. The resurgence of tuberculosis: is your laboratory ready? Journal of clinical microbiology 1993;31(4):767.

27.   Cruciani M, Scarparo C, Malena M, Bosco O, Serpelloni G, Mengoli C. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. Journal of clinical microbiology 2004;42(5):2321-5.

28.   Pras E, Schumacher HR, Kastner DL, Wilder RL. Lack of evidence of mycobacteria in synovial tissue from patients with rheumatoid arthritis. Arthritis & Rheumatism 1996;39(12):2080-1.

29.   Dix-Peek SI, Vrettos BC, Hoffman EB. Tuberculosis of the elbow in children. Journal of shoulder and elbow surgery 2003;12(3):282-6.

30.   Lee A, Campbell J, Hoffman E. Tuberculosis of the knee in children. Journal of Bone & Joint Surgery, British Volume 1995;77(2):313-8.

31.   Colmenero J, Jiménez-Mejías M, Reguera J, et al. Tuberculous vertebral osteomyelitis in the new millennium: still a diagnostic and therapeutic challenge. European Journal of Clinical Microbiology and Infectious Diseases 2004;23(6):477-83.

32.   Mondal A. Cytological diagnosis of vertebral tuberculosis with fine-needle aspiration biopsy. The Journal of Bone & Joint Surgery 1994;76(2):181-4.

33.   Negi S, Gupta S, Khare S, Lal S. Comparison of various microbiological tests including polymerase chain reaction for the diagnosis of osteoarticular tuberculosis. Indian journal of medical microbiology 2005;23(4):245.

34.   Tiwari V, Jain A, Verma R. Application of enzyme amplified mycobacterial DNA detection in the diagnosis of pulmonary & extra-pulmonary tuberculosis. Indian Journal of Medical Research 2003;118:224-8.

35.   Sun Ys, Lou Sq, Wen Jm, et al. Clinical value of polymerase chain reaction in the diagnosis of joint tuberculosis by detecting the DNA of Mycobacterium tuberculosis. Orthopaedic surgery 2011;3(1):64-71.

36.   Berk RH, Yazici M, Atabey N, Ozdamar OS, Pabuccuoglu U, Alici E. Detection of Mycobacterium tuberculosis in Formaldehyde Solution – Fixed, Paraffin – Embedded Tissue by Polymerase Chain Reaction in Pott's Disease. Spine 1996;21(17):1991-5.

37.   Pandey V, Chawla K, Acharya K, Rao S, Rao S. The role of polymerase chain reaction in the management of osteoarticular tuberculosis. International Orthopaedics 2009;33(3):801-5.

38.   Cox HS, Daniels JF, Muller O, et al, editors. Impact of decentralized care and the Xpert MTB/RIF test on rifampicin-resistant tuberculosis treatment initiation in Khayelitsha, South Africa. Open Forum Infectious Diseases; 2015: Oxford University Press.

39.   WHO. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MT. World Health Organisation 2013.

40.   Kramer N, Rosenstein E. Rheumatologic manifestations of tuberculosis. Bulletin on the rheumatic diseases 1997;46(3):5.

41.   Singh UB, Bhanu NV, Suresh VN, Arora J, Rana T, Seth P. Utility of polymerase chain reaction in diagnosis of tuberculosis from samples of bone marrow aspirate. The American journal of tropical medicine and hygiene 2006;75(5):960-3.

42.   Rachow A, Zumla A, Heinrich N, et al. Rapid and accurate detection of Mycobacterium tuberculosis in sputum samples by Cepheid Xpert MTB/RIF assay–a clinical validation study. PloS one 2011;6(6):e20458.

43.   Theron G, Venter R, Calligaro G, et al. Xpert MTB/RIF Results in Patients With Previous Tuberculosis: Can We Distinguish True From False Positive Results? Clinical Infectious Diseases 2016:civ1223.

44.   Puri L, Oghor C, Denkinger CM, Pai M. Xpert MTB/RIF for tuberculosis testing: access and price in highly privatised health markets. The Lancet Global Health 2016;4(2):e94-e5.

45.   Dheda K, Theron G, Welte A. Cost-effectiveness of Xpert MTB/RIF and investing in health care in Africa. The Lancet Global Health 2014;2(10):e554-e6.

46.   Theron G, Pooran A, Peter J, et al. Do adjunct tuberculosis tests, when combined with Xpert MTB/RIF, improve accuracy and the cost of diagnosis in a resource-poor setting? European Respiratory Journal 2012;40(1):161-8.

47.   Pantoja A, Fitzpatrick C, Vassall A, Weyer K, Floyd K. Xpert MTB/RIF for diagnosis of tuberculosis and drug-resistant tuberculosis: a cost and affordability analysis. European Respiratory Journal 2013;42(3):708-20.

48.   Vassall A, van Kampen S, Sohn H, et al. Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. PLoS Med 2011;8(11):e1001120.

49.   Menzies NA, Cohen T, Lin H-H, Murray M, Salomon JA. Population health impact and cost-effectiveness of tuberculosis diagnosis with Xpert MTB/RIF: a dynamic simulation and economic evaluation. PLoS Med 2012;9(11):e1001347.

50.   Theron G, Peter J, Dowdy D, Langley I, Squire SB, Dheda K. Do high rates of empirical treatment undermine the potential effect of new diagnostic tests for tuberculosis in high-burden settings? The Lancet Infectious diseases 2014;14(6):527-32.

51.   Hanrahan C, Clouse K, Bassett J, et al. The patient impact of point-of-care vs. laboratory placement of Xpert® MTB/RIF. The International Journal of Tuberculosis and Lung Disease 2015;19(7):811-6.

52.   Calligaro GL, Theron G, Khalfey H, et al. Burden of tuberculosis in intensive care units in Cape Town, South Africa, and assessment of the accuracy and effect on patient outcomes of the Xpert MTB/RIF test on tracheal aspirate samples for diagnosis of pulmonary tuberculosis: a prospective burden of disease study with a nested randomised controlled trial. The Lancet Respiratory Medicine 2015;3(8):621-30.

53.   Philipsen R, Sánchez C, Maduskar P, et al. Automated chest-radiography as a triage for Xpert testing in resource-constrained settings: a prospective study of diagnostic accuracy and costs. Scientific reports 2015;5.

54.   Hillemann D, Rusch-Gerdes S, Boehme C, Richter E. Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. Journal of clinical microbiology 2011;49(4):1202-5.

55.   Theron G, Peter J, Dheda K. Xpert MTB/RIF test for tuberculosis. Lancet 2011;378(9790):481; author reply 2-3.

56.   Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev 2014;1.

57.   Kaur R, Kachroo K, Sharma JK, Vatturi SM, Dang A. Diagnostic accuracy of xpert test in tuberculosis detection: A systematic review and meta-analysis. Journal of global infectious diseases 2016;8(1):32.

58.   Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. The Lancet Infectious diseases 2011;11(11):819-24.

59.   Reither K, Manyama C, Clowes P, et al. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in children: A prospective, multi-centre evaluation. Journal of Infection 2015;70(4):392-9.

60.   Wang X, Pappoe F, Huang Y, et al. Xpert MTB/RIF Assay for Pulmonary Tuberculosis and Rifampicin Resistance in Children: a Meta-Analysis. Clinical laboratory 2014;61(11):1775-85.

61.   Reither K, Manyama C, Clowes P, et al. Xpert MTB/RIF assay for diagnosis of pulmonary tuberculosis in children: A prospective, multi-centre evaluation. Journal of Infection 2015;70(4):392-9.

62.   Marcy O, Ung V, Goyet S, et al. Performance of Xpert MTB/RIF and alternative specimen collection methods for the diagnosis of tuberculosis in HIV-infected children. Clinical Infectious Diseases 2016;62(9):1161-8.

63.   Penz E, Boffa J, Roberts D, et al. Diagnostic accuracy of the Xpert® MTB/RIF assay for extra-pulmonary tuberculosis: A meta-analysis. The International Journal of Tuberculosis and Lung Disease 2015;19(3):278-84.

64.   Floss HG, Yu T-W. Rifamycin mode of action, resistance, and biosynthesis. Chemical reviews 2005;105(2):621-32.

65.   Monni T, Visser A, Visser H, Motsitsi S. Clinical utility of tissue polymerase chain reaction in the diagnosis of spinal tuberculosis. SA Orthopaedic Journal 2012;11:23-7.

66.   Gu Y, Wang G, Dong W, et al. Xpert MTB/RIF and GenoType MTBDRplus assays for the rapid diagnosis of bone and joint tuberculosis. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases 2015;36:27-30.

67.   Standards for the Reporting of Diagnostic accuracy studies (STARD). 2008. Available from: http://www.stard-statement.org/.

68.   Negi S, Gupta S, Khare S, Lal S. Comparison of various microbiological tests including polymerase chain reaction for the diagnosis of osteoarticular tuberculosis. Indian J Med Microbiol 2005;23(4):245-8.

69.   Davies AP, Newport LE, Billington OJ, Gillespie SH. Length of time to laboratory diagnosis of Mycobacterium tuberculosis infection: comparison of in-house methods with reference laboratory results. The Journal of infection 1999;39(3):205-8.

70.   Sun Y, Zhang Y, Lu Z. [Clinical study of polymerase chain reaction technique in the diagnosis of bone tuberculosis]. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases 1997;20(3):145-8.

71.   Rasit AH, Razak M, Ting FS. The pattern of spinal tuberculosis in Sarawak General Hospital. The Medical journal of Malaysia 2001;56(2):143-50.

72.   Hella JJ, Hiza HC, Mfinanga E, Reither K. Paraspinal abscess secondary to tuberculous spondylitis diagnosed by Xpert MTB/RIF assay in rural Tanzania. BMJ case reports 2013;2013.

73.   A C, RB D, JZ M. Clinical characteristics and treatment outcomes of patients with isoniazid-monoresistant tuberculosis. Clin Infect Dis 2009.;48:179–85.

74.   Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. Clinical infectious diseases 2012;55(8):1088-95.

75.   Held M, Laubscher M, Dix-Peek S, Zar H, Dunn R. Accuracy of GeneXpert PCR Testing in Osteoarticular TB. Bone & Joint Journal Orthopaedic Proceedings Supplement 2014;96(SUPP 19):59-.

76.   Laubscher M, Held M, Zar H, Dunn R, editors. GeneXpert polymerase chain reaction for spinal tuberculosis-An accurate and rapid diagnostic test. International Meeting on Advanced Spine Techniques; 2014; Valencia.

Appendices

Ethics approval

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/research/humanethics/forms

06 June 2013

HREC REF: 264/2013

Prof R Dunn
Orthopaedic Surgery
OMB

Dear Prof Dunn

PROJECT TITLE: GENEXPERT ASSAY AS DIAGNOSTIC TOOL OF MUSCULOSKELETAL TB

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 4th June 2013.

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

Approval is granted for one year till the 30th June 2014

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/research/humanethics/forms)

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

Please quote the HREC. REF in all your correspondence.

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Information sheet for patients

INFORMATION FOR STUDY: GeneXpert as diagnostic tool of musculoskeletal tuberculosis

Institution	Individuals
Groote Schuur Hospital	**Prof. Dunn, M. Held, M. Laubscher**

THE INFROMATION BELOW WILL BE SUPPLIED TO ALL PATIENTS TAKING PART IN THIS STUDY.

Your symptoms

You/your child have been admitted and examined by our doctors. Because of your symptoms the doctors suspect you may have TB. Depending on your symptoms, the normal standard of care for TB investigations in our institution usually includes a chest x-ray, and HIV test, blood tests and collection of tissue to look for TB.

What is this study about?

We are carrying out medical research to find better ways of preventing and treating TB in the future for everybody's benefit. We want to find ways to diagnose TB quicker and more precisely. To achieve this we would like to assess if the new test we are performing on all of our patients with suspected TB is accurate enough to make this possible (GeneXpert). This test is performed on all tissues we collect during biopsies of our patients, regardless of their participation in this study. To help us learn more we are asking our patients to allow us to use the data we are collecting from their samples for research purposes. There will be no personal data included in the database.

What will it involve for me/my child?

You/your child will be treated no different to anyone who does not take part in this study. The sample we collect will be assessed in the laboratory in the same way as if you would not take part in this study.
If there are any other research activities that our staff would like you to participate in, staff shall explain and ask your permission first.

Are there any risks or disadvantages to me or my child in taking part?

You/your child will have exactly the same risks as someone - with your condition - not taking part in this study.

Are there any benefits to me/my child of taking part?

There are no additional benefits for you/your child.

What happens if I refuse to participate?
All participation in research is voluntary. You are free to decide if you want to take part. If you do agree you can change your mind at any time and withdraw from the research. This will not affect your/your child's care now or in the future.

What happens to the samples?

They will be sent to the national health laboratory. Your samples will be treated the same way as samples of patients who are not participating in the study.

Who will have access to information about me/my child in this research?

The doctors who are also involved in treating your medical condition. Any additional staff involved (Research assistance, statisticians) with the research project will see your data WITHOUT your personal details.

Who has allowed this research to take place?

Our departmental research committee and the local ethics committee have looked carefully at this work and agreed that the research is important, that it will be conducted properly and participants' safety and rights have been respected.

What if I have any questions?
You may ask any of our staff questions at any time. The principle investigator of this study is:
Prof. Robert Dunn, Head: Division of Orthopaedic Surgery, Spine Deformity Service Red Cross Children's Hospital
Secretaries: University of Cape Town, Mrs Priest, tel. 021 404 5108

If you want to ask someone independent anything about this research please contact
If you have any questions, concerns or complaints about this research study, its procedures, risks and benefits, or alternative courses of treatment, you should ask the Chairperson of the Human Research Ethics Committee, Prof Blockman: 021 406 6492.

Authors' Guidelines

Abstract

- ☐ Structured use concise prose (Aims, Patients (Materials) and Methods, Results, Conclusion)
- ☐ Take home message – one sentence summing up the bottom line message, why is this paper important
- ☐ No level of evidence
- ☐ Clear hypothesis/question asked
- ☐ The design and the results briefly described
- ☐ Where this paper fits into the current knowledge stated

Introduction

- ☐ Background given
- ☐ The question being asked/hypothesis tested included
- ☐ Study design stated

Patients and Methods

- ☐ How many patients and why clearly stated
- ☐ Was a power study carried out? If so, include the details
- ☐ How were the patients chosen? Inclusion/exclusion criteria clearly stated
- ☐ Were they randomised? How?
- ☐ Demographics included
- ☐ What tests were carried out? State measurements/units used
- ☐ What outcome scores were used? Were they validated?
- ☐ State who recorded results, were they blinded?
- ☐ How were the controls chosen?
- ☐ Explicitly state the number of patients, cases, joints involved etc
- ☐ Information regarding bilateral cases included
- ☐ Where percentages are quoted, ensure that the absolute numbers have been given
- ☐ Details of patients lost to follow-up included, details given
- ☐ What period of time does the study cover? Why?
- ☐ Include life table/survival analysis included where appropriate
- ☐ State ethical approval/informed consent received if needed

Statistics

- ☐ State the tests used and include references
- ☐ Exact p values included for all statistical values, and the test stated
- ☐ State the level of significance

Results

- ☐ State absolute number where percentages are quoted
- ☐ Means and ranges, or medians and interquartile ranges stated
- ☐ Presented clearly and in a logical order
- ☐ Numbers/outcomes/tests/follow-up all match text and tables/figures
- ☐ Units given for any results

Discussion

- ☐ It is appropriate to the question?
- ☐ State if / how strongly your results support your conclusion? How strongly?
- ☐ State how your results fit into the current knowledge
- ☐ Will your results change clinical practice? If so, state how

References

- ☐ Are they from studies within the last 10 years?
- ☐ Are they inclusive?
- ☐ Reduce bias wherever possible
- ☐ Reduce the number cited to only those that are fully relevant

Tables

- ☐ Do not repeat results presented in the text, only new or additional information
- ☐ Ensure the legends comprehensive and clearly state what the table shows

Figures

- ☐ Only provide a maximum of ten (counting a, b, c separately)
- ☐ Ensure legends are comprehensive and clearly state what the figure shows and state what any additional notes or lettering represent
- ☐ Ensure that all axis labels have been applied to all charts or graphs
- ☐ Stains given if presenting histology
- ☐ Magnification given if presenting histology
- ☐ Submitted individually. Please split composite images into their separate components

General

- ☐ Ensure it is no longer than 4,000 words
- ☐ Ensure that all abbreviations have been spelt out at first use
- ☐ The manuscript has been blinded and any institutional or author names removed
- ☐ A letter of transmittal has been signed by all authors