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Diagnostic Accuracy of the Xpert MTB/RIF Assay for Extrapulmonary Tuberculosis in Children with Musculoskeletal Infections

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Abstract

Background—Xpert MTB/RIF (Xpert) is useful for the diagnosis of extrapulmonary TB in adults, but there is limited evidence on its usefulness in children. We aimed to investigate the accuracy of Xpert for the diagnosis of extrapulmonary TB in children with musculoskeletal infections.

Methods—The diagnostic accuracy of Xpert was compared with 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 with 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.

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Ethics approval

The study was approved by the human research ethics committee of the Faculty of Health Sciences, University of Cape Town [HREC REF: 264/2013]. This was in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Conclusions—Xpert confirmed extrapulmonary TB of bone and joints more accurately and faster than culture and should be used as a first line test. Histology remains a useful test for musculoskeletal TB in children.

Keywords

Xpert; Tuberculosis; child; extrapulmonary; accuracy

Introduction

The World Health Organization (WHO) estimates 9 million newly diagnosed tuberculosis cases in 2013, 25% in Africa and more than 500 cases per 100000 inhabitants in South Africa; childhood TB is estimated to account for 15-20% of the total caseload in African countries.(1) Extrapulmonary disease accounts for 10% to 42% of TB cases, of which around 10-25% have musculoskeletal TB. (1-3)

At Red Cross Children's Hospital in Cape Town, the largest children's hospital in sub-Saharan Africa, approximately 20 children younger than 12 years of age are treated for musculoskeletal TB each year (60% spinal TB, 20% in knee, 16% in hip and less than 5% TB of the ankle or upper limb).(4) The sensitivity of TB culture compared with 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).(4-7) Non-automated nucleic acid amplification tests in synovial joint biopsies of children have a sensitivity of only 40% when compared with culture or histology,(8) 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.(6, 8-11) Additionally, MDR TB has a prevalence of 5% in musculoskeletal TB in our population(12) making a correct diagnosis on tissue biopsy imperative to identify resistance and initiate optimal treatment.

Xpert MTB/RIF is a rapid, automated, onsite nucleic acid amplification test and 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%(13) and in a recent meta-analysis(14) 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.(15) We previously reported on a sensitivity of 95.6% and specificity of 96.2% in 71 spinal samples from adults when compared to culture or histology as a reference standard.(12) Another study(16) compared Xpert in 60 orthopedic fluid samples with culture and found a sensitivity of 63.6%. Currently no large studies have assessed the accuracy of Xpert in childhood extrapulmonary TB, or specifically in osteoarticular TB. Children are known to present with paucibacillary disease compared to adults, which might influence the accuracy of diagnostic tests. We

therefore aimed to assess the diagnostic accuracy of Xpert in children with suspected extrapulmonary disease in form of musculoskeletal TB.

Methods

The diagnostic accuracy of Xpert was evaluated prospectively in consecutive 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 included. 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 a chest radiograph, suggestive of TB or a radiograph of an affected joint, showing erosions and osteopenia involving both sides of the 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. As part of the routine clinical workup, radiologically predetermined areas of disease were biopsied surgically through an open approach, under sterile conditions and general anesthesia. Adhering to local hospital guidelines, Xpert, culture and histology were done for all patients. Informed consent was taken from a parent or legal guardian. Spinal biopsies were performed by a subspecialist spinal unit. A subspecialist pediatric orthopedic 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 MTB/RIF 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 testing. The specimen was mixed with Xpert SR lysis buffer at a ratio of 1:3. Two milliliters 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, Sparks 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 Centers 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 epithelioid 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.

Statistical Analysis

The accuracy of Xpert MTB/RIF (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 analyzed using descriptive statistics, mean and 95% confidence intervals were used in normally distributed data, and 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 $\alpha = 0.05$.

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 co-infected, 27/30 (90%) were HIV uninfected.

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) monoresistance, 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 extrapulmonary 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,(17) the sensitivity was lower than that published for musculoskeletal samples in adults. (12, 15, 18) 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-infected 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-infected 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.

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References

1. World Health Organization. Global Tuberculosis report. 2014
2. 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; 46:186. [PubMed: 22448057]
3. Trecarichi E, Di Meco E, Mazzotta V, Fantoni M. Tuberculous spondylodiscitis: epidemiology, clinical features, treatment, and outcome. *Eur Rev Med Pharmacol Sci*. 2012; 16:58–72.
4. Maqungo S, Oleksak M, Dix-Peek S, Hoffman E. Tuberculosis of the foot and ankle in children. *SA Orthopaedic Journal*. 2012; 11:23–28.
5. Dix-Peek SI, Vrettos BC, Hoffman EB. Tuberculosis of the elbow in children. *Journal of shoulder and elbow surgery*. 2003; 12:282–286. [PubMed: 12851583]
6. Campbell J, Hoffman E. Tuberculosis of the hip in children. *Journal of Bone & Joint Surgery, British Volume*. 1995; 77:319–326.
7. Lee A, Campbell J, Hoffman E. Tuberculosis of the knee in children. *Journal of Bone & Joint Surgery, British Volume*. 1995; 77:313–318.
8. Hoffman EB, Allin J, Campbell JAB, Leisegang FM. Tuberculosis of the Knee. *Clinical orthopaedics and related research*. 2002; 398:100–106. [PubMed: 11964637]
9. Watts HG, Lifeso RM. Tuberculosis of bones and joints. *The Journal of bone and joint surgery American volume*. 1996; 78:288–298. [PubMed: 8609123]
10. Rajasekaran S, Shanmugasundaram T. Prediction of the angle of gibbus deformity in tuberculosis of the spine. *The Journal of Bone & Joint Surgery*. 1987; 69:503–509. [PubMed: 3571308]
11. Mohideen M, Rasool M. Tuberculosis of the hip joint region in children. *SA Orthopaedic Journal*. 2013; 12:38–43.
12. 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:1366–1369. [PubMed: 25274923]
13. 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; 11:819–824. [PubMed: 21764384]
14. 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; 70:392–399. [PubMed: 25312863]
15. 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; 11:23–27.
16. 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. [PubMed: 26004172]
17. Zar HJ, Workman L, Isaacs W, et al. Rapid molecular diagnosis of pulmonary tuberculosis in children using nasopharyngeal specimens. *Clinical infectious diseases*. 2012; 55:1088–1095. [PubMed: 22752518]
18. 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; 20:145–148. [PubMed: 10072817]

Table 1

Sites of biopsies.

Site	Number (%)
Spine	14 (12)
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

Table 2

Characteristics of children and biopsy sites (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)	
Male patients (%)		60 (58.8)	7 (53.9)	3/9 (33.3)	50/80 (62.5)	0.224
Age (months) –		66.8	51.1	60.2	68.3	0.479
Median (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 samples (%)	Sensitivity (N, %, CI 95%)	Specificity (N, %, CI 95%)	PPV (N, %, CI 95%)	NPV (N, %, CI 95%)
Xpert	17 (15.6)	17/23	86/86	17/17	86/92
		73.9 (51.6 – 89.8)	100.0 (95.7 – 100.0)	100.0 (81.6 – 100.0)	93.5 (86.3 – 97.6)
AFB only *	15 (13.8)	15/23	85/85	15/15	85/93
		65.2 (42.7 – 83.6)	100.0 (95.4 – 100.0)	100.0 (79.6 – 100.0)	91.4 (83.8 – 96.2)
Histology	23 (21.9)	23/23	82/82	23/23	82/82
		100.0 (85.7 – 100.0)	100.0 (95.5 – 100.0)	100.0 (85.7 – 100.0)	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	<0.00	< 0.00	<0.00

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