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Diagnostic value of Xpert MTB/RIF Ultra for osteoarticular tuberculosis

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SUMMARY

Objectives: The diagnosis of osteoarticular tuberculosis (TB) remains challenging and results in under- or over-diagnosis. The aim of the present study was to evaluate performance of the novel next-generation Xpert MTB/RIF Ultra (Xpert Ultra) in comparison to culture and Xpert MTB/RIF (Xpert) for osteoarticular TB diagnosis in high burden settings.

Methods: Osteoarticular TB suspected cases were enrolled consecutively during June 2017 to June 2018 at Beijing Chest Hospital and their pus specimens were subjected to smear, culture, Xpert and Xpert Ultra. Drug susceptibility testing (DST) was conducted for all of the recovered isolates. The performances of Xpert Ultra and Xpert were evaluated using composite reference standard (CRS) as gold standard, which included clinical, laboratory, histopathological, radiological and ≥6 months' follow-up data.

Results: In total, 186 patients were recruited, and 132 of them were diagnosed with osteoarticular TB according to CRS. The direct head-to-head performance comparison for *M. tuberculosis* detection showed that Xpert Ultra (90.91%, 120/132) produced a higher sensitivity than Xpert (78.79%, 104/132, $P = 0.006$) and culture (39.39%, 52/132, $P < 0.001$). When Xpert Ultra outcomes were integrated, the percentage of confirmed osteoarticular TB case increased from 84.09% (111/132) to 93.94% (124/132). The specificities of Xpert and Xpert Ultra were 100% (34/34) and 97.06% (33/34), respectively. Both Xpert Ultra and Xpert accurately identified all of the 9 rifampicin (RIF)-resistant and 38 RIF-sensitive cases defined by phenotypic DST. Therefore, Xpert Ultra was 100% concordant with phenotypic DST for the detection of RIF resistance.

Conclusions: Xpert Ultra detected significantly more osteoarticular TB cases than Xpert or culture, making it a useful tool for rapid diagnosis of osteoarticular TB.

Introduction

Tuberculosis (TB) is one of the top ten causes of deaths and the leading cause from a single infectious agent, with an estimated 1.3 million deaths in 2017.[1] Extrapulmonary tuberculosis (EPTB) accounted for 14% of the 6.4 million incident cases.[1] Osteoarticular TB is ranked as the third most frequent form of EPTB.[2] Early diagnosis and initiation of appropriate therapy are the best ways to prevent severe sequelae and mortality for osteoarticular TB patients. Therefore, continual progress in osteoarticular TB diagnosis is highly important.

The Xpert MTB/RIF (Xpert) (Cepheid, Sunnyvale USA) assay, can detect *Mycobacterium tuberculosis* (Mtb) and its rifampicin (RIF) susceptibility within 2 h.[3] Besides pulmonary TB, the World Health Organization (WHO) also endorsed this technique for EPTB diagnosis in 2013.[4] Xpert sensitivity was >80% and specificity was ≥90% with pus and tissue specimens of bone or joint for osteoarticular TB diagnosis.[5–10] A next generation Xpert MTB/RIF assay, termed Xpert MTB/RIF Ultra (Xpert Ultra) (Cepheid, Sunnyvale USA), was developed for increased sensitivity. Xpert Ultra had a substantially lower limit of detection (LOD) i.e. 15.6 CFU/ml in contrast with Xpert (112.6 CFU/ml).[11] Several studies also demonstrated that Xpert Ultra had higher sensitivity in diagnosing PTB,[12–14] EPTB,[15–17] and pediatric TB[18] in contrast with Xpert. As a new diagnostic tool, Xpert Ultra shows tremendous potential for TB diagnosis, especially for cases with paucibacillary characterizations. However, data on the diagnostic performance of Xpert Ultra in osteoarticular TB remains limited. The aim of the present study was to analyze the diagnostic value of Xpert Ultra for osteoarticular TB comparing with culture and Xpert in a high TB burden and low HIV burden setting.

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Material and methods

Ethical approval

The ethical approvals for this study were obtained from the Beijing Chest Hospital Ethics Committee. A written informed consent was acquired from each participant.

Study design and participants

Adults with suspected osteoarticular TB were prospectively and consecutively enrolled during June 2017 to June 2018 at Beijing Chest Hospital (Beijing, China), which is the only national level TB referral center in China. All the patients with clinical features suggestive of osteoarticular TB were enrolled, and included local symptoms such as pain, swelling in the joints, tenderness, effusion, restriction of movements, and systematic symptoms such as fever, loss of weight/appetite, elevated erythrocyte sedimentation rate (ESR), and other clues of TB such as cough, breathlessness, and history of TB. All the patients were followed up for a minimum of 6 months. Each pus specimen collected during operation from each patient was subjected to smear microscopy, culture, Xpert and Xpert Ultra, while tissue specimen was subjected to histopathological examination, simultaneously. Drug susceptibility testing (DST) was conducted for all of the recovered isolates.

Patient categories

Patients were divided into 4 groups according to the composite reference standard (CRS),[10] which was composed of clinical, laboratory, histopathological, and radiological examinations and follow-up data. (1) Definite or confirmed osteoarticular TB: bacteriological evidence was acquired by any of the smear microscopy, culture or Xpert; (2) probable osteoarticular TB: bacteriological evidence was negative; clinical symptoms, radiological findings, and histopathological examinations were suggestive of TB; (3) possible osteoarticular TB: bacteriological evidence was negative; clinical symptoms were suggestive of TB; histopathology examination and/or radiological examination were mildly suggestive of TB; the patient responded well to the empirical anti-tubercular treatment during the follow-up term; and (4) non-osteoarticular TB: diagnosed as other disease, or the laboratory testing was not suggestive of TB, and the patient improved without receiving anti-tubercular treatment.

Smear and culture

Direct smear was prepared and stained with auramine and examined by light-emitting diode microscopy. The smear was read and interpreted in accordance with the WHO guidelines.[19] After processing with N-acetyl-L-cysteine and sodium hydroxide (NALC–NaOH) and centrifugation, the resuspended pellet was subjected to cultivation on both solid Lowenstein-Jensen medium (Encode Medical Engineering Co., Ltd, China) and liquid medium using mycobacteria growth indicator tube (MGIT) 960 system (Becton, Dickinson and Company, USA). The time to positivity was recorded. For all the isolates, MPT64 antigen testing was performed to confirm the presence of Mtb complex.

Histopathological examination

The tissue specimens were fixed in neutral formalin, dehydrated and subsequently paraffin-embedded. Four-micrometer sections were stained with hematoxylin and eosin solution and observed by light microscopy for patho-morphological changes. Acid-fast bacilli (AFB) or its DNA were detected from the fixed specimens either by ZN staining or by molecular testing as described before.[20] The pathological diagnostic categories included (1) Confirmed TB: chronic granulomatous inflammation with or without caseous necrosis were observed, and AFB or its DNA were detected in the lesion; (2) suggestive of TB: typical chronic granulomatous inflammation with caseous necrosis observed but negative bacteriological examination; and (3) non-osteoarticular TB: neither granulomatous inflammation nor caseous necrosis was observed, and no bacteriological evidence was present.

Xpert and Xpert Ultra

The Xpert and Xpert Ultra assays were performed as per the manufacturer's instructions. Briefly, 1 ml pus specimen was mixed with 2 ml sample reagent, vortexed for at least 10 s, and incubated at room temperature for 10 min. The mixture was vortexed for another 10 s and incubated at room temperature for 5 min. 2 ml of the mixture was transferred into the cartridge and loaded into the GeneXpert instrument. The automatic detection procedure was then run. For an invalid result, a repeat Xpert/ Xpert Ultra test was performed on the same sample. Semi-quantitative estimation of Mtb load was also determined by Xpert Ultra as "high", "medium", "low", "very low" or "trace", depending on cycle threshold (C_T) value.

Drug susceptibility testing

Culture positive samples were also subjected for drug susceptibility testing (DST) with proportion method using Lowenstein-Jensen medium. The critical concentration of 40 μg/ml was used for RIF.

Statistical analyses

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different assays were calculated against the reference standard. We used SPSS version 19.0 (IBM, Armonk, NY, USA) to compare baseline clinical characteristics and demographic data by diagnosis via Manne-Whitney *U* test for continuous variables and Chi-squared test for categorical variables. Differences were considered statistically significant at $P < 0.05$.

Results

Patient characteristics

In total, 186 osteoarticular TB suspected cases were recruited. 20 participants were excluded from the analysis due to lack of definite diagnosis ($n = 9$), indeterminate Xpert results ($n = 5$), indeterminate Xpert Ultra results ($n = 4$) or absence of enough specimen ($n = 2$). Thus, the final sample size for analysis was 166 patients (Fig. 1), which included 132 (79.52%) osteoarticular TB patients (including 111 definite cases, 9 probable and 12 possible cases) and 34 (20.48%) non-osteoarticular TB patients (including 27 suppurative spondylitis, 3 rheumatoid arthritis, 2 brucellosis and 2 cancer cases). Osteoarticular TB patients were younger than the non-osteoarticular TB patients ($P < 0.005$). High proportion of osteoarticular TB patients (25.00%) also combined with pulmonary TB in contrast with the non-osteoarticular TB patients (6.06%, $P = 0.015$). The recruited non-osteoarticular TB patients had higher diabetes mellitus comorbidity than the TB group ($P < 0.001$), although the reason was not under investigation in this assay. All of the patients were HIV-uninfected. Demographic and clinical characteristics are shown in Table 1.

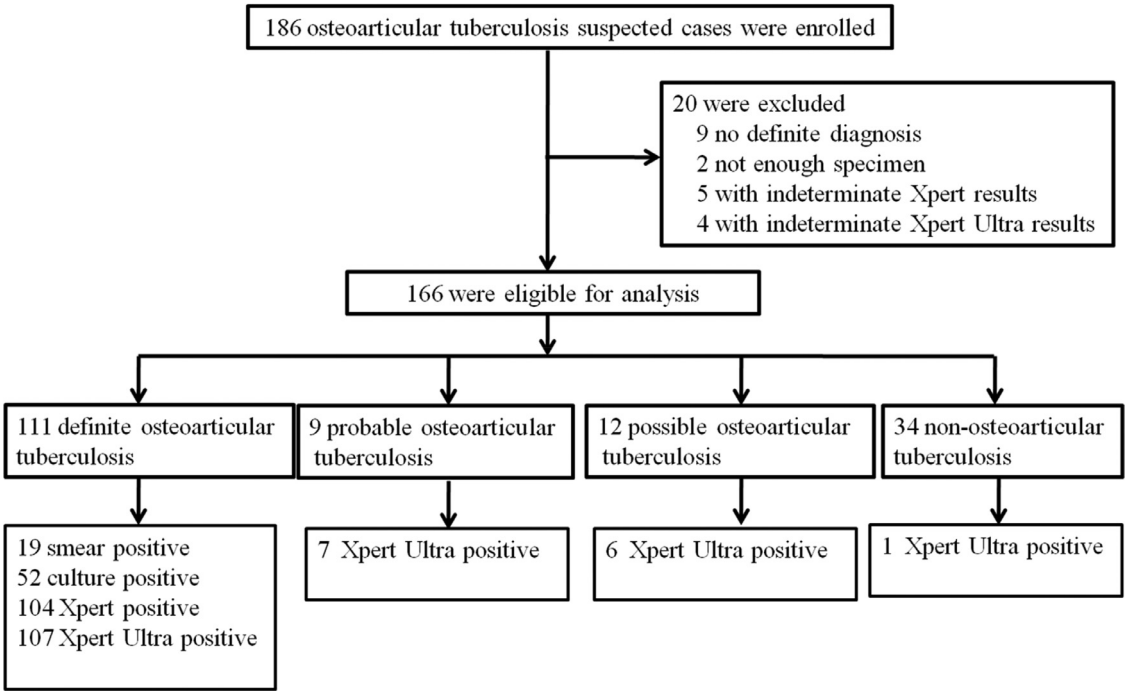

Fig. 1. Recruitment and diagnostic classification of the participants.

Table 1
Demographic and clinical characteristics of the participants.

Characteristics	Osteoarticular TB ($n = 132$)	Non-osteoarticular TB ($n = 34$)	P value
Age, median (range), yr	51 (16–86)	58 (14–82)	**0.005**
Gender			**0.037**
Male	59(44.70)	22(64.71)	
Female	73(55.30)	12(35.29)	
Underlying condition			
Diabetes mellitus	14(10.61)	12(35.29)	**<0.001**
Hypertension	33(25.00)	12(35.29)	0.229
Chronic kidney disease	1(0.76)	0(0)	0.611
Rheumatologic disease	2(1.52)	0(0)	0.470
Combined TB			
Pulmonary TB	33(25.00)	2(6.06)	**0.015**
Tuberculous meningitis	2(1.52)	0(0)	0.470
Tuberculous pleurisy	5(3.79)	0(0)	0.249
Infected site			
Cervical vertebra	2(1.52)	1(2.94)	0.578
Thoracic vertebrae	36(27.27)	8(23.53)	0.659
Lumbar vertebrae	35(26.52)	14(41.18)	0.095
Hip joint	9(6.82)	0(0)	0.117
Knee joint	8(6.06)	2(5.88)	0.969
Elbow joint	5(3.79)	2(5.88)	0.588
Shoulder joint	3(2.27)	1(2.94)	0.821
Sacroiliac joint	3(2.27)	1(2.94)	0.821
Ankle joint	2(1.52)	1(2.94)	0.578
Femur	5(3.79)	0(0)	0.249
Tarsal	4(3.03)	0(0)	0.304
Humerus	4(3.03)	0(0)	0.304
Rib	3(2.27)	2(5.88)	0.272
Ilium	3(2.27)	0(0)	0.375
Other bones	10(7.58)	2(5.88)	0.734

Other bones including tibia, calcaneus, sternum, ulna, publis and sciatic bone.

Performance of Xpert Ultra in osteoarticular TB diagnosis

All of the 132 osteoarticular TB patients enrolled had undergone 2–4 weeks of anti-TB treatment before specimen collection. Among these osteoarticular TB patients, 19 were positive for the smear test, 52 by any culture method, 104 by Xpert, and 120 by Xpert Ultra assay in pus. The direct head-to-head performance comparison for Mtb detection showed that Xpert Ultra (90.91%, 120/132) produced a higher sensitivity than Xpert (78.79%, 104/132, $P = 0.006$), culture (39.39%,52/132, $P < 0.001$) and smear (14.39%,19/132, $P < 0.001$), respectively (Table 2). Stratified analysis of osteoarticular TB cases according to the smear and culture status was conducted. Sensitivity of Xpert Ultra was higher than Xpert among both smear-negative cases (89.38%, 101/113 vs 75.22%, 85/113; $P = 0.005$) and culture-negative cases (87.5%, 70/80 vs 67.5%, 54/80; $P = 0.002$). Xpert Ultra and Xpert had the same

Table 2
Performance of different methods for osteoarticular tuberculosis diagnosis.

Patient group	Smear	Culture	Xpert	Xpert Ultra	*P* value
Culture positive osteoarticular TB					
Sensitivity	16/52(30.77)	–	50/52(96.15)	50/52(96.15)	1
Specificity	34/34(100)	–	34/34 (100)	33/34(97.06)	0.314
PPV	16/16(100)	–	50/50(100)	50/51(98.04)	0.320
NPV	34/70(48.57)	–	34/36(94.44)	34/36(94.44)	1
Definite osteoarticular TB					
Sensitivity	19/111(17.12)	52/111(46.85)	104/111(93.69)	107/111(96.40)	0.354
Specificity	34/34(100)	34/34(100)	34/34(100)	33/34(97.06)	0.314
PPV	19/19(100)	52/52(100)	104/104(100)	107/108(99.07)	0.325
NPV	34/126(26.98)	34/93(36.56)	34/41(82.93)	33/37(89.19)	0.427
Probable osteoarticular TB					
Sensitivity	–	–	–	7/9(77.78)	
Specificity	–	–	–	33/34(97.06)	
PPV	–	–	–	7/8(87.50)	
NPV	–	–	–	33/35(94.29)	
Possible osteoarticular TB					
Sensitivity	–	–	–	6/12(50.00)	
Specificity	–	–	–	33/34(97.06)	
PPV	–	–	–	6/7(85.71)	
NPV	–	–	–	33/39(84.62)	
Osteoarticular TB total					
Sensitivity	19/132(14.39)	52/132 (39.39)	104/132 (78.79)	120/132 (90.91)	0.006
Specificity	34/34(100)	34/34(100)	34/34(100)	33/34(97.06)	0.314
PPV	19/19(100)	52/52(100)	104/104(100)	120/121(99.17)	0.353
NPV	34/147(23.13)	34/114(29.82)	34/62(54.84)	33/45(73.33)	0.051

P values represent the difference between Xpert and Xpert Ultra.

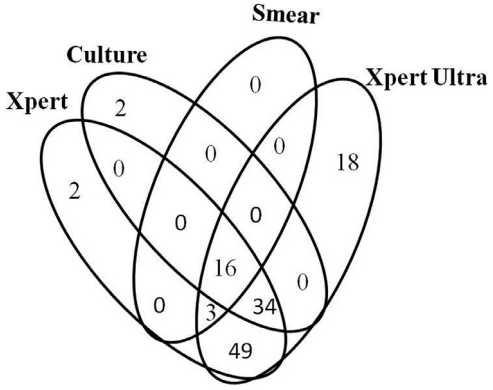

Fig. 2. Venn diagram of the overlap of between methods for osteoarticular TB diagnostics.

sensitivity among smear-positive (both 100%, 19/19) and culture-positive cases (both 96.15%, 50/52). The specificities of smear, culture, Xpert and Xpert Ultra were 100% (34/34), 100% (34/34), 100% (34/34) and 97.06% (33/34), respectively. One fungal infection case produced positive Xpert Ultra result.

When Xpert Ultra outcomes were also integrated, 7 out of the 9 (77.78%) probable and 6 of the 12 (50.00%) possible osteoarticular TB cases were re-classified as definite cases, the percentage of definite osteoarticular TB patients showed an obvious increase from 84.09% (111/132) to 93.94% (124/132) ($P=0.011$). Ultimately, a total of 124 patients had evidence of Mtb in pus specimens, which meant at least one positive outcome from smear, culture, Xpert or Xpert Ultra. Among these 124 patients, 18 were detected by Xpert Ultra assay only, compared to 0, 2 and 2 from smear, culture and Xpert assay, respectively (Fig. 2).

Performance of Xpert Ultra in RIF resistance detection

52 participants had pus culture-positive outcomes and phenotypic DST results. Among them, Xpert Ultra provided interpretable RIF resistance detection results for 47 participants and 3 trace results, whereas Xpert provided interpretable results for 50 participants. Among the 47 cases with phenotypic DST outcomes and eligible Xpert and Xpert Ultra results, both Xpert Ultra and Xpert correctly identified all of the 9 RIF-resistant and 38 RIF-sensitive cases defined by phenotypic DST. Therefore, the sensitivity and specificity for both Xpert Ultra and Xpert was 100%, respectively. Besides, among 80 culture-negative osteoarticular TB patients, Xpert Ultra provided interpretable RIF resistance detection results for 49 patients including 4 RIF-resistant cases, while the counterpart for Xpert were 54 patients and 3 RIF-resistant cases.

Table 3
Correlation between Xpert Ultra semi-quantitative and smear results.

Xpert Ultra semi-quantitative	Smear microscopy				% of smear positive specimens
	Negative	1+	2+	Total	
Positive high	4	3	1	8	50.00
Positive medium	8	7	0	15	46.67
Positive low	49	8	0	57	14.04
Positive very low	16	0	0	16	0
Positive trace	24	0	0	24	0
Negative	12	0	0	12	0
Total	113	18	1	132	

Relationship between the semi-quantitative readouts of Xpert Ultra and bacilli-load in the sample

The semi-quantitative readouts for the positive Xpert Ultra samples in this study were as follows: 6.67% (8/120) “high”, 12.50% (15/120) “medium”, 47.50% (57/120) “low”, 13.33% (16/120) “very low” and 20.00% (24/120) “trace”. Smear-positive specimens accounted for 50.00% (4/8), 46.67% (7/15), and 14.04% (8/57) of the Xpert Ultra-positive readouts at “high”, “medium”, and “low” outcomes, respectively (Table 3). Specimens with Xpert Ultra-positive readouts at “very low” ($n=16$), “trace” ($n=24$), or “negative” ($n=12$) results were all smear negative. Time to positivity of

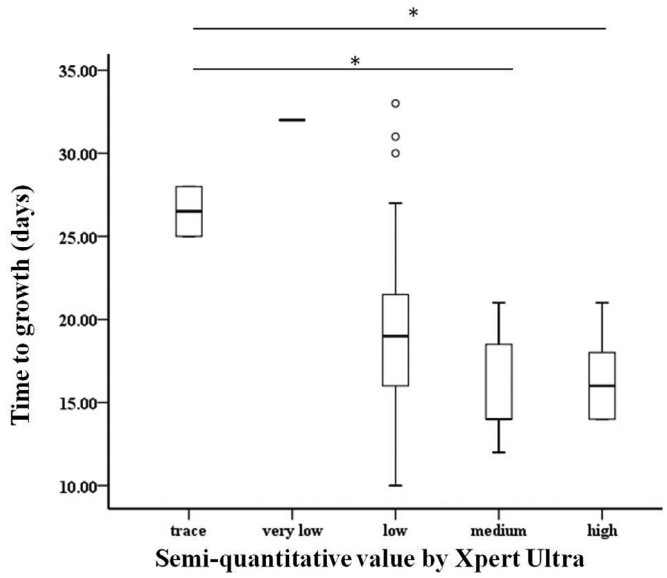

Fig. 3. Turn-around time of positive culture for pus samples classified according to the semi-quantitative value provided by Xpert Ultra.
Boxes represent 25th, 50th and 75th percentiles of the data. The length of the box is the interquartile range. The lower and upper whiskers represent minimum and maximum.
Individual data points represent outliers.
* represents statistically difference between "trace" and "medium" category ($P = 0.004$), and between "trace" and "high" category ($P = 0.004$) by Manne–Whitney U test.

MGIT960 is generally used to reflect the bacilli-load in the samples. Regarding the time required for culture-positive growth in this assay (Fig. 3), significant differences were found between specimens that produced category "trace" outcomes (26.5 ± 2.12 days) in contrast with "medium" (16.00 ± 3.32 days, $P = 0.004$) and "high" category outcomes (16.50 ± 2.74 days, $P = 0.004$).

Discussion

Osteoarticular TB is an ancient disease that has disfigured and disabled many patients around the world by causing skeletal deformities and neurological sequelae.[21] An early and accurate diagnosis is therefore highly important to avoid these severe consequences. Xpert Ultra has shown improved sensitivity for diagnosis of pulmonary TB.[12–14,18] Three studies have verified that the Xpert Ultra also outperformed other tests currently used for EPTB diagnosis.[15–17] In this study, we focused on osteoarticular TB, which has never been well evaluated with Xpert Ultra. The prospective design and ≥6 months of follow-up step were important strengths of this study.

In this assay, Xpert Ultra (90.91%) demonstrated higher sensitivity than Xpert (78.79%, $P < 0.05$), acquiring an incremental benefit of 12.12%. The result is in line with the recent reports that utilized the non-sterile fluids (including 8 joint fluids) and obtained 15.7–27% higher sensitivities with Xpert Ultra than Xpert.[16] In this study, the sensitivity of Xpert Ultra (90.91%) was also significantly higher than culture (39.39%, $P < 0.001$). Although Xpert Ultra (15.6 CFU/mL)[11] has a similar limit of detection in vitro with liquid culture (10–50 CFU/mL),[22] Xpert Ultra has the added advantage of being able to detect non-viable Mtb bacilli in patients who had commenced anti-TB treatment prior to sample collection.[11] All of the patients enrolled in our study had undergone 2–4 weeks of anti-TB treatment before specimen collection, which may result in the low sensitivity of culture. Furthermore, the value of concomitant use of different tests was also considered. Because of the high sensitivity of Xpert Ultra assay, concatenating it with culture or Xpert did not show any obvious benefit. In our assay, the Xpert assay greatly changed the composition percentage of confirmed categories. Without Xpert Ultra results, 84.09% of the osteoarticular TB were classified as confirmed cases that increased to 93.94% after the integration of Xpert Ultra outcomes. In clinical practice, high sensitivity of Xpert Ultra could facilitate diagnosis of osteoarticular TB at earlier stages of disease. Owing to shorter turnaround time, a timely Xpert Ultra assay may change the algorithm of osteoarticular TB patient care.

Drug-resistant osteoarticular TB is an emerging health problem. From a public health perspective, reducing the time between diagnosis and treatment initiation has direct benefits for both the patient and the community.[23] Phenotypic DST remains the gold standard for drug sensitivity testing, which depends on culturing of Mtb. Due to the paucibacillary nature of osteoarticular TB, the sensitivity of culture is relatively low. As a consequence, diagnosis of drug-resistant osteoarticular TB is often difficult and delayed. In this study, both Xpert Ultra and Xpert correctly identified all of the 9 RIF-resistant and 38 RIF-sensitive cases defined by phenotypic DST. Considering the accuracy, convenience, and rapid turnaround time, both Xpert Ultra and Xpert could shorten the whole course of medical care and decrease the risk of disabling sequelae caused by osteoarticular TB.

An additional semi-quantitative category of "Trace" was introduced for Xpert Ultra to identify the paucibacillary samples, which indicates that the amplification of IS6110/IS1081 sequence was successful but not the *rpoB* gene.[11] The "Trace" category is the result of improved LOD of the Xpert Ultra. In our study, "Trace" played an important role in improved performance of Xpert Ultra. 24 out of 120 samples detected by Xpert Ultra were detected in trace amounts. Among these 24 samples, 15 were positive by Xpert and only 3 by culture. However, the "Trace" category likely contributes to the decreased specificity of the test. The association of the clinical manifestations of the patient with Xpert Ultra "Trace" results would help to distinguish ongoing active paucibacillary tuberculosis from detection of dead bacilli from previous infections.[13]

Like pulmonary TB,[13] the Xpert Ultra semi-quantitative results were correlated with bacilli-load in the pus specimen in this assay. Both, smear outcomes and time to positivity of liquid culture, are commonly used as indirect indicators for bacterial load and are correlated with the positive outcome levels. This correlation indicated that besides identifying the highly infectious patients (those with Xpert Ultra positive Medium and High), Xpert Ultra can also pick up the cases with less infectiousness, which is very meaningful for TB control.

In conclusion, Xpert Ultra detected significantly more osteoarticular TB than Xpert or culture, making it a useful tool for rapid diagnosis of osteoarticular TB.

Conflict of interests

No conflict of interests to disclose.

Acknowledgment

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References

1. World Health Organization. *Global tuberculosis report 2018*. Geneva, Switherland: World Health Organization; 2018.
2. Qian Y, Han Q, Liu W, Yuan WE, Fan C. Characteristics and management of bone and joint tuberculosis in native and migrant population in shanghai during 2011 to 2015. *BMC Infect Dis* 2018;**18**(1):543.
3. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *New Engl J Med* 2010;**363**(11):1005–15.
4. World Health Organization Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF system for the diagnosis of pulmonary and extra-pulmonary TB in adults and children. *Policy Update*. Geneva, Switzerland: WHO; 2013.
5. Gu Y, Wang G, Dong W, Li Y, Ma Y, Shang Y, et al. Xpert MTB/RIF and genotype MTBDRplus assays for the rapid diagnosis of bone and joint tuberculosis. *Int J Infect Dis* 2015;**36**:27–30.
6. Held M, Laubscher M, Zar HJ, Dunn RN. GeneXpert polymerase chain reaction for spinal tuberculosis: an accurate and rapid diagnostic test. *Bone Joint J* 2014;**96-B**(10):1366–9.
7. Tang L, Feng S, Gao R, Han C, Sun X, Bao Y, et al. A comparative study on the role of xpert MTB/RIF in testing different types of spinal tuberculosis tissue specimens. *Genet Test Mol Biomarkers* 2017;**21**(12):722–6.
8. Wen H, Li P, Ma H, Lv G. Diagnostic accuracy of xpert MTB/RIF assay for musculoskeletal tuberculosis: a meta-analysis. *Infect Drug Resist* 2017;**10**:299–305.
9. Massi MN, Biatko KT, Handayani I, Pratama MY, Septriani S, Nurdin GM, et al. Evaluation of rapid genexpert MTB/RIF method using DNA tissue specimens of vertebral bones in patients with suspected spondylitis TB. *J Orthop* 2017;**14**(1):189–91.
10. Wang G, Dong W, Lan T, Fan J, Tang K, Li Y, et al. Diagnostic accuracy evaluation of the conventional and molecular tests for spinal tuberculosis in a cohort, head-to-head study. *Emerg Microbes Infect* 2018;**7**(1):109.
11. Chakravorty S, Simmons AM, Rowneki M, et al. The new xpert MTB/RIF Ultra: improving detection of mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. *MBio* 2017;**8**(4) pii: e00812-17.
12. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF ultra for detection of mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Lancet Infect Dis* 2018;**18**:76–84.
13. Opota O, Zakham F, Mazza-Stalder J, Nicod L, Greub G, Jaton K. Added value of xpert MTB/RIF ultra for diagnosis of pulmonary tuberculosis in a low-prevalence setting. *J Clin Microbiol* 2019;**57**(2) pii: e01717-18.
14. Wang G, Wang S, Jiang G, Yang X, Huang M, Huo F, et al. Xpert MTB/RIF ultra improved the diagnosis of paucibacillary tuberculosis: a prospective cohort study. *J Infect* 2019;**78**(4):311–16.
15. Bahr NC, Nuwagira E, Evans EE, et al. Diagnostic accuracy of xpert MTB/RIF ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. *Lancet Infect Dis* 2018;**18**:68–75.
16. Perez-Risco D, Rodriguez-Temporal D, Valledor-Sanchez I, Alcaide F. Evaluation of the xpert MTB/RIF ultra assay for direct detection of mycobacterium tuberculosis complex in smear-negative extrapulmonary samples. *J Clin Microbiol* 2018;**27**(9) 56pii: e00659-18.
17. Wu X, Tan G, Gao R, Yao L, Bi D, Guo Y, et al. Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis. *Int J Infect Dis* 2019;**81**:91–6.
18. Sabi I, Rachow A, Mapamba D, et al. Xpert MTB/RIF ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. *J Infect* 2018;**77**(4):321–7.
19. International Union against Tuberculosis and Lung Disease. *Technicalguide: sputum examination for tuberculosis by direct microscopy in low income countries*. 5th ed. Paris, France: International Union against Tuberculosis and Lung Disease; 2000.
20. Wei G, Mu J, Wang G, Huo F, Dong L, Li Y, et al. The reliability analysis of Xpert–positive result for smear-negative and culture-negative specimen collected from bone and joint tuberculosis suspects. *J Thorac Dis* 2016;**8**(6):1205–9.
21. Sharma K, Sharma M, Batra N, Sharma A, Dhillon MS. Diagnostic potential of multi-targeted LAMP (loop-mediated isothermal amplification) for osteoarticular tuberculosis. *J Orthop Res* 2017;**35**(2):361–5.
22. Lawn SD, Mwaba P, Bates M, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. *Lancet Infect Dis* 2013;**13**(4):349–61.
23. Jain AK, Jaggi KR, Bhayana H, Saha R. Drug-resistant spinal tuberculosis. *Indian J Orthop* 2018;**52**(2):100–7.