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ORIGINAL ARTICLE

Utility of Xpert MTB/RIF Ultra and digital chest radiography for the diagnosis and treatment of TB in people living with HIV: a randomised controlled trial (XACT-TB)

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Background: TB is a leading cause of morbidity among HIV positive individuals. Accurate algorithms are needed to achieve early TB diagnosis and treatment. We investigated the use of Xpert MTB/RIF Ultra in combination with chest radiography for TB diagnosis in ambulatory HIV positive individuals.

Methods: This was a randomised controlled trial with a 2-by-2 factorial design. Outpatient HIV clinic attendees with cough were randomised to four arms: Arm 1—Standard Xpert/no chest radiography (CXR); Arm 2—Standard Xpert/CXR; Arm 3—Xpert Ultra/no CXR; and Arm 4—Xpert Ultra/CXR. Participants were followed up at days 28 and 56 to assess for TB treatment initiation.

Results: We randomised 640 participants. Bacteriologically confirmed TB treatment initiation at day 28 were: Arm 1 (8.4% [14/162]), Arm 2 (6.9% [11/159]), Arm 3 (8.2% [13/159]) and Arm 4 (5.6% [9/160]) and between Xpert Ultra group (Arms 3 and 4) (6.9% [22/319]) vs Standard Xpert group (Arms 1 and 2) (7.8% [25/321]), risk ratio 0.89 (95% CI 0.51 to 1.54). By day 56, there were also similar all-TB treatment initiations in the x-ray group (Arms 2 and 4) (16.0% [51/319]) compared with the no x-ray group (Arms 1 and 3) (13.1% [42/321]), risk ratio 1.22 (95% CI 0.84 to 1.78); however, the contribution of clinically diagnosed treatment initiations were higher in x-ray groups (50.9% vs 19.0%).

Conclusions: Xpert Ultra performed similarly to Xpert MTB/RIF. X-rays are useful for TB screening but further research should investigate how to mitigate false-positive treatment initiations.

Keywords: diagnosis, HIV, radiography, TB

Introduction

TB is a leading cause of morbidity and mortality among people living with HIV in Africa. In 2019, there were an estimated 600 000 HIV positive people with TB, of whom 200 000 died, the highest in absolute terms of any world region.[1] Autopsy studies also show that TB is an important cause of death in HIV positive individuals; TB was a cause of death among 43% of HIV positive individuals dying in hospitals in Africa.[2,3] Systematic TB screening and diagnosis using accurate tests is important to achieve timely treatment initiation and to reduce TB disease burden among HIV positive individuals.

Modern tests widely used for TB diagnosis in HIV positive individuals are the Xpert MTB/RIF assay and urine lipoarabinomannan (LAM) assay. Xpert assay has a higher diagnostic yield over smear microscopy but has lower accuracy in those with HIV infection.[4–7] The Xpert MTB/RIF Ultra is a second generation assay with an improved limit of detection (LOD) for *Mycobacterium tuberculosis* of 15.6 colony forming units per millilitre (CFU/ml) compared

with the first generation Xpert with a LOD of 143.4 CFU/ml.[8] Xpert Ultra has a superior sensitivity of 87.6% (95% CI 75.4 to 94.1%) in HIV positive individuals compared with 74.9% (95% CI 58.7 to 86.2%) for Xpert, but with a lower specificity of 92.8% (95% CI 82.3 to 97.0%) vs 99.7% (95% CI 98.6 to 100%).[9] There is need for evidence whether this improved accuracy results in better clinical outcomes for HIV positive individuals. Use of urine-LAM for TB diagnosis in HIV positive individuals admitted to hospital has been shown to lead to improved detection of TB disease and survival.[10]

Chest radiography has historically been used to diagnose pulmonary TB but the availability of mobile digital chest x-ray machines has led to renewed interest in incorporating it in the TB diagnostic pathway.[11] In a systematic literature review and meta-analysis, chest radiography was shown to have improved sensitivity for TB screening in HIV positive individuals (86% [95% CI 69.7 to 92.9%]) compared with symptom screening alone 52% (95% CI 38 to 66%).[12] Modern x-ray machines with computer-aided diagnosis (CAD) software are now available, allowing for a high volume of x-rays to be analysed.[11] However, there is still limited evidence for appropriate placement and impact on patient outcomes of digital chest radiography in the care of patients with respiratory symptoms, especially in the era of new and rapid TB diagnostic tests.

Effective TB screening and diagnosis algorithms with demonstrable impact on patient outcomes are required for improved care of HIV positive individuals. The aim of this study was to investigate the impact on treatment initiation, hospitalisation and survival of the use of Xpert Ultra compared with Xpert MTB/RIF ('Standard Xpert') in ambulatory HIV positive individuals presenting with cough in antiretroviral therapy (ART) clinics in Blantyre, Malawi. The hypothesis was that use of TB symptom screening to identify adults with suspected active TB and subsequently testing them for TB with Xpert Ultra can increase bacteriological confirmation and treatment initiation, and could potentially reduce mortality. We also explored the benefits of additional use of chest radiography (with CAD).

Methods

Study design

The XACT-TB trial was a multi-site, individually randomised controlled trial (RCT) with a 2-by-2 factorial design. The four arms of the trial were: Arm 1—'Standard Xpert/no chest radiography' (standard of care); Arm 2—'Standard Xpert/chest radiography'; Arm 3—'Xpert Ultra/no chest radiography'; and Arm 4—'Xpert Ultra/chest radiography'. The clinical trial was registered with the International Standard RCT number ISRCTN77241966 on 2 September 2019 (available at https://www.isrctn.com/ISRCTN77241966).

The study was conducted at three ART clinics in Blantyre, Malawi, namely, Queen Elizabeth Central Hospital (QECH), Bangwe and Limbe Health Centre from 1 January 2020 to 31 January 2021, with follow-ups completed on 30 April 2021. The routine practice in these ART clinics is that at each visit individuals are asked for the presence of any of the following symptoms—cough of any duration, fever, night sweats and weight loss—and requested to submit sputum samples for testing with Standard Xpert. Patients with TB symptoms and CD4 count $\leq$200 cells/mm^3 also receive testing with urine LAM assay, but this is inconsistently applied due to limited CD4 count testing.

Participants

We recruited ambulant adults aged $\geq$18 y living with HIV and presenting with cough (any duration) and able to submit sputum (spontaneously or induced). We excluded those with danger signs (unable to walk unaided, confused or agitated, breathless when speaking or at rest), those taking TB treatment or who were unable to submit sputum (or refused sputum induction).

Implementation of randomisation schedule

A random allocation sequence was generated by a statistician in advance using computer-generated random numbers. Randomisation was 1:1:1:1 and block-randomised with a variable block size. Paper slips with a randomisation number and intervention were printed in double and inserted in an opaque envelope for the clinic and the laboratory to allow for sequential allocation concealment.

Study procedures

All participants answered a detailed questionnaire on their past medical and drug history, functional status and their place of residence. Each participant's weight and height were measured. Trained research assistants instructed participants to submit two spot sputum samples. The first was tested using either Standard Xpert or Xpert Ultra cartridges depending on the arm allocation, and TB culture was performed on the second sample. Testing using urine-LAM assay was provided to participants as part of routine care.

Randomisation to Xpert Ultra or Standard Xpert was only known by the laboratory technicians. The laboratory technicians were provided with paper slips in envelopes that contained a randomisation number and the method of sputum test allocation.

Sputum samples were analysed using the Xpert Ultra or Standard Xpert cartridges run on GeneXpert Edge instruments, which produce automated readouts for *M. tuberculosis* detection and rifampicin resistance. The semi-quantitative scale for Xpert Ultra results was negative, trace, very low, low, medium or high.[13] The semi-quantitative scale for Xpert results was negative, very low, low, medium or high.[14] However, results issued from the laboratory were reported only as 'TB test positive' for all results that were positive on Xpert Ultra or Standard Xpert, or 'TB test negative' if the results were negative on either test. The attending clinicians did not know if the result was from Xpert Ultra or Standard Xpert.

Liquid culture was performed using Mycobacteria Growth Indicator Tube (MGIT) with a BACTEC 960 instrument (BD Microbiology Systems, Sparks, MD, USA) and solid culture on Lowenstein-Jensen (LJ) medium. Cultures that were positive for growth of acid-fast bacilli underwent confirmation of *M. tuberculosis* complex by MPT64/MPB64 antigen detection. All samples with positive culture growth also underwent Line Probe Assay testing using the GenoType MTBDRplus version 2.0 (Hain Lifescience GmbH,

Nehren, Germany) for detection of MTB complex and rifampicin and/or isoniazid resistance.

Chest radiography screening and interpretation procedures

Allocation to chest radiography was known at the clinic by checking the clinic randomisation paper slip, that is, research assistants and clinicians knew if participants needed referral for a chest x-ray or not and also accessed x-ray findings for clinical decision making. Participants were referred for a chest x-ray after submitting their sputum samples. QECH and Bangwe ART clinics had digital radiography machines on site and participants received a chest x-ray assessment the same day. However, those randomised to receive an x-ray at Limbe clinic had to be transported once a week to receive their imaging at the QECH radiology unit. QECH had stationary digital x-ray equipment while the Bangwe clinic had portable x-ray equipment, the MinXray HF120/60HPPWV (MinXray, Inc. Northbrook, IL, USA).[11]

Chest radiographs were interpreted by attending clinicians as 'CXR abnormal consistent with TB', 'CXR abnormal not consistent with TB' and 'normal CXR'. X-ray images were reviewed on a computer equipped with Microdicom DICOM Viewer (version 3.3.2, Sofia, Bulgaria) by the study team. Digital x-ray images were also analysed by the Qure.ai qXR v. 3.0 software.[13] The Qure.ai report was not available routinely and was not used for clinical decision making. Qure.ai qXR v. 3.0 analyses lung fields of a posteroanterior chest x-ray for the presence of abnormalities and outputs a TB score of between 0 and 1 as well as a qualitative summary of the chest x-ray into the following categorisations: 'radiological signs of TB', 'abnormal CXR' and 'normal CXR'.

Follow-up procedures

Participants were followed up at days 28 and 56 to assess various clinical outcomes (if initiated on TB treatment, date when treatment was initiated, hospital admissions and vital status).

TB case definitions

Bacteriologically confirmed TB was defined as a positive result on either Xpert, Xpert Ultra or culture (MGIT or LJ). Clinical diagnosis of TB was when one had a compatible clinical illness or radiological disease and/or the decision of the responsible clinical team to commence TB treatment in the absence of any positive bacteriological tests for TB.

Outcome definitions

The primary outcome was cumulative incidence of bacteriologically confirmed TB treatment initiation by day 28 from recruitment among individuals with TB symptoms. The secondary outcomes included the cumulative incidence of all TB treatment initiations (of both bacteriologically confirmed and clinically diagnosed TB cases) at days 28 and 56, time from enrolment to TB diagnosis and TB treatment initiation and all-cause mortality at day 56. We also assessed yield of index test (Standard Xpert or Xpert Ultra) and calculated sensitivity and specificity compared with TB culture. Consistent with the factorial design, comparisons on these outcomes were made for participants grouped either as the Xpert group (combination of arms 1 and 2) vs the Xpert Ultra group (combination of arms 3 and 4) or the chest radiography group (combination of arms 2 and 4) vs the no chest radiography group (combination of arms 1 and 3). Results were also presented for each of the four arms.

Sample size and statistical analysis

For the sample size calculation, it was assumed that TB treatment initiations would be 75% of the bacteriologically confirmed TB for the Xpert group and 95% of the bacteriologically confirmed TB for the Xpert Ultra group.[15,16] Fifty-nine participants with culture positive TB were required per group to detect this difference at 80% power and a two-sided alpha of 0.05 with a 1:1 allocation between the groups. The assumed prevalence of culture positive TB of 10% among HIV positive participants with any TB symptom,[15] therefore, 1180 participants were required. A proportion of 6% was factored in for missing data and the final number of participants required was 626 per Xpert group. To also investigate the exploratory secondary objective to compare TB treatment initiations between chest radiography and no chest radiography arms, we required a 2-by-2 factorial design. We assumed bacteriologically confirmed TB treatment initiation with chest radiography to be 87% and 83% if no routine chest x-ray screening.[16] Based on sample size calculation for the primary objective (626 per Xpert group), each of the four arms of the trial would have 313 participants.

Because of the COVID-19 pandemic, there were disruptions to the study. Upon the recommendation of the trial steering committee, we decided to stop recruitment after we reached 640 participants out of the planned 1252 participants.

Analysis was based on intention to treat. The primary and secondary outcomes were expressed as cumulative risk and risk ratios and 95% CIs were determined for comparing each intervention group with the no-intervention groups: Poisson regression models were used. For graphical presentation of the time to TB treatment initiation, Nelson–Aalen plots were used.

Additional analyses were conducted to assess the ability of chest radiography screening to aid in TB diagnosis under different scenarios, that is, the yield of TB if laboratory testing followed categorisation by Qure.ai as 'abnormal' or 'abnormal consistent with TB'. Receiver operator curve (ROC) analysis was conducted on the quantitative Qure.ai scores to determine a cut-off point that achieves minimal target product profiles (TPP) for a triage test for TB in this population (i.e., 90% sensitivity and 30% false positive percentage).[17]

Results

Enrolment into the study

Of the 10 371 ambulatory patients that were assessed for eligibility, 640 (6.1%) were recruited into the study (Figure 1). These were subsequently randomised as follows: 162 participants were randomised to Arm 1, 159 to Arm 2, 159 to Arm 3 and 160 to Arm 4. The numbers of participants who received an allocated intervention were: 158 of 162 for Arm 1, 155 of 159 for Arm 2, 156

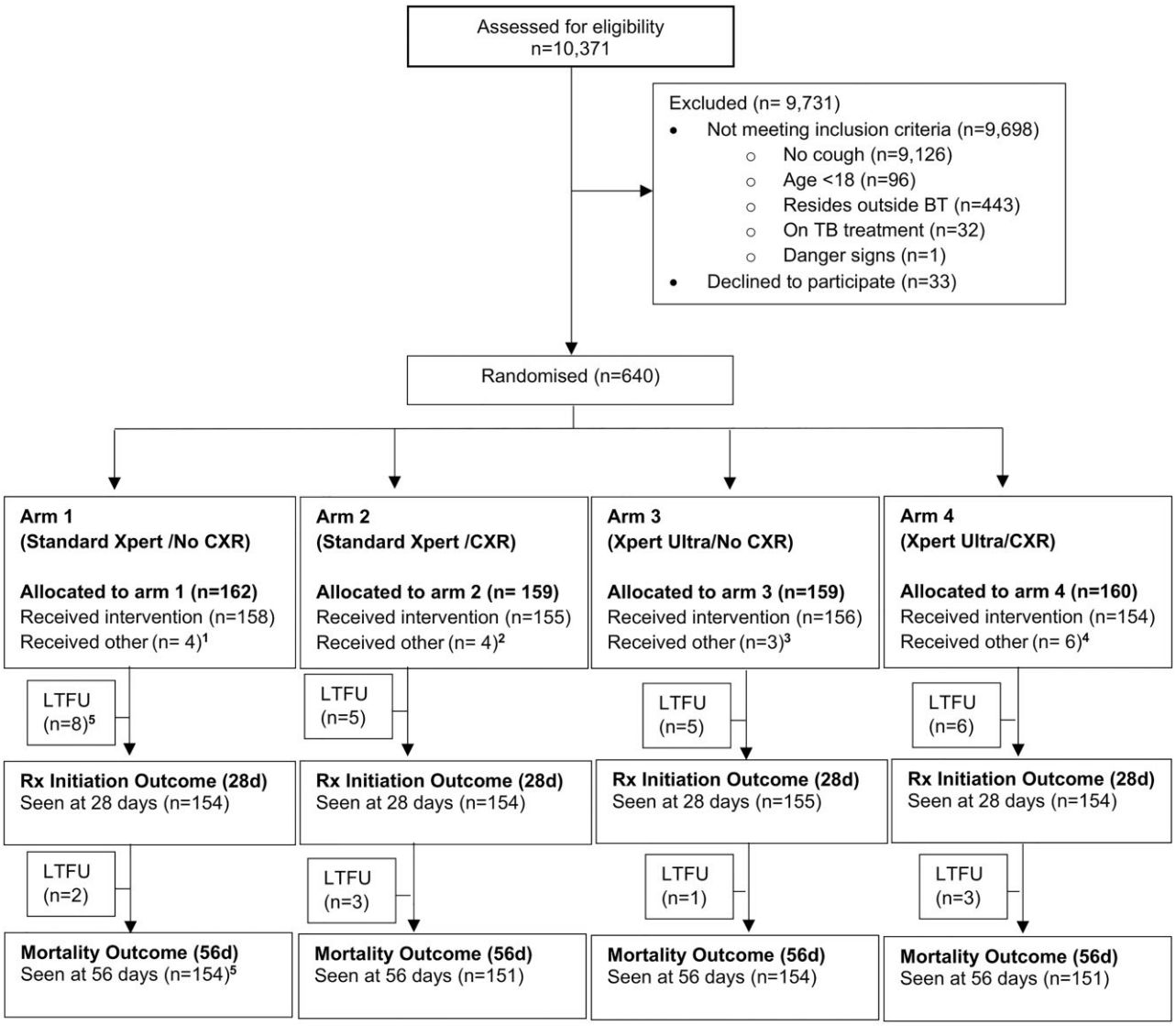

Figure 1. Recruitment and follow-up flowchart. CXR, chest x-ray; LTFU, lost to follow-up. 1Four participants received Xpert MTB/RIF Ultra instead of Xpert MTB/RIF. 2Three participants received Xpert MTB/RIF Ultra instead of Xpert MTB/RIF and one did not receive a chest x-ray. 3Three participants received Xpert MTB/RIF instead of Xpert MTB/RIF Ultra. 4Two participants received Xpert MTB/RIF instead of Xpert MTB/RIF Ultra and four did not receive a chest x-ray. 5There were two who had died before day 28 and were considered lost to follow-up for the TB treatment initiation outcome but were included in the day 56 outcome.

of 159 for Arm 3 and 154 of 160 for Arm 4. Reasons for the few non-compliances are included in Figure 1.

Baseline characteristics

Table 1 summarizes the characteristics of the participants that were randomized. The mean age and median body mass index (BMI) were similar across the arms. There was a range of 4.4% to 6.3% between the arms for those who had been treated for TB in the past 2 y and 19.4% to 27.0% for those who had been treated for TB over a duration >2 y. All the participants included in the analysis were receiving ART. TB preventative therapy use was low across the arms, ranging from 19.4% to 26.5%.

Index test results and diagnostic performance

The results of yield of TB disease by Xpert and Xpert Ultra tests are shown in Table 2. The yield of index test positive results in the Standard Xpert group (combination of Arms 1 and 2) was 25/321 (7.8%). The sensitivity of standard Xpert compared with TB culture was 65.0% (13/20; 95% CI 40.8 to 84.6%) and specificity was 97.0% (292/301; 95% CI 94.4 to 98.6%). The yield of index test positive results in the Xpert Ultra group (combination

Table 1. Baseline characteristics of patients randomised to study arms

Variable	Arm 1 (standard Xpert/no x-ray) N (%)	Arm 2 (standard Xpert and x-ray) N (%)	Arm 3 (Xpert Ultra/no x-ray) N (%)	Arm 4 (Xpert Ultra and x-ray) N (%)
Total	162	159	159	160
Gender				
Male	78 (48.2)	78 (49.1)	69 (43.4)	58 (36.3)
Female	84 (51.8)	81 (50.9)	90 (56.6)	103 (63.7)
Age (y)				
Mean (SD)	41.2 (10.1)	41.7 (10.5)	41.2 (10.3)	41.2 (10.6)
18–24	12 (7.4)	7 (4.4)	15 (9.4)	11 (6.9)
25–34	27 (16.7)	32 (20.1)	22 (13.8)	35 (21.9)
35–44	68 (42.0)	67 (42.1)	69 (43.4)	54 (33.8)
45–54	41 (25.3)	39 (24.5)	39 (24.5)	44 (27.5)
>55	14 (8.6)	14 (8.8)	14 (8.8)	16 (10.0)
BMI (kg/m^2)				
Median (IQR)	22.3 (5.4)	22.2 (4.7)	22.3 (5.6)	22.8 (7.2)
Min-18.5	25 (15.4)	25 (15.7)	19 (12.0)	20 (12.5)
18.5–24.9	94 (58.0)	100 (62.9)	95 (59.8)	80 (50.0)
25.0–29.9	33 (20.4)	23 (14.5)	27 (17.0)	39 (24.4)
≥30	10 (6.2)	11 (6.9)	18(11.3)	21 (13.1)
TB history				
No	120 (74.1)	109 (68.6)	117 (73.6)	121 (75.6)
Recent TB (≤2 y)	9 (5.6)	7 (4.4)	10 (6.3)	8 (5.0)
Old TB (>2 y)	33 (20.4)	43 (27.0)	31 (19.5)	31 (19.4)
ART regimen				
1st line (INSTI-based)	136 (84.0)	140 (88.1)	138 (86.8)	142 (88.8)
Other 1st line	8 (4.9)	7 (4.4)	4 (2.5)	3 (1.9)
2nd line	16 (9.9)	9 (5.7)	10 (6.3)	10 (6.3)
3rd line	2 (1.2)	3 (1.9)	7 (4.4)	5 (3.1)
ART duration, y				
<1	31 (19.1)	28 (17.6)	32 (20.1)	42 (26.3)
1–10	83 (51.2)	70 (44.0)	78 (49.1)	70 (43.8)
>10	48 (29.6)	60(37.7)	48 (30.2)	48 (30.0)
Unknown	0 (0)	1 (0.6)	1 (0.6)	0 (0)
Ever used TB preventative therapy				
Yes (isoniazid)	43 (26.5)	41 (25.8)	33(20.8)	31 (19.4)
No	107 (66.1)	107 (67.3)	112 (70.4)	116 (72.5)
Unknown	12 (7.4)	11 (6.9)	14 (8.8)	13 (8.1)
Urine TB-LAM status				
Positive	3 (1.9)	4 (2.5)	2 (1.3)	3 (1.9)
Negative	125 (77.2)	118 (74.2)	121 (76.1)	121 (75.6)
Not done	34 (21.0)	37 (23.3)	36 (22.6)	36 (22.5)
Recruitment Site				
QECH	94 (58.0)	93 (58.4)	86 (54.1)	91 (56.9)
Bangwe	29 (17.9)	33 (20.8)	35 (22.0)	32 (20.0)
Limbe	39 (24.1)	33 (20.8)	38 (23.9)	37 (23.1)

Abbreviations: ART, antiretroviral therapy, BMI, body mass index; INSTI, Integrase Strand Transfer Inhibitor; LAM, lipoarabinomannan; QECH, Queen Elizabeth Central Hospital.

Table 2. Incidence of TB treatment initiation, hospitalisations and mortality

Category	N	Risk ratio (95% CI)	p
Index test (Xpert/Xpert Ultra) positive TB			
Standard Xpert vs Xpert Ultra			
Standard Xpert group (Arm 1 and 2)	22/321 (6.9)	1	0.743
Xpert Ultra group (Arm 3 and 4)	24/319 (7.5)	1.10 (0.63 to 1.92)	
No x-ray vs x-ray			
No x-ray group (Arm 1 and 3)	29/321 (9.0)	1	0.068
X-ray group (Arm 3 and 4)	17/319 (5.3)	0.60 (0.33 to 1.05)	
Individual arms			0.327
Arm 1 (Standard Xpert/no x-ray)	14/162 (8.6)	1	
Arm 2 (standard Xpert and x-ray)	8/159 (5.0)	0.58 (0.25 to 1.35)	
Arm 3 (Xpert Ultra/no x-ray)	15/159 (9.4)	1.09 (0.54 to 2.19)	
Arm 4 (Xpert Ultra and x-ray)	9/160 (5.6)	0.65 (0.29 to 1.46)	
Bacteriologically confirmed TB treatment initiations at day 28[a]			
Standard Xpert vs Xpert Ultra			
Standard Xpert group (Arm 1 and 2)	25/321 (7.8)	1	0.666
Xpert Ultra group (Arm 3 and 4)	22/319 (6.9)	0.89 (0.51 to 1.54)	
No x-ray vs x-ray			
No x-ray group (Arm 1 and 3)	27/321 (8.4)	1	0.301
X-ray group (Arm 3 and 4)	20/319 (6.3)	0.74 (0.42 to 1.30)	
Individual arms			
Arm 1 (Standard Xpert/no x-ray)	14/162 (8.6)	1	0.722
Arm 2 (Standard Xpert and x-ray)	11/159 (6.9)	1.80 (0.37 to 1.71)	
Arm 3 (Xpert Ultra/no x-ray)	13/159 (8.2)	0.95 (0.46 to 1.95)	
Arm 4 (Xpert Ultra and x-ray)	9/160 (5.6)	0.65 (0.29 to 1.46)	
All TB treatment initiations at day 56			
Xpert vs Xpert Ultra			
Standard Xpert group (Arm 1 and 2)	49/321 (15.3)	1	0.598
Xpert Ultra group (Arm 3 and 4)	44/319 (13.8)	0.90 (0.62 to 1.31)	
No x-ray vs x-ray			
No x-ray group (Arm 1 and 3)	42/321 (13.1)	1	0.299
X-ray group (Arm 3 and 4)	51/319 (16.0)	1.22 (0.84 to 1.78)	
Individual arms			
Arm 1 (Standard Xpert/no x-ray)	19/162 (11.7)	1	0.310
Arm 2 (Standard Xpert and x-ray)	30/159 (18.9)	1.60 (0.94 to 2.73)	
Arm 3 (Xpert Ultra/no x-ray)	23/159 (14.5)	1.23 (0.69 to 2.17)	
Arm 4 (Xpert Ultra and x-ray)	21/160 (13.1)	1.11 (0.62 to 2.00)	
Hospitalisations and death at day 56			
Xpert vs Xpert Ultra			0.194
Standard Xpert group (Arm 1 and 2)	18/321 (5.6)	1	
Xpert Ultra group (Arm 3 and 4)	11/319 (3.5)	0.61 (0.29 to 1.28)	
No x-ray vs x-ray			
No x-ray group (arm 1 and 3)	12/321 (3.7)	1	0.336
X-ray group (Arm 3 and 4)	17/319 (5.3)	1.42 (0.69 to 2.93	
Individual arms			
Arm 1 (Standard Xpert only/no x-ray)	7/162 (4.3)	1	0.414
Arm 2 (Standard Xpert and x-ray)	11/159 (6.9)	1.60 (0.63 to 4.02)	
Arm 3 (Xpert Ultra/no x-ray)	5/159 (3.1)	0.72 (0.24 to 2.25)	
Arm 4 (Xpert Ultra and x-ray)	6/160 (3.8)	0.86 (0.29 to 2.53)	

[a]Includes TB culture results available at 28 d. All patients who did not start treatment at 28 d were excluded from the numerator regardless of index test result.

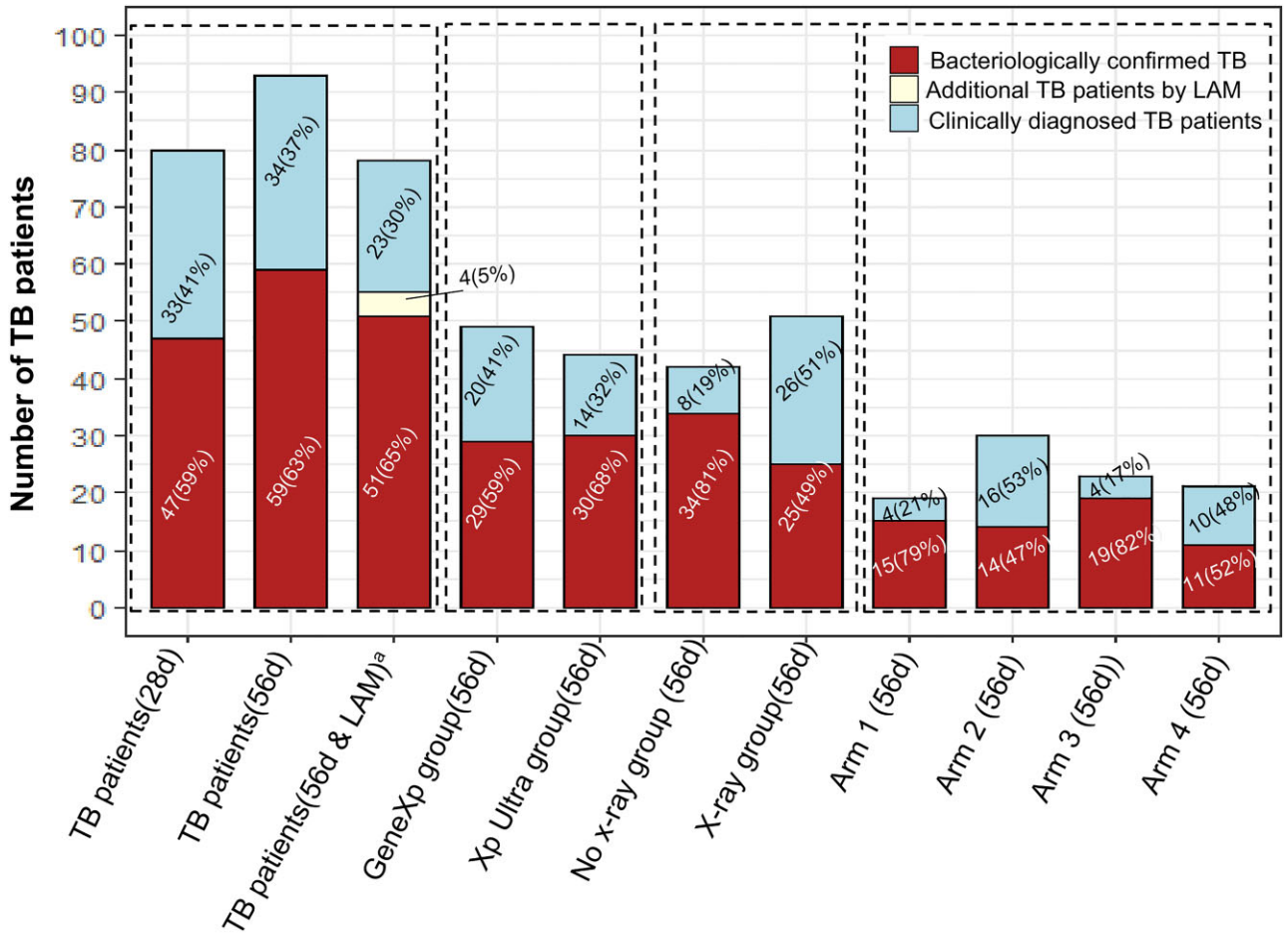

Figure 2. Composition of TB treatment initiation.

of Arms 3 and 4) was 24/319 (7.5%). The sensitivity of the Xpert Ultra test compared with TB culture was 53.9% (7/13; 95% CI 25.1 to 80.8%) and specificity was 94.4% (289/306; 95% CI 91.3 to 96.7%).

TB treatment initiation

The cumulative incidence of bacteriologically confirmed TB treatment initiations at day 28 was 7.8% (25/321) in the Standard Xpert group (combination of Arms 1 and 2) vs 6.9% (22/319) in the Ultra group (combination of Arms 3 and 4), with a risk ratio of 0.89 (95% CI 0.51 to 1.54; p=0.66) (Table 2). There was little difference across the four arms (8.6% in Arm 1, 6.9% in Arm 2, 8.2% in Arm 3 and 5.6% in Arm 4; p=0.72). By x-ray group, 6.3% in the x-ray group (combination of Arms 2 and 4) and 8.4% in the no x-ray group (combination of Arms 1 and 3) had bacteriologically confirmed TB disease, with a risk ratio of 0.74 (95% CI 0.42 to 1.30; p=0.30).

The cumulative incidence of all TB treatment initiations at day 56 (both bacteriologically confirmed and clinically diagnosed) was 15.3% (49/321) in the Xpert group and 13.8% (44/319) in the Ultra group, a risk ratio of 0.90 (95% CI 0.62 to 1.31; p=0.60) (Table 2). There were also no significant differences in the no x-ray group (13.1% [42/321]) vs the x-ray group (16.0% [51/319]), risk ratio 1.22 (95% CI 0.84 to 1.78; p=0.30).

Timing and composition of TB treatment initiation

There were fewer participants that were initiated on treatment after day 28 in all arms (Figures 2 and S1). By day 28, 80 participants had been initiated on TB treatment across all arms (Figure 2). A greater proportion of these were following bacteriological confirmation (47/80; 58.8%) (Figure 2). There were 93 participants who had been initiated on treatment by day 56 and a greater proportion following bacteriological confirmation (63.4% [59/93]) (Figure 2). None of the patients had rifampicin or isoniazid mono-resistance or multidrug-resistant TB. A comparison between x-ray vs no x-ray groups showed that in the arms with x-ray, a greater proportion of the treatment initiations had clinically diagnosed TB (50.9% vs 19.0%) (Figure 2). This was also observed with Arms 2 and 4 that had x-ray allocations.

There were 12 patients with a positive urine LAM test out of the 497 patients tested; 8 patients with a positive LAM also had a positive Xpert MTB/RIF or TB culture result, while the other 4 had

Table 3. Efficiency of TB screening using chest radiography

Category	Number screened	Confirmed TB detected	Bacteriologically confirmed TB missed	Clinically diagnosed TB detected	Clinically diagnosed TB missed
All X-ray screened and TB tested	290	25	NA	25	NA
Qure.ai					
Abnormality consistent with TB	49/290 (16.9)	20/25 (80.0)	5/25 (20.0)	12/25 (48.0)	13/25 (52.0)
Any abnormality	115/290 (39.7)	21/25 (84.0)	4/25 (16.0)	21/25 (84.0)	4/25 (16.0)
Cut-off score of 0.25	69/290 (23.8)	20/25 (80.0)	5/25 (20.0)	17/25 (68.0)	8/25 (22.0)
Independent medical doctors					
Abnormality consistent with TB	46/290 (15.9)	15/25 (60.0)	10/25 (40.0)	9/25 (36.0)	16/25 (64.0)
Any abnormality	68/290 (23.4)	18/25 (72.0)	7/25 (28.0)	15/25 (60.0)	10/25 (40.0)

Abbreviation: NA, not applicable.

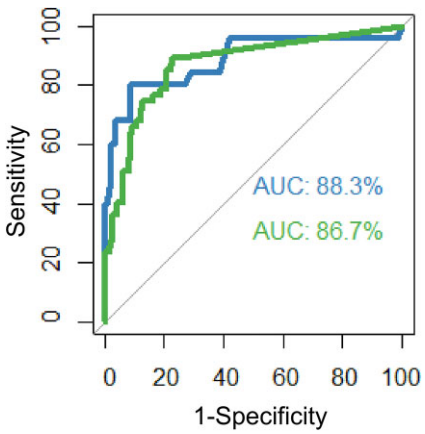

Figure 3. ROC curve for use of Qure.ai score for diagnosis of TB disease.

a negative microbiological result. The additional contribution of urine LAM testing to all patients treated for TB and tested for LAM was 4/78 (5.1%) (Figure 2). Because of the trial design, only 2 of these 4 additional patients received chest radiography and their x-rays were normal.

Efficiency of TB screening using chest radiography

There were 290 participants who received chest radiography screening (i.e., from the x-ray group). Of those who received chest radiography, 25/290 (8.6%) had bacteriologically confirmed TB and 25/290 (8.6%) were started on TB treatment based on clinical decision alone (Table 3). Table 3 shows expected findings if subsequent laboratory testing was conditional on different findings of x-ray abnormalities as assessed by Qure.ai system or by independent medical doctors. Qure.ai identified more bacteriologically confirmed TB cases (20/25 [80.0%]) than human readers (15/25 [60.0%]) (Table 3).

Following ROC curve analysis of quantitative Qure.ai scoring, a cut-off value of 0.25 achieved a sensitivity ≥89% for bacteriologically confirmed TB and ≥90% for all TB treatment initiations, both at a false positive percentage of ≥30% (Figure 3). Using a cut-off value of 0.25 would reduce the number of laboratory tests by three-quarters (68/290 [23.8%]), while maintaining good detection of bacteriologically confirmed TB patients (20/25 [80.0%]) (Table 3).

The radiological and laboratory findings of patients who received a chest radiography examination and were started on TB treatment were grouped into eight categories (shown in Figure S2).

Hospitalisation and mortality

By day 56 there were 26/640 (4.3%) hospitalisations. By arm, the hospitalisations were as follows: 7/162 (4.3%) for Arm 1, 11/159 (6.9%) for Arm 2, 5/159 (3.1%) for Arm 3 and 6/160 (3.8%) for Arm 4 (Table 2). By day 56 there were 8/640 (1.3%) deaths overall. Composite outcome of deaths and hospitalisations is shown in Table 2.

Discussion

The main finding of this trial is that Xpert MTB/RIF Ultra resulted in similar bacteriologically confirmed TB treatment initiations to Xpert MTB/RIF (6.9% and 7.8%, respectively). We hypothesised that Xpert Ultra would result in more bacteriologically confirmed TB treatment initiations compared with Xpert due to a higher sensitivity. This study is not the first to demonstrate a lack of impact of a superior diagnostic test on patient-related factors and clinical outcomes. Studies evaluating standard Xpert against microscopy showed no difference on mortality, successful treatment outcome or the proportion of participants who were treated for TB.[7,18–20] The diagnostic accuracy of the two tests was similar in this population: 65.0% (95% CI 40.8 to 84.6%) for Xpert and 53.9% (95% CI 25.1 to 80.8%) for Xpert Ultra. The advantage of the Xpert Ultra assay is an earlier time to result (<80 min), but TB diagnostic algorithms incorporating Xpert Ultra must carefully consider the population (TB prevalence, multidrug-resistant risk, previous TB disease), how to handle results (exclusion or inclusion of trace results, actions following indeterminate rifampicin

resistance testing) and balance in risk of false positive TB treatment initiation.[8,19]

The role of chest radiography in TB diagnosis was investigated in this trial. By day 56, all TB treatment initiations were similar by x-ray group (16.0%) and no x-ray group (13.1%), although the x-ray group had a higher proportion of clinically diagnosed treatment initiations (50.9% vs 19.0%). It was also demonstrated that the use of computer-aided interpretation of x-ray images using Qure.ai software would identify more bacteriologically confirmed TB cases (19/24 [79.2%]) than human readers (14/24 [58.3%]). This adds evidence to the WHO's recommendation that 'in populations in which TB screening is recommended, CAD software programmes may be used in place of human readers for interpreting digital chest X-rays for screening and triage for TB disease'.[21] The additional benefit of CAD is its ability to reproducibly process a large volume of x-rays. Ultra-portable X-ray equipment is now becoming available, allowing for further ease of use, as are a variety of software packages for interpretation of digital chest x-ray images.[11]

A limitation of this study is that the data available led to a significantly smaller sample size (low numbers of participants recruited due to COVID-19). With the reduced sample size of 640, also assuming a 10% culture positive prevalence, the power to detect differences between Xpert groups was reduced to 47%. Another limitation was that x-ray using CAD was used retrospectively, limiting full evaluation of its role in clinical decision making in real time. Full cost-effectiveness analyses of combination of tests were not conducted in this trial; however, estimates of unit costs are increasingly becoming available to assist in deciding on algorithms of TB screening.[22,23] The results of this trial are generalisable to low-income countries with a high TB burden.

Conclusions

Xpert MTB/RIF Ultra resulted in a statistically similar proportion of bacteriologically confirmed TB treatment initiation in ambulatory HIV positive individuals with cough. Chest x-ray screening is useful and CAD software outperformed human readers; further research should investigate how to mitigate the impact of x-rays on increasing false positive treatment initiations. The design of algorithms for TB screening and diagnosis should take into consideration the ease of implementation, cost and impact on clinical outcomes.

Authors' contributions: MM, GN, KM, AC, ELC, PM and MN were involved in the study conception and the study design. MM, CM, GN, TL, ELC, PM and MN obtained the funding. MM, HHT, CM, RS, GN, TL, TDS, KM, AC, PM and MN implemented the study. MM, TDS, KM and MN coordinated and conducted experiments. MM, HHT, CM, RS, GN, TDS, KM, AC, ELC, PM and MN were involved in data management and planning the analysis. MM and MN performed the analysis. All the authors were involved in interpreting the data. MM and MN wrote the first draft. All the authors read and approved the final version of the manuscript.

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Competing interests: The authors declare that they have no competing interests.

Ethical approval: The trial was approved by the College of Medicine Research Ethics Committee (Reg No: P.07/19/2751). Written informed consent was given by all the participants involved in the study. The study was conducted in accordance with the relevant national guidelines and regulations and the ethical standards of the Helsinki Declaration.

Data availability: The trial protocol and dataset supporting the conclusions of this article are available in the figshare repository (https://figshare.com/account/home#/projects/115032).

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