

Xpert MTB/RIF versus sputum microscopy as the initial diagnostic test for tuberculosis: a cluster-randomised trial embedded in South African roll-out of Xpert MTB/RIF



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Summary

Background In South Africa, sputum smear microscopy has been replaced with Xpert MTB/RIF as the initial diagnostic test for tuberculosis. In a pragmatic parallel cluster-randomised trial, we evaluated the effect on patient and programme outcomes.

Methods We randomly allocated 20 laboratories (clusters) in medium-burden districts of South Africa to either an Xpert (immediate Xpert) or microscopy (Xpert deferred) group (1:1), stratified by province. At two primary care clinics per laboratory, a systematic sample of adults giving sputum for tuberculosis investigation was assessed for eligibility. The primary outcome was mortality at 6 months from enrolment. Masking of participants' group allocation was not possible because of the pragmatic trial design. The trial is registered with the ISRCTN registry (ISRCTN68905568) and the South African Clinical Trial Register (DOH-27-1011-3849).

Findings Between June and November, 2012, 4972 people were screened, and 4656 (93·6%) enrolled (median age 36 years; 2891 [62%] female; 2212 [62%] reported being HIV-positive). There was no difference between the Xpert and microscopy groups with respect to mortality at 6 months (91/2324 [3·9%] vs 116/2332 [5·0%], respectively; adjusted risk ratio [aRR] 1·10, 95% CI 0·75–1·62).

Interpretation Xpert did not reduce mortality at 6 months compared with sputum microscopy. Improving outcomes in drug-sensitive tuberculosis programmes might require not only better diagnostic tests but also better linkage to care.

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Introduction

Improved diagnosis of tuberculosis is a global priority for tuberculosis control. Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) is a rapid, molecular, cartridge-based test that represents an important advance in tuberculosis diagnostics, with consistently better sensitivity than sputum smear microscopy, and an immediate rifampicin resistance result.^{1–3} The 2013 WHO guidelines include a conditional recommendation for Xpert MTB/RIF as the initial diagnostic test in all adults with suspected tuberculosis, acknowledging resource implications.⁴ The impact of Xpert MTB/RIF will, however, depend on the system in which it is used^{5,6} and countries will need evidence about patient, programme, and cost-effectiveness outcomes to inform policy recommendations for programmatic implementation of the test in their settings.⁷

In 2011, South Africa, with the third largest number of tuberculosis cases globally, made a policy decision to replace sputum smear microscopy with Xpert MTB/RIF as the first-line test for tuberculosis across the entire national laboratory service.⁸ The South African Xpert MTB/RIF programme is the largest in the world, accounting for more than half of all cartridges procured globally in 2013.

Here, we use a pragmatic cluster-randomised trial embedded in the South African national roll-out of Xpert MTB/RIF to assess the effect of Xpert MTB/RIF use versus sputum smear microscopy use on mortality, proportion test positive, proportion treated and the initial loss to follow-up in people being investigated for tuberculosis⁹

Methods

Study design

The XTEND study was a pragmatic, two-arm, parallel, cluster-randomised trial to assess the effect of Xpert MTB/RIF implementation in South Africa. A cluster was defined as a laboratory and two primary care clinics served by, but not co-located with, that laboratory. Further details of how clinics and laboratories were selected is in the appendix.

The XTEND protocol was approved by the ethics committees of the University of the Witwatersrand; the University of Cape Town; the London School of Hygiene & Tropical Medicine; and WHO. The study protocol is available online. The study is registered with the ISRCTN trials register (ISRCTN68905568) and the South African Clinical Trials Register (DOH-27-1011-

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For the **study protocol** see
http://www.auruminstitute.org/index.php?option=com_phocadownload&view=category&id=8&Itemid=417

Research in context

Evidence before this study

The aim of the XTEND study was to assess the effects of replacing smear microscopy with Xpert MTB/RIF on outcomes in people being investigated for tuberculosis, in the context of South African national roll-out of this test. South Africa was the first country to replace smear microscopy with Xpert MTB/RIF nationwide, and thus no similar data were available at the time we were planning the trial. We, therefore, did not do a systematic review as part of planning the trial. Subsequently, we searched PubMed for studies published from Jan 1, 2010, to Oct 31, 2014 using the terms tuberculosis AND randomised controlled trial AND (Xpert* OR Genexpert OR MTB/RIF), which identified only the TB-NEAT trial. This trial compared Xpert MTB/RIF with smear microscopy, with same-day results for both testing procedures; all participants also underwent same-day chest radiography. The primary outcome was morbidity, measured by TBscore, which was not different between study groups. The proportion of participants starting treatment for tuberculosis was high (more than 40% by day 56), and in many patients the

decision to treat was based on radiological evidence, and did not differ by study group. There was no difference in mortality (8% by day 56 in both groups).

Added value of this study

The XTEND trial provides robust evidence that Xpert MTB/RIF did not affect patient-relevant outcomes in the context of routine implementation in South Africa.

Implications of all the available evidence

Xpert MTB/RIF did result in a 50% increase in the number of people with bacteriological confirmation of tuberculosis, meaning that nurses could start treatment immediately for more people. 16% of people with a positive tuberculosis test result (by either method) did not start treatment at the clinic where the test was taken: tuberculosis programmes need to close this gap. The risk of death by 6 months' was higher in people who did not know their HIV status, or who were HIV-positive but were not taking antiretroviral therapy. People being tested for tuberculosis should know their HIV status, and health systems need to facilitate prompt linkage to HIV care for those who test positive.

3849). An independent Data Safety and Monitoring Board monitored the study every year.

Setting and participants

From March, 2011, Xpert MTB/RIF was rolled out in four phases: (1) a pilot phase in high-burden districts, followed by full implementation in laboratories in (2) high-burden, (3) medium-burden, and (4) low-burden districts. This trial was embedded in phase 3 of the national roll-out. Laboratories identified by the National Health Laboratory Service in July, 2011, as sites for a GeneXpert 16-module instrument (using data on the number of specimens submitted for sputum microscopy), were eligible for inclusion. Laboratories were ineligible if they were part of other Xpert MTB/RIF evaluations, already had a GeneXpert instrument, did not comply with standard of care tuberculosis diagnostics or were likely to be closed down for operational reasons during the study. In collaboration with the National Department of Health and the National Health Laboratory Service, we identified 20 laboratories in medium-burden districts in four provinces. Laboratories were grouped into four strata, based on province, and randomised by a statistician using Stata (version 11, StataCorp LP, College Station, Texas) to either the Xpert (immediate implementation) or the microscopy (with Xpert implementation deferred) study group.

From the primary care clinics currently served by the included laboratories, we selected two clinics per laboratory after a review of the number of sputum specimens sent to the National Health Laboratory Service in 2009–10. Clinics were not eligible for selection if they sent sputum specimens to other laboratories that use

Xpert MTB/RIF; were currently or planning to use Xpert MTB/RIF as a point-of-care test in the next 12 months; were located on the same site as the associated laboratory (because this does not reflect typical clinics in South Africa); or were conducting other research projects related to tuberculosis. Hospital sites were excluded because it was likely that testing was done on site, rather than at a separate laboratory.

Participants were eligible for inclusion if they were aged 18 years or older, not on tuberculosis treatment, had had a sputum specimen requested by clinic staff to investigate for possible tuberculosis, resident in the clinic catchment area, and not planning to relocate within the next 8 months. All participants gave informed written and witnessed verbal consent. Study staff recruited participants from a systematic sample of clinic attendees who satisfied inclusion criteria. Identical methods for recruitment and similar calendar recruitment periods were used in each study group. At clinics investigating fewer than five patients per day for tuberculosis, sequential patients referred to study staff were screened for enrolment. At clinics investigating 6–10 and 11–15 persons with suspected tuberculosis per day, every second or third person was screened for enrolment, respectively. At enrolment, personal identifiers, such as South African identity number, locator information (including participant-nominated contacts), demographic and clinical data relevant to tuberculosis and mortality risk, were collected including presence of tuberculosis symptoms, such as cough, weight loss, night sweats, and fever. Tracking details (name, date, barcode) of the sputum specimen requested by clinic staff at enrolment were recorded.

Procedures

GeneXpert 16-module instruments were introduced into laboratories assigned to the Xpert group (equivalent to the “intervention”). Laboratories assigned to the microscopy group continued to use quality-assured smear microscopy during enrolment of study participants (equivalent to the control condition), but implemented Xpert MTB/RIF about 6 months after enrolment was completed. All laboratory staff received training from the National Health Laboratory Service on specimen processing, instrument operation and maintenance, and documentation of results. Clinic staff were trained by the National Department of Health on the South African algorithm for Xpert MTB/RIF use (appendix). Additional information on Xpert MTB/RIF implementation is provided in the appendix.

All patient management, including investigations for tuberculosis, selection of tuberculosis treatment regimens, directly observed therapy, adherence monitoring, and antiretroviral therapy, was by health service staff in line with routine practice, and the study was designed in a way that would not affect practice in either group. In the Xpert group, people with suspected tuberculosis had one spot-sputum specimen collected for Xpert MTB/RIF testing at the associated laboratory. In the microscopy group, people requiring investigation for tuberculosis had two sputum specimens collected for fluorescence microscopy. Subsequent clinical management of people who were index-test negative was at the discretion of clinic staff, guided by the relevant diagnostic algorithm, which, for HIV-positive people, included chest radiography, trial of antibiotics, and collection of sputum for mycobacterial culture (appendix). In both study groups, sputum specimens were transported to the laboratories at least once per day and the target turnaround time for results was 48 h.

Consistent with the parallel design, participants were enrolled over a similar calendar period across all clusters. In the Xpert group, recruitment started a median of 3·6 months (range 1·0–7·4) after Xpert MTB/RIF was implemented, so that clinic staff had an opportunity to become familiar with the new procedures before enrolment started. In the microscopy group, enrolment began at the earliest opportunity.

In the microscopy group, two specimens were requested, versus one in the Xpert arm; thus any specimen collected within 4 days of enrolment was regarded as an index specimen, in both study groups. Study staff, who were unaware of the index test result, telephoned participants 1 week and 1, 2, and 4 months after enrolment to maintain contact and update locator information. Participants who were successfully contacted by telephone were sent a mobile phone airtime voucher of small value (less than US\$2) as an incentive to remain contactable during the study. If participants were not contactable after 3 attempts, we contacted the participant’s nominated contact or made a home visit to re-establish contact. Before the 6-month interview, study staff reviewed patients’ clinic records for information such as

results of index and subsequent tests and tuberculosis treatment and/or antiretroviral therapy start dates. If a participant had attended another clinic or a hospital during this time, as reported through the interview with the participant or a nominated contact (if the participant had died), or from information obtained from a record review at the enrolment clinic, study staff attempted to source patient data from these clinics and hospitals.

At 6 months from enrolment, study staff interviewed participants by telephone, or at a home visit, to ascertain whether the participant had started tuberculosis treatment or antiretroviral therapy and, if so, treatment start dates. Participants whose index test result was positive and who had not started tuberculosis treatment were referred for treatment.

All data (including participant identifiers, enrolment and follow-up questionnaires, visit contact records, and information from case notes) were collected using a custom-designed data collection application on a smart-phone (appendix).

Primary health clinic staff identified people to be investigated for tuberculosis, collected sputum specimens, and labelled sputum jars and specimen request forms with a unique laboratory barcode, which was recorded in the tuberculosis register. Study staff obtained the laboratory barcodes of sputum specimens from the tuberculosis register on the day of enrolment. Immediately before the 6-month follow-up interview, study staff accessed results of the index test, where available, so that if a participant had a positive index test result but had not started treatment, appropriate advice could be given. The National Health Laboratory Service Corporate Data Warehouse assisted with data matching and retrieval of sputum results from all participants. Where matching was unsuccessful, results were obtained directly from laboratories or from the tuberculosis register.

We recorded deaths through reports from participant-nominated contacts, clinic staff, and by accessing the Department of Home Affairs vital statistics database using participants’ South African identification numbers. An Endpoints Committee, unaware of group allocation, assigned vital status for a small number of participants where data were conflicting. Participants starting tuberculosis treatment were identified by self-report at the 6-month follow-up interview and through record review at the index clinic or other facilities.

Study outcomes

The primary outcome was mortality, measured 6 months after enrolment. Secondary outcomes included (1) proportion with a positive index test result; (2) in participants with a positive result, initial loss to follow-up, defined as the proportion not started on tuberculosis treatment within 28 days of enrolment; (3) proportion of the overall cohort starting tuberculosis treatment by 6 months from enrolment. In a post-hoc analysis of data from participants who were treated for tuberculosis, we

See Online for appendix

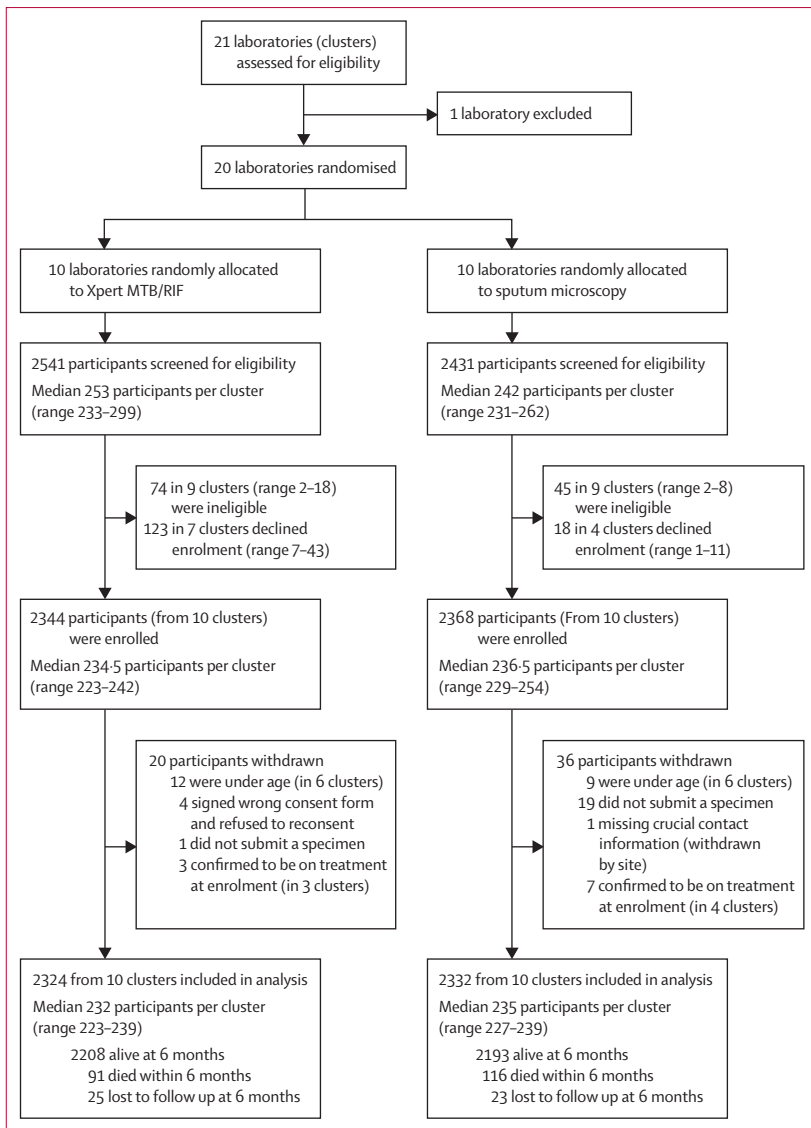


Figure 1: Trial profile

compared the proportion with microbiologically confirmed tuberculosis, defined as any positive Xpert MTB/RIF, microscopy or culture result, in each study group.

Statistical analysis

To inform the sample size calculation we reviewed the limited epidemiological and interventional research data that were available. In a study from KwaZulu-Natal of people being investigated for tuberculosis, HIV prevalence was 84% among those for whom HIV status was known. Data from the Western Cape and KwaZulu-Natal in South Africa suggested a risk of death of 2–11% in people being investigated for tuberculosis at 2 months' follow-up. We also used data (provided by Prof Mark Nicol, University of Cape Town) from a pilot study in two

primary health clinics in the Western Cape. Preliminary results show that mortality at 2 months' follow-up in a group randomly allocated testing with Xpert MTB/RIF was 74% lower than that in a group diagnosed with microscopy (0.8% vs 3.1%, respectively). On the basis of these data, we estimated that, in our study, Xpert MTB/RIF might be associated with a 50% reduction in mortality by 6 months from enrolment.

We assumed mortality of 5% in the microscopy group. Therefore, with 10 clusters per group, 220 people per cluster, and a coefficient of variation of 0.25, we estimated that there would be approximately 90% power to detect a 50% reduction in mortality in the Xpert group.

We analysed data using methods appropriate to the trial design, with a small number of clusters (see appendix), as described in a predefined analysis plan.⁹ Briefly, we adopted a cluster-level analysis taking into account the stratified randomisation. We used log-transformed cluster-level risk or rates to estimate geometric means for the two study groups. An approximate SE for the log (risk or rate ratio) based on geometric means of cluster risks or rates was calculated by two-way ANOVA on stratum, group, and the interaction between stratum and group.

As is not uncommon in cluster-randomised trials with a small number of clusters, we noted baseline imbalance between study groups in some factors associated with mortality, and we, therefore, adjusted for these individual-level baseline factors using a two-stage approach.⁹ In stage 1, a regression model at the individual level, including terms for the adjustment factors and strata, but not study group, was fitted, and the expected number of outcomes accumulated at the cluster level. In stage 2, at the cluster level, linear regression of the log (observed/expected outcomes) on stratum and group was used to estimate the risk/rate ratio. We calculated an approximate SE for the log (risk ratio) using similar approach as before. A post-hoc sensitivity analysis for the primary outcome was also done with logistic regression random-effects regression.

Role of the funding source

The funder played no role in the study design, implementation, data collection, analysis or decision to publish. The corresponding author confirms that he had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From June to November, 2012, we screened 4972 adults, of whom 119 (2.4%) were ineligible, 141 (2.8%) declined enrolment, and 4712 were enrolled (figure). Subsequently, 56 participants were withdrawn, leaving 4656 participants (median age 36 years, 62% female; table 1) in the analysis for the primary outcome.

Around three quarters (3542 [76%]) of participants reported knowing and were willing to share their HIV

status, of whom 2206 (62%) were HIV positive, with no difference between study groups. Of those who reported being HIV positive, 33% (729/2206) had ever been on antiretroviral therapy and the median self-reported CD4 cell count (n=1130) was 310 cells per μL (IQR 183–470). Baseline variables were similar by study group, except that participants in the Xpert group were less likely than those in the microscopy group to have a body-mass index $<18.5 \text{ kg/m}^2$ and more likely to report no tuberculosis symptoms (table 1).

Vital status at 6 months from enrolment was known for 4608 (99.0%) participants and 207 deaths occurred at a relatively constant rate during follow-up (data not shown). Based on the total cohort of 4656 participants, the 6-month mortality risk was 3.9% (91/2324) and 5.0% (116/2332) in the Xpert and microscopy groups, respectively, giving a risk ratio, adjusted for randomisation stratum only, of 0.86 (95% CI 0.56–1.28; $p=0.43$; table 2, appendix table 1). After adjusting for age group, sex, body-mass index (BMI) group, number of tuberculosis symptoms, self-reported HIV status, and randomisation stratum, the risk ratio was 1.10 (95% CI 0.75–1.62; $p=0.61$). Sensitivity analyses excluding those with unknown vital status at 6 months (n=48), or assuming those with unknown vital status were deaths, gave similar results. In a post-hoc sensitivity analysis using a logistic regression random-effects model, we obtained an odds ratio, adjusted for randomisation stratum, of 0.79 (95% CI 0.57–1.09; $p=0.16$) and a fully adjusted (for the same factors as previously mentioned) odds ratio of 1.03 (95% CI 0.74–1.44; $p=0.87$).

In an analysis which was not prespecified but was done to help explain our results, individual-level risk factors associated with an increased risk of death included being simultaneously HIV-positive (self-reported) and antiretroviral therapy naïve (aOR 3.32, 95% CI 2.03–5.41), or not knowing HIV status (2.41, 1.47–3.98), versus being HIV negative; lower BMI ($<18.5 \text{ kg/m}^2$ vs $18.5\text{--}24.9 \text{ kg/m}^2$: aOR 1.40, 95% CI 0.94–2.07), increasing age (<30 versus ≥ 50 years: aOR 2.53, 95% CI 1.59–4.04) and increasing number of tuberculosis symptoms (of current cough, night sweats, fever, or weight loss (table 3). The coefficient of variation for the primary outcome, taking into account the stratified design, was 0.24.

Results of the index test were known for 94.7% (n=4411) of participants. The proportion with a positive index test result was higher in the Xpert group than in the microscopy group and, after adjustment for baseline imbalance, there was a 49% greater proportion with a positive index test result in the Xpert group (table 2). In the Xpert group, 4.0% (8/200) of participants with a positive Xpert MTB/RIF test had a positive rifampicin-resistance result.

By 6 months from enrolment, 541 (11.6%) participants investigated for tuberculosis had started tuberculosis treatment, with rates not varying between groups

	Xpert (n=2324)	Microscopy (n=2332)
Age (years)	35 (28–45)	37 (29–48)
Female sex	1493 (64.2%)	1398 (59.9%)
Born in South Africa	2148 (92.4%)	2218 (95.1%)
Single marital status	1233 (53.1%)	1305 (56.0%)
Main income source		
Employed	968 (41.6%)	1093 (46.9%)
Government grant	485 (20.9%)	363 (15.6%)
Other	871 (37.5%)	876 (37.6%)
Dwelling		
House	1211 (52.1%)	1557 (66.8%)
Traditional	601 (25.9%)	378 (16.2%)
Other	512 (22.0%)	397 (17.0%)
HIV status, self-report		
Negative	639 (27.5%)	697 (29.9%)
Positive*	1052 (45.3%)	1154 (49.5%)
Unknown	633 (27.2%)	481 (20.6%)
CD4 count (self-report [cells/ μL])†	303 (171–457)	315 (192–480)
Ever taken ART (if known HIV positive)	352/1052 (33.5%)	377/1154 (32.7%)
Time on ART (years)	1.8 (0.7–3.7)	1.2 (0.4–3.0)
Has had previous tuberculosis	344 (14.8%)	374 (16.0%)
BMI (kg/m^2)		
<18.5	202 (8.7%)	288 (12.4%)
$18.5\text{--}25.0$	1062 (45.7%)	1077 (46.2%)
>25.0	1060 (45.6%)	967 (41.5%)
Karnofsky score		
≤ 80	960 (41.3%)	981 (42.1%)
90	941 (40.5%)	1055 (45.2%)
100	423 (18.2%)	296 (12.7%)
Symptoms reported		
Cough	1879 (80.9%)	1952 (83.7%)
Night sweats	946 (40.7%)	1152 (49.4%)
Weight loss	1030 (44.3%)	1210 (51.9%)
Fever	886 (38.1%)	1206 (51.7%)
Total number of symptoms‡		
0	227 (9.8%)	139 (6.0%)
1	547 (23.5%)	456 (19.6%)
2	752 (32.4%)	628 (27.0%)
≥ 3	798 (34.3%)	1109 (47.5%)
Main reason for visit		
TB symptoms	1689 (72.7%)	1735 (74.4%)
HIV care	194 (8.3%)	215 (9.2%)
HIV test	140 (6.0%)	150 (6.4%)
General/chronic	164 (7.1%)	158 (6.8%)
Antenatal clinic	126 (5.4%)	50 (2.1%)
Other	11 (0.5%)	24 (1.0%)

Data are n(%) or median (IQR) or n/N (%) unless otherwise stated. TB=tuberculosis. ART=antiretroviral therapy *Percentage HIV positive out of those with known HIV status 62.2% (1052/1691) and 62.3% (1154/1851) in the Xpert and microscopy arms, respectively. † In the Xpert group n=521, and in the microscopy group n=609. ‡Of current cough, night sweats, fever, or weight loss.

Table 1: Baseline characteristics of the enrolled population

	Xpert*	Microscopy*	Ratio measure (95% CI)			
			Unadjusted	p	Adjusted	p
Mortality risk 0–6 months	91/2324 (3.9%)	116/2332 (5.0%)	0.86 (0.56–1.28)	0.43	1.10 (0.75–1.62) †	0.61
Tested positive for tuberculosis	200/2176 (9.2%)	174/2235 (7.8%)	1.27 (0.81–2.00)	0.27	1.49 (1.00–2.23) ‡	0.05
Initial loss to follow-up§	34/200 (17.0%)	26/174 (14.9%)	0.97 (0.48–1.96)	0.93	0.96 (0.48–1.93) ¶	0.91
Treated for tuberculosis	250/2324 (10.8%)	291/2332 (12.5%)	0.88 (0.60–1.29)	0.48	1.04 (0.76–1.43) ‡	0.79
Tuberculosis microbiologically confirmed	196/250 (78.4%)	189/291 (65.0%)	1.21 (0.99–1.47)	0.06	1.20 (0.98–1.47) ‡	0.07

Data are n/N (%). *Summary ignores cluster. †Adjusted for age group, sex, body-mass index group, number of tuberculosis symptoms, and HIV status. ‡Adjusted for age group, sex, body-mass index group, and number of tuberculosis symptoms. §Because there was no loss to follow-up in two clusters, we added one event to all clusters. ¶Adjusted for body-mass index group and number of tuberculosis symptoms.

Table 2: Effect of Xpert MTB/RIF on the primary and secondary outcomes

	Number of deaths (%)	OR*	Adjusted OR† (95% CI)	p
Province				0.54
A	55/1752 (3.1%)	1	1	
B	32/462 (6.9%)	2.30	1.34 (0.82–2.15)	
C	76/1762 (4.3%)	1.39	0.96 (0.66–1.40)	
D	30/435 (6.9%)	2.29	1.04 (0.63–1.70)	
Positive index test result				0.06
No	159/4037 (3.9%)	1	1	
Yes	34/374 (9.1%)	2.25	1.49 (0.99–2.24)	
Sex				0.001
Male	114/1675 (6.8%)	1	1	
Female	79/2739 (2.9%)	0.42	0.59 (0.43–0.82)	
Age (years)				0.001
<30	32/1302 (2.5%)	1	1	
30–34.9	29/704 (4.1%)	1.64	1.34 (0.80–2.29)	
35–39.9	37/621 (6.0%)	2.32	2.02 (1.23–3.34)	
40–49.9	42/899 (4.7%)	1.80	1.59 (0.98–2.59)	
≥50	53/885 (6.0%)	2.58	2.53 (1.59–4.04)	
Number of symptoms‡				<0.0001
0	2/350 (0.6%)	0.18	0.23 (0.05–0.98)	
1	16/957 (1.7%)	0.52	0.59 (0.33–1.06)	
2	41/1298 (3.2%)	1	1	
3	62/1073 (5.8%)	1.80	1.58 (1.04–2.38)	
4	72/733 (9.8%)	3.12	2.51 (1.66–3.80)	
Body-mass index (kg/m ²)				0.003
<18.5	41/468 (8.6%)	1.68	1.40 (0.94–2.07)	
18.5–24.9	108/2020 (10.4%)	1	1	
25–29.9	26/1015 (4.1%)	0.49	0.60 (0.38–0.94)	
≥30	18/909 (3.7%)	0.38	0.57 (0.33–0.97)	
HIV status, by self-report				<0.0001
Negative	24/1276 (1.9%)	1	1	
Positive, not on ART	89/1398 (6.4%)	3.15	3.32 (2.03–5.41)	
Positive, on ART	26/688 (3.8%)	1.85	1.79 (0.99–3.21)	
Unknown	54/1049 (5.2%)	2.78	2.41 (1.47–3.98)	

ART=antiretroviral therapy. *Adjusted for province. †Adjusted for all variables in table. ‡Number of symptoms from: current cough, night sweats, fever, and weight loss.

Table 3: Risk factor analysis for mortality by 6 months from enrolment

(table 2). Of the 541 participants who had started treatment for tuberculosis, a greater proportion in the Xpert than the microscopy group had microbiological

confirmation (table 2). The median time to starting tuberculosis treatment was 7 days in the Xpert group and 10 days in the microscopy group.

Initial loss to follow-up was documented for 16.0% (60/374) of participants with a positive index test result, which was similar by study group (table 2).

Discussion

We had a rare opportunity to embed a randomised evaluation into South Africa’s national roll-out of Xpert MTB/RIF, allowing us to assess the effectiveness of the test under programmatic conditions. In people tested for tuberculosis, most of whom were HIV-positive, we found that, compared with sputum microscopy, implementation of Xpert MTB/RIF did not reduce mortality at 6 months, the proportion of people who had started tuberculosis treatment at 6 months, nor the time to treatment for people with a positive test result, although it did result in a higher proportion of positive test results.

Mortality in our study population was high, and, contrary to previous assumptions,^{11, 12} not reduced after implementation of Xpert MTB/RIF. Our study was not designed to determine causes of death, but the strong association of death with unknown HIV status and, for HIV-positive participants, the protective effect of antiretroviral therapy, suggest that HIV-related immunosuppression had an important role. That the deaths occurred at a relatively constant rate during the 6 months of follow-up suggests opportunities for interventions to reduce mortality, and easily-determined markers (older age, multiple tuberculosis symptoms, as well as unknown HIV status and not taking antiretroviral therapy) could identify those at highest risk. In future analyses, we will explore adherence to the recommended post-test diagnostic algorithms, and individual and health system barriers to algorithm adherence. Our data support the policy that people being investigated for tuberculosis should know their HIV status, and linkage to HIV care should be facilitated for those who are HIV positive.

The 49% increase in the proportion with a positive index test result in the Xpert group is better than results from meta-analysis of laboratory-based evaluations of

Xpert versus microscopy, perhaps because of high HIV prevalence in our population.³ This finding suggests an important advantage of Xpert MTB/RIF testing for the health system, because increased detection rates will facilitate rapid treatment of microbiologically confirmed tuberculosis by nurses, with the potential to reduce transmission. An increase of this magnitude has the potential to result in cost savings both for patients and for tuberculosis programmes, and we are currently undertaking economic analyses to investigate this.

However, this increase in detection rates did not translate into an increased number of people receiving tuberculosis treatment by 6 months, suggesting that Xpert MTB/RIF replaced, rather than supplemented, empirical treatment (ie, treatment without microbiological confirmation). Given the pragmatic trial design, mycobacterial cultures were only performed if requested as routine by clinic staff, and thus we cannot report what proportion of people with microbiologically confirmed tuberculosis started treatment in either group, nor what proportion of people without microbiologically confirmed tuberculosis received unnecessary empirical treatment.

It might be argued that the location of laboratory services in South Africa, usually separate from clinics, limits the potential benefits of Xpert MTB/RIF. Collocation of clinics and laboratory services could allow for in-session results, potentially reducing initial loss to follow-up and time to treatment. However, our results are consistent with those of the TB-NEAT trial,¹⁰ in which people being investigated for tuberculosis in primary care clinics in four African countries were individually randomised to on-site Xpert MTB/RIF or sputum microscopy, both providing same-day results.¹⁰ In TB-NEAT, although the proportion of participants receiving same-day diagnosis and same-day treatment initiation was higher with Xpert MTB/RIF than with microscopy, the proportion of patients who had started tuberculosis treatment by 56 days was similar (43% vs 42%), and there was no difference in morbidity or mortality. The very high proportion of patients starting empirical treatment was probably attributable to same-day chest radiography, provided for all patients. In our trial, chest radiography was at the discretion of clinic staff for investigation of HIV-positive people with negative test results, and often required travel to another clinic, as is common in resource-limited settings.

Our study enrolled people being investigated for tuberculosis in typical primary care clinics. However, a limitation of this approach is that, with a 7% prevalence of rifampicin resistance in tuberculosis cases in South Africa,¹³ we had too few rifampicin-resistant results to assess the effect of Xpert MTB/RIF on outcomes for this type of tuberculosis.

The replacement of sputum microscopy with Xpert MTB/RIF might have a greater effect on case detection and time to appropriate drug treatment, and thus

potentially on transmission of drug-resistant tuberculosis.² Further research is needed to guide policy on tuberculosis diagnostics in settings with high prevalence of drug-resistant tuberculosis.

Given that our trial was embedded within the national roll-out of Xpert MTB/RIF, we believe that our results reflect programmatic performance of the test in the South African health system, and are likely to be generalisable to settings with a similar health system to that in South Africa and that also have high tuberculosis and HIV prevalence. Our trial was pragmatic and did not attempt to influence adherence to diagnostic algorithms, nor provide a diagnostic gold standard; this approach led to a further limitation in that we do not know the true prevalence of tuberculosis (pulmonary or extrapulmonary) in our participants. Our regular retention calls to participants were designed to maintain contact and were not intended to influence clinical care. However, these activities could have influenced study outcomes, particularly initial loss to follow-up and time to treatment, but since they were identical in both study groups they are unlikely to have had an effect on between-group comparisons. Imbalance of some individual-level factors (BMI and number of symptoms reported) at baseline is not uncommon in cluster-randomised trials with a relatively small number of clusters. In our study, the observed baseline imbalance suggested that patients selected by clinic staff to have sputum sent for tuberculosis testing were slightly healthier in the Xpert group. A possible explanation is that staff in clinics assigned to the Xpert group had recently been trained on the Xpert diagnostic algorithm by National Department of Health staff, as part of the process of Xpert roll-out. This may have resulted in clinic staff initiating tuberculosis testing among a wider range of patients, including healthier patients, than happened in the microscopy group.

In conclusion, implementation of Xpert MTB/RIF in South Africa appears unlikely to improve control of drug-sensitive tuberculosis without improvements to the health system, in particular changes to reduce initial loss to follow-up and time to treatment initiation. We noted that unknown HIV status, or positive status but no antiretroviral therapy, were important determinants of mortality, which supports the policy that all people being investigated for tuberculosis should know their HIV status, and suggests the need to improve linkage between investigation for tuberculosis and HIV care. Our evaluation of Xpert MTB/RIF, conducted under programmatic conditions, has highlighted the importance of addressing health system weaknesses in order to maximise the effect of new diagnostics. WHO recommendations should be updated to reflect the impact of implementing Xpert MTB/RIF under programmatic conditions on patient-relevant outcomes, and consider the broader context of the health system in which tests are used.

Contributors

The study was designed by all authors. GJC, KMcC, KLF, VC, and ADG were responsible for data collection. GJC, KMcC, KLF, and ADG were responsible for the analysis. All authors contributed to interpretation and writing of the manuscript.

Declaration of interests

We declare no competing interests.

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References

- Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; **363**: 1005–15.
- Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011; **377**: 1495–505.
- Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2014; **1**: CD009593.
- WHO. 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 extrapulmonary tuberculosis in adults and children. Policy Update. 2013 Prepublication. <http://www.stoptb.org/wg/gli/assets/documents/WHO%20Policy%20Statement%20on%20Xpert%20MTB-RIF%202013%20pre%20publication%2022102013.pdf> (accessed May 22, 2014).
- Small PM, Pai M. Tuberculosis diagnosis—time for a game change. *N Engl J Med* 2010; **363**: 1070–71.
- 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? *Lancet Infect Dis* 2014; **14**: 527–32.
- Squire SB, Ramsay AR, van den Hof S, et al. Making innovations accessible to the poor through implementation research. *Int J Tuberc Lung Dis* 2011; **15**: 862–70.
- WHO. Global tuberculosis report 2013. Geneva: World Health Organization, 2013.
- Hayes RJ, Moulton LH. Cluster randomised trials. London: Chapman & Hall, 2009.
- Theron G, Zijenah L, Chanda D, et al. Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. *Lancet* 2014; **383**: 424–35.
- Menzies NA, Cohen T, Lin HH, 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**: e1001347.
- Dowdy DW, Davis JL, den Boon S, Walter ND, Katamba A, Cattamanchi A. Population-level impact of same-day microscopy and Xpert MTB/RIF for tuberculosis diagnosis in Africa. *PLoS One* 2013; **8**: e70485.
- Scott LE, Cunningham BE, Van Rie A, et al. Exploring the value of the Xpert MTB/RIF probe frequencies and cycle threshold data for surveillance purposes. 19th Conference on Retroviruses and Opportunistic Infections. Seattle, WA, USA; March 5–8, 2012. Abstr #924.