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# Characteristics and Early Outcomes of Patients With Xpert MTB/RIF-Negative Pulmonary Tuberculosis Diagnosed During Screening Before Antiretroviral Therapy

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**Background.** A proportion of patients with tuberculosis diagnosed by sputum culture during screening before antiretroviral therapy (ART) have false-negative Xpert MTB/RIF assay results (Xpert-negative tuberculosis). We determined the characteristics and early ART program outcomes of such patients.

**Methods.** Adult patients who enrolled in a South African township ART service were systematically screened for pulmonary tuberculosis regardless of symptoms by testing paired sputum samples with Xpert MTB/RIF and liquid culture. The ART service provided follow-up for all patients, and early (90-day) programmatic outcomes were determined.

**Results.** Among 602 patients screened, 523 had  $\geq 1$  Xpert and culture result, yielding 89 culture-positive tuberculosis diagnoses. Of these, 37 (42%) of the patients with tuberculosis were Xpert-negative when a single sputum sample was tested, compared with 25 (28%) when 2 samples were tested. Compared with patients with Xpert-positive tuberculosis, those with Xpert-negative tuberculosis (using either definition) had substantially higher CD4 cell counts, lower plasma viral loads, higher hemoglobin concentrations, and higher body mass index. Their tuberculosis was also less advanced, with a lower frequency of prolonged cough ( $\geq 2$  weeks), less extensive radiographic abnormalities, and a lower frequency of detectable lipoarabinomannan antigenuria and mycobacteriuria. Xpert-negative cases were all sputum smear negative with prolonged time to culture positivity (median, 21 days). Despite greater delays in starting tuberculosis treatment, Xpert-negative patients were less likely to die during follow-up.

**Conclusions.** Compared to patients with Xpert-positive tuberculosis diagnosed during pre-ART screening, Xpert-negative cases had less advanced immunosuppression and less advanced tuberculosis and did not have adverse outcomes despite substantial delays in starting tuberculosis treatment.

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The Xpert MTB/RIF assay represents a major breakthrough in diagnostics for tuberculosis [1–3]. This rapid molecular assay can be used close to the point of care by operators with minimal technical expertise, enabling diagnosis of tuberculosis and simultaneous assessment of rifampicin resistance within 2 hours when testing unprocessed sputum samples or specimens from extrapulmonary sites [1]. Testing a single sputum sample detects 98%–100% of smear-positive pulmonary tuberculosis and between 57% and 83% of sputum smear-negative disease in adults presenting with suspected tuberculosis [1]. The assay was

endorsed by the World Health Organization (WHO) in December 2010 as a replacement for sputum smear microscopy in resource-limited settings, especially in clinical populations with high rates of multidrug-resistant tuberculosis or human immunodeficiency virus (HIV)–associated tuberculosis [4].

Some countries such as South Africa have embarked on national implementation of the Xpert MTB/RIF assay. However, much research is needed to guide this process and to address the many operational questions that are emerging. An important issue that has not yet been addressed is the implication of false-negative Xpert MTB/RIF results (hereafter referred to as “Xpert-negative tuberculosis”). Although much attention has focused on the challenges of sputum smear-negative HIV-associated tuberculosis [5], we now need to characterize this new subpopulation of patients with Xpert-negative tuberculosis.

We reported elsewhere on the utility of the Xpert MTB/RIF assay for screening patients for HIV-associated tuberculosis before starting antiretroviral therapy (ART) in a South African township [6]. Although the assay increased case finding by 45% compared with sputum smear microscopy, sputum samples from more than one-quarter of culture-positive cases nevertheless tested Xpert-negative even when paired sputum samples were tested [6]. In this study, we report on the clinical, hematological, microbiological, and radiological characteristics of patients with Xpert-negative tuberculosis diagnosed in this clinical setting, and we relate Xpert status to subsequent clinical and programmatic outcomes. To broaden the applicability of these data to settings where only 1 test is performed, we analyzed data in 2 ways, first defining patients as Xpert-negative by a negative test on a single sputum sample and then by negative tests on 2 samples. These data will help to shape policies regarding screening algorithms for HIV-associated tuberculosis.

## METHODS

The ART service in Gugulethu township, Cape Town, South Africa, has been described in detail elsewhere, and the major burden of tuberculosis in these patients’ population has been characterized in detail [7–9]. The current cohort of patients were recruited between 12 March 2010 and 20th April 2011, and the cases of some of these patients have been described elsewhere [6, 10]. Consecutive eligible HIV-infected patients were recruited from among patients newly referred to the clinic for ART. Study eligibility criteria included age >18 years, being ART naive and having no current tuberculosis diagnosis. All participants provided written informed consent and the study was approved by the research ethics committees of the University of Cape Town, Cape Town, South Africa, and the London School of Hygiene and Tropical Medicine, London, United Kingdom.

At their first visit to the clinic, patients were prospectively recruited, clinically characterized and clinically staged according to WHO criteria [11]. They were then screened for tuberculosis and underwent routine baseline laboratory investigations. Demographic details were recorded, and a standardized symptom-screening questionnaire (which included the WHO symptom screen for HIV-associated tuberculosis [12]) was completed. Two sputum samples were requested from each patient; a spot specimen was first obtained, followed by a second that was induced using nebulized 3% hypertonic saline. If necessary, both specimens were induced. Urine samples were also collected and stored at  $-20^{\circ}\text{C}$ . Blood CD4 cell counts and plasma viral load were measured for all patients with routine laboratory services. Chest radiographs were obtained and analyzed by an experienced reader certified in the use of the chest radiograph reading and recording system [13, 14].

## Laboratory Procedures

Sputum specimens were processed using standardized protocols and quality assurance procedures by a centralized accredited laboratory, which has previously participated in international evaluations of the Xpert MTB/RIF assay conducted by the Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland [2, 3]. Samples were decontaminated with N-acetyl-L-cysteine and sodium hydroxide and concentrated by centrifugation. Smears prepared from the sputum pellets were stained with auramine O fluorescent stain for fluorescence microscopy, and equal volumes of the remaining pellet were tested by liquid culture and the Xpert MTB/RIF assay. All smears graded as scanty, 1+, 2+, or 3+ were defined as “smear positive.” Culture was performed using mycobacterial growth indicator tubes (MGIT; Becton Dickinson), which were incubated for up to 6 weeks. Cultures positive for acid-fast bacilli were identified as the *Mycobacterium tuberculosis* complex using the MTBDR<sub>plus</sub> assay (Hain Lifesciences). Xpert MTB/RIF assays were carried out according to the manufacturer’s instructions. The results of all tests were read by technologists blinded to the outcomes of the other assays. To assess possible culture contamination as a cause of positive cultures that arose from sputum samples that tested Xpert-negative, isolates underwent molecular typing using mycobacterial interspersed repetitive-unit variable-number tandem repeat (MIRU-VNTR) analysis [15].

Frozen urine samples were thawed and analyzed for the presence of lipoarabinomannan (LAM) using the commercially available Clearview TB-ELISA (Alere), with strict adherence to the manufacturer’s instructions. The thawed urine samples (2.0 mL) were also concentrated by centrifugation, resuspended in 0.75 mL of phosphate buffer, and then tested

using the Xpert MTB/RIF assay according to the manufacturer's instructions.

### Patient Outcomes

Patients were followed up by the routine ART service, and patients with a diagnosis of tuberculosis (including those diagnosed by Xpert MTB/RIF) were referred to tuberculosis clinics within the township for treatment. ART service patient records were reviewed to determine clinical outcomes. A record was made of the number of patients who underwent tuberculosis treatment and ART and when the treatment was initiated. Program losses due to death, follow-up or transfer were also ascertained. The clinical courses of patients who had a negative symptom screen at baseline were also reviewed to determine whether they developed symptoms before starting tuberculosis treatment.

### Definitions and Analysis

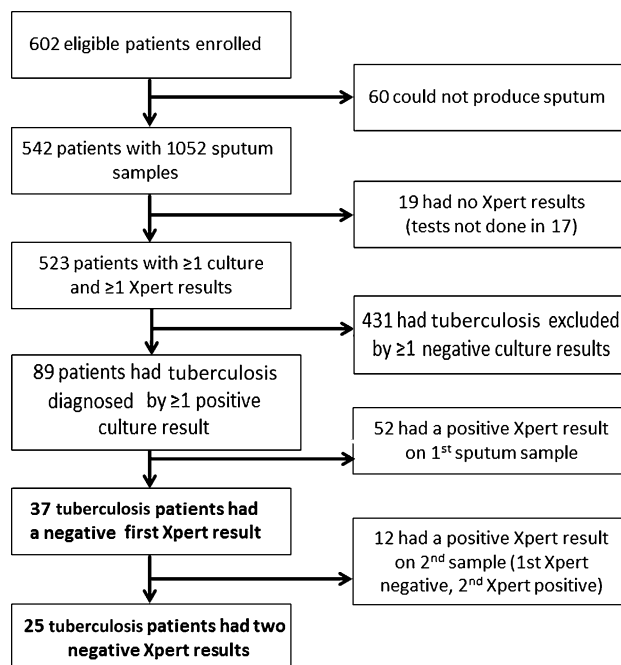
Patients were defined as having tuberculosis if *M. tuberculosis* was cultured from  $\geq 1$  sputum sample. Patients with tuberculosis were then categorized in 2 different ways based on the analysis of Xpert MTB/RIF results from only the first sputum sample or from both samples. In the latter analysis, patients were classified as Xpert-positive if either or both samples tested positive and Xpert-negative if both tests were negative. Patients were characterized using simple descriptive statistics, and the sensitivity of the Xpert assay was calculated for patients stratified by CD4 cell counts and sputum smear status. Characteristics and 30- and 90-day outcomes of patients with Xpert-positive or Xpert-negative tuberculosis were compared using the Wilcoxon rank-sum test, Student *t* test, and  $\chi^2$  and Fisher's exact tests, as appropriate. Logistic regression analysis was used to identify factors independently associated with Xpert-negative disease. All statistical tests were 2 sided at  $\alpha = .05$ .

## RESULTS

### Tuberculosis Diagnoses

Of 604 consecutive patients who were eligible and invited, 602 agreed to participate. Sputum samples were obtained from 542 (90.0%) patients, and culture and Xpert MTB/RIF results were available from  $\geq 1$  sputum sample for 523 patients (Figure 1). *M. tuberculosis* was cultured from at least 1 sample from 89 patients, which represented a 17.0% prevalence (95% confidence interval, 13.9–20.5) of culture-proven pulmonary tuberculosis among these patients. Twenty-four (27.0%) cases were sputum smear positive, and 65 (73%) were smear negative.

Of the cases of culture-confirmed tuberculosis ( $n = 89$ ), 37 (41.6%) were Xpert-negative and 52 (58.4%) were Xpert-positive based on the results of the first sputum sample



**Figure 1.** Flow diagram showing numbers of patients studied, tuberculosis cases diagnosed and numbers of cases diagnosed by Xpert MTB/RIF testing of 1 or 2 sputum samples.

(Figure 1). Based on the results of 2 samples, 25 (28.1%) were Xpert-negative (both samples negative), and 64 (71.9%) were Xpert-positive (1 or 2 samples positive).

Patients with a diagnosis of tuberculosis had a median age of 33.4 years (interquartile range [IQR], 28.3–40.4); 62% were female, the median body mass index was 21.2 kg/m<sup>2</sup> (IQR, 19.2–25.0) and 21.4% of cases had retreatment disease. The median CD4 cell count was 131 cells/ $\mu$ L (IQR, 55–204), median plasma viral load was 4.8 log<sub>10</sub> copies/mL (IQR, 4.4–5.3) and 47% of cases had a history of WHO stage 3 or 4 (AIDS) disease. Although a positive WHO symptom screen (any cough, fever, weight loss or night sweats) was recorded in a majority (82.0%) of patients, only 24.7% had cough of  $\geq 2$  weeks duration.

### Characteristics of Patients With Xpert-Negative Tuberculosis

Patients with Xpert-positive or Xpert-negative tuberculosis were defined in 2 ways, initially using the first sputum sample result and then using both sputum sample results. Regardless of the way patients were defined, there were notable differences between Xpert-negative and Xpert-positive patients (Table 1). Xpert-negative patients had less advanced HIV infection with higher CD4 cell counts and lower plasma viral loads. They were also far less likely to report a cough of  $\geq 2$  weeks duration and they had a higher median body mass index, higher median hemoglobin concentration, and a lower

**Table 1. Characteristics of Patients (n = 89) With Tuberculosis Diagnosed During Screening Before Antiretroviral Therapy and Stratified According to Whether Patients Had Positive or Negative Xpert MTB/RIF Results With Testing of 1 or 2 Sputum Samples**

Variable	Results of Xpert Test (1 Sample)			Results of Xpert Test (2 Samples)		
	Negative (n = 37)	Positive (n = 52)	P	Negative (n = 25)	Positive (n = 64)	P
<b>Patient characteristics</b>						
Age, median (IQR), years	34.3 (28.6–40.4)	32.3 (27.5–39.4)	.566	32.1 (28.7–38.0)	33.5 (26.8–40.7)	.927
Female, No. (%)	22 (59.5)	33 (63.5)	.702	16 (64.0)	39 (60.9)	.789
BMI, median (IQR), kg/m <sup>2</sup>	22.1 (20.4–27.2)	20.8 (18.8–23.0)	.049	22.1 (20.6–28.5)	21.0 (18.8–23.8)	.037
History of tuberculosis, No. (%)	10 (27.0)	9 (17.3)	.270	5 (20.0)	14 (21.9)	.846
<b>Blood test results<sup>a</sup></b>						
Hemoglobin, g/dL	11.6 (10.3–12.8)	9.5 (8.1–11.6)	<b>.002</b>	11.6 (10.4–13.1)	10.1 (8.5–11.7)	<b>.029</b>
White blood cell count, × 10 <sup>9</sup> cells/L	5.7 (4.1–6.7)	5.7 (4.9–9.7)	.230	5.5 (4.1–6.5)	5.7 (4.9–8.7)	.285
Absolute neutrophil count, × 10 <sup>9</sup> cells/L	2.9 (2.0–4.1)	3.8 (2.6–7.4)	<b>.008</b>	2.9 (2.0–3.9)	3.6 (2.5–6.5)	<b>.022</b>
Absolute lymphocyte count, × 10 <sup>9</sup> cells/L	1.8 (1.3–2.4)	1.4 (0.7–1.8)	<b>.011</b>	1.8 (1.4–2.4)	1.4 (0.7–1.9)	<b>.021</b>
ALT, IU/L	23 (15–32)	21.5 (14–44)	.765	20 (14–31)	23 (14–40)	.319
Platelets, × 10 <sup>9</sup> cells/L	272 (209–324)	328.5 (220–419)	.128	273.5 (240.5–314.5)	328 (210–419)	.367
CD4 cell count, median, cells/μL	183 (112–213)	100 (35–186)	<b>.020</b>	189 (137–215)	106 (37–185)	<b>.006</b>
CD4 cell count, cells/μL <sup>b</sup>			<b>.058</b>			<b>.012</b>
<50	5 (13.5)	15 (29.4)		3 (12.0)	17 (27.0)	
50–99	4 (10.8)	10 (19.6)		2 (8.0)	12 (19.1)	
100–149	6 (16.2)	11 (21.6)		2 (8.0)	15 (23.8)	
150–199	9 (24.3)	4 (7.8)		7 (28.0)	6 (9.5)	
≥200	13 (35.1)	11 (21.6)		11 (44.0)	13 (20.6)	
Log viral load, median (IQR), copies/mL <sup>b</sup>	4.7 (4.4–5.1)	4.9 (4.7–5.4)	<b>.033</b>	4.4 (4.2–4.7)	5.1 (4.7–5.5)	<b>&lt;.001</b>
WHO stage at enrollment, No. (%)			.529			.924
1 or 2	21 (56.8)	26 (50.0)		13 (52.0)	34 (53.1)	
3 or 4	16 (43.2)	26 (50.0)		12 (48.0)	30 (46.9)	
<b>Symptoms</b>						
Any cough, fever, night sweats, or weight loss (≥1 symptom)	26 (70.3)	47 (90.4)	<b>.015</b>	18 (72.0)	55 (85.9)	.124
Current cough ≥2 weeks	5 (13.5)	17 (32.7)	<b>.039</b>	2 (8.0)	20 (31.3)	<b>.028</b>

Blood test results are all median (interquartile range) values except for the CD4 categories, which are numbers (%). P values of <.05 have been indicated in bold. Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; IQR, interquartile range; WHO, World Health Organization.

<sup>a</sup> Blood test results were available for 85 patients.

<sup>b</sup> CD4 cell counts and viral loads were available for 88 patients.

absolute neutrophil count. In a multivariate analysis of patient characteristics, 3 variables remained strongly associated with Xpert status in the final model (Table 2). Xpert-negative patients were more likely to have higher CD4 cell counts, a lower plasma viral load, and a lower blood neutrophil count.

In view of the strong association with CD4 cell count, we next examined how the sensitivity of Xpert MTB/RIF varied across a range of CD4 cell count strata (Figure 2). This confirmed that the Xpert MTB/RIF assay had the highest sensitivity among those with the lowest CD4 cell counts with testing of either 1 (Figure 2A) or 2 (Figure 2B) sputum samples.

### Results of Other Diagnostic Tests

We next explored the relationship between sputum Xpert status and the results of other tuberculosis investigations (Table 3). None of the Xpert-negative cases were sputum smear positive, whereas more than one-third of the Xpert-positive cases were smear positive. Moreover, the median time to positivity for detection of growth of *M. tuberculosis* in automated liquid culture was substantially longer for patients with Xpert-negative tuberculosis than for those with Xpert-positive tuberculosis. The proportions of patients with detectable LAM antigenuria and *M. tuberculosis* bacteriuria detected using the Xpert MTB/RIF assay

**Table 2. Univariate and Multivariate Analysis Using Logistic Regression Showing Association Between Patient Characteristics and Risk of Xpert-Negative Tuberculosis Among Patients With Culture-Positive Disease for Whom Complete Data Were Available (n = 85)**

Risk Factor	Crude Odds Ratio	95% CI	P	Adjusted Odds Ratio	95% CI	P
<b>Age, years</b>						
≤30	1.0	...	...	...	...	...
>30	1.11	.42–2.98	.831	...	...	...
<b>Sex</b>						
Male	1.0	...	...	...	...	...
Female	1.14	.44–2.97	.788	...	...	...
<b>Body mass index, kg/m<sup>2</sup></b>						
<18	1.0	...	...	1.0	...	...
18–25	4.62	.55–38.65	.079	2.06	.18–23.98	.458
>25	8.31	.91–75.73	...	4.15	.31–55.41	...
<b>CD4, cells/μL</b>						
<150	1.0	...	...	1.0	...	...
≥150	5.95	2.14–16.61	.001	4.54	1.17–17.72	.023
<b>Viral load, log copies/mL</b>						
≥4.5	1.0	...	...	1.0	...	...
<4.5	6.02	2.16–16.73	.001	6.42	1.56–26.44	.007
<b>Hemoglobin, g/dL</b>						
<11	1.0	...	...	...	...	...
≥11	3.08	1.14–8.31	.022	1.40	.31–6.35	.662
<b>Absolute neutrophil count, × 10<sup>9</sup> cells/L</b>						
≥3.5	1.0	...	...	1.0	...	...
<3.5	2.1	.82–5.92	.109	4.87	1.26–18.89	.016
<b>Absolute lymphocyte count, × 10<sup>9</sup> cells/L</b>						
<1.5	1.0	...	...	1.0	...	...
≥1.5	1.96	.75–5.17	.167	1.20	.31–4.56	.794
<b>Previous tuberculosis treatment</b>						
No	1.0	...	...	...	...	...
Yes	1.12	.36–3.52	.845	...	...	...
<b>WHO symptom screen</b>						
Positive	1.0	...	...	...	...	...
Negative	2.38	.77–7.30	.136	1.38	.26–7.16	.703
<b>Cough ≥2 weeks</b>						
Yes	1.0	...	...	1.0	...	...
No	5.23	1.12–24.35	.014	3.53	.60–20.91	.135

Abbreviations: CI, confidence interval; WHO, World Health Organization.

were substantially lower among patients with Xpert-negative tuberculosis (Table 3).

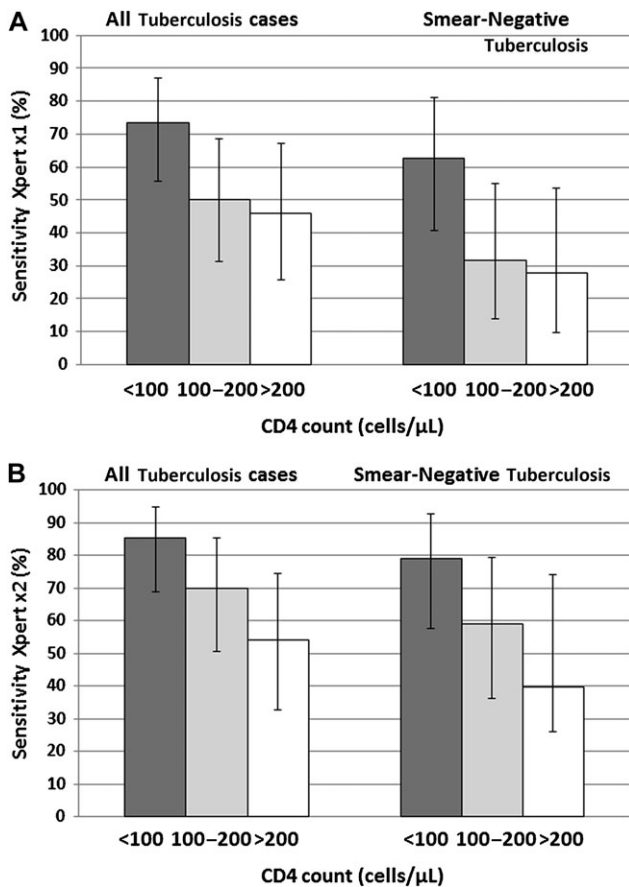
After comparing pulmonary radiographic appearances in the 2 groups, we observed that smaller proportions of patients with Xpert-negative tuberculosis had hilar and mediastinal lymphadenopathy or pleural abnormalities, and these patients also had a lower median number of radiographic zones affected by parenchymal abnormalities (Table 3). However, there was no statistical evidence to support these trends.

#### Patient Outcomes

We next compared the early ART program outcomes of patients with Xpert-positive and Xpert-negative tuberculosis, ascertaining

outcomes after 30 and 90 days from the time of program enrollment when the tuberculosis screening was done (Table 4). Although the timing of ART initiation did not differ between the 2 groups, Xpert-negative patients were much less likely than Xpert-positive cases to start tuberculosis treatment within 30 days of follow-up and were more likely to start ART before tuberculosis treatment. However, although the median time to starting tuberculosis treatment was much longer for the Xpert-negative compared with the Xpert-positive group (32 vs 9 days, respectively;  $P < .001$ ), Xpert-negative patients did not have adverse outcomes. Of the 6 deaths that occurred, all were in the Xpert-positive group (Table 4). Similar proportions in the 2 groups were lost to follow-up.





**Figure 2.** Sensitivity (with 95% confidence intervals) of Xpert MTB/RIF for diagnosing human immunodeficiency virus–associated tuberculosis during screening of patients before antiretroviral therapy, stratified by CD4 cell count and sputum smear status. Results displayed are from screening with 1 (A) or 2 (B) Xpert tests.

### Follow-up of Asymptomatic Patients and Strain Typing

Diagnoses of tuberculosis in asymptomatic individuals that were obtained using liquid culture could arise erroneously from laboratory contamination, so we determined the clinical course for such patients. Although 16 patients (18.0%) had a negative symptom screen at baseline, 12 (75%) of these developed tuberculosis symptoms during the first 8 weeks of follow-up and 4 (all Xpert-negative) remained asymptomatic until initiation of tuberculosis treatment. Two of these patients had abnormal chest radiographs with clearly discernible parenchymal opacities. Of the remainder, 1 patient had 2 positive cultures, with the shortest time to positivity being 15 days, and the remaining patient had a single positive culture at 28 days. MIRU-VNTR strain typing of isolates from all patients ( $n = 9$ ) with culture-positive Xpert-negative tuberculosis in whom there were paired *M. tuberculosis* isolates revealed identical banding patterns within each pair.

## DISCUSSION

Tuberculosis is a leading cause of morbidity, mortality and nosocomial disease transmission in ART programs in sub-Saharan Africa [8, 16–19]. The Xpert MTB/RIF assay represents a major step forward in screening for tuberculosis in this setting, greatly increasing case finding compared with smear microscopy [6]. Nevertheless, in the present study, 42% of culture-positive cases tested Xpert-negative on the first sputum sample and 28% of cases tested Xpert-negative on 2 samples. Careful comparison of patients with Xpert-positive tuberculosis showed that those with Xpert-negative tuberculosis (defined either by 1 or by 2 negative tests) had less advanced HIV-associated immunodeficiency and less advanced tuberculosis disease. In addition, despite much greater delays in starting tuberculosis treatment, patients with Xpert-negative tuberculosis did not have inferior early ART program outcomes and were less likely to die. Thus, the early clinical consequences of the false-negative Xpert tests were not grave, potentially affording time for reinvestigation.

Not only did patients with Xpert-negative tuberculosis have less advanced immunodeficiency, but they also had better prognostic indices, including a higher median body mass index and higher hemoglobin concentrations. These findings were robust whether Xpert-negative tuberculosis was defined based on 1 or 2 negative tests. Higher CD4 cell counts, lower plasma viral load and lower blood neutrophil counts were each strong independent predictors of negative Xpert results. Thus, although the Xpert assay missed a proportion of tuberculosis diagnoses, these were typically in patients with less advanced immunodeficiency. The reason underlying the higher neutrophil counts in those with Xpert-positive tuberculosis is unclear, possibly reflecting greater systemic immune activation or copathology, but there is also increasing evidence for an important role of neutrophils in the host inflammatory response to *M. tuberculosis* [20].

Other investigations for tuberculosis all strongly suggested that patients with Xpert-negative tuberculosis had a lower mycobacterial burden and less advanced tuberculosis disease. All these cases were smear-negative, the median time to culture positivity was prolonged, and detection of the LAM antigen and *M. tuberculosis* in urine samples was infrequent, suggesting a low frequency of disseminated tuberculosis [21]. Pulmonary radiographic findings correlate poorly with mycobacterial burden in immunocompromised patients because abnormalities are attenuated by the diminished host inflammatory response. However, a trend of less extensive radiographic abnormalities was observed in those with Xpert-negative disease. Collectively, the results of these diagnostic tests and the favorable prognostic features all suggest that Xpert-negative patients have

**Table 3. Results of Microbiological and Radiological Investigations for Tuberculosis Among All Patients With Tuberculosis and Those With Xpert-Negative or Xpert-Positive Tuberculosis, as Defined by Results From 2 Sputum Samples**

Results of Investigation	Patients With Tuberculosis			P
	All Patients (n = 89)	Xpert-Negative (n = 25)	Xpert-Positive (n = 64)	
<b>Microbiological findings</b>				
Smear positive	24/89 (27.0)	0/25 (0)	24/64 (37.5)	<.001
Time to culture positivity, median (IQR), d	16 (11–21)	21 (17–25)	13.5 (10–18)	<.001
Urine LAM ELISA-positive	23/84 (27.4)	2/25 (8.0)	21/59 (35.6)	.014
Urine Xpert-positive	17/85 (20.0)	2/25 (8.0)	15/60 (25.0)	.084
<b>Chest radiographic findings<sup>a</sup></b>				
Any abnormality	64/86 (74.4)	17/24 (70.8)	47/62 (75.8)	.783
Hilar or mediastinal lymphadenopathy	25/86 (29.1)	4/24 (16.7)	21/62 (33.9)	.185
Pleural abnormality	17/86 (19.7)	2/24 (8.3)	15/62 (24.2)	.134
Volume loss	12/86 (13.9)	3/24 (12.5)	9/62 (14.5)	1.00
Cavitation	2/86 (2.3)	0/24 (0)	2/62 (3.2)	1.00
Parenchymal abnormality	60/86 (69.8)	17/24 (70.8)	43/62 (69.4)	1.00
Parenchymal abnormality, median No. (%) of zones	3 (1.5–5)	2 (1–4)	3 (2–5)	.199

Abbreviations: ELISA, enzyme-linked immunosorbent assay; IQR, interquartile range; LAM, lipoarabinomannan.

<sup>a</sup> Chest radiographs were obtained from 86 patients. All values are proportions (%) unless otherwise stated.

a lower mycobacterial burden and less advanced tuberculosis and HIV disease.

The analytic limit of detection (95% sensitivity for detection) of the Xpert MTB/RIF assay has been defined in laboratory experiments as 131 colony-forming units (CFU)/mL of sputum [22]. This compares with a threshold of approximately 10 000 CFU/mL for detection by smear microscopy. Thus, the finding of Xpert-negative culture-positive disease defines a subgroup of patients with very low concentrations of *M. tuberculosis* in sputum. These patients are therefore likely to have a lower tuberculosis transmission risk compared with Xpert-positive cases. Three groups of patients might be defined as being broadly associated with incrementally lower mycobacterial load: those with culture-positive Xpert-positive smear-positive tuberculosis, those with culture-positive Xpert-positive smear-negative tuberculosis, and those with culture-positive Xpert-negative smear-negative tuberculosis, with the third category defining Xpert-negative disease.

The natural history of HIV progression and CD4 cell count decline is typically associated with increasing extrapulmonary tuberculosis, diminishing frequency of pulmonary cavitation and lower bacillary load in sputum samples [20]. However, we studied a very homogeneous population in which advanced immunodeficiency was universal and pulmonary cavitation was rare. This may partly explain the somewhat counterintuitive finding that the Xpert MTB/RIF assay had the greatest sensitivity among patients with the lowest CD4 cell counts. These patients may have unchecked mycobacterial replication, leading to higher concentrations of organisms in sputum. This is also

suggested by a similar trend in the sensitivity of smear microscopy in this patient population [10].

Although patients with Xpert-negative tuberculosis had much longer delays in receiving tuberculosis treatment, their prognosis was not adversely affected, which was most likely due to their better prognostic characteristics. Furthermore, patients in this study with Xpert-negative tuberculosis had the diagnostic “safety net” of sputum cultures, which ultimately revealed their diagnoses and resulted in the initiation of treatment. It is not known whether these cases would have been diagnosed and what their outcomes would have been in the absence of additional cultures. If the Xpert MTB/RIF assay were to be used for pre-ART routine tuberculosis screening in this setting, it would miss tuberculosis diagnoses in a proportion of cases with a low mortality risk and with a low risk of nosocomial tuberculosis transmission. Subsequent progression of symptoms during follow-up of such patients may alert healthcare workers to the need for repeated diagnostic screening, which might include serial screening with Xpert MTB/RIF.

Patients with HIV-associated tuberculosis and very low CD4 cell counts have a high mortality risk, yet these are the very patients in whom establishing a tuberculosis diagnosis is often the most challenging and slow [23]. With the low sensitivity of diagnostic tools in resource-limited settings, it has been suggested that the use of empiric tuberculosis treatment in patients with very advanced immunodeficiency and a high tuberculosis risk might be an effective strategy to reduce the mortality risk [24]. Randomized controlled trials are being



**Table 4. Early Antiretroviral Therapy Program Outcomes in all Patients With Tuberculosis and Those With Xpert-Negative and Xpert-Positive Tuberculosis, as Defined by Results From 2 Sputum Samples**

Outcome	Patients With Tuberculosis			P
	All Patients (n = 89)	Xpert-Negative (n = 25)	Xpert-Positive (n = 64)	
<b>30-day follow-up</b>				
Started ART	25 (28.1)	7 (28.0)	18 (28.1)	.991
Started tuberculosis treatment	50 (56.2)	7 (28.0)	43 (67.2)	.001
Started ART and tuberculosis treatment	14 (15.7)	1 (4.0)	13 (20.3)	.101
Started ART before starting tuberculosis treatment	13 (14.6)	7 (28.0)	6 (9.4)	.025
Transferred out	0	0	0	...
Lost to follow-up	9 (10.1)	4 (16.0)	5 (7.8)	.261
Death	6 (6.7)	0	6 (9.4)	.179
Alive and in program	74 (83.2)	21 (84.0)	53 (82.8)	1
<b>90-day follow-up</b>				
Started ART	57 (64.0)	18 (72.0)	39 (60.9)	.328
Started tuberculosis treatment	66 (74.2)	17 (68.0)	49 (76.6)	.407
Started ART and tuberculosis treatment	48 (53.9)	13 (52.0)	35 (54.7)	.819
Started ART before starting tuberculosis treatment	18 (20.2)	11 (44.0)	7 (10.9)	<.001
Transferred out	1 (1.1)	0	1 (1.6)	1
Unavailable for follow-up	12 (13.5)	4 (16.0)	8 (12.5)	.733
Death	6 (6.7)	0	6 (9.4)	.179
Alive and in program	70 (78.7)	21 (84.0)	49 (76.6)	.570
<b>Timing of treatment<sup>a</sup></b>				
Time to starting ART, median (IQR), days	32 (27–48)	37.5 (28–62)	31 (27–48)	.4187
Time to starting tuberculosis treatment, median (IQR), days	14 (8–30)	32 (26–48)	9 (6–18)	<.0001

Variables are numbers (%) unless otherwise stated.

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range.

<sup>a</sup> Based on patients who started treatment within 90 days of follow-up.

conducted to determine if this strategy is truly effective. However, we found that the Xpert MTB/RIF assay had the highest sensitivity among patients with the lowest CD4 cell counts, with tests on 2 samples detecting ~85% of sputum culture-positive disease among those with CD4 cell counts <100 cells/ $\mu$ L. Thus, the rationale for empiric treatment may be somewhat diminished in settings where Xpert MTB/RIF becomes available. In ART programs in sub-Saharan Africa where tuberculosis is a major cause of death [16], high early mortality may be effectively reduced by using the assay as a screening tool.

This is the first study to report on the characteristics and early outcomes of patients with Xpert-negative tuberculosis. The strengths of this study include the careful characterization of patients with tuberculosis diagnosed during the screening of unselected consecutive patients, analyses based on the testing of 1 and 2 sputum samples and comparison with numerous other tuberculosis diagnostics. Outcomes of ART program losses were also carefully ascertained in this cohort [25], yet deaths among those lost to follow-up cannot be definitively excluded. Weaknesses include the fact that it is not known what the

outcomes of patients with Xpert-negative tuberculosis would have been in the absence of sputum cultures. It is impossible to definitively exclude any cases of culture contamination, yet most patients with culture-positive disease who were asymptomatic rapidly developed symptoms during follow-up, and there was no evidence to suggest contamination based on the molecular typing of paired isolates. The data from the present study should not be extrapolated to clinical populations of symptomatic patients with suspected tuberculosis attending tuberculosis clinics, because Xpert-negative patients in this setting may have different characteristics.

In conclusion, the Xpert MTB/RIF assay missed 28%–42% of culture-positive tuberculosis diagnoses (depending on the number of samples tested) during the pre-ART screening of patients regardless of symptoms. Any adverse consequences of missed or delayed diagnoses in patients with Xpert-negative tuberculosis, however, were offset by the fact that these patients were less immunocompromised, had less advanced tuberculosis disease, had a low mortality risk and were likely to have a lower tuberculosis transmission risk. Good short-term prognosis affords time for further investigation of such patients.

## Notes

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