The long-term risk of tuberculosis among individuals with Xpert Ultra "trace" screening results: a longitudinal follow-up study

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Content: summary (250 words), text (3,288words), 20 references, 5 figures, 2 tables, and supplementary materials

Key words: community screening; tuberculosis; incidence; Xpert MTB/RIF Ultra; trace; molecular diagnostic testing

Research in context

Evidence before this study

Recent advances in tuberculosis research have shifted the disease framework from a binary classification of latent versus active tuberculosis to a continuum of disease states. They have also led to a better understanding of the dynamic disease course of early tuberculosis, which can either progress to culture-positive disease or regress spontaneously over time. “Trace" results from Xpert MTB/RIF Ultra (“Ultra”) are sometimes perceived as false positives in individuals who subsequently test negative on additional diagnostic assays. However, some of these individuals may have early tuberculosis that falls below the detection threshold of existing diagnostic tests and could progress to microbiologically detectable disease over time. To investigate this, we searched PubMed for studies published up to February 7, 2025, using the terms “tuberculosis” AND (“Xpert OR “Xpert Ultra” OR “Ultra”) AND “Trace” and also reviewed the reference lists of relevant search results. Two prevalence surveys that used Xpert Ultra as a confirmatory test for individuals with symptoms or an abnormal chest X-ray found that 20% and 46% of those with trace-positive sputum had positive cultures. In a study conducted in Uganda where Ultra was used as an initial screening test, only 14% of individuals with a trace-positive result had positive sputum cultures. However, no prior studies have prospectively examined the incidence of tuberculosis among individuals with a trace-positive Ultra result during systematic screening who are otherwise microbiologically negative and not started on treatment.

Added value of this study

In this study, individuals with Ultra trace-positive screening results who were not started on treatment after extensive diagnostic testing were followed for up to two years with repeated testing. About 25% developed tuberculosis during follow-up, and the 2-year cumulative hazard of incident tuberculosis was substantial at 35% (95% confidence interval 19-52%). Those who had a normal chest X-ray at enrollment were at significantly lower risk of developing tuberculosis. Incident tuberculosis risk was similar between those who reported symptoms at the time of enrollment and those who did not.

Implications of all the available evidence

The high incidence of tuberculosis observed among people with trace results in this study support provision of treatment to most individuals who receive trace results during tuberculosis screening. These results also demonstrate that X-ray could be a useful tool to guide treatment decision-making for individuals with trace-positive sputum.

Summary

Background: Systematic screening for tuberculosis using Xpert Ultra generates “trace” results of uncertain significance. Additional microbiological testing in this context is often negative, but individuals with trace results might have early disease or elevated risk of tuberculosis.

Methods: We screened for tuberculosis with Xpert Ultra in Uganda, enrolling individuals with trace-positive results and Ultra-negative controls. Participants without tuberculosis on extensive initial evaluation were followed, with repeat testing at 1, 3, and 6 months after trace results, and at 12 and 24 months for all participants. We estimated cumulative cause-specific hazards of incident tuberculosis, considering a definition of tuberculosis that included clinician judgment and one based strictly on microbiological results. We compared participants with Ultra-trace versus Ultra-negative sputum, and subgroups of participants with Ultra-trace sputum.

Findings: Of 129 participants with trace-positive screening results, 45 (35%) were recommended for treatment upon enrollment, and eight were lost to follow-up within three months. Of 76 remaining participants followed for median 697 (interquartile range 179-714) days, 20 (26%) were recommended for tuberculosis treatment. The cumulative hazard of clinician-defined incident tuberculosis was 26% (95% confidence interval: 14-38%) at one year and 35% (19-52%) at two years, versus 2% (0-5%) at two years for controls. Hazards were similar for microbiologically defined incident tuberculosis. Incident tuberculosis was strongly associated with abnormal baseline chest X-ray (hazard ratio 15.0 [3.4-65.1]) but not with baseline symptoms.

Interpretation: Individuals with trace-positive sputum during screening, particularly those with abnormal chest imaging, are at substantial risk of incident tuberculosis over the subsequent two years.

Funding: National Institutes of Health

Introduction

Despite being a curable disease, tuberculosis continues to be the leading single-agent infectious cause of death worldwide, accounting for 1.25 million deaths in 2023.[1] The World Health Organization estimates that about 25% of individuals who develop tuberculosis are never diagnosed and started on treatment.[1] Detecting tuberculosis at early stages through effective diagnostic strategies could reduce transmission and prevent severe outcomes.

Tuberculosis programs are increasingly conducting community-based tuberculosis screening,[2] and during such screening efforts, Xpert MTB/RIF Ultra ("Ultra"; Cepheid), a molecular diagnostic tool for tuberculosis, is widely used as a confirmatory test after positive screening results. In screening contexts, a large proportion of positive Ultra results are in the “trace” category.[3-5] Trace results, which represent the lowest level of *M. tuberculosis (Mtb)* DNA detection, are reported when the Ultra assay detects one of its multicopy amplification targets (*IS1660* or *IS1810*) but not its single-copy *rpoB* gene target.[6] Studies that have used Ultra as an initial or confirmatory test during general-population screening in high-burden settings have reported that 24% to 46% of positive results are trace-positive.[3-5,7] Due to lower tuberculosis prevalence in general populations compared to symptomatic patients tested at health facilities, the positive predictive value of a trace result may be lower in these screening contexts, but it is uncertain what proportion of trace results reflect underlying tuberculosis disease.

We previously reported on systematic screening for tuberculosis that we conducted in Uganda, using Ultra as an initial test regardless of symptoms, with detailed baseline evaluation of participants who received trace results.[5] Among the first 92 such participants, only 22 (24%) had microbiologically confirmed tuberculosis, with just 13 (14%) having a positive culture. However, tuberculosis has a dynamic and varied disease course, with progression and regression of bacterial burden occurring across different states of infection and disease.[8-10] We hypothesized that some individuals with a trace-positive sputum result who are otherwise microbiologically negative may have early (or otherwise minimal-burden) disease that could progress over time, such that they could potentially benefit from preventive or empirical treatment. Therefore, within the same cohort after full enrollment, we aimed to estimate the incidence of tuberculosis disease among participants with Ultra trace-positive screening results who had completed detailed clinical evaluations for tuberculosis with negative results and no recommendation for treatment.

Methods

Symptom-Neutral Tuberculosis Screening and Participant Recruitment

We conducted Xpert Ultra-based systematic screening for tuberculosis in Kampala, Uganda, from February 2021 to April 2024, enrolling individuals with trace-positive results as well as Ultra-positive and Ultra-negative controls. Detailed procedures for recruitment and initial evaluation have been described previously.[5] Briefly, participants for tuberculosis screening were recruited primarily through community-based screening events and door-to-door screening and to a lesser extent through contact investigations. Xpert Ultra sputum testing was offered to any individuals aged ≥15 years who were not on active tuberculosis treatment, regardless of their symptom status. From among these screening participants, we then recruited all participants who had tested trace-positive on Xpert Ultra screening, as well as age- and sex-matched participants with negative screening results ("negative controls") and consecutive participants with positive (>trace) screening results ("positive controls"; recruited until reaching 110 participants), for further diagnostic evaluation. To enhance our sample size, we also enrolled people who were found to have trace-positive sputum during community-based, symptom-agnostic Ultra testing that was offered in high-risk areas of Kampala during a national screening campaign.[11]

Evaluation and Follow-Up of Study Participants

Upon enrollment, participants with a trace-positive screening result received extensive diagnostic testing, including repeat sputum Xpert Ultra testing, two sets of sputum liquid and solid mycobacterial cultures, HIV testing (using a serial rapid testing algorithm,[12] if not known positive), chest X-ray (CXR), and chest computed tomography (CT). Participants with HIV also received urine lipoarabinomannan (LAM) testing (Determine TB LAM; Abbott). Baseline and longitudinal study results were reviewed by an independent physician panel comprising experienced Ugandan pulmonologists and radiologists ("consultants"), who could recommend additional tuberculosis diagnostic testing and/or initiation of tuberculosis treatment as they deemed appropriate. Participants who were advised to start tuberculosis treatment were referred to a health facility for treatment initiation. Participants who were not recommended for tuberculosis treatment, or who declined treatment that had been recommended, were followed with scheduled re-evaluations at 1, 3, 6, 12, and 24 months (**Figure 1**), in addition to unscheduled re-evaluations if they reported changes in health status. At each scheduled follow-up visit, participants were assessed for symptoms and asked to provide expectorated sputum for repeat testing by Ultra. Sputum cultures and chest X-rays were also repeated at the 3- and 12-month follow-up visits, with additional cultures at 6 and 24 months added partway through the study (in November 2023). Both negative- and positive-control participants underwent the same diagnostic evaluations at baseline. Negative-control participants not recommended for treatment were followed for up to two years, with

symptom assessment at 6 months and repeat sputum testing at 12 and 24 months. Positive-control participants were referred for treatment initiation.

Interpretation of Chest Imaging by Radiologists and CAD Software

Chest X-ray and CT images of study participants were reviewed by two independent radiologists, with a third radiologist reviewing images where the initial readings were discrepant. The radiologists, blinded to clinical information, rated the consistency with current tuberculosis and (separately) with prior tuberculosis on four-point scales, as detailed previously.[5] In addition, all baseline chest X-rays were retrospectively analyzed using CAD software (qXR v4, Qure.ai, India); CAD results were unavailable during treatment decision-making.

Statistical Analysis

Our primary analysis defined tuberculosis based on treatment recommendation. Under this definition, all participants for whom consultants recommended treatment or an external clinician prescribed treatment were counted as having tuberculosis, including those diagnosed clinically; microbiological results, including positive results that clinicians or consultants disregarded as false-positive, did not affect outcome classification. In secondary analyses, we defined tuberculosis microbiologically as having at least one of: a sputum Ultra result greater than trace, a sputum culture positive for *M. tuberculosis* complex, or (among people with HIV) a positive urine LAM result. For survival analyses using this microbiological definition of tuberculosis, we censored participants who initiated treatment without microbiological positivity, while those who were clinically diagnosed but declined the recommended treatment remained eligible to experience incident microbiologically defined tuberculosis; their further follow-up time was included only as follow-up for onset of microbiological positivity.

We first estimated the cumulative proportion of participants with trace sputum screening results who were diagnosed with tuberculosis (i.e., recommended for treatment) over a follow-up period of up to two years, with a corresponding binomial 95% confidence interval (CI). Diagnoses were stratified by microbiological status. To be counted as negative in this analysis, participants were required to have completed at least three months of follow-up.

Then, focusing on participants who were not diagnosed with tuberculosis at baseline, we used survival analysis to evaluate whether a trace screening result was associated with an elevated incidence of tuberculosis during follow-up. We compared the cumulative cause-specific hazards of developing tuberculosis between participants with trace versus negative initial Ultra screening results, reporting cumulative hazards and hazard ratios (HR) with 95% CIs.[13] We performed this analysis for both the

treatment-recommendation and the microbiological definitions of tuberculosis, using the corresponding definition of tuberculosis to also define the cohort without tuberculosis at baseline. We present results for the two definitions in parallel.

Additionally, we compared the cumulative cause-specific hazards of developing tuberculosis between subgroups of participants with trace screening results who had different baseline characteristics. For each baseline risk factor — male sex, HIV infection, positive symptom status (based on a four-symptom assessment), and history of prior tuberculosis treatment — we calculated hazard ratios comparing those with and without the risk factor, with corresponding 95% CIs. For participants who completed chest X-ray (CXR) or CT at baseline, we additionaly estimated hazard ratios comparing those with and without abnormal baseline chest imaging by each of several definitions, namely (a) CXR read by radiologists as having any abnormalities, (b) CXR read by radiologists as suggestive of tuberculosis, (c) CXR with qXR tuberculosis score ["CAD score"] ≥0.2, (d) CXR with CAD score ≥0.5, and (e) CT read as suggestive of tuberculosis. All comparisons between subsets of participants with trace screening results were performed for each of two definitions of tuberculosis in parallel.

Finally, we compared baseline demographic, clinical, and radiographic characteristics among the following five groups of participants: 1) positive control participants, 2) participants with trace-positive sputum (PWTS) recommended for treatment at baseline ("Trace, prevalent TB"), 3) PWTS recommended for treatment during follow-up ("Trace, incident TB"), 4) PWTS not recommended for treatment and followed for at least three months ("Trace, no TB"), and 5) negative control participants. The CAD scores of baseline chest X-rays were compared between five groups of participants using Kruskall-Wallis tests followed by Dunn tests with Bonferroni correction.[14] We also estimated the area under the curve (AUC) for the CAD score at baseline in diagnosing tuberculosis disease (at baseline or during follow-up) among PWTS who were either diagnosed or followed for ≥ three months, using both definitions of tuberculosis diagnosis (treatment recommendation versus microbiological positivity).

This analysis was conducted after all participants had sufficient time to complete six months of follow-up, with culture results from samples collected at six-month visits finalized. Statistical significance was determined as a two-sided alpha <0.05. Analyses were performed using STATA version 16.1 and R version 4.3.2.

Ethics Considerations

Informed consent (or adolescent assent with parental consent) was obtained from all study participants. The study was approved by the Institutional Review Boards of the Johns Hopkins University School of Medicine and the Makerere University School of Public Health. Study progress was monitored semi-

annually by an Observational Study Monitoring Board (OSMB). In June 2024, based on preliminary findings, the OSMB recommended that participants with initial trace screening results, no history of prior tuberculosis treatment, and no tuberculosis diagnosis during the study be referred for tuberculosis preventive treatment when they completed study follow-up, and these recommendations were implemented accordingly.

Results

A total of 31,505 individuals aged ≥15 years participated in tuberculosis screening, including 16,568 recruited through event-based screening, 14,782 through door-to-door screening, and 155 through contact investigation. Of these, 31,321 (>99%) successfully provided sputum samples, and 31,150 had valid Xpert results. Among those with valid results, 297 (1.0%) tested positive, including 125 (42% of positives) with trace-positive results. We recruited 111 (89%) of these individuals and additionally recruited 18 participants who were found to have trace-positive sputum during a national screening campaign[11] (**Figure 2**). We also enrolled 138 matched Xpert-negative controls and 110 (76% of 144 eligible) Xpert-positive (>trace) controls.

Among 129 participants with trace-positive sputum (PWTS), the median age was 33 (interquartile range [IQR]: 24-39), 5% (19/129) had HIV infection, 18% (23/129) had a history of prior tuberculosis, and 47% (44/93 who were assessed) reported a cough at screening (**Table 1**). Of 129 PWTS, 45 (35%) were recommended tuberculosis treatment based on additional testing performed at baseline, including 36 participants with microbiological confirmation and nine participants who were clinically diagnosed. Of 84 PWTS not recommended for treatment based on baseline data, eight participants were lost to follow-up before three months. The remaining 76 were followed for incident tuberculosis over a median of 697 days (IQR 179–714), and of these, 20 (26% of 76) were recommended to receive tuberculosis treatment during follow-up, including 16 with microbiological confirmation and four clinically diagnosed. Therefore, among 121 PWTS with sufficient follow-up data for evaluation, 65 (54%, 95% CI 44-63%) were recommended for tuberculosis treatment either at baseline or during follow-up. Three deaths occurred among study participants: one PWTS living with HIV died of tuberculosis at 12 months after missing their six-month study visit and stopping antiretroviral treatment; one negative-control participant died during childbirth; and a positive-control participant died shortly after enrollment, before initiating treatment.

Among PWTS not diagnosed with tuberculosis through baseline evaluation, the cumulative cause-specific hazard of incident tuberculosis was 26% (95% CI 14-38) at one year and 35% (95% CI 19-52) at two years when estimated based on treatment recommendation. Corresponding cumulative hazards

based on microbiological positivity were 26% (95% CI 13-39) at one year and 39% (95% CI 21-57) at two years. The two-year cumulative hazard of incident tuberculosis among Xpert-negative controls was 2% (0-5), based on treatment recommendation or 1% (0-4) based on microbiological positivity (**Figure 3 a-b**). Among PWTS, incident tuberculosis was strongly associated with abnormal baseline CXR (HR 15.0 [95% CI 3.4-65.1] as interpreted by human readers, **Figure 3 c-d**) but not with symptoms (HR 1.3 95% CI 0.5-3.3) (**Supplementary figure 1**). Sex, HIV status, and history of prior tuberculosis were also not significantly associated with incident tuberculosis among PWTS not diagnosed with tuberculosis at baseline (**Supplementary table 1**).

Of 377 study participants, 331 (88%) completed a chest X-ray at enrollment. The median CAD scores (by qXR v4) were highest among Ultra-positive controls (median 0.97 [IQR 0.86–0.99]) and lowest among negative controls (median 0.17 [IQR 0.06–0.35]). Among 113 PWTS, those with prevalent tuberculosis and those with incident tuberculosis (by treatment recommendation-based definition) had similar median CAD scores (median 0.84 [IQR 0.52–0.97] versus 0.87 [IQR 0.70–0.92]), but those who were followed for ≥3 months without any tuberculosis diagnosis had lower CAD scores (median 0.30 [IQR 0.09–0.76], in comparison with PWTS with prevalent [p<0.01] or incident [p=0.03] tuberculosis). Among PWTS not diagnosed with tuberculosis, elevated CAD scores occurred mainly in those with history of prior tuberculosis treatment (**Figure 4**).

Baseline CXR interpretation by qXR v4 achieved an AUC of 0.78 (95% CI 0.69-0.86) for predicting prevalent or incident tuberculosis among PWTS using the treatment recommendation-based definition, and 0.77 (95% CI: 0.68-0.86) when defining tuberculosis by microbiological positivity (**Figure 5**). Using the microbiological definition of tuberculosis, a CAD score threshold of 0.5 resulted in a sensitivity of 75% and a specificity of 64%, and a threshold of 0.2 had a sensitivity of 86% and specificity of 45%. Results were similar when a treatment recommendation-based definition was used (**Supplementary Table 2)**. Accuracy was higher among PWTS who did not have a prior history of tuberculosis treatment (AUC 0.84 [95% CI 0.76-0.93] for treatment recommendation; AUC 0.85 [95% CI 0.76-0.93] for microbiological positivity).

Of 63 PWTS with prevalent or incident tuberculosis (by treatment recommendation-based definition) who completed a CXR at baseline, only four (6%) had very low qXR scores (<0.1); all four of their baseline X-rays were also interpreted as normal by radiologists. One of these participants had a normal baseline chest CT but had untreated HIV and was started on tuberculosis treatment by her HIV clinician. The remaining three participants, all of whom had culture-confirmed tuberculosis, had subcentimeter nodules identifiable on their baseline chest CTs: a segmental nodular consolidation in a participant with positive culture at baseline, and scattered solid nodules in the remaining two participants who were

diagnosed at 3 months and 6 months. Nearly all PWTS who were diagnosed with tuberculosis at baseline or during follow-up (61 out of 64, 95%) had abnormal chest CTs at baseline (**Table 2**), compared to 36% of PWTS who were not diagnosed.

Discussion

In this population-representative cohort of people with Ultra-trace-positive sputum in a high-burden setting, the two-year risk of developing tuberculosis was high, even after a baseline evaluation that was interpreted as excluding tuberculosis disease. Approximately one-third were diagnosed through additional testing shortly after their trace result, and of the remainder, the cumulative two-year hazard of incident tuberculosis was 35%. Among PWTS who were not diagnosed with tuberculosis at baseline, those with abnormal initial chest imaging had a particularly high risk of developing tuberculosis during follow-up, whereas baseline symptoms showed no prognostic value.

Our report highlights that individuals who receive trace results from tuberculosis screening are at substantial risk of (prevalent or incident) tuberculosis. Furthermore, while this study used sputum Ultra as an initial test, most screening programs utilize Ultra as a confirmatory test only among individuals with abnormal chest X-ray and/or symptoms.[3,4] This screening step increases the prevalence of tuberculosis among those who qualify for testing with Ultra, and thus the positive predictive value of the Ultra test. When X-ray is used for screening, our results suggest that the screening step may also increase the probability that those with trace results have or will soon develop tuberculosis, even if further microbiological testing at the time of screening would be negative. Thus, our results support treatment of most individuals who receive trace results during tuberculosis case-finding activities, particularly when those trace results are preceded by chest X-ray screening.

Our results are aligned with the current understanding of the tuberculosis disease spectrum and provide further insights into the natural course of the disease. Early tuberculosis has a dynamic disease course, and disease burden can fluctuate over time.[8,10,15] Some individuals with culture-positive tuberculosis may experience spontaneous improvement and become culture-negative.[9] As often occurs after treatment, this immune-driven reduction in disease burden could leave nonviable *Mtb* that are detectable on molecular tests even after the burden of viable bacteria becomes too small for culture to detect.[16] However, culture-negative individuals who once had enough mycobacterial burden to produce a positive Ultra result, and who have not received sterilizing treatment, could be at high risk of progressing (or progression again) to culture-positive disease. Although our study confirms an elevated risk among people with trace-positive sputum, some lower-burden tuberculosis may be cured with

shortened regimens,[17] and the appropriate treatment strategy for this form of paucibacillary tuberculosis is uncertain.

Our results also demonstrate the potential utility of chest imaging in interpreting Ultra trace results. More than half of PWTS without prevalent tuberculosis had baseline CXRs interpreted as normal by radiologists, and this subset had a substantially lower risk of developing tuberculosis (cumulative hazard 6% at 2 years) — potentially too low to justify empiric multi-drug treatment, although still above the risk in contacts for whom preventive therapy is recommended.[18] It should be noted that radiologists interpreting chest X-ray images in this study had access to paired chest CT images, and the estimated hazard ratio (>10) may overestimate the ability of X-rays to discriminate early tuberculosis-related changes. However, X-ray abnormality scores interpreted by CAD software still demonstrated utility in identifying prevalent or incident tuberculosis among PWTS, particularly among those without prior tuberculosis treatment. Thus, as has been described among other at-risk populations,[9,19] chest X-ray appears to have utility among people with trace sputum for identifying culture-negative tuberculosis that is likely to progress.

This study has several limitations. Although we had an overall high rate of recruitment and retention, there could have been selection bias, as PWTS who were at higher risk of tuberculosis may have been more likely to participate in screening, enroll into the study, and complete follow-up. The distinction between incident and prevalent tuberculosis is not always clear, and tuberculosis that was active but not currently microbiologically positive may have been inconsistently classified. We did not induce sputum, which could have led to underestimation of culture-positive prevalence or incidence. Under our primary definition of tuberculosis, clinical diagnoses may have led to overestimation of prevalence, biased the cohort being followed for incidence toward lower-risk individuals, and/or overestimated incidence among that cohort. It is reassuring that incidence estimates were similar under our secondary, microbiological definition, although censoring individuals who started treatment and classifying false-positive microbiological results as positives may have led to under- or overestimation of incidence, respectively.

In summary, we conducted community-based tuberculosis screening using sputum Ultra in Kampala, Uganda, and found that more than half of the individuals who received a trace result were confirmed to have tuberculosis within two years. Risk was elevated even among those not initially thought to have tuberculosis after extensive baseline diagnostic procedures.Tuberculosis diagnosis during follow-up was strongly associated with baseline chest imaging abnormalities but not with baseline symptoms. Our findings provide compelling evidence supporting tuberculosis treatment for most individuals with trace-

positive screening results. Further research is needed to determine the most appropriate treatment strategy for these individuals.

Contributors

JS performed the data analysis and wrote the original draft of the manuscript. MN coordinated field data collection activities in Uganda. AN contributed to data management and data curation. PB and CV also contributed to data curation. JM, JA, RK, FK and MM enrolled participants and collected data in Uganda. CEK contributed to the laboratory processing of study samples. DWD contributed to the study's conception and critically revised the manuscript. AK contributed to the study's conception and supervised the data collection process in Uganda. EAK conceptualized the study, acquired funding and resources, supervised the data collection and development of the analytic plan, verified the underlying data, and critically revised the manuscript. All authors had full access to all the data in the study and accept responsibility for the decision to submit the manuscript for publication.

Data sharing

The deidentified dataset used for this study and a data dictionary will be available upon a reasonable request. Data sharing will be limited to non-commercial research use only. Requests should include a proposal outlining the intended use and methodology and will be subject to review and approval. Proposals can be directed to ekendall@jhmi.edu.

Declaration of interests

Authors report no conflicts of interest.

Acknowledgements

This work was supported by the National Institutes of Health (grant numbers R01HL153611 [to E. A. K.], R01HL138728 [to D. W. D.], T32AI007291 and K23AI185268 [to J.S.]). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Figure 1. Study procedures for evaluation and follow-up after a trace Xpert Ultra result during community-wide screening. Community-based tuberculosis screening was conducted in Kampala, Uganda, from February 2021 to April 2024, using Xpert Ultra as the initial test. Participants with a trace-positive result were enrolled and underwent a detailed assessment, including symptom evaluation, additional microbiological testing (repeat sputum Ultra, two sets of sputum liquid and solid mycobacterial cultures, and urine LAM for people with HIV), chest X-ray, and chest CT. Those not initiated on treatment were followed at 1, 3, 6, 12, and 24 months, with repeat testing as outlined below. In November 2023, sputum cultures at 6 and 24 months were added to all subsequent follow-up.

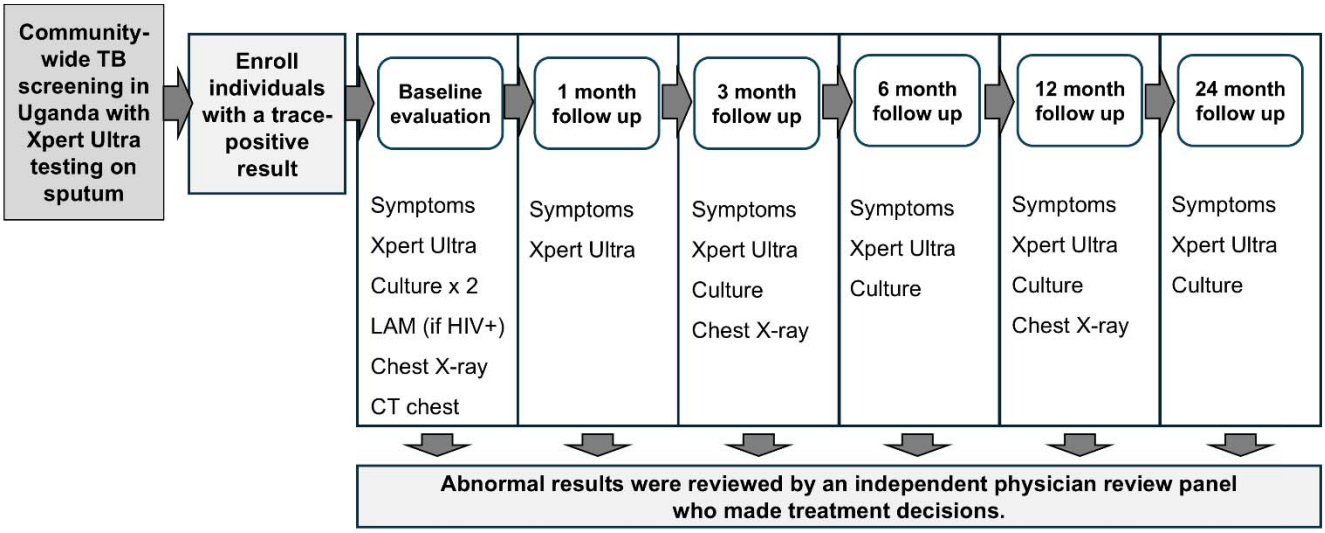


Figure 2. Recruitment and diagnostic outcomes of study participants with Ultra trace-positive sputum. The flow diagram illustrates the recruitment process and diagnostic outcomes of study participants. Most participants were recruited from community-based tuberculosis screening conducted by the study team using Ultra as the initial test, and a minority of participants were recruited after undergoing similar symptom-neutral screening with Ultra through a national screening campaign. Among the 129 participants with trace-positive screening results, 45 and 20 were recommended for treatment at baseline or during follow-up, respectively, including 36 and 16, respectively, with microbiological confirmation by Ultra, culture or urine lipoarabinomannan. There were 2 participants at baseline and 4 during follow-up with presumed false-positive microbiological results, for whom treatment was not recommended; these were classified as having baseline or incident tuberculosis in a secondary analysis that used a strictly microbiological definition, but they are not classified as "recommended for treatment" in this figure.

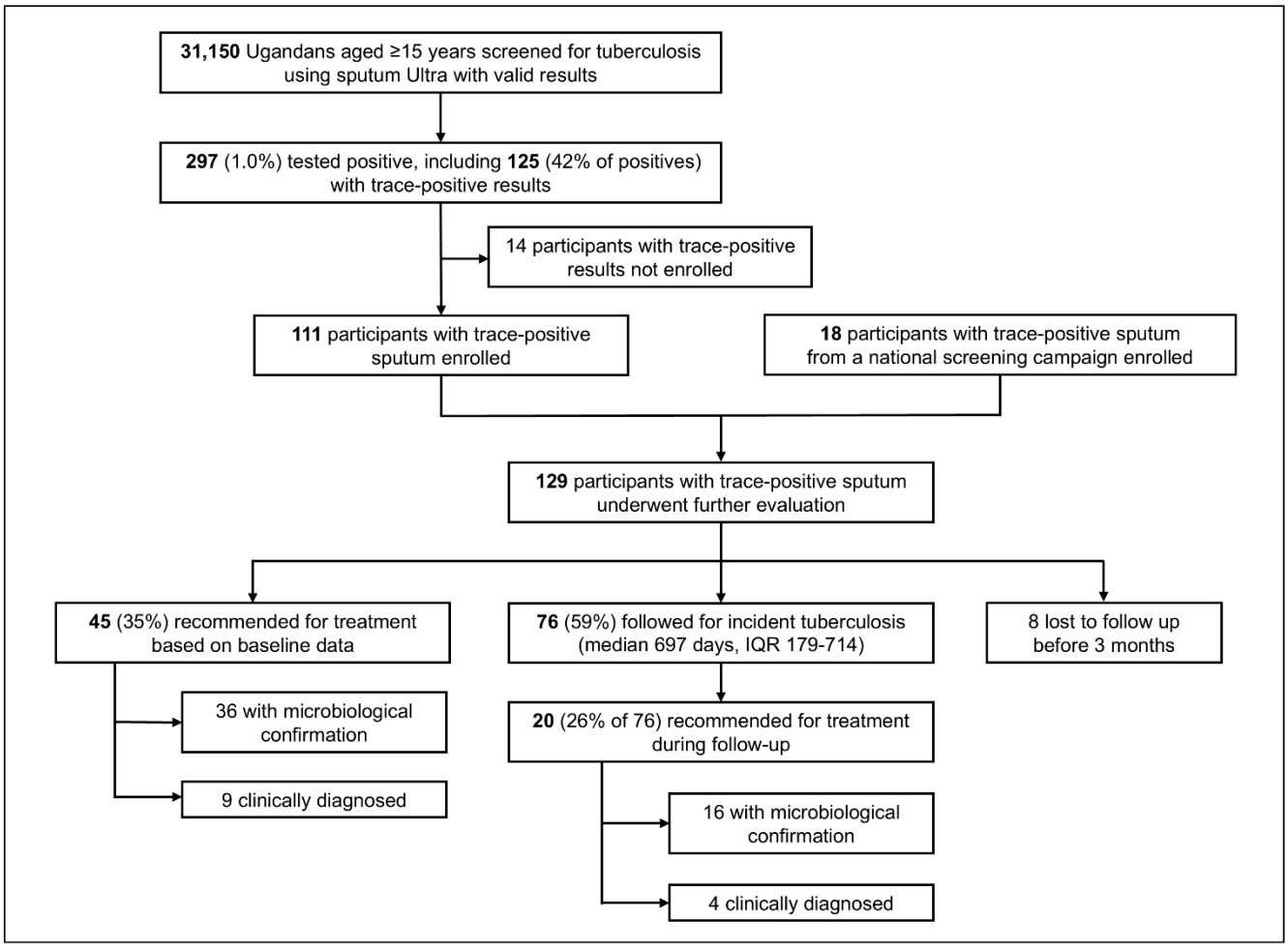


Table 1. Characteristics individuals screened for tuberculosis in community settings in Kampala, Uganda, according to initial sputum Ultra results and treatment recommendation.

	Positive Ultra (n=110)	Trace Ultra (n=121)[#]			Negative Ultra (n=138)
		Treatment recommended at baseline (n=45)	Treatment recommended during follow-up (n=20)	Treatment not recommended[#] (n=56)	
Age (years), median (IQR)[†]	35 (28, 42)	35 (24, 42)	32 (27, 38)	33.5 (25, 38)	32 (24, 39)
Female, n (%)[†]	26 (24%)	13 (29%)	8 (40%)	25 (45%)	59 (43%)
HIV positive, n (%)[†]	23 (21%)	9 (20%)	4 (20%)	6 (11%)	11 (8%)
Recent household tuberculosis exposure, n (%)[††]	15 (14%)	7 (16%)	4 (20%)	7 (13%)	7 (5%)
Screened during contact investigation, n (%)	5 (5%)	2 (4%)	0 (0%)	3 (5%)	0 (0%)
Prior tuberculosis, n (%)	20 (18%)	4 (9%)	7 (35%)	12 (21%)	6 (4%)
Current smoking, n (%)	36 (33%)	14 (31%)	8 (40%)	8 (14%)	15 (11%)
Underweight (body mass index <18.5 kg/m^2), n/N (%)	27/102 (25%)	17/45 (38%)	2/19 (11%)	4/46 (9%)	11/124 (9%)
Cough at screening, n/N (%)	67/94 (71%)	21/35 (60%)	5/11 (46%)	18/41 (44%)	34/119 (29%)
Cough at enrollment, n (%)	98 (89%)	37 (82%)	14 (70%)	37 (66%)	29 (21%)
Weeks of cough,[‡] median (IQR)	5.5 (3, 16)	4 (2, 8)	2 (1, 6)	3 (1, 12)	2 (1, 4)
Cough severity on 0-100 visual analog scale, [‡, 20] median (IQR)	50 (30, 70)	40 (20, 60)	30 (10, 50)	30 (20, 50)	25 (10, 30)
Any tuberculosis symptom at screening*, n (%)	68/94 (72%)	22/35 (63%)	6/11 (55%)	20/41 (49%)	39/119 (33%)
Any tuberculosis symptom at enrollment, n (%)**	104 (95%)	40 (89%)	18 (90%)	47 (84%)	57 (41%)
Positive QuantiFERON, n/N (%)	59/67 (88%)	35/40 (88%)	13/16 (81%)	32/53 (60%)	50/105 (48%)
CRP ≥ 5.0 mg/L, n/N (%)	56/103 (54%)	19/44 (43%)	4/19 (21%)	13/56 (23%)	21/135 (16%)

[†] Matching constrained the age and sex of negative controls to be similar to, and HIV prevalence to be no greater than, that of PWTS as a whole.
[††] Within one year
[‡] If cough reported.
* any cough, fever, night sweats, or current weight loss at the time of screening
** any cough, fever, night sweats, or weight loss (5kg over 12 month) at the time of enrollment
[#] This excludes 8 participants who completed less than 3 months of follow-up.
HIV = human immunodeficiency virus, IQR = interquartile range, CRP = C-reactive protein

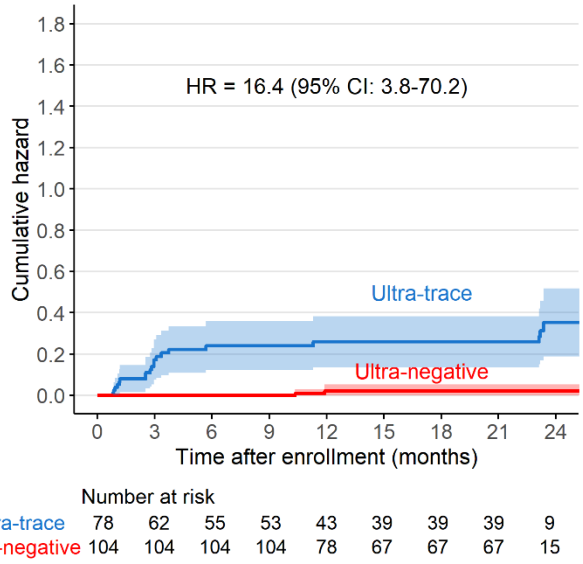
Table 2. Chest computed tomography characteristics of individuals screened for tuberculosis, according to initial sputum Ultra results and treatment recommendation.

	Positive Ultra (n=101)	Trace Ultra (n=123)			Negative Ultra (n=107)
		Diagnosed at baseline (n=44)	Diagnosed during follow-up (n=20)	Not diagnosed (n=53)	
Any abnormality	96 (95%)	41 (93%)	20 (100%)	19 (36%)	23 (21%)
Consolidation	55 (54%)	20 (45%)	5 (25%)	4 (8%)	2 (2%)
Ground glass opacity	32 (32%)	11 (25%)	3 (15%)	3 (6%)	4 (4%)
Nodule (≤3cm)	89 (88%)	34 (77%)	20 (100%)	14 (26%)	8 (7%)
Mass (>3cm)	3 (3%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)
Septal thickening	77 (76%)	24 (55%)	12 (60%)	12 (23%)	5 (5%)
Bronchial or bronchovascular abnormality	79 (78%)	25 (57%)	11 (55%)	12 (23%)	6 (6%)
Cavitation	53 (52%)	10 (23%)	3 (15%)	2 (4%)	0 (0%)
Parenchymal fibrosis	61 (60%)	23 (52%)	11 (55%)	15 (28%)	7 (7%)
Volume loss	34 (34%)	11 (25%)	5 (25%)	7 (13%)	1 (1%)
Emphysematous changes	20 (20%)	9 (20%)	3 (15%)	9 (17%)	5 (5%)
Pleural thickening or fibrosis	20 (20%)	9 (20%)	3 (15%)	9 (15%)	5 (5%)
Mediastinal or hilar lymphadenopathy	14 (14%)	6 (14%)	3 (15%)	0 (0%)	0 (0%)
Cardiac abnormality	0 (0%)	0 (0%)	0 (0%)	1 (2%)	2 (2%)
Suggestive of active tuberculosis*	83 (82%)	34 (77%)	13 (65%)	5 (9%)	3 (3%)
Suggestive of prior tuberculosis*	59 (58%)	26 (59%)	13 (65%)	13 (25%)	7 (7%)

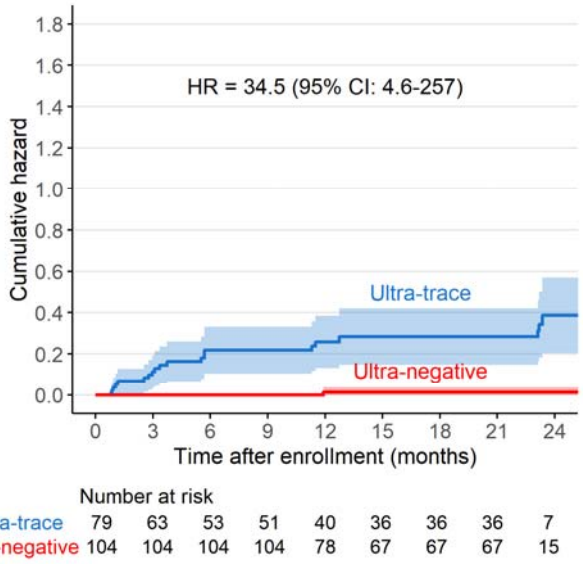
*Rated by a majority of radiologists as at least somewhat suggestive of current active or prior tuberculosis.

Figure 3. **Cumulative cause-specific hazards of receiving a tuberculosis treatment recommendation (left) or developing a positive microbiological result for tuberculosis (right).** The upper panels (a, b) present the cumulative hazard of incident TB stratified by participants' initial Xpert Ultra results during community-wide tuberculosis screening. The lower panels (c, d) show results only for participants with initial trace-positive Ultra results, stratified by chest X-ray results at enrollment as interpreted by human readers.

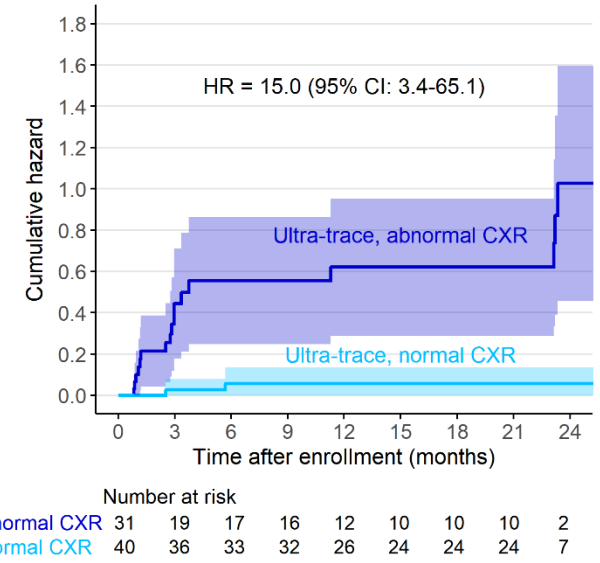
a) Treatment recommendation, by initial Ultra



b) Microbiological positivity, by initial Ultra



c) Treatment recommendation, by initial CXR



d) Microbiological positivity, by initial CXR

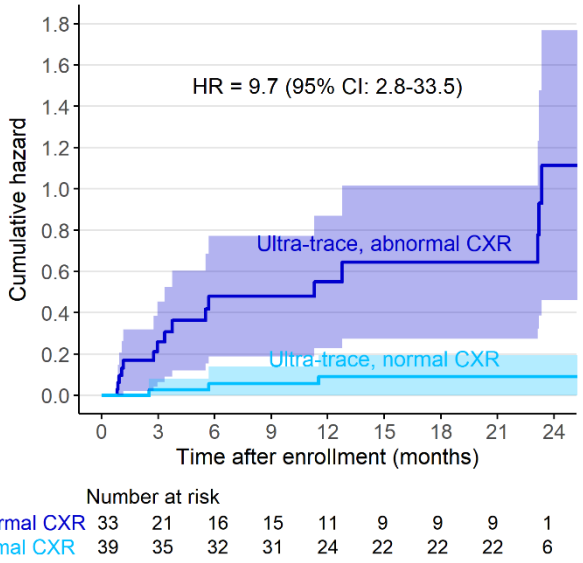
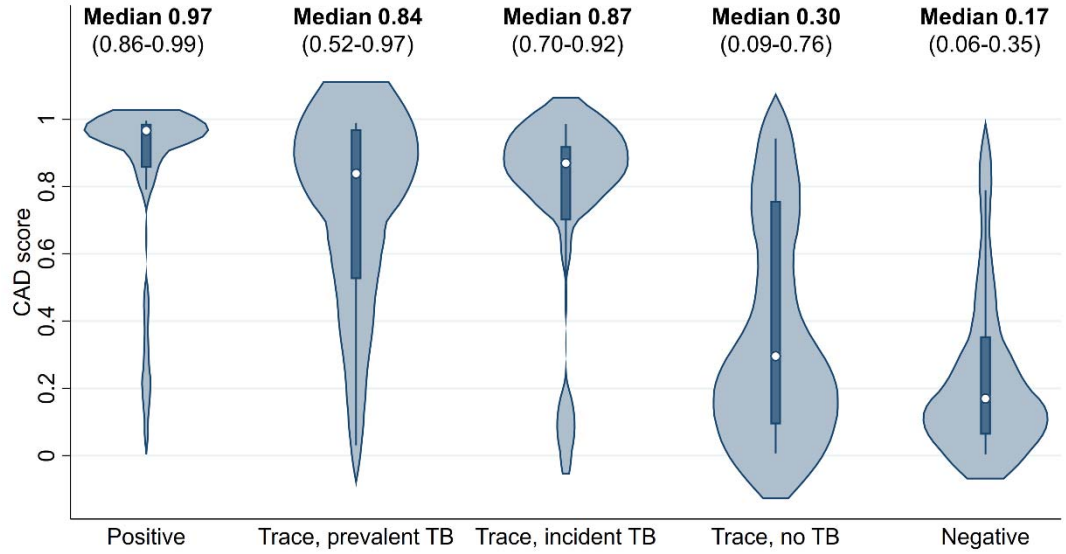


Figure 4. Distribution of CAD-interpreted chest X-ray scores by initial sputum Ultra results and tuberculosis treatment recommendation. Five different groups are shown: Ultra-positive at baseline, Ultra-trace and recommended for tuberculosis treatment at baseline ("prevalent TB"), Ultra-trace and recommended for tuberculosis treatment during follow-up ("incident TB"), Ultra-trace and never recommended for tuberculosis treatment ("no TB"), and Ultra-negative at baseline. Results are shown (a) among all study participants and (b) limited to participants without a prior history of tuberculosis. Curves represent the distribution of CAD scores. Dots indicate median values, boxes show interquartile ranges, and whiskers extend to the upper and lower adjacent values for CAD scores in each group. The medians and interquartile ranges of CAD scores for each group are additionally described above each plot.

a) All participants



b) Participants without a prior history of tuberculosis

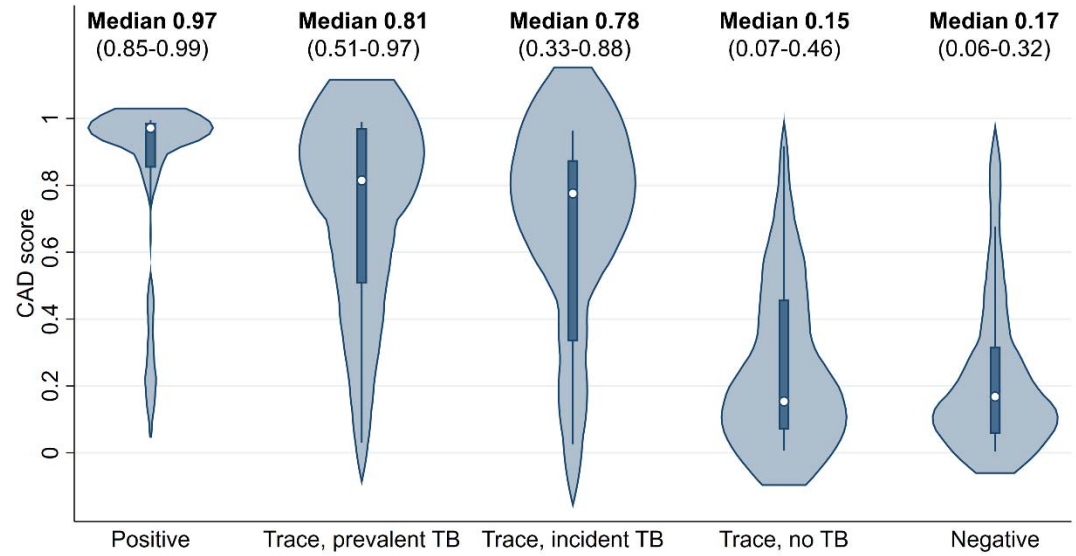
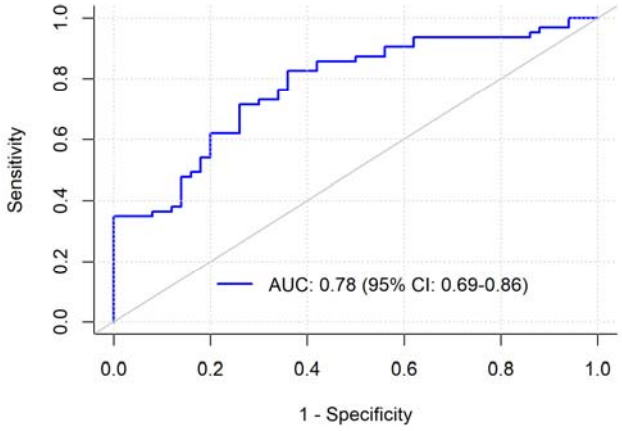
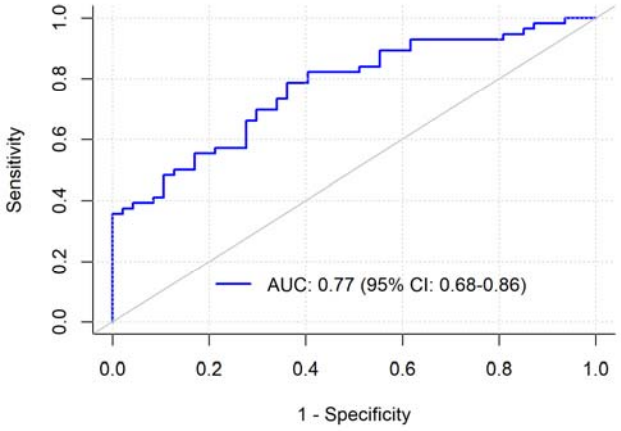


Figure 5. Receiver operating characteristic (ROC) curves of computer-aided detection software (qXR v4) interpretations of baseline chest X-rays for predicting tuberculosis disease. The reference standard for these plots is a treatment recommendation (left panels) or microbiological positivity (right panels), among all individuals with trace-positive screening results (a, b) or only those with no prior history of tuberculosis (c, d). This analysis included participants who completed chest X-rays at enrollment and were either diagnosed with tuberculosis (at baseline or during follow-up) or followed for at least 3 months without a tuberculosis diagnosis. Abbreviations: AUC (area under the curve); CI (confidence interval)

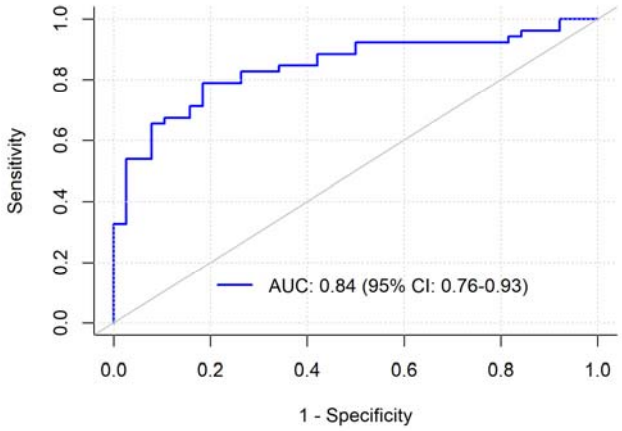
a) Treatment recommendation, all participants



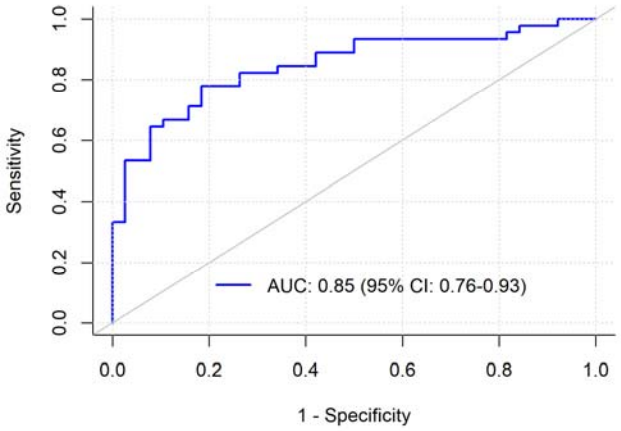
b) Microbiological positivity, all participants



c)Treatment recommendation, no prior tuberculosis



d) Microbiological positivity, no prior tuberculosis



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