

Clinical factors associated with negative GeneXpert Ultra results among adults with pulmonary TB disease

Sunita Nolan

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Public Health

University of Washington

2026

Committee:

Paul K. Drain, MD, MPH, FIDSA

Adrienne Shapiro, MD, PhD, MSc

Julianne Meisner, PhD, MS, BVM&S

Program Authorized to Offer Degree:

Department of Epidemiology

©Copyright 2026
Sunita Nolan

Abstract

Background: Xpert MTB/RIF Ultra is a sputum diagnostic test for pulmonary tuberculosis (TB) disease, but false negative results may occur. Understanding the characteristics associated with Ultra-negative pulmonary TB disease (UN-PTB) can minimize risks of missed or delayed diagnosis in TB endemic regions.

Methods: We conducted a secondary analysis of the PROVE TB 1 and 2 studies, which were prospective cohorts of adults being evaluated at several clinics or a hospital for pulmonary TB disease in KwaZulu-Natal, South Africa. For both studies, participants at risk of pulmonary TB disease were enrolled and followed for at least two months. At time of initiation of anti-TB therapy, TB nurses collected sputum specimens for Xpert Ultra, as well as solid and liquid *Mycobacterium tuberculosis* (Mtb) culture. Participants could have been either microbiologically or clinically diagnosed with pulmonary TB, and those Ultra-negative were considered to have UN-PTB. Logistic regression was used to estimate adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for factors associated with UN-PTB.

Results: Among 424 adults with pulmonary TB disease, with a median age of 37 years, 44% female, and 77% were living with HIV. 199 (47%) people had UN-PTB at baseline, including 5 participants with positive Mtb cultures. Among those with UN-PTB, 100 (50%) were diagnosed empirically at baseline and 99 (50%) were diagnosed during follow-up. Compared to participants with Ultra-positive PTB, those with UN-PTB were more likely to be female, living with HIV, and hospitalized at enrollment. In multivariable analyses, UN-PTB was associated with hospitalization at enrollment (aOR 2.66, 95% CI 1.72–4.15), chest X-ray findings not indicative of TB (aOR 2.09, 95% CI 1.14–3.88), living with HIV (aOR 1.85, 95% CI 1.10–3.15), and female sex (aOR 1.73, 95% CI 1.14–2.64). Cough was associated with a significantly lower odds of UN-PTB (aOR 0.47, 95% CI 0.23–0.89).

Conclusions: In this South African cohort, roughly half of people diagnosed with pulmonary TB disease had UN-PTB. UN-PTB was more common among patients who were hospitalized, living with HIV, female, or had chest X-ray findings not suggestive of TB. These findings identify clinical and diagnostic gaps that could be addressed to reduce the risk of missed or delayed TB diagnosis.

Introduction

Tuberculosis (TB) causes disease in over 10 million people each year, and is the leading infectious cause of mortality worldwide, despite being largely preventable and curable. Of the estimated 10.7 million TB cases in 2024, only 8.3 million cases were diagnosed and reported, leaving a global gap of 2.4 million undiagnosed or unreported cases.¹ TB is both underdiagnosed and underreported due to limited access to health care and imperfect performance of available diagnostic tests.² Current diagnostic tools for TB are insensitive and/or inaccessible (e.g., prohibitively expensive, require specialized lab equipment available only in a centralized lab setting), contributing to underdiagnosis of TB.

WHO recommends sputum GeneXpert MTB/RIF and MTB/RIF Ultra as an initial diagnostic test for TB in adults with signs and symptoms of pulmonary TB rather than smear microscopy, a widely used inexpensive test with low sensitivity and specificity.³ Xpert Ultra is an automated, real-time nucleic acid amplification test that can detect genomic fragments of *Mycobacterium tuberculosis* (Mtb) within two hours.⁴ Compared with smear microscopy, molecular testing substantially improves TB detection.⁴ Xpert Ultra is an updated version of the Xpert MTB/RIF assay with improved sensitivity for detecting TB disease, particularly among individuals with low bacillary burden. A recent review reported pooled sensitivity of approximately 88% for Xpert Ultra compared with 83% for Xpert MTB/RIF when using culture as the reference standard.⁵ Diagnoses from Xpert Ultra form the basis of this analysis, although the assay is not universally used or available.

However, studies have found that although Xpert's sensitivity is high overall, sensitivity can be lower in some populations. For example, findings show that Xpert sensitivity is suboptimal among people with smear-negative pulmonary TB disease, people with low bacillary loads, and in people living with HIV when compared to culture^{3,6-8}. In high burden settings, clinicians often initiate empiric TB treatment when Xpert results are negative but clinical suspicion is high.⁹ Studies from high burden setting show that empirically diagnosed, sputum Xpert-negative patients may differ in clinical presentation from those with molecularly confirmed TB, including higher HIV prevalence, shorter duration of cough, and lower inflammatory markers.^{6,9-11} Together, these findings suggest that TB diagnosis reflects both molecular test performance and clinical recognition of disease severity and risk.

Additionally, as the availability of molecular testing has grown, clinicians may be recalibrating their thresholds for empiric treatment, making it increasingly important to understand which patients are less likely to be detected by these assays. Characterizing the clinical features associated with UN-PTB may help identify patients whose disease is less clinically apparent at presentation and therefore at greater risk of delayed diagnosis. This may provide insight into TB profiles that are less likely to be detected by current molecular diagnostics and inform decisions about developing novel, rapid POC diagnostic tests for TB that are needed to identify active cases and close the diagnostic gap. Using data from two prospective cohort studies conducted in a high TB- and HIV-burden setting in South Africa, this study sought to characterize clinical profiles associated with UN-PTB among adults with pulmonary TB disease.

Methods

Study setting

This study was conducted using data from the PROVE TB 1 and PROVE TB 2 studies, which enrolled participants from Harry Gwala Regional Hospital (formerly Edendale Hospital) and affiliated outpatient primary health care clinics in the uMgungundlovu district of KwaZulu-Natal, South Africa. This region is characterized by a high burden of TB and HIV. The study was implemented through a collaboration between the University of Washington, the Human Sciences Research Council (HSRC), and Umkhuseli Innovation and Research Management (UIRM).

Study participants

The PROVE TB 1 and 2 cohorts enrolled adult participants presenting for TB between October 2019 and February 2021 (PROVE 1) and May 2023 and July 2024 (PROVE 2). PROVE TB 1 enrolled hospitalized and ambulatory adults and included participants with and without known TB at enrollment. PROVE TB 2 enrolled adults to participating study sites with at least one TB-related symptom within the preceding two weeks, a chest X-ray suggestive of TB, or who independently requested TB testing. For this analysis, participants were included if they were diagnosed with TB at baseline evaluation or within two months of follow-up and had a sputum Xpert Ultra result available. The PROVE TB 1 cohort enrolled 684 participants and the PROVE TB 2 cohort enrolled 846 participants. For this analysis, we restricted to participants diagnosed with pulmonary TB by month two of follow-up, yielding a combined analytic sample of 424 participants.

Study design

This study was a secondary analysis combining data from the PROVE TB 1 and PROVE TB 2 studies, two prospective cohort diagnostic evaluation studies designed to assess novel TB diagnostics in high burden settings. This analysis uses baseline and follow-up data collected as part of the parent cohorts to examine clinical and patient characteristics associated with negative sputum Xpert Ultra results among individuals ultimately diagnosed with pulmonary TB disease. In the parent studies, TB disease classification was determined using a microbiological reference standard (MRS) and a clinical reference standard (CRS). The MRS classified participants as TB-positive based on microbiologic confirmation (positive Xpert Ultra result or positive culture). Under the CRS, participants were considered TB-positive if they either met the MRS definition or were empirically initiated on TB treatment despite negative microbiologic testing. Tuberculosis diagnosis followed the parent study TB classification based on the CRS. For this analysis pulmonary TB disease was defined according to the parent study's final classification at baseline or by two-month follow-up. UN-PTB was defined as pulmonary TB disease with a negative baseline Xpert Ultra result, regardless of culture result, participants with negative Xpert Ultra but positive Mtb culture were classified as UN-PTB for this analysis.

The primary exposures of interest included baseline demographic, clinical, and laboratory characteristics hypothesized to be associated with UN-PTB. Key exposure domains included demographic characteristics (e.g., age, sex), general health indicators (e.g., body mass index, Karnofsky performance score), TB-related symptoms and clinical findings, chest X-ray findings, HIV status and immunologic markers, prior TB treatment history, and baseline sputum AFB smear results. All exposure variables were assessed using data collected at enrollment through standardized CRFs, clinical evaluation, and laboratory testing.

The primary outcome for this study was negative sputum Xpert Ultra result, defined as a negative Xpert Ultra assay performed on sputum samples among participants subsequently diagnosed with pulmonary TB. For descriptive analyses, participants were categorized in mutually exclusive categories defined by

diagnostic pathway: (1) molecular diagnosis at baseline (Xpert Ultra positive), (2) empiric clinical diagnosis at baseline despite negative Xpert Ultra, and (3) diagnosis of any kind during follow-up after a baseline clinical determination of no TB and negative Xpert Ultra test. Diagnostic pathway groups were defined based on clinical decision making.

All baseline characteristics were considered candidate exposure variables. Variables included in the final multivariable model were selected a priori based on clinical relevance, prior literature, and findings from the descriptive analyses.

Data collection

Data for this analysis were obtained from the PROVE TB 1 and PROVE TB 2 study databases. Baseline data were collected at enrollment and included demographic information, medical history, TB symptoms, HIV status, and clinical findings. Laboratory data included Xpert Ultra results, sputum AFB smear results, sputum TB culture results, and CD4 count among people living with HIV. Culture results were categorized according to the available data in the analytic dataset. An unavailable culture result could potentially reflect failure to collect a sputum specimen, culture not being performed, specimen or processing issues, or an ordered culture for which no result was available. Chest X-ray procedures differed between the parent studies. In PROVE TB 1, chest X-ray findings were abstracted from available clinical evaluations and were not systematically obtained for all participants. In PROVE TB 2, chest X-rays were obtained when possible if not already performed as part of standard clinical care. Follow-up data were abstracted at two months after baseline to ascertain TB diagnoses among participants with initially negative molecular diagnostic tests. Participants who did not receive a baseline TB diagnosis did not return to study sites for follow-up clinical evaluation. Data collection for PROVE TB 1 and 2 included enrollment and informed consent, baseline symptom assessment and clinical evaluation, collection of sputum samples for testing, HIV and laboratory evaluation when applicable, and follow-up to determine TB diagnosis and treatment initiation through telephone or chart review.

Data analysis

Descriptive analyses were conducted among participants diagnosed with pulmonary TB disease. Categorical variables were summarized using frequencies and proportions and continuous variables were summarized using medians and interquartile ranges.

We first described and compared baseline characteristics across three diagnostic groups: (1) participants with a positive Xpert Ultra result at baseline, (2) participants empirically diagnosed with pulmonary TB disease at baseline despite a negative Xpert Ultra result, and (3) participants diagnosed with pulmonary TB disease within two months of follow-up after a negative baseline Xpert Ultra test. Differences across diagnostic groups were assessed using Chi-square tests or Fisher's exact tests for categorical variables and Kruskal-Wallis rank-sum tests for continuous variables. These analyses were exploratory and intended to identify clinically meaningful patterns in patient characteristics and clinical presentation across diagnostic pathways. Findings from these analyses, together with prior literature and clinical relevance, informed the selection of predictor variables for multivariable analyses. To identify these profiles, we then used logistic regression to estimate odds ratios for demographic and clinical factors associated with UN-PTB. The primary outcome was a binary indicator of sputum negative versus sputum positive Xpert Ultra result.

To preserve model stability, variables representing key clinical constructs were selected a priori for inclusion in the multivariable model. The final model adjusted for age, sex, HIV status, hospitalization at enrollment, chest X-ray findings, and cough. These variables were selected based on prior literature, clinical relevance, and findings from the descriptive analyses. Odds ratios (ORs), adjusted odds ratios (aORs), and 95% confidence intervals were estimated. Sensitivity analyses were conducted by adding body mass index (BMI), prior TB treatment history, and baseline sputum acid-fast bacilli (AFB) smear status individually to the primary multivariable model to assess the robustness of the observed associations.

Results

A total of 1,530 adults presenting for TB evaluation were enrolled in the PROVE TB 1 and 2 studies. Of these participants, 424 individuals were diagnosed with pulmonary TB disease by baseline or by two months of follow-up and were included in the analytic sample. Among these 424 participants, 199 (47%) had UN-PTB at baseline and 225 (53%) had a positive baseline Xpert Ultra result. Among participants with UN-PTB, 100 (50%) were diagnosed empirically at baseline and 99 (50%) were diagnosed during follow-up. Five participants with UN-PTB had a positive sputum Mtb culture.

Baseline demographic and clinical characteristics differed across the three diagnostic pathway groups (Table 1). Participants with UN-PTB were more likely to be female, HIV positive, and hospitalized at enrollment compared to those with a positive Xpert Ultra result at baseline. Participants with a positive baseline Xpert Ultra result were more likely to present with cough, night sweats, and weight loss. Additionally, those with chest X-rays indicative of TB disease were more likely to be diagnosed at baseline by either molecular testing or empirically than during follow-up.

Table 1. Baseline demographic and clinical characteristics of adults with pulmonary tuberculosis, stratified by diagnostic modality

Characteristic	Xpert positive N = 225 ¹	Empiric TB at baseline N = 100 ¹	Diagnosed at follow-up N = 99 ¹	Overall N = 424 ¹	p-value ²
Demographics					
Age	36 (29, 48)	38 (30, 45)	38 (30, 45)	37 (29, 47)	>0.9
Sex					
Female	81 (36%)	51 (51%)	53 (54%)	185 (44%)	
Male	144 (64%)	49 (49%)	46 (46%)	239 (56%)	0.003
Current smoker	59 (26%)	23 (23%)	14 (14%)	96 (23%)	0.057
Symptoms					
Cough	207 (92%)	81 (81%)	87 (88%)	375 (88%)	0.016

Characteristic	Xpert positive N = 225 ¹	Empiric TB at baseline N = 100 ¹	Diagnosed at follow-up N = 99 ¹	Overall N = 424 ¹	p-value ²
Coughing blood	10 (9.6%)	5 (8.3%)	4 (6.6%)	19 (8.4%)	0.8
Unknown	121	40	38	199	
Fever and chills	137 (61%)	55 (55%)	58 (59%)	250 (59%)	0.6
Night sweats	158 (70%)	58 (58%)	56 (57%)	272 (64%)	0.021
Loss of appetite	57 (55%)	37 (62%)	26 (43%)	120 (53%)	0.10
Unknown	121	40	38	199	
Fatigue	172 (76%)	75 (75%)	69 (70%)	316 (75%)	0.4
Shortness of breath	53 (51%)	23 (38%)	33 (54%)	109 (48%)	0.2
Unknown	121	40	38	199	
Chest pain	54 (52%)	22 (37%)	30 (49%)	106 (47%)	0.2
Unknown	121	40	38	199	
Loss of ≥ 5 kg	174 (77%)	64 (64%)	59 (60%)	297 (70%)	0.002
Vital signs					
BMI	20.1 (17.8, 23.4)	21.0 (18.7, 24.6)	21.2 (18.4, 25.2)	20.5 (18.2, 24.2)	0.055
Unknown	5	2	5	12	
HIV status					<0.001
Negative	69 (31%)	20 (20%)	10 (10%)	99 (23%)	
Positive	156 (69%)	80 (80%)	89 (90%)	325 (77%)	
Hospitalization status					<0.001
Hospitalized at enrollment	86 (38%)	61 (61%)	67 (68%)	214 (50%)	

Characteristic	Xpert positive N = 225 ¹	Empiric TB at baseline N = 100 ¹	Diagnosed at follow-up N = 99 ¹	Overall N = 424 ¹	p-value ²
Not hospitalized at enrollment	139 (62%)	39 (39%)	32 (32%)	210 (50%)	<0.001
Karnofsky score	70 (60, 80)	70 (50, 83)	70 (50, 80)	70 (50, 80)	
Unknown	1	0	1	2	
X-ray indicating TB					
No	52 (23%)	33 (33%)	41 (41%)	126 (30%)	
No recent chest x-ray	123 (55%)	42 (42%)	53 (54%)	218 (52%)	
Yes	48 (22%)	25 (25%)	5 (5.1%)	78 (18%)	
Unknown	2	0	0	2	
Laboratory results					
Lab AFB					
AFB negative	74 (44%)	60 (100%)	59 (97%)	193 (67%)	
AFB positive	93 (56%)	0 (0%)	2 (3.3%)	95 (33%)	
Unknown	58	40	38	136	
Lab culture result					
Negative	13 (13%)	56 (93%)	54 (89%)	123 (55%)	
Positive MOTT*	0 (0%)	2 (3.3%)	4 (6.6%)	6 (2.7%)	
Positive MTB	91 (88%)	2 (3.3%)	3 (4.9%)	96 (43%)	
Unknown	121	40	38	199	
Lab CD4 count (among people living with HIV)	128 (58, 250)	140 (40, 365)	161 (41, 410)	141 (51, 330)	0.3

Characteristic	Xpert positive N = 225 ¹	Empiric TB at baseline N = 100 ¹	Diagnosed at follow-up N = 99 ¹	Overall N = 424 ¹	p-value ²
Unknown	117	36	38	191	
Lab HGB result	11.45 (9.60, 12.80)	11.15 (9.45, 13.20)	11.00 (8.50, 12.60)	11.30 (9.30, 12.80)	0.4
Unknown	59	16	20	95	

¹Median (Q1, Q3); n (%)

²Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test

*mycobacteria other than tuberculosis

We used multivariable logistic regression to identify demographic and clinical factors associated with UN-PTB among the analytic sample (Table 2). After adjustment for age, sex, HIV status, hospitalization at enrollment, chest X-ray findings, and cough, several demographic and clinical characteristics remained independently associated with UN-PTB. Participants hospitalized at enrollment had higher odds of UN-PTB (aOR 2.66, 95% CI 1.72–4.15), as did participants with chest X-rays that did not indicate TB disease (aOR 2.09, 95% CI 1.14–3.88). HIV-positive participants had higher odds of UN-PTB than HIV-negative participants (aOR 1.85, 95% CI 1.10–3.15). Similarly, Female participants had higher odds of UN-PTB compared to male participants (aOR 1.73, 95% CI 1.14–2.64). In contrast, participants reporting cough had lower odds of UN-PTB (aOR 0.47, 95% CI 0.23–0.89). Age was not associated with UN-PTB after adjustment (aOR 1.00, 95% CI 0.99–1.02).

Table 2. Univariate and multivariable predictors of GeneXpert Ultra negativity among adults with pulmonary tuberculosis

Characteristic	OR (95% CI)	p-value	aOR (95% CI)	Adjusted p-value
Demographics				
Age	1.00 (0.98, 1.01)	0.86	1.00 (0.99, 1.02)	0.86
Female sex	1.94 (1.32, 2.88)	< 0.01	1.73 (1.14, 2.64)	0.01
Vital signs				
BMI	1.05 (1.01, 1.09)	0.01	–	–
HIV positive	2.49 (1.55, 4.07)	< 0.01	1.85 (1.10, 3.15)	0.02
Hospitalized at enrollment	2.91 (1.97, 4.34)	< 0.01	2.66 (1.72, 4.15)	< 0.01
CXR not indicating TB	2.27 (1.28, 4.09)	0.01	2.09 (1.14, 3.88)	0.02
No recent CXR	1.23 (0.73, 2.11)	0.43	1.50 (0.85, 2.70)	0.17
Prior TB treatment	1.55 (1.04, 2.32)	0.03	–	–
Symptoms				

Characteristic	OR (95% CI)	p-value	aOR (95% CI)	Adjusted p-value
Demographics				
Cough	0.47 (0.25, 0.86)	0.02	0.47 (0.23, 0.89)	0.02
Weight loss	0.47 (0.31, 0.72)	< 0.01	–	–
Night sweats	0.57 (0.38, 0.85)	< 0.01	–	–
Laboratory results				
Positive AFB	0.01 (0.002, 0.04)	< 0.01	–	–
CD4 < 200 uL	0.66 (0.39, 1.26)	0.13	–	–
The model adjusted for age, sex, HIV status, hospitalization at enrollment, cough, and chest X-ray results				
Outcome: GeneXpert Ultra negativity at baseline (reference = GeneXpert Ultra positive).				

Sensitivity analyses were performed by individually adding BMI and prior TB treatment to the primary multivariable model (Table 3). Largely, the associations from the primary model remained consistent across all sensitivity analyses. Adjustment for prior TB treatment did not change the primary associations. Adjustment for BMI attenuated the association between female sex and UN-PTB, while BMI was independently associated with higher odds of UN-PTB.

Table 3. Sensitivity analyses of factors associated with UN-PTB

Demographic and Clinical Characteristic	Primary model aOR (95% CI)	+ BMI	+ Prior TB treatment
Age	1.00 (0.99, 1.02)	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)
Female sex	1.73 (1.14, 2.64)	1.48 (0.94, 2.34)	1.73 (1.14, 2.65)
HIV positive	1.85 (1.10, 3.15)	1.92 (1.14, 3.29)	1.74 (1.03, 2.98)
Hospitalized at enrollment	2.66 (1.72, 4.15)	2.61 (1.68, 4.11)	2.64 (1.71, 4.11)
CXR not indicating TB	2.09 (1.14, 3.88)	1.91 (1.03, 3.55)	2.08 (1.13, 3.87)
No recent CXR	1.50 (0.85, 2.70)	1.33 (0.75, 2.36)	1.43 (0.83, 2.35)
Cough	0.47 (0.23, 0.89)	0.51 (0.26, 0.98)	0.47 (0.23, 0.90)
BMI	–	1.05 (1.00, 1.10)	–
Prior TB treatment	–	–	1.30 (0.84, 2.03)
Positive AFB smear	–	–	–
Outcome: GeneXpert Ultra negativity at baseline. Each sensitivity model added a single variable to the primary model.			

Discussion

Although Xpert Ultra is a highly sensitive diagnostic test, achieving microbiologic confirmation remains challenging in some populations, and decisions to ultimately diagnose and initiate TB treatment often rely on clinical decision making in addition to molecular test results. In this study, 47% of adults ultimately diagnosed with pulmonary TB disease had UN-PTB at baseline. Female sex, HIV positivity, hospitalization at study enrollment, and chest X-ray not indicative of TB disease were all associated with higher odds of UN-PTB. In contrast, patients presenting with classic pulmonary TB disease symptoms such as cough, weight loss of ≥ 5 kg, or night sweats were less likely to have UN-PTB. These findings were further illustrated by the uneven distribution of patient characteristics across diagnostic groups

where we found a higher proportion of female, HIV positive, and hospitalized patients to have an empiric or delayed diagnosis (UN-PTB) compared to those with baseline Xpert-Ultra confirmation.

The association between HIV positivity and UN-PTB is likely explained by both biological and clinical factors. HIV-associated cases of TB disease often have lower rates of molecular confirmation because severe immunosuppression might lead to paucibacillary disease and an inability to produce adequate sputum samples for diagnostic testing.¹² This aligns with our finding that people living with HIV were more likely to have UN-PTB. Indeed, among the 121 participants in our cohort with UN-PTB and available baseline culture results, only 5 individuals were culture-positive for *Mtb*, further highlighting the challenges of achieving microbiologic confirmation in this population. Additionally, the high prevalence of HIV in our study population may have also influenced clinical decision making. In high TB- and HIV-burden settings, clinicians may have a lower threshold to initiate TB treatment despite negative molecular testing because of the severe consequences of delayed treatment. This practice is supported by a 2019 study from Uganda that found clinical diagnoses of Xpert-negative patients ultimately identified relatively few additional culture-confirmed cases but still captured a subset of true TB disease cases that would have been missed with reliance on molecular testing alone⁹.

Notably, hospitalization at enrollment was independently associated with high odds of UN-PTB and was the strongest predictor in the adjusted model and across sensitivity models. A possible explanation for this association is that hospitalized patients represent a clinically complex population where TB disease diagnosis is particularly challenging. Hospitalized patients may have multiple co-infections, atypical clinical presentations, or competing diagnoses that complicate diagnostic evaluation and increase reliance on clinical judgement in the absence of molecular confirmation. Patients who are severely ill may have difficulty producing adequate sputum for microbiologic testing which potentially limits the ability to obtain molecular confirmation with sputum-based diagnostics.. This challenge may be particularly important among people living with HIV. HIV-associated TB disease may be characterized by lower bacillary burden in sputum specimens, and it is well documented that patients who are acutely ill may have difficulty producing sputum for microbiologic testing. Studies among hospitalized people living with HIV in high TB-burden settings have demonstrated substantial challenges obtaining sputum specimens and supports the use of non-sputum-based diagnostic approaches in this population.^{13,14} When there is uncertainty about a TB diagnosis in a hospitalized patient, clinicians may also be less willing to delay treatment among patients at high risk of mortality without an alternative, treatable diagnosis. This is especially true in high TB- and HIV-burden settings where there is significant mortality in untreated, smear negative patients.^{15,16} However, empiric TB treatment also carries the risk of incorrectly attributing illness to TB when another underlying condition may be responsible for illness which potentially delays identification and appropriate treatment. In this study, the exact reasons for hospitalization were not documented so it is challenging to draw conclusions about why this association was so strong in this group. Although hospitalization would be considered a marker of severe illness, CD4 count and Karnofsky scores were similar across all three diagnostic groups, which suggests that there may be other factors driving the higher odds of UN-PTB among hospitalized participants.

The sex differences in baseline UN-PTB in this study were pronounced. Female participants were more frequently diagnosed empirically or at follow-up than male participants. Differences in health-seeking behavior may contribute to this association. Prior studies in South Africa have demonstrated lower engagement with health services and delays in care-seeking among men, while women with TB disease symptoms have been found to be more likely to seek care than man. Earlier inagement with care could

result in women being evaluated for TB at an earlier stage of disease, when pulmonary involvement and bacillary burden may be lower and sputum production more limited.¹⁷ A potential mechanism is that biological differences in pulmonary TB disease presentation may influence the likelihood of achieving microbiologic confirmation. A prior study found that women with pulmonary TB disease are less likely to present with cavitary or smear-positive disease than men which could reduce the bacillary load in sputum samples.¹⁸ Lower bacillary burden may in turn reduce the odds of detection by Xpert Ultra. Differences in sputum specimen quality may further contribute to this association. Prior studies have reported that women were more likely to produce salivary or otherwise lower-quality sputum samples, which may contain fewer bacteria and reduce the sensitivity of smear microscopy and molecular diagnostic tests.^{19,20} These mechanisms may be related since earlier presentation among women could contribute to differences in disease severity, sputum production, and bacillary burden at the time of diagnostic evaluation. Future studies could collect symptom duration data prior to presentation to evaluate whether differences in timing of care-seeking contributed to the higher odds of UN-PTB among women in this cohort.

Our study found that several pulmonary TB disease signs and symptoms, including night sweats, weight loss of ≥ 5 kg, and cough were all associated with lower odds of UN-PTB. These findings likely reflect differences in disease presentation and bacillary burden among those who did or did not test positive with Xpert Ultra at baseline. Presence of these hallmark TB disease symptoms is associated with greater bacillary burden which improves performance of sputum-based molecular tests.^{21,22} In contrast, chest X-rays not suggestive of TB were associated with higher odds of UN-PTB. Although chest X-ray is an important tool for TB screening and diagnosis, X-ray findings can frequently appear atypical of pulmonary TB disease or even normal among people living with HIV, especially in stages of advanced immunosuppression.²³ Given that a large proportion of our study sample were living with HIV, atypical TB X-ray presentations may have contributed to this association.

Although the two UN-PTB diagnostic groups were similar across many demographic and clinical characteristics, there were notable differences that may contribute to explaining why some participants were treated empirically at baseline while others were not diagnosed until follow-up. In particular, chest X-ray findings suggestive of TB disease were substantially more common among participants diagnosed empirically at baseline than among those diagnosed at follow up (approximately 25% vs 5%). HIV prevalence also differed between groups, with a slightly higher proportion of participants living with HIV in the delayed diagnosis group. In contrast, baseline culture positivity was similarly distributed and uncommon in both groups, with only 2 participants in the empiric group and 3 in the delayed group having a positive Mtb culture. Symptom profiles between UN-PTB groups were similar as well. Differences in the clinical information available to clinicians, such as chest X-rays, may have contributed to whether UN-PTB was diagnosed and treated at baseline or during follow-up. However, this study determined follow-up diagnoses through telephone interviews and medical record review and did not collect detailed clinical information for those who were not diagnosed at baseline, limiting our ability to identify more specific factors that prompted subsequent TB disease diagnosis. Future studies with longitudinal clinical follow-up could help clarify pathways leading to delayed TB diagnosis after an initial negative Xpert Ultra result. This diagnostic uncertainty has been observed in other studies. A 2025 study from South Africa and Uganda found that approximately half of patients with uncertain molecular TB results who were initially managed without TB treatment were ultimately diagnosed within one year, with

most diagnoses occurring within the first three months of follow-up through a combination of repeat microbiologic testing and clinical judgement.²⁴

The low frequency of documented microbiologic confirmation among patients with UN-PTB also raises important questions about diagnostic certainty and the potential for overdiagnosis of TB disease. In this study, baseline sputum culture results were unavailable for a substantial proportion of participants with UN-PTB, and among those with available results, culture confirmation was uncommon. This challenge goes beyond our study. In 2024, only 64% diagnosed and reported TB disease cases had bacteriologically confirmed disease, globally. The WHO has emphasized the need to improve bacteriologic confirmation and notes that low levels of confirmation may reflect overdependence on clinical diagnosis and potential overdiagnosis.¹ Clinical diagnosis and empiric treatment remain important when TB disease is strongly suspected despite negative molecular testing, especially because even highly sensitive molecular tests may miss disease in populations with low bacillary burden or difficulty producing sputum. However, a conservative approach to initiating TB treatment is not without consequences. Some patients empirically treated may have other infections causing their symptoms, and inappropriately attributing illness to TB could delay proper identification and treatment of the true underlying condition. These diagnostic challenges highlight the need for better diagnostic tools that can provide microbiologic confirmation among those who can't be readily diagnosed with sputum-based testing. The WHO recommends incorporating non-sputum approaches for populations in whom sputum-based diagnosis is particularly challenging. Urine LF-LAM for people living with HIV who have TB disease symptoms, are seriously ill, or have advanced HIV disease.³ Expanding access to non-sputum approaches may help balance competing risks encountered after a negative sputum Xpert Ultra result.

Strengths and Limitations

A key strength of this study was the inclusion of both Xpert-Ultra confirmed and clinically diagnosed pulmonary TB cases, allowing us to characterize UN-PTB within a population that reflects real-world TB diagnosis. This is particularly relevant in high TB- and HIV- burden settings, where treatment decisions often require clinical judgement in the absence of molecular confirmation. Additionally, the prospective cohort design and standardized baseline data collection allowed for detailed characterization and comparison of participant demographic and clinical factors across diagnostic pathway groups.

This study also has several limitations. First, this was a secondary analysis of two existing cohorts which limits available variables and limits assessment of factors like reason for hospitalization or detailed clinical evaluation following baseline evaluation among those with delayed diagnosis. Second, because most participants in this study were living with HIV, these findings may not be generalizable to populations with different epidemiological characteristics. Finally, baseline sputum culture results were unavailable for a substantial portion of participants, and the analytic dataset did not allow for distinction of the reasons for unavailable results.

Conclusion

This study identified clinical characteristics associated with UN-PTB among adults with pulmonary TB disease. Female sex, HIV positivity, hospitalization at enrollment, and chest X-ray findings not indicative of TB disease were associated with higher odds of UN-PTB, while cough was associated with lower odds. Among participants with UN-PTB and available baseline culture results, culture positivity was uncommon, highlighting the substantial role of clinical decision making in diagnosing this group.

Importantly, the presence of culture-confirmed TB disease among this subset of patients demonstrates that clinical diagnoses following a negative Xpert Ultra result can identify true TB disease cases that might have been missed if relying on Xpert Ultra confirmation alone; however the low frequency of documented microbiologic confirmation also raises the possibility that some clinically diagnosed cases may represent TB disease overdiagnosis. These findings underscore the need for improved diagnostic tools and strategies following a negative Xpert Ultra result, particularly among hospitalized patients and people living with HIV, including continued clinical evaluation, close follow-up, and expanded use of new diagnostic strategies that don't rely only on sputum. These strategies may help balance the competing risks of delaying treatment among patients with true TB disease and unnecessary treatment of patients whose illness may be attributable to another condition.

Acknowledgements

This study was a secondary analysis of data collected through the PROVE TB and PROVE TB 2 studies, which were funded by the Bill & Melinda Gates Foundation. We are very grateful to the participants of both studies and to the study teams whose work made this analysis possible. We especially thank Paul Drain, Doug Wilson, and Adrienne Shapiro for their leadership of the parent studies and their support of this work. We also acknowledge Shannon Bosman, Alastair van Heerden, Ronit Dalmat, Yugashnee Pillay, Ben Grant, John Connelly, Amy Steadman, Stewart Chang, David Boyle, Charlotte Ahls, Brad Wagner, David Beebe, and Barry Lutz for their contributions to the PROVE TB studies. We are grateful to Jennifer Morton, Andrea Deisher, Samantha Aucock, Haylea Hannah, Zanele Magcaba, Zinhle Ngcobo, Nonhle Bridget Mapanga, Princess Hector, Zarna Marfatia, Justice Quame-Amaglo, Susan Morrison, William Ngwane, Subashinie Sidhoo, Sindiswe Ngcobo, Harald Haugen, Elena Rechkina, and Daphne Hamilton for their contributions to study coordination, clinical research, laboratory activities, and study implementation. We also want to thank the data management and analysis teams for their work managing, preparing, and supporting the study data used in this analysis. We also thank the teams at the University of Washington, Umkhuseli Innovation and Research Management, Human Sciences Research Council, Harry Gwala Regional Hospital, and the National Health Laboratory Service for their contributions to the PROVE TB studies.

References

1. Global Tuberculosis Report 2025. Accessed August 5, 2026. <https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2025>
2. *WHO Global Tuberculosis Report 2020*. 1st ed. World Health Organization; 2020.
3. World Health Organization. *WHO Consolidated Guidelines on Tuberculosis: Rapid Diagnostics for Tuberculosis Detection; Module 3: Diagnosis*. Third edition. World Health Organization; 2024.
4. WHO Global TB Programme, ed. *Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children*. World Health Organization, Global TB Program; 2013.
5. Horne DJ, Kohli M, Zifodya JS, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults - Horne, DJ - 2019 | Cochrane Library. Accessed March 12, 2026. <https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD009593.pub4/full>
6. Lee HN, Kim JI, Kim YH. Clinical and CT characteristics of Xpert MTB/RIF-negative pulmonary tuberculosis. *PLOS ONE*. 2021;16(5):e0250616. doi:10.1371/journal.pone.0250616
7. Velen K, Podewils LJ, Shah NS, et al. Performance of GeneXpert MTB/RIF for Diagnosing Tuberculosis Among Symptomatic Household Contacts of Index Patients in South Africa. *Open Forum Infect Dis*. 2021;8(4):ofab025. doi:10.1093/ofid/ofab025
8. Wang J, Zhang X, Huo F, et al. Analysis of Xpert MTB/RIF results in retested patients with very low initial bacterial loads: A retrospective study in China. *J Infect Public Health*. 2023;16(6):911-916. doi:10.1016/j.jiph.2023.04.004
9. Kendall EA, Kamoga C, Kitonsa PJ, et al. Empiric treatment of pulmonary TB in the Xpert era: Correspondence of sputum culture, Xpert MTB/RIF, and clinical diagnoses. *PLoS ONE*. 2019;14(7):e0220251. doi:10.1371/journal.pone.0220251
10. Yu S, Gao Y, Lu J, et al. Clinical profiles and related factors in tuberculosis patients with positive sputum smear mycobacterium tuberculosis tests. *Sci Rep*. 2024;14(1):20376. doi:10.1038/s41598-024-71403-5
11. Theron G, Peter J, van Zyl-Smit R, et al. Evaluation of the Xpert MTB/RIF Assay for the Diagnosis of Pulmonary Tuberculosis in a High HIV Prevalence Setting. *Am J Respir Crit Care Med*. 2011;184(1):132-140. doi:10.1164/rccm.201101-0056OC
12. Dhana A, Hamada Y, Kengne AP, et al. Tuberculosis screening among HIV-positive inpatients: a systematic review and individual participant data meta-analysis. *Lancet HIV*. 2022;9(4):e233-e241. doi:10.1016/S2352-3018(22)00002-9

13. Gupta-Wright A, Corbett EL, Oosterhout JJ van, et al. Rapid urine-based screening for tuberculosis in HIV-positive patients admitted to hospital in Africa (STAMP): a pragmatic, multicentre, parallel-group, double-blind, randomised controlled trial. *The Lancet*. 2018;392(10144):292-301. doi:10.1016/S0140-6736(18)31267-4
14. Cossa M, Ndege R, Meggi B, et al. Expanding Xpert MTB/RIF Ultra and lateral flow urine lipoarabinomannan testing for diagnosis of tuberculosis among adults living with HIV admitted to hospitals in Tanzania and Mozambique (EXULTANT): a randomised controlled trial. *Lancet Infect Dis*. 2026;0(0). doi:10.1016/S1473-3099(26)00133-7
15. MacPherson P, Dimairo M, Bandason T, et al. Risk factors for mortality in smear-negative tuberculosis suspects: a cohort study in Harare, Zimbabwe. October 2011. doi:10.5588/ijtld.11.0056
16. Nakiyingi L, Bwanika JM, Kirenga B, et al. Clinical Predictors and Accuracy of Empiric Tuberculosis Treatment among Sputum Smear-Negative HIV-Infected Adult TB Suspects in Uganda. *PLOS ONE*. 2013;8(9):e74023. doi:10.1371/journal.pone.0074023
17. Smith A, Burger R, Claassens M, Ayles H, Godfrey-Faussett P, Beyers N. Health care workers' gender bias in testing could contribute to missed tuberculosis among women in South Africa. *Int J Tuberc Lung Dis*. 2016;20(3):350-356. doi:10.5588/ijtld.15.0312
18. Min J, Park JS, Kim HW, et al. Differential effects of sex on tuberculosis location and severity across the lifespan. *Sci Rep*. 2023;13:6023. doi:10.1038/s41598-023-33245-5
19. Meyer AJ, Atuheire C, Worodria W, et al. Sputum quality and diagnostic performance of GeneXpert MTB/RIF among smear-negative adults with presumed tuberculosis in Uganda. *PLOS ONE*. 2017;12(7):e0180572. doi:10.1371/journal.pone.0180572
20. Khan MS, Dar O, Sismanidis C, Shah K, Godfrey-Faussett P. Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomised controlled trial. *The Lancet*. 2007;369(9577):1955-1960. doi:10.1016/S0140-6736(07)60916-7
21. Beynon F, Theron G, Respeito D, et al. Correlation of Xpert MTB/RIF with measures to assess Mycobacterium tuberculosis bacillary burden in high HIV burden areas of Southern Africa. *Sci Rep*. 2018;8(1):5201. doi:10.1038/s41598-018-23066-2
22. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. *Lancet Infect Dis*. 2018;18(1):76-84. doi:10.1016/S1473-3099(17)30691-6
23. Yoo SD, Cattamanchi A, Boon S den, et al. Clinical significance of normal chest radiographs among HIV-seropositive patients with suspected tuberculosis in Uganda. *Respirol Carlton Vic*. 2011;16(5):836-841. doi:10.1111/j.1440-1843.2011.01981.x

24. Dalmat RR, Visek C, Budiawan E, et al. Cumulative TB disease burden following sputum Xpert Ultra 'Trace' results in clinical settings: Results from a multi-site observational clinical study. *medRxiv*. Preprint posted online July 15, 2025:2025.07.14.25331384.
doi:10.1101/2025.07.14.25331384