A clinical prediction score including trial of antibiotics and C-reactive protein to improve the diagnosis of tuberculosis in ambulatory people with HIV

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Abstract

Background - The use of a ‘trial of antibiotics’ as empiric therapy for bacterial pneumonia, as a diagnostic tool for tuberculosis in people with HIV (PWH) was removed from WHO recommendations in 2007, based on expert opinion. Current guidelines recommend antibiotics only after 2 Xpert MTB/RIF tests (if available), chest X-ray, and clinical assessment have suggested that tuberculosis is unlikely. Despite this, a ‘trial of antibiotics’ remains common in algorithms in low resource settings, but its value is uncertain. C-reactive protein (CRP), which has been proposed as a ‘rule-out’ test for tuberculosis, may be an objective marker of response to antibiotics.

Methods - We performed a passive case finding cohort study of adult PWHs with a positive WHO symptom screen. All participants received antibiotics at first visit according to the local protocol and were reviewed to ascertain clinical response. Point-of-care CRP was measured at both visits. All patients had sputum tested with Xpert MTB/RIF Ultra (Ultra) and the reference standard was based on 2 sputum mycobacterial cultures. We explored multivariable prediction models (MPM) for tuberculosis based on 1 or 2 visits.

Results - Seventy-five of 207 patients (36%) had confirmed tuberculosis. Clinical response to antibiotics after 2 days was a good predictor of disease. A MPM based on 2 visits, without CRP, had acceptable discrimination (c-statistic 0.75) and calibration (GOF p = 0.07). Addition of CRP after antibiotics improved the model moderately (c-statistic 0.78). CRP at first visit was not an independent predictor of tuberculosis.

Conclusion - In adults PWH seeking care for symptoms suggestive of tuberculosis, lack of response to antibiotics is a strong predictor of disease and is likely to be useful, particularly when access to Ultra is limited. CRP adds value when measured after antibiotics but is of limited value at first visit.

Key words – Xpert MTB/RIF Ultra, C-reactive protein, diagnostic accuracy, WHO algorithm, multivariable prediction models
Background

Limitations of current diagnostics remain a challenge in the fight against tuberculosis. A 'trial-of-
antibiotics' (ToA) is defined as a course of broad-spectrum antibiotics, with negligible
Mycobacterium tuberculosis activity, given to patients suspected of having tuberculosis (1). Patients
with a clinical response to antibiotics, which is not consistently defined, are considered unlikely to
have tuberculosis and vice versa. The 2003 World Health Organization (WHO) guidelines for
diagnosing tuberculosis, which did not differentiate by HIV status, included a ToA after 3 negative
sputum smears, with clinical improvement ruling out tuberculosis (1). In 2007 the revised WHO
guideline for HIV-prevalent and resource-constrained settings (2) stated that the primary role of
antibiotics should not be as a diagnostic aid; instead they should be used to treat concomitant
bacterial infection in people with HIV/AIDS (PWH) with cough. The level of evidence for this
recommendation was IV (expert opinion based on evaluation of other evidence). The 2007 algorithm
was shown to expedite the diagnosis of smear-negative pulmonary tuberculosis and increase
diagnostic accuracy compared to the 2003 algorithm (3), although the diagnostic accuracy remained
suboptimal (4).

In 2016, WHO published a revised algorithm for diagnosing tuberculosis in ambulatory PWH (5). All
patients should have Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) testing of sputum, although the
WHO has subsequently endorsed the more sensitive Xpert MTB/RIF Ultra (Ultra) assay. If Xpert is
negative or unavailable, the algorithm recommends further investigations (chest X-ray, repeat Xpert
MTB/RIF, and mycobacterial culture if available) at the first visit, with antibiotics only advised if
tuberculosis remains unlikely at this point. Patients with a response to antibiotics are presumed not
to have active tuberculosis and should initiate isoniazid preventive therapy; patients with no or
partial response to antibiotics should have further tuberculosis investigations, rather than empiric
anti-tuberculosis treatment. To our knowledge, this algorithm has not been formally validated.

Despite being removed from WHO guidelines in 2007, ToA is common in diagnostic algorithms in
resource-limited settings, although its value remains uncertain. A recent systematic review of 8
studies including 2586 participants, found a pooled sensitivity and specificity of a ToA of 0.72 and
0.77 respectively (6).

The specificity of symptom screening for tuberculosis in PWH, which is used as the entry criteria for
diagnostic algorithms is 0.71 for patients on antiretroviral therapy (ART) (sensitivity 0.51) and 0.28
for ART-naive patients (sensitivity 0.89) (7), and so large numbers of patients without tuberculosis
enter the algorithm but are Xpert negative. This poses a huge challenge in resource constrained
settings. Multivariable prediction models (MPM) have been proposed as a way to pre-screen
symptomatic patients and focus investigations on those with increased probability of disease (8-10),
but none have been externally validated.

C-reactive protein (CRP), which is now available as a point-of-care (POC) test costing <$2, has been
evaluated as an initial test for tuberculosis in patients with HIV. A systematic review and meta-
analysis of diagnostic accuracy studies found that a value <10 mg/L has a sensitivity of 0.93 and
specificity of 0.61 among outpatients with HIV (11). Analysis of three studies including only patients
self-reporting symptoms found a higher sensitivity (range 0.96-0.97) and lower specificity (range
0.33-073). CRP has not been evaluated as an additional variable to clinical prediction rules or as an
objective marker of response to antibiotics for the diagnosis of HIV-associated tuberculosis.
We performed a passive case-finding cohort study of PWH meeting entry criteria for the 2016 WHO algorithm, in a setting where local standard operating procedures (SOP) are for all patients to receive antibiotics at the first visit. We aimed to develop MPMs including subjective response to antibiotics and POC measurements of CRP.

The study is reported according to the Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) statement(12), and the Standards for Reporting of Diagnostic Accuracy Studies (STARD) statement(13).

Objectives

To determine the independent predictors of tuberculosis at index visit and following a trial of empiric antibiotics among adult PWH seeking clinic care with compatible symptoms.

To develop multivariable prediction models (MPM) for tuberculosis based on independent predictors at index and return visit.

Methods

Study participants and data collected

We recruited HIV positive patients at a community healthcare clinic in Johannesburg, South Africa with a positive WHO symptom screen (any one of current cough, fever, night sweats, or weight loss) based on research team capacity, from the queue of adults actively seeking care for their symptoms. Recruitment was therefore non-consecutive and non-random. We included patients regardless of ART status, receipt of isoniazid preventive therapy, or prior tuberculosis. We excluded those who received antibiotics within 14 days to minimise the possibility of referral bias, as well as those currently taking tuberculosis treatment.

At the first visit, all patients were systematically evaluated for tuberculosis with vital signs, body mass index (BMI), sputum smear microscopy, Ultra, and liquid mycobacterial culture (Mycobacterial growth indicator tube (MGIT, BACTEC™ MGIT 960™ TB System). Sputum induction with nebulised hypertonic saline (5%) was used when participants were unable to produce sputum spontaneously. Ultra was repeated if the first result was indeterminate. CRP was measured at the point of care (Abbot Afinion AS100 analyser, the range of values was 5–>200 mg/L), and antibiotics for respiratory pathogens (amoxicillin, amoxicillin-clavulanate, or azithromycin) were dispensed as part of usual clinical care. Serum and urine samples were stored. After a lead in period of 20 patients, MN who has 20 years of nursing experience assessing patients for tuberculosis, was asked if tuberculosis was likely or unlikely after clinically assessing the patient and reviewing the CRP results.

Patients were followed-up after 2-5 days when results of the Ultra were known. Vital signs were repeated, and they were asked if their symptoms were better, about the same, or worse; a clinical response to antibiotics was defined as symptoms being reported as better. CRP and sputum mycobacterial culture were repeated for all patients. All patients with positive Ultra or tuberculosis culture on sputum were initiated on anti-tuberculosis therapy according to national guidelines and those who remained symptomatic were referred to their clinic for follow-up care.

Outcomes

The primary outcome was active tuberculosis defined as ≥1 sputum culture positive for Mycobacterium tuberculosis. Patients with 2 negative cultures or those with one negative, one
contaminated culture, and who were asymptomatic after 6 weeks without anti-tuberculous therapy were considered not to have tuberculosis.

**Predictors**

There is no consensus on the best method for selecting candidate variables for MPM, but suggested approaches include using literature review, clinical knowledge, and studying the distribution of predictors in the study data. *A priori*, we considered predictors known to be associated with prevalent and/or incident tuberculosis amongst PWH at index visit: age, sex, ART status, CD4 count, duration of symptoms, temperature, number of tuberculosis symptoms, current smoking status, BMI, and CRP.

We defined ART status as: no ART or on ART <3 months vs. on ART for >3 months at time of presentation. Patients who had interrupted ART for >3 months prior to presentation were categorised as ART <3 months.

We also considered factors that may be predictive of tuberculosis at a second visit following a course of oral antibiotics: CRP, change in CRP, and subjective improvement of symptoms. Change in CRP was measured on a logarithmic scale due to the exponential decline in CRP when patients are treated for community acquired pneumonia (14).

**Sample size**

It is recommended, to ensure predictive accuracy, that the total number of candidate predictors is limited so that there are at least 10 outcomes for each candidate predictor studied (15). We predicted a tuberculosis prevalence rate of 35% and chose a sample size of 200 to ensure at least 70 outcomes so that up to 7 candidate predictors could be included in the final models.

**Statistical analysis methods**

Baseline characteristics were described as proportions or medians. We used simple logistic regression to assess the ability of selected variables to predict tuberculosis.

Model development used a backward stepwise approach. A multiple logistic regression model using only the *a priori* selected variables was created (supplementary appendix). Variables with the highest p-value were removed sequentially with reduced models being compared to the original model using the log likelihood ratio test. Model development was complete if removal of a variable led to a statistically significant difference (p<0.05) between the reduced model and the original model or when all predictors in a reduced model had p <0.1. We considered models based on information available at visit 1 and visit 2 (excluding the Ultra result).

Final models were visually assessed by a calibration plot, and by the Hosmer–Lemeshow goodness of fit test (GOF) (16). An estimate of the c-statistic was used to assess discrimination (values 0.7–0.8 are deemed acceptable, 0.8–0.9 good, and ≥0.9 outstanding) (17). Internal validation used 1000 bootstrap resamples (18). A risk score was generated for each patient which is converted to predicted risk using the equation predicted risk = 1/(1+e⁻risk score).

To externally validate the clinical prediction model developed by Hanifa et al (8), we obtained the measured predictors and outcome values in the participants of our study. We then quantified the discrimination and calibration of the XPHACTOR rule in our participants, using a calibration plot, GOF, and an estimate of the c-statistic.

**Missing data**
The full data-set was used as less than 5% of values were missing. In a sensitivity analysis we used multiple imputation with chained equations. The upper limit of detection of the CRP assay is 200 mg/L. Values >200 were imputed as 300 based on the range of CRP values >200 seen in a similar patient group (19).

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Ethics

All participants signed informed consent. The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand, protocol number M180401.

Results

Two hundred and seventeen participants were recruited between June 2018 and February 2019. Ten were excluded from the final analysis as they did not attend visit 2 and therefore had only 1 available sputum culture (figure 1). One hundred and thirty-eight participants (76%) were female and median age was 36 years. Median CD4 count was 185 cells/µL, 116 (56%) had been diagnosed with HIV within the past month and most (95%) had cough (table 1).

Seventy-five (36%) participants had confirmed tuberculosis of whom 9 (12%) were sputum smear positive (including scanty) and 62 (83%) were Ultra positive (including trace) (table 2). Two participants had confirmed rifampicin mono-resistant tuberculosis and 1 had multi-drug resistance. Sensitivity and specificity of Ultra, compared to the outcome defined above, was 0.83 (95% CI 0.72, 0.90) and 0.99 (0.96, 1.00) respectively; against a reference standard of 1 sputum culture it was 0.89 (0.79, 0.96) and 0.97 (0.93, 0.99). The equivalent figures for CRP ≥10 mg/L were 0.95 (0.87, 0.98) and 0.26 (0.19, 0.34). No improvement in symptoms after antibiotics had a sensitivity and specificity of 0.43 (0.32,0.55) and 0.86 (0.78, 0.91) respectively (table 3).

Multiple variables were associated with tuberculosis on simple logistic regression. The strongest association was with the nurse’s opinion of likely tuberculosis (OR 6.5, 95% CI 3.11- 13.6). Other strong associations were with no improvement in symptoms after antibiotics (OR 4.53, 2.32- 8.85) and CRP at visit 2 (OR 1.11, 1.06-1.16 per 10-unit increase) (table 4).
|                                | All participants | Confirmed TB | Not TB |
|--------------------------------|------------------|--------------|--------|
|                                | N=207            | N=75         | N=132  |
| Female sex                     | 138 (67)         | 54 (72)      | 84 (64)|
| Age, years                     | 36 (31-41)       | 35 (30.5-39) | 37 (31-41) |
| HIV/ART status                 |                  |              |        |
| Months since HIV diagnosis     | 0 (0-45)         | 0 (0-6.5)    | 0 (0-59) |
| Diagnosed with HIV <1 month    | 116 (56)         | 51 (68)      | 65 (49) |
| Ever been on ART               | 82 (40)          | 22 (29)      | 60 (45) |
| Currently on ART               | 80 (39)          | 21 (28)      | 59 (45) |
| ART status < 3 months          | 145 (70)         | 61 (81)      | 84 (64) |
| Current CD4 count cell/µL      | 185 (51-242)     | 114 (46-259) | 227 (55-416) |
| TB history                     |                  |              |        |
| Previous TB                    | 34 (17)          | 9 (12)       | 25 (19) |
| Previous IPT                   | 4 (2)            | 2 (3)        | 2 (2)  |
| Current IPT                    | 10 (5)           | 2 (3)        | 8 (6)  |
| WHO symptoms                   |                  |              |        |
| Duration of symptoms ≥ 14 days | 94 (47)          | 44 (59)      | 50 (38) |
| Cough                          | 196 (95)         | 73 (97)      | 123 (93) |
| Fever                          | 124 (60)         | 52 (69)      | 72 (55) |
| Night sweats                   | 155 (75)         | 61 (81)      | 94 (71) |
| Weight loss                    | 186 (90)         | 73 (97)      | 113 (86) |
| Number of symptoms             |                  |              |        |
| 1                              | 8 (4)            | 1 (1)        | 7 (5)  |
| 2                              | 41 (20)          | 10 (13)      | 31 (23) |
| 3                              | 62 (30)          | 22 (29)      | 44 (33) |
| 4                              | 96 (46)          | 46 (61)      | 50 (38) |
| Medical history                |                  |              |        |
| Current smoker                 | 60 (29)          | 23 (31)      | 37 (28) |
| Diabetes                       | 1 (0.5)          | 1 (1)        | 0 (0)  |
| Measurements                   |                  |              |        |
| BMI, kg/m²                     | 20.1 (18.3-22.3) | 19.3 (18.2-21.7) | 20.5 (18.7-22.8) |
| Pulse rate, bpm                | 76 (68-82)       | 78 (72-88)   | 74 (68-82) |
| Respiratory rate, bpm          | 14 (14-16)       | 16 (14-16)   | 14 (14-16) |
| Temperature, Celsius           |                  |              |        |
| 36.0-37.4                      | 184 (89)         | 62 (83)      | 122 (92) |
| 37.5-38.9                      | 19 (14)          | 10 (13)      | 9 (7)   |
| 39.0-40.0                      | 4 (2)            | 3 (4)        | 1 (1)   |
| C-reactive protein, mg/L       | 57 (16.8-115)    | 74 (45-126)  | 38 (9-94.5) |
| Visit 2                        |                  |              |        |
| Days after visit 1             | 2 (2-4)          | 2 (2-4)      | 2 (2-4) |
| Symptoms improved              | 155 (75)         | 42 (56)      | 113 (86) |
| C-reactive protein, mg/L       | 34 (9-91)        | 81 (38-123)  | 16 (5-56) |
| Log C-reactive protein change | -0.103 (0.105 - 0.721) | -0.008 (-0.206 - 0.34) | 0.22 (0 - 1.07) |

Table 1 Baseline characteristics of 207 adult participants with HIV and symptoms suggestive of tuberculosis. Missing values: Previous TB (4), Duration of symptoms ≥ 14 days (6), night sweats (1), current smoker (3), diabetes (3), C-reactive protein visit 1 (3), symptoms improved (1), C-reactive protein visit 2 (4), log C-reactive protein change (6)
|                         | Confirmed TB, n | No TB, n |
|-------------------------|----------------|----------|
| **Sputum smear positive** |                |          |
| Negative                | 66             | 131      |
| Scanty                  | 1              | 1        |
| 1+                      | 0              | 0        |
| 2+                      | 4              | 0        |
| 3+                      | 4              | 0        |
| **Xpert MTB/RIF Ultra** |                |          |
| Negative                | 13             | 131      |
| Trace                   | 1              | 1*       |
| Positive                | 61             | 0        |
| **Xpert MTB/RIF Ultra** |                |          |
| Rifampicin resistance   | 4              | 0        |
| **Positive sputum culture** |            |          |
| Visit 1                 | 65             | 0        |
| Visit 2                 | 65             | 0        |
| **HAIN resistance**     |                |          |
| Rifampicin mono resistance | 2            | 0        |
| Isoniazide mono resistance | 0             | 0        |
| Multidrug resistance    | 1              | 0        |

Table 1 Sputum results from 207 participants with HIV and symptoms suggestive of tuberculosis. *patient was asymptomatic without anti-tuberculosis treatment after 6 weeks.
| Index test                                    | Sensitivity (95%CI) | Specificity (95%CI) | Positive predictive value (95%CI) | Negative predictive value (95%CI) | Likelihood ratio +ve (95%CI) | Likelihood ratio –ve (95%CI) |
|----------------------------------------------|---------------------|---------------------|----------------------------------|----------------------------------|-------------------------------|-------------------------------|
| Smear (including scanty)                     | 0.12 (0.06,0.22)    | 0.99 (0.96,1.00)    | 0.90 (0.55,1.00)                 | 0.66 (0.59,0.73)                 | 15.84 (2.05, 122.6)          | 0.89 (0.81,0.97)              |
| Xpert MTB/RIF Ultra (including trace) *      | 0.89 (0.79, 0.96)   | 0.97 (0.93, 0.99)   | 0.94 (0.84, 0.98)                | 0.95 (0.90, 0.98)                | 29.89 (11.3, 78.8)           | 0.11 (0.06, 0.22)             |
| Xpert MTB/RIF Ultra (including trace)        | 0.83 (0.72,0.90)    | 0.99 (0.96,1.00)    | 0.98 (0.91,1.00)                 | 0.91 (0.85,0.95)                 | 109.12 (15.44,771,03)        | 0.17 (0.11,0.29)              |
| CRP ≥10 mg/L visit 1                         | 0.95 (0.87,0.98)    | 0.26 (0.19,0.34)    | 0.42 (0.34,0.49)                 | 0.89 (0.75,0.97)                 | 1.28 (1.14,1.43)             | 0.21 (0.08,0.57)              |
| Symptoms not improved after antibiotics      | 0.43 (0.32,0.55)    | 0.86 (0.78,0.91)    | 0.63 (0.48, 0.76)                | 0.73 (0.65, 0.80)                | 3.00 (1.84, 4.91)            | 0.66 (0.54, 0.82)             |
| CRP ≥10 mg/L after antibiotics               | 0.94 (0.86,0.98)    | 0.37 (0.28,0.46)    | 0.45 (0.37,0.53)                 | 0.92 (0.81,0.98)                 | 1.49 (1.29,1.72)             | 0.15 (0.06,0.40)              |

Table 2: Diagnostic accuracy of sputum tests and C-reactive protein in 207 adult participants with HIV and symptoms suggestive of tuberculosis. Reference standard of 2 sputum cultures unless stated. *reference standard culture as visit 1 only (8 participants excluded due to contaminated cultures)
|                               | Unadjusted odds ratio | 95% CI       |
|-------------------------------|-----------------------|--------------|
| **Visit 1**                   |                       |              |
| Age                           | 0.97                  | 0.93-1.01    |
| Sex                           |                       |              |
| Female                        | Referent group        |              |
| Male                          | 1.21                  | 0.66-2.22    |
| ART status                    |                       |              |
| < 3 months                    | Referent group        |              |
| > 3 months                    | 0.40                  | 0.20-0.79    |
| Current CD4 count*            | 0.0998                | 0.0997-0.0999|
| Number of symptoms            | 1.80                  | 1.25-2.58    |
| Duration of symptoms          |                       |              |
| < 14 days                     | Referent group        |              |
| ≥ 14 days                     | 2.26                  | 1.26-4.05    |
| Smoking status                |                       |              |
| Non smoker                    | Referent group        |              |
| Current smoker                | 1.21                  | 0.65-2.25    |
| BMI                           | 0.90                  | 0.83-0.98    |
| Temperature, Celsius          |                       |              |
| ≤ 37.4                        | Referent group        |              |
| ≥ 37.5                        | 2.32                  | 0.95-5.67    |
| C-reactive protein*           | 1.04                  | 1.00-1.07    |
| Nurse opinion                 |                       |              |
| Unlikely TB                   | Referent group        |              |
| Likely TB                     | 6.51                  | 3.11-13.6    |
| **Visit 2**                   |                       |              |
| Symptoms change               |                       |              |
| Improved                      | Referent group        |              |
| Not improved                  | 4.53                  | 2.32-8.85    |
| C-reactive protein*           | 1.11                  | 1.06-1.16    |
| C-reactive protein change (log scale) | 0.46             | 0.29-0.73  

Table 3 Simple logistic regression showing factors associated with tuberculosis in 207 adult participants with HIV

CD4 count, number of symptoms, BMI, and C-reactive protein were modelled linearly

*per 10-unit increase

The final model using information available at visit 1 included ART status, number of symptoms, duration of symptoms, and temperature, table 5. The model’s c-statistic was 0.70; the equivalent in bootstrap validation was 0.68, with an optimism estimate of 0.042, indicating good stability of the model in internal validation, figure 2. There was no evidence of poor calibration based on the GOF test (p= 0.63). However, visual inspection of the calibration plot shows underestimation of risk in the mid-range of values, figure 3.
The final model based on information available at visit 2 included change in symptoms after antibiotics, CRP at visit 2, number of symptoms, duration of symptoms, and ART status, table 6. The model’s c-statistic was 0.78; the equivalent in bootstrap validation was 0.76 with an optimism estimate of 0.046, indicating good stability of the model in internal validation, figure 4. There was no evidence of poor calibration based on the GOF test (p= 0.07). Visual inspection of the calibration plot indicates good agreement between the rate of tuberculosis estimated by the model and the tuberculosis frequency observed in the study population, figure 5.

Number of symptoms and temperature were modelled linearly

\[ \text{Risk score} = -19.8 - 0.72 \times (\text{if ART status >3 months}) + (0.48 \times \text{No of symptoms}) + 0.71 \times (\text{if cough >14 days}) + (0.48 \times \text{temperature}) \]

The final model based on information available at visit 2 included change in symptoms after antibiotics, CRP at visit 2, number of symptoms, duration of symptoms, and ART status, table 6. The model’s c-statistic was 0.78; the equivalent in bootstrap validation was 0.76 with an optimism estimate of 0.046, indicating good stability of the model in internal validation, figure 4. There was no evidence of poor calibration based on the GOF test (p= 0.07). Visual inspection of the calibration plot indicates good agreement between the rate of tuberculosis estimated by the model and the tuberculosis frequency observed in the study population, figure 5.

| ART status       | Adjusted Odds ratio (95% CI) | Beta coefficient (log [adjusted OR]) |
|------------------|------------------------------|-------------------------------------|
| < 3 months       | 1                            | 0                                   |
| > 3 months       | 0.49 (0.24 - 0.10)           | -0.72                               |
| Number of symptoms | 1.62 (1.09 - 2.41)        | 0.48                                 |
| Duration of symptoms |                          |                                      |
| <14 days         | 1                            | 2.03 (1.10 - 3.76)                  | 0                                   |
| ≥ 14 days        | 0.48 (0.97 - 2.66)           | 0.71                                 |

**Table 5** Multiple logistic regression model predicting tuberculosis based on data available at presentation in 207 adults with HIV and a positive symptom screen

Number of symptoms and temperature were modelled linearly

\[ \text{Risk score} = -19.8 - 0.72 \times (\text{if ART status >3 months}) + (0.48 \times \text{No of symptoms}) + 0.71 \times (\text{if cough >14 days}) + (0.48 \times \text{temperature}) \]

The final model based on information available at visit 2 included change in symptoms after antibiotics, CRP at visit 2, number of symptoms, duration of symptoms, and ART status, table 6. The model’s c-statistic was 0.78; the equivalent in bootstrap validation was 0.76 with an optimism estimate of 0.046, indicating good stability of the model in internal validation, figure 4. There was no evidence of poor calibration based on the GOF test (p= 0.07). Visual inspection of the calibration plot indicates good agreement between the rate of tuberculosis estimated by the model and the tuberculosis frequency observed in the study population, figure 5.

| Symptom change       | Adjusted Odds ratio (95% CI) | Beta coefficient (log [adjusted OR]) |
|----------------------|------------------------------|-------------------------------------|
| Improved             | 3.24 (1.51 - 6.94)           | 1.17                                 |
| Not improved         | 1                            | 0                                   |
| CRP at visit 2*      | 1.07 (1.02 - 1.12)           | 0.07                                 |
| Number of symptoms   | 1.46 (0.95 - 2.23)           | 0.38                                 |
| Duration of symptoms |                              |                                      |
| <14 days             | 2.18 (1.12 - 4.23)           | 0.78                                 |
| ≥ 14 days            | 0.50 (0.22 - 1.09)           | -0.70                                |

**Table 6** Multiple logistic regression model predicting tuberculosis based on data available at a second visit following antibiotics in 207 adults with HIV and a positive symptom screen

Number of symptoms was modelled linearly

\[ \text{Risk score} = -2.81 +1.17 \times (\text{if symptoms not improved}) + (0.007 \times \text{CRP at visit 2}) + (0.38 \times \text{number of symptoms}) + 0.78 \times (\text{if symptom duration ≥ 14 days}) - 0.70 \times (\text{if ART status >3 months}) \]
We explored a model with data available at visit 2 without CRP, which included change in symptoms after antibiotics, number of symptoms, duration of symptoms, and ART status, table 7. The model’s c-statistic was 0.75; the equivalent in bootstrap validation was 0.73 with an optimism estimate of 0.037, indicating good stability of the model in internal validation, figure 6. There was no evidence of poor calibration based on the GOF test (p = 0.60). Visual inspection of the calibration plot indicates reasonable agreement between the rate of tuberculosis estimated by the model and the tuberculosis frequency observed in the study population, figure 7.

|                        | Adjusted Odds ratio (95% CI) | Beta coefficient (log [adjusted OR]) |
|------------------------|------------------------------|-------------------------------------|
| Symptom change         |                              |                                     |
| Improved               | 1                            | 0                                   |
| Not improved           | 4.50 (2.19-9.23)              | 1.50                                |
| Number of symptoms     | 1.70 (1.12-2.57)              | 0.53                                |
| Duration of symptoms   |                              |                                     |
| <14 days               | 1                            | 0                                   |
| ≥ 14 days              | 2.09 (1.10-3.97)              | 0.74                                |
| ART status             |                              |                                     |
| < 3 months             | 1                            | 0                                   |
| >3 months              | 0.54 (0.25-1.14)              | -0.62                               |

Table 7 Multiple logistic regression model predicting tuberculosis based on data available at a second visit following antibiotics in 207 adults with HIV and a positive symptom screen (excluding C-reactive protein)

Risk score = -2.90 + 1.50 (if symptoms not improved) + 0.53X number of symptoms + 0.74 (if symptom duration ≥ 14 days) – 0.62 (if ART status > 3 months)

The XPHACTOR clinical prediction rule was not adequately validated in our cohort. Discrimination was poor (c-statistic 0.65) although calibration was good, GOF test p= 0.96, figure 8.

**Discussion**

Since 2007, WHO algorithms have discouraged the use of a ToA as a diagnostic strategy for ambulatory PWH suspected of having tuberculosis. Our study suggests that lack of clinical response to antibiotics, as assessed by improvement in symptoms, is the strongest independent predictor of tuberculosis in patients seeking care for their symptoms (passive case finding). The best clinical prediction model based on 2-visits (excluding CRP), included change in symptoms after antibiotics, number and duration of symptoms, and ART status. It showed acceptable discrimination (c-statistic 0.75) with good calibration (GOF p=0.60). A systematic review of 2586 patients found the sensitivity and specificity of ToA to be 0.72 and 0.77 respectively (6) and noted substantial heterogeneity due to inconsistent methodologies across studies. In our study, ToA had a lower sensitivity (0.43) but
higher specificity (0.86). This suggests that response to antibiotics may be an important part of diagnostic algorithms, particularly when access to Ultra is limited. Further information regarding the diagnostic accuracy and clinical impact of ToA as a diagnostic aid will come from an ongoing randomized controlled trial (https://clinicaltrials.gov/ct2/show/NCT03545373).

This is the first study to evaluate CRP as an objective marker of response to antibiotics in PWH who are suspected of having tuberculosis. Change in CRP and absolute CRP were both predictors of tuberculosis in simple logistic regression analysis, but only the absolute value after antibiotics was retained in the final predictive models. Including CRP in the final model based on 2-visits slightly improved discrimination (c-statistic 0.78) with good calibration (GOF p=0.07).

CRP has been proposed as a useful initial test in the evaluation of PWH suspected of having tuberculosis. In our study, the sensitivity and specificity of CRP ≥10 was 0.95 (0.87,0.98) and 0.26 (0.19,0.34) respectively, which is compatible with the findings of a meta-analysis which showed a range of sensitivity and specificity of 0.96-0.97 and 0.33-0.73 respectively in patients self-reporting symptoms compatible with tuberculosis (11). CRP <10 mg/L is most likely to be useful as a ‘rule out’ test, allowing resources such as Ultra to be allocated to patients with a higher probability of disease. However, in our passive case finding cohort, the negative predictive value (NPV) of 0.89 (0.75,0.97) when prevalence is 36% is unlikely to be high enough to confidently exclude disease. When analysed as a continuous variable, CRP at presentation was a significant predictor of tuberculosis on simple logistic regression (OR 1.04 per 10-unit increase 95% CI 1.00-1.07) but was not retained in a diagnostic model, also suggesting that it may not be useful in the initial evaluation of passive case finding patients.

There have been 3 attempts to develop MPMs for adult patients presenting with symptoms of tuberculosis. The studies by Balcha et al(9) and Rudolf et al(10), from Ethiopia and Bissau respectively included Karnofsky status, mid-upper arm circumference (MUAC), peripheral lymphadenopathy, anaemia, cough, dyspnoea, chest pain, and BMI as predictors. Each showed reasonable discrimination, but their sample sizes were relatively small and neither has been externally validated. The XPHACTOR rule is based on ART status, CD4 count, BMI, and number of WHO symptoms and shows acceptable discrimination (c-statistic 0.75) and calibration (GOF p=0.31). Our study was unable to validate the XPHACTOR rule, showing poor discrimination (AUC 0.65). In keeping with standard validation procedures, our study population was similar but different. Both studies were carried out in the same city but differed in respect to the prevalence of tuberculosis, the number of symptoms, and whether presentation was self-directed. Our results suggest that different models may be necessary for active and passive case finding approaches. We were also unable to develop a model with good discrimination and calibration based on data available at visit 1 only. The best model, which did not include CRP, showed only moderate discrimination, c-statistic 0.70, with no evidence of poor calibration (GOF p=0.63).

The sensitivity of Ultra is insufficiently high to exclude tuberculosis when negative, particularly in high prevalence settings. Current WHO guidance when Xpert is negative is to investigate further with chest X-ray, clinical assessment and a repeat Xpert MTB/RIF using a fresh specimen. The prevalence of culture confirmed tuberculosis in participants with negative Ultra in our study was 9%, suggesting that further investigation is likely to be needed, although an algorithm for ruling out disease in a proportion of these patients would be useful to save resources.
Our study has a number of limitations. The sample was taken from a single clinic. All patients self-presented with symptoms suggestive of tuberculosis (passive case finding) and the tuberculosis prevalence was high (36%), therefore our findings may not be generalizable to active case finding populations or where the prevalence of tuberculosis is lower. In addition, the WHO symptom screen is known to have imperfect sensitivity and so some patients with active tuberculosis will not be captured by this passive case finding methodology. The time between index and return visit was relatively short (median 2 days) due to the standard operating procedures of this urban clinic. It may not be practical to receive Ultra results and arrange return visits within 2 days in more rural clinics. The choice of antibiotic prescribed for each patient was made by independently of the study and not recorded. Therefore a proportion of participants received azithromycin which is known to have anti-inflammatory properties which may reduce CRP though a mechanism that is unrelated to treatment of pneumonia. A strength of the study was that we obtained 2 sputum mycobacterial cultures from each patient, ensuring a highly sensitive and specific reference standard.

Future work should be to validate our findings in different populations. In settings where access to Ultra is limited, symptomatic improvement to a ToA, possibly including repeat CRP, should be evaluated. Ultra could be targeted to patients with higher probability of tuberculosis at visit 2. Our study lacked sufficient power to determine predictors of tuberculosis when Ultra is negative (13 cases). Future work should investigate predictors of tuberculosis and rules for prioritizing further testing, with repeat Ultra, chest X-ray, and culture in this group. Particularly given that the prevalence of tuberculosis remains high (9%). It would also be important to test whether similar strategies apply to patients at index visit, if they have received antibiotics prior to presentation, a population that was excluded from our study. Practical considerations for the implementation of ToA as a diagnostic strategy include the need for an uninterrupted supply of antibiotics and the possible increase in clinic workload. However, this may be minimized if patients are asked to return for a second visit only if their symptoms persist.

In conclusion, a clinical response to antibiotics is useful for diagnosing tuberculosis in PWH who are identified by passive case finding, and is a strategy that could be implemented immediately. CRP after antibiotics adds value but is not essential. CRP at index presentation is of limited value in this population.

Figure legends

Figure 4 Participant flow diagram for 217 adults with HIV and symptoms suggestive of tuberculosis.

Ultra +ve includes trace

Figure 2 Receiver operator characteristic curve for a clinical prediction model for tuberculosis based on data available at index visit in 207 adults with HIV and a positive symptom screen

Figure 3 Calibration plot of the multivariate logistic regression model aimed at establishing a clinical prediction rule for the diagnosis of tuberculosis at index visit among 207 adults with HIV and a positive symptom screen

Figure 4 Receiver operator characteristic curve for a clinical prediction model for tuberculosis based on data available at a second visit following antibiotics index visit in 207 adults with HIV and a positive symptom screen
Figure 5 Calibration plot of the multivariate logistic regression model aimed at establishing a clinical prediction rule for the diagnosis of tuberculosis at a second visit following antibiotics among 207 adults with HIV and a positive symptom screen.

Figure 6 Receiver operator characteristic curve for a clinical prediction model for tuberculosis based on data available at a second visit following antibiotics index visit in 207 adults with HIV and a positive symptom screen (excluding C-reactive protein).

Figure 7 Calibration plot of the multivariate logistic regression model aimed at establishing a clinical prediction rule for the diagnosis of tuberculosis at a second visit following antibiotics among 207 adults with HIV and a positive symptom screen (excluding C-reactive protein).

Figure 8 Receiver operator characteristic curve for validation of the XPHACTOR clinical prediction model for tuberculosis based in 207 adults with HIV and a positive symptom screen.

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Figure 2

AUC: 0.697
Figure 4
Figure 5
Figure 6

![Graph showing the Receiver Operating Characteristic (ROC) curve with an AUC of 0.753.](graph.png)
Figure 7

[Graph showing predicted probability by prediction rule against observed probability, with data points scattered across the graph.]
Figure 8

[Graph showing an ROC curve with the area under the curve (AUC) labeled as 0.654]