Optimizing Tuberculosis Diagnosis in HIV-Infected Inpatients Meeting the Criteria of Seriously Ill in the WHO Algorithm

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Declaration

I, *Dr Rulan Griesel*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Background

The WHO algorithm for the diagnosis of tuberculosis in seriously ill HIV-infected patients lacks a firm evidence base. We aimed to develop a clinical prediction rule for the diagnosis of tuberculosis and to determine the diagnostic utility of the Xpert MTB/RIF assay in seriously ill HIV-infected patients.

Methods

We conducted a prospective study among HIV-infected inpatients with any cough duration and WHO-defined danger signs. Culture-positive tuberculosis from any site was the reference standard. *A priori* selected variables were assessed for univariate associations with tuberculosis. The most predictive variables were assessed in a multivariate logistic regression model and used to establish a clinical prediction rule for diagnosing tuberculosis.

Results

We enrolled 484 participants: median age 36 years, 65·5% female, median CD4 count 89 cells/μL, and 35·3% on antiretroviral therapy. Tuberculosis was diagnosed in 52·7% of participants. The c-statistic of our clinical prediction rule (variables: cough ≥14 days, unable to walk unaided, temperature >39oC, chest radiograph assessment, haemoglobin, and white cell count) was 0·811 (95%CI 0·802, 0·819). The classic tuberculosis symptoms (fever, night sweats, weight loss) added no discriminatory value in diagnosing tuberculosis. Xpert MTB/RIF assay sensitivity was 86·3% and specificity was 96·1%.

Conclusion

Our clinical prediction rule had good diagnostic utility for tuberculosis among seriously ill HIV-infected inpatients. Xpert MTB/RIF assay, incorporated into the updated 2016 WHO algorithm, had high sensitivity and specificity in this population. Our findings could facilitate improved diagnosis of tuberculosis among seriously ill HIV-infected inpatients in resource-constrained settings.

Acknowledgments and Contributions

- Prof G Maartens, Prof M Mendelson, and Dr H van der Plas designed the parent study, developed the protocol, and procured funding.
- Dr H van der Plas initiated the enrollment of participants and data collection.
- Dr W Sikhondze assited with patient enrollment and data collection.
- Dr MX Rangaka developed the statistical analysis plan for the parent study.
- Prof M Nicol assited with sample analysis and storage, and contributed to the design of the parent study.
- Prof AP Kengne asssited with the statistical analyses of this publication.
- Ms A Stewart was responsible for statistical analysis of this publication.
- Dr R Griesel collected data for the parent study, assited with the statistical analyses of this publication, and prepared the first draft of the publication together with Prof G Maartens.
- All authors read and approved the final manuscript.
- Chest radiographic interpretation was done by Prof H Goodman, Division of Radiology, Department of Radiation Medicine at the University of Cape Town.
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Clinical Infectious Diseases

MAJOR ARTICLE

Optimizing Tuberculosis Diagnosis in Human Immunodeficiency Virus–Infected Inpatients Meeting the Criteria of Seriously Ill in the World Health Organization Algorithm

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Background. The World Health Organization (WHO) algorithm for the diagnosis of tuberculosis in seriously ill human immunodeficiency virus (HIV)–infected patients lacks a firm evidence base. We aimed to develop a clinical prediction rule for the diagnosis of tuberculosis and to determine the diagnostic utility of the Xpert MTB/RIF assay in seriously ill HIV-infected patients.

Methods. We conducted a prospective study among HIV-infected inpatients with any cough duration and WHO-defined danger signs. Culture-positive tuberculosis from any site was the reference standard. A priori selected variables were assessed for univariate associations with tuberculosis. The most predictive variables were assessed in a multivariate logistic regression model and used to establish a clinical prediction rule for diagnosing tuberculosis.

Results. We enrolled 484 participants. The median age was 36 years, 65.5% were female, the median CD4 count was 89 cells/μL, and 35.3% were on antiretroviral therapy. Tuberculosis was diagnosed in 52.7% of participants. The c-statistic of our clinical prediction rule (variables: cough ≥14 days, unable to walk unaided, temperature >39°C, chest radiograph assessment, hemoglobin, and white cell count) was 0.811 (95% confidence interval, .802–.819). The classic tuberculosis symptoms (fever, night sweats, weight loss) added no discriminatory value in diagnosing tuberculosis. Xpert MTB/RIF assay sensitivity was 86.3% and specificity was 96.1%.

Conclusions. Our clinical prediction rule had good diagnostic utility for tuberculosis among seriously ill HIV-infected inpatients. Xpert MTB/RIF assay, incorporated into the updated 2016 WHO algorithm, had high sensitivity and specificity in this population. Our findings could facilitate improved diagnosis of tuberculosis among seriously ill HIV-infected inpatients in resource-constrained settings.

Keywords. HIV; tuberculosis diagnosis; WHO algorithm; inpatients; Xpert MTB/RIF assay.

Tuberculosis remains a major cause of death among human immunodeficiency virus (HIV)–infected adults in resource-constrained countries in the antiretroviral therapy (ART) era [1]. Delayed or missed diagnosis contributes to tuberculosis mortality [2, 3]. World Health Organization (WHO) 2007 guidelines to diagnose smear-negative tuberculosis [4] included an algorithm for seriously ill patients with cough of 2–3 weeks and ≥1 danger sign (respiratory rate >30 breaths/minute, heart rate >120 beats/minute, temperature >39°C, and being unable to walk unaided).

A modified WHO algorithm for seriously ill patients improved clinical outcomes in 2 African cohort studies [5, 6]. However, no study has explored the ability of the 2007 WHO seriously ill algorithm to diagnose tuberculosis, which has several limitations. First, pulmonary tuberculosis commonly presents with cough duration of <14 days [7, 8]. Second, other classic tuberculosis symptoms (fever, night sweats, and weight loss), which have high negative predictive value in active case finding [9], were not included. Third, it preceded the Xpert MTB/RIF assay, which is more sensitive than smear microscopy in HIV-infected patients [10]. Finally, hemoglobin concentration and white cell count (WCC), which predict tuberculosis among HIV-infected patients [11–13], could add diagnostic value.

We conducted a prospective cohort study to attempt to improve the ability of the 2007 WHO algorithm to diagnose tuberculosis in seriously ill HIV-infected patients by evaluating any cough duration, classic tuberculosis symptoms, chest radiographic features, hemoglobin, WCC, and the Xpert MTB/RIF assay.

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METHODS

Study Population

We conducted a prospective cohort study in 2 regional hospitals in Cape Town, South Africa serving high-burden HIV and tuberculosis communities, GF Jooste Hospital (November 2011–February 2013) and Khayelitsha District Hospital (March 2013–October 2014). Inclusion criteria were as follows: HIV infected, ≥18 years of age, admitted within 24 hours, coughing for any duration, and ≥1 WHO danger sign. Exclusion criteria were antituberculosis therapy that is current or completed in the previous month or defaulted within the past 6 months, exacerbation of cardiac failure or chronic obstructive pulmonary disease, and inability to produce a spontaneous or induced sputum sample. Participants were followed up at 28 and 56 days after discharge.

Data Collection

Demographic data and clinical features (at the time of admission) were recorded on a standardized case record form. Chest radiographs performed on admission were assessed by the study medical officer for features suggestive of pulmonary tuberculosis and/or bacterial pneumonia and/or *Pneumocystis jirovecii* pneumonia (PJP). Chest radiographs were also retrospectively reviewed by a blinded specialist radiologist, who documented the presence of specific radiographic features (enlarged hilar or mediastinal lymph nodes, pleural effusions, interstitial ("ground glass") infiltration, nodularity, cavitation, diffuse micronodular infiltration, linear/reticulonodular infiltration, consolidation, and features of previous tuberculosis), and then classified radiographs as likely, possible, or unlikely for pulmonary tuberculosis and/or bacterial pneumonia and/or PJP. CD4 cell count was obtained on admission if none was available from the prior 6 months, and hemoglobin and WCC were done on admission. Sputum induction, using an ultrasonic nebulizer and hypertonic saline, was done on participants unable to spontaneously produce sputum. Three sputum samples from each participant were sent for Gram stain, culture, and sensitivity (1 sample); and auramine smear microscopy for acid-fast bacilli, and mycobacterial culture (BACTEC MGIT 960; Becton Dickinson, Franklin Lakes, New Jersey) (2 samples). The sputum pellet on one of the samples for mycobacterial culture was split after decontamination for Xpert MTB/RIF assay (Cepheid, Sunnyvale, California). Mycobacterial blood cultures (BacT/Alert MP; bioMérieux, Durham, North Carolina) were sent from all participants. Extrapulmonary samples (eg, pleural fluid) were sent for mycobacterial culture when appropriate.

Case Definitions and Procedures

Tuberculosis was defined as a positive culture for *Mycobacterium tuberculosis* from any site. The study criteria for initiating antituberculosis therapy included positive microbiological diagnosis (auramine stain and/or Xpert MTB/RIF assay and/or culture); and/or a radiological diagnosis (chest radiograph showing mediastinal and/or hilar lymph nodes or miliary infiltrates; abdominal ultrasound showing multiple enlarged lymph nodes and/or multiple splenic hypoechoic lesions and/or pericardial effusion); and/or pleural effusion/ascites showing a lymphocytic exudate; and/or no clinical improvement after 3–5 days of antibiotic therapy. Decisions to start antituberculosis therapy were made by study staff.

The diagnosis of bacterial pneumonia was made with consistent symptoms and evidence of consolidation on chest radiograph. Bacterial pneumonia was treated with a broad-spectrum β-lactam antibiotic (eg, ceftriaxone, amoxicillin-clavulanate), and a macrolide was added with CRB-65 score >2 (1 point for each of: confusion, respiratory rate ≥30/min, blood pressure systolic <90 mm Hg or diastolic ≤60 mm Hg, age ≥65 years [14, 15].

PJP was diagnosed with a cough duration ≤12 weeks, bilateral interstitial infiltration on chest radiograph, and hypoxia (defined as oxygen saturation of ≤92%) or dyspnea. PJP was treated with high-dose trimethoprim-sulfamethoxazole, and prednisone if hypoxia was present.

Statistical Analysis

A sample size of 473 was sufficient to detect an estimated 30% prevalence of culture-positive tuberculosis with 5% precision. This sample size incorporated the need for at least 10 culture-positive tuberculosis events per predictive variable for multivariate logistic regression analysis [16].

Analyses were performed using Stata version 12.1 software (StataCorp, College Station, Texas). Missing data were imputed using chained equations and 20 iterations. Baseline characteristics were described as proportions or medians. We used univariate associations to assess the ability of the following a priori selected variables to predict tuberculosis: age, sex, cough duration, individual WHO danger signs, classic tuberculosis symptoms (fever, night sweats, and weight loss), radiologist assessment of tuberculosis on chest radiograph (categorized as likely or possible), hemoglobin, and WCC [17]. A backward stepwise approach proposed by Collet [18] was used to select the most predictive variables in establishing a multivariate logistic regression model.

The model was visually assessed by a calibration plot, and by the Hosmer–Lemeshow test. An estimate of the c-statistic was used to assess discrimination (values 0.7–0.8 are deemed acceptable and 0.8–0.9 good) [19]. Internal validation used 1000 bootstrap resamples [20]. A clinical prediction rule with score chart was constructed utilizing a standard method [21]. We used the clinical prediction rule to predict the probability of having tuberculosis, and compared it with the reference standard of culture-positive tuberculosis. We calculated the diagnostic accuracy for the range of possible scores from the clinical prediction rule.

We explored associations of individual chest radiograph features recorded by the radiologist with culture-positive tuberculosis, reported as odds ratios with 95% confidence intervals (CIs).

We calculated the diagnostic accuracy of sputum smear and Xpert MTB/RIF assay for diagnosing culture-positive tuberculosis.

Ethics Approval

Approval for the study was obtained from the University of Cape Town Human Research Ethics Committee. All eligible participants signed informed consent before enrollment into the study. Confused participants were enrolled and given the option to continue with participation once orientated; their data were removed from the study if consent was declined.

RESULTS

Participant Characteristics

We screened 2054 patients and enrolled 484 (Figure 1). Table 1 shows participants' baseline characteristics. All participants were commenced on antibiotic therapy at admission or at the referral site. Fifty-three percent (255/484) of participants had culture-positive tuberculosis, 50.6% (245/484) bacterial pneumonia, and 10.5% (51/484) PJP. Antituberculosis therapy was empirically started in 56 (11.6%) participants with negative sputum smears and Xpert MTB/RIF assays, of whom 22 (43.1%) had culture-positive tuberculosis. Other diagnoses (*Cryptococcus neoformans*, *Emmonsia parva*, nontuberculous mycobacteria, and malignancy) were made in 8.5% (41/484). Clinically diagnosed coinfections were common: tuberculosis with bacterial pneumonia in 25.8% (125/484) and tuberculosis with PJP in 4.3% (21/484). Different sites of culture-positive specimens (Supplementary Table 1) and pulmonary/extrapulmonary/disseminated tuberculosis are given in the Supplementary Data. The diagnostic accuracy of Xpert MTB/RIF assay and smear is shown in Table 2.

The yield of sputum culture was higher with sputum induction than spontaneous sputum production (56.1% [175/312] vs 45.5% [74/163]; $P = .027$). Similarly, the yield of smear and Xpert MTB/RIF assay was higher with sputum induction than spontaneous sputum production (32.7% [102/312] vs 30.1% [49/163], $P = .553$ and 51.9% [162/312] vs 41.7% [68/163], $P = .035$, respectively).

Seventy-two participants with negative sputum smears had features of tuberculosis on chest radiograph diagnosed by medical officers, fulfilling the criteria of the 2007 WHO algorithm for seriously ill inpatients to start empiric antituberculosis therapy with a sensitivity of 49.1% and specificity of 91.5% for the diagnosis of culture-positive tuberculosis.

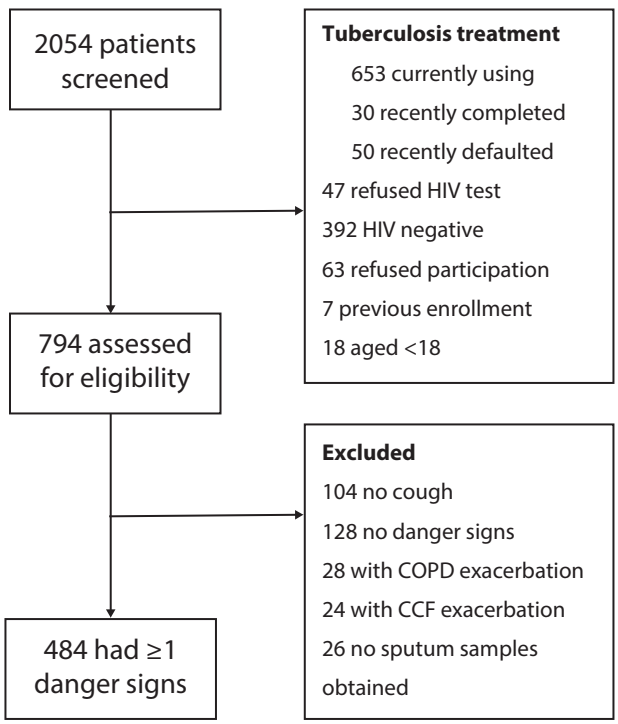

Figure 1. Flow diagram for participant inclusion into the study. Abbreviations: CCF, congestive cardiac failure; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus.

Clinical Prediction Rule

Univariate associations with culture-positive tuberculosis are shown in Table 3. In multivariate logistic regression (Supplementary Table 2) the most significant predictors of culture-positive tuberculosis were being unable to walk unaided, radiologist assessment of "likely tuberculosis" on chest radiograph, and anemia. Raised WCC was a significant negative predictor of tuberculosis. The only classic tuberculosis symptom that showed a significant association with the diagnosis of tuberculosis was weight loss, but it was not significant on multivariate analysis. Cough duration ≥14 days was predictive of tuberculosis, but 28.6% (73/255) of culture-positive tuberculosis participants had cough duration <14 days.

The calibration curve (Figure 2*A*) for the final model followed the ideal calibration line, indicating good agreement between the rate of tuberculosis estimated by the model and the tuberculosis frequency observed in the study population, confirmed by the Hosmer-Lemeshow χ^2 statistic of 10.65 ($P = .385$). The model's c-statistic was 0.834 (95% CI, .798–.871) (Figure 2*B*). The equivalents in bootstrap validation were 0.836 (95% CI, .800–.871), with an optimism estimate of 0.011 (95% CI, .010–.012), indicating good stability of the model in internal validation.

The clinical prediction rule with score chart was developed using the 6 selected variables (Table 4). Hemoglobin and WCC were categorized as tertiles. The diagnostic accuracy assessment based on different scores obtained from the clinical prediction rule is summarized in Table 5. The clinical prediction rule model showed a c-statistic of 0.811 (95% CI, .802–.819).

Chest Radiograph Assessment

The radiologist assessed 416 participants' chest radiographs: 151 (36.3%) were categorized as likely, 223 (53.6%) as possible,

Table 1. Baseline Characteristics of 484 Seriously Ill Human Immunodeficiency Virus–Infected Participants Presenting With a Cough of Any Duration and ≥1 World Health Organization Danger Sign

Characteristic	No. (%) or Median (25th–75th Percentile)
Baseline variables	
Age, y	36 (30–42)
Sex, female	317 (65.5)
Body mass index[a], kg/m^2	20 (18–39)
CD4 count[b], cells/μL	89 (34–210)
Cough duration[c], d	14 (7–21)
Cough duration ≥14 d	296 (61.2)
Using ART	171 (35.3)
Duration on ART, y	2.3 (0.2–5)
Previous tuberculosis	236 (48.8)
Sputum induction[d]	312 (65.7)
WHO danger signs	
Respiratory rate >30 breaths/min	315 (65.1)
Heart rate >120 beats/min	383 (79.1)
Temperature >39°C	81 (16.7)
Unable to walk unaided	259 (53.5)
Tuberculosis symptoms	
Fever[e]	394 (81.6)
Night sweats[f]	314 (65.3)
Weight loss	442 (91.3)
Laboratory investigations	
Hemoglobin[g] g/dL	9.5 (7.8–11.2)
WCC[h], ×10^9/L	8.6 (5.6–13.0)

Abbreviations: ART, antiretroviral therapy; WCC, white cell count; WHO, World Health Organization.

[a]Seventeen missing values.

[b]One missing value.

[c]Two missing values.

[d]Nine missing values.

[e]One missing value.

[f]Three missing values.

[g]Three missing value.

[h]Three missing values.

and 42 (10.0%) as unlikely tuberculosis. Univariate analysis of individual chest radiograph features revealed that diffuse micronodular infiltration, enlarged hilar or mediastinal lymph nodes, and nodularity >3 mm were predictors of culture-positive tuberculosis (Table 6). Bacterial pneumonia was a likely diagnosis in 9.6% (40/416) and PJP in 9.4% (39/416). Medical officers' categorization of likely tuberculosis on chest radiograph had an odds ratio of 9.4 (95% CI, 5.7–15.6) for culture-positive tuberculosis.

Table 2. Diagnostic Accuracy Evaluation of Sputum Auramine Smear and Xpert MTB/RIF Assay by the Reference Standard of Positive Culture From Any Site (n = 255) Among 484 Seriously Ill Human Immunodeficiency Virus–Infected Participants Presenting With a Cough of Any Duration and ≥1 World Health Organization Danger Sign

Parameter	Xpert MTB/RIF	Auramine Smear
Sensitivity, %	86.3 (81.5–90.3)	57.0 (50.7–63.2)
Specificity, %	96.1 (92.6–98.2)	98.7 (96.2–99.7)
Positive predictive value, %	96.1 (92.7–98.2)	98.0 (94.2–99.6)
Negative predictive value, %	86.2 (81.4–90.2)	67.2 (61.9–72.2)
Positive likelihood ratio	21.9 (11.5–41.6)	43.3 (14.0–134.0)
Negative likelihood ratio	0.14 (.11–.19)	0.44 (.38–.50)

Values in parentheses indicate the 95% confidence interval.

DISCUSSION

Our study is the first to evaluate the clinical, radiographic, and laboratory diagnosis of tuberculosis in a prospective cohort of

Table 3. Univariate Associations With Culture-Positive Tuberculosis Among 484 Seriously Ill Human Immunodeficiency Virus–Infected Participants Presenting With a Cough of Any Duration and ≥1 World Health Organization Danger Sign

Variable	OR	(95% CI)	Wald P Value
Age[a]	0.98	(.96–1.00)	.044
Sex			
Female	Referent group		
Male	0.95	(.65–1.39)	.799
Cough duration ≥14 d			
No	Referent group		
Yes	2.47	(1.70–3.60)	<.001
WHO danger signs			
Respiratory rate >30 breaths/min			
No	Referent group		
Yes	0.78	(.54–1.14)	.207
Heart rate >120 beats/min			
No	Referent group		
Yes	1.53	(.98–2.37)	.060
Temperature >39°C			
No	Referent group		
Yes	1.86	(1.13–3.07)	.014
Unable to walk unaided			
No	Referent group		
Yes	2.77	(1.92–4.01)	<.001
Tuberculosis symptoms			
Fever			
No	Referent group		
Yes	1.07	(.67–1.69)	.783
Night sweats			
No	Referent group		
Yes	1.18	(.81–1.72)	.384
Weight loss			
No	Referent group		
Yes	3.08	(1.54–6.17)	.002
Increasing number of tuberculosis symptoms	1.28	(1.01–1.64)	.041
Radiographic assessment of tuberculosis			
Unlikely	Referent group		
Possible	2.13	(1.00–4.54)	.052
Likely	11.01	(4.92–24.66)	<.001
Laboratory investigations			
Hemoglobin, g/dL[b]	0.69	(.63–.76)	<.001
WCC, ×10^9/L[c]	0.90	(.87–.93)	<.001

Abbreviations: CI, confidence interval; OR, odds ratio; WCC, white cell count; WHO, World Health Organization.

[a]Per 1 year increase.

[b]Per 1 g/dL increase.

[c]Per 1 × 10^9/L increase.

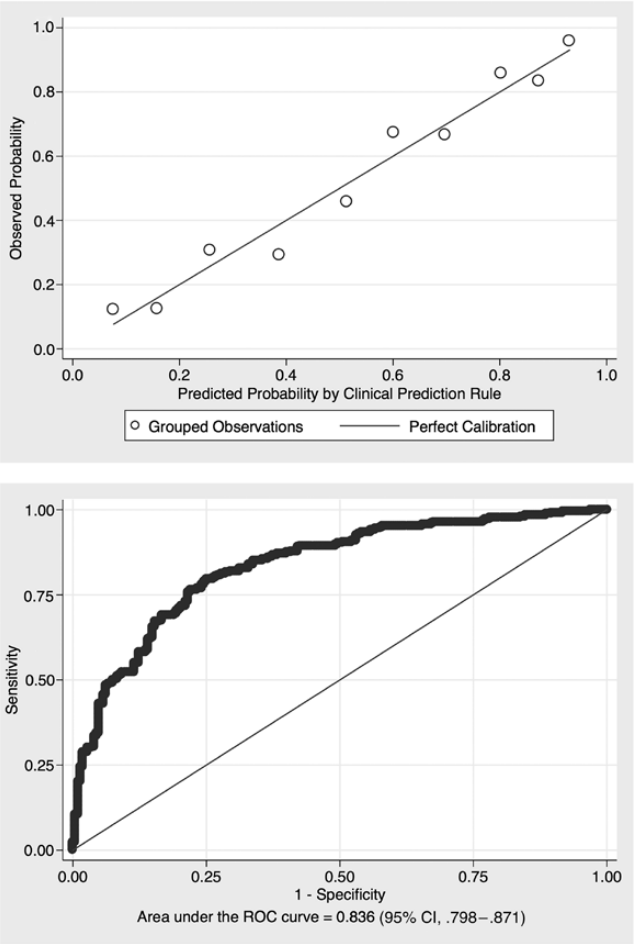

Figure 2. Upper, Calibration plot for the assessment of variables included in a multivariate logistic regression model aimed at establishing a clinical prediction rule for the diagnosis of tuberculosis among 484 seriously ill human immunodeficiency virus (HIV)–infected participants presenting with a cough of any duration and 1 or more World Health Organization (WHO) danger sign. The line shows perfect calibration between observed and predicted tuberculosis. Lower, Discrimination of the multivariate logistic regression model aimed at establishing a clinical prediction rule for the diagnosis of tuberculosis among 484 seriously ill HIV-infected participants presenting with a cough of any duration and ≥1 WHO danger sign. Abbreviations: CI, confidence interval; ROC, receiver operating characteristic.

inpatients fulfilling the criteria of the 2007 WHO algorithm for seriously ill HIV-infected patients. In 2016 (when enrollment into our study was completed), the WHO updated the algorithm by including classic tuberculosis symptoms and the Xpert MTB/RIF assay, and by making cough of any duration an inclusion criterion [22]. We had already incorporated all of these features into our study; therefore, we are also able to evaluate the updated WHO algorithm, but it should be noted that cough is no longer a requirement. We developed a clinical prediction rule with good diagnostic performance for tuberculosis using 6 variables, all of which are readily obtainable in most resource-constrained settings. The most significant predictors of tuberculosis were a radiologist assessment of likely tuberculosis on chest radiograph and anemia, while a raised WCC was a strong negative predictor of tuberculosis. The classic tuberculosis symptoms added no discriminatory value in diagnosing tuberculosis. Scores of 3 or 4 in our clinical prediction rule could be used to start empiric antituberculosis therapy in seriously ill patients as the sensitivity of these scores is around 90%. However, no single feature of our clinical prediction rule should be used in decisions to treat empirically for tuberculosis. The Xpert MTB/RIF assay, which has not previously been evaluated in seriously ill inpatients, had a high sensitivity of 86.3% in our participants. Implementation of our clinical prediction rule in resource-constrained settings could augment the 2016 WHO algorithm, inform the development of future algorithms, and ensure timely initiation of empiric antituberculosis treatment in seriously ill HIV-infected patients.

The 2007 WHO algorithm for seriously ill patients requires cough duration of 2–3 weeks. Although we found that cough duration of ≥14 days was predictive of tuberculosis, 28.6% of participants with confirmed tuberculosis had cough duration <14 days. Most of the data on cough duration of >14 days as a trigger for tuberculosis investigations are from studies of ambulatory patients and may not be generalizable to seriously ill inpatients. Two African studies found a high prevalence of tuberculosis among inpatients presenting acutely with pneumonia [7, 8]. These findings together with ours suggest that any cough duration was an appropriate improvement made in the 2016 WHO algorithm for the diagnosis of tuberculosis among seriously ill patients.

The classic tuberculosis symptoms fever and weight loss were sensitive, but not specific for the diagnosis of tuberculosis among HIV-infected inpatients with negative sputum smears [23]. The differential diagnosis in HIV-infected inpatients with cough and danger signs is wide, and many of these disorders and/or advanced HIV disease could reduce the diagnostic utility of the classic tuberculosis symptoms, which could explain why these were not independently associated with tuberculosis in our study. Two of the WHO danger signs, being unable to walk unaided and temperature >39°C, were significant predictors of tuberculosis in our cohort.

The WHO has recently reiterated the importance of using chest radiographic assessment to facilitate the rapid initiation of antituberculosis therapy among seriously ill HIV-infected patients [24]. Our study supports this recommendation, with a radiologist assessment of likely tuberculosis being the strongest predictor of tuberculosis in our clinical prediction rule. Other studies of hospitalized HIV-infected participants with smear-negative pulmonary tuberculosis have also reported the diagnostic value of chest radiography [17, 23, 25, 26]. The commonest individual radiographic feature associated with culture-positive tuberculosis in our study was enlarged hilar or mediastinal lymph nodes. Cavitation was not a significant clinical predictor of culture-positive tuberculosis, which might be explained by the low median CD4 count and high prevalence

Table 4. Clinical Prediction Rule for the Diagnosis of Culture-Positive Tuberculosis Among 484 Seriously Ill Human Immunodeficiency Virus–Infected Participants Presenting With a Cough of Any Duration and ≥1 World Health Organization Danger Sign

Variable	Category	Points
Cough duration	≥14 d	1
Temperature >39°C	Yes	1
Unable to walk unaided	Yes	1
Tuberculosis on chest radiograph	Possible	1
	Likely	5
Hemoglobin, g/dL	3.3–8.3	3
	8.4–10.6	2
White cell count, $\times 10^9$/L	1–6.5	1
	11.2–40.4	–2

of previous tuberculosis. Other studies of inpatients from high-burden settings have also reported that intrathoracic lymphadenopathy was a good predictor of tuberculosis, but cavitation was not [25, 26].

Our finding that anemia was associated with tuberculosis is in keeping with other studies of inpatients and outpatients [12, 26]. A study from Cameroon found anemia and leukopenia to be independent predictors of extrapulmonary involvement in patients with pulmonary tuberculosis; many patients in our cohort had disseminated disease, which could explain why hemoglobin and WCC were good predictors of tuberculosis [13].

Sputum Xpert MTB/RIF assay performed well in our cohort, with a sensitivity of 86.3%, which is somewhat higher than the sensitivity of 79% reported in HIV-infected patients in a systematic review [10]. The high sensitivity of Xpert MTB/RIF assay we found could be explained by the high proportion of participants who had sputum induction, which increased the yield of Xpert MTB/RIF assay in our study (contrary to the findings of a study in ambulatory patients) [27]. We found a higher yield of sputum culture with sputum induction than spontaneous sputum production, which is consistent with other studies [27, 28]. In view of these findings, we call for greater access to sputum induction in resource-constrained settings.

Our study has some limitations. First, our findings may not be generalizable to patients without cough or in settings with different prevalence of tuberculosis and other pulmonary opportunistic infections. Two studies evaluated the prevalence of culture-positive tuberculosis in HIV-infected inpatients with WHO danger signs and negative sputum smears, reporting 23% in a Ugandan study [6] (51% of whom had WHO danger signs) and 23% in a study from South Africa [5], which is similar to the 22.7% culture-positive prevalence in our participants with negative sputum smears, suggesting that our findings are generalizable to sub-Saharan Africa. Tuberculosis is the leading cause of hospitalization among HIV-infected adults worldwide, with similar proportions in low- to middle-income countries, and it is thus conceivable that our findings may also be generalizable outside of Africa [29]. Second, all our participants had 1 or more WHO danger signs, which limited our ability to assess their value for the diagnosis of tuberculosis. Third, a specialist radiologist's assessments of the chest radiographs performed well in our clinical prediction rule, but in resource-constrained settings nonspecialist doctors usually read chest radiographs and their interpretation is likely to be less accurate. Fourth, the diagnoses of bacterial pneumonia and PJP were clinical and not based on microbiological reference standards. We attempted to confirm bacterial pneumonia with blood and sputum cultures, but these were almost all negative because of antibiotic use immediately before inclusion into our study. Fifth, the reference standard for diagnosing tuberculosis was culture, which is not 100% sensitive. Finally, while bootstrap resampling has several advantages over other internal validation methods, it is not enough to demonstrate the external applicability of the derived prediction rule. Because internal validation in general is optimistic, a drop in the performance of the prediction rule could be observed when it is applied to different settings. While our measure of optimism suggests that such a drop would be marginal, external validation, ideally conducted by different investigators, is needed to confirm the performance of our prediction rule before wide uptake in routine practice. Strengths of

Table 5. Diagnostic Accuracy Assessment of a Clinical Prediction Rule for the Diagnosis of Culture-Positive Tuberculosis Among 484 Seriously Ill Human Immunodeficiency Virus–Infected Participants Presenting With a Cough of Any Duration and ≥1 World Health Organization Danger Sign

Total Score	Probability of Tuberculosis	Sensitivity	Specificity	Positive Likelihood Ratio	Negative Likelihood Ratio
≤1	0.1%	100%	0.0%	1	…
2	0.2%	95.2%	28.8%	1.34	0.17
3	0.5%	92.2%	45.4%	1.69	0.17
4	1.1%	87.2%	59.3%	2.14	0.23
5	2.5%	78.6%	67.7%	2.43	0.31
6	5.6%	66.0%	80.3%	3.36	0.42
7	12.2%	50.8%	90.5%	5.35	0.54
8	24.4%	39.1%	93%	5.58	0.65
9	43.0%	31.2%	96.4%	8.67	0.71
10	63.7%	19.5%	97.9%	9.13	0.82
11	80.4%	5.9%	99.6%	13.44	0.95
12	90.5%	0.2%	100%	…	1

Table 6. Univariate Analysis of Individual Chest Radiograph Variables as Predictors of Culture-Positive Tuberculosis, Among the 416 Seriously Ill Human Immunodeficiency Virus–Infected Participants Presenting With a Cough of Any Duration and ≥1 World Health Organization Danger Sign and With a Specialist Radiologist Report

Chest Radiograph Variables	no./No. (%)	Crude OR	(95% CI)	Wald *P* Value
Consolidation	217/413 (52.5)	0.86	(.57–1.29)	.456
Diffuse micronodular infiltration	29/416 (6.9)	6.45	(2.20–18.87)	.001
Linear/reticulonodular infiltration	226/415 (54.5)	0.93	(.63–1.37)	.713
Enlarged hilar or mediastinal lymph nodes	210/414 (50.7)	2.34	(1.58–3.47)	<.001
Pleural effusion	195/414 (47.1)	1.24	(.84–1.82)	.284
Interstitial infiltration	259/415 (62.4)	0.92	(.62–1.37)	.685
Nodularity (>3 mm)	217/415 (52.3)	2.21	(1.49–3.28)	<.001
Cavitation	249/415 (60.0)	1.01	(.68–1.49)	.969

Abbreviations: CI, confidence interval; OR, odds ratio.

our study are its prospective design, the use of multiple mycobacterial cultures and sputum induction to establish a reference standard for tuberculosis, and a higher than expected number of tuberculosis events, which increased our power to develop a clinical prediction rule.

In conclusion, we developed a clinical prediction rule with good performance characteristics for diagnosing tuberculosis in seriously ill HIV-infected patients and showed that the Xpert MTB/RIF assay had high sensitivity. These findings, if validated in different settings, could contribute to the development of an improved algorithm for tuberculosis in seriously ill inpatients.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. G. M., M. M., M. N., and M. X. R. designed the study. R. G., H. v. d. P., and W. S. collected the data. G. M., M. M., R. G., A. S., and A. P. K. analyzed and interpreted the data. R. G. wrote the first draft. All authors reviewed, revised, and approved the final report.

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Appendix I

Reviewers comments addressed
As requested by the editor, we have shortened the paper to under 3000 words (total 2885 words). Note that the revised paper only shows changes made in response to reviewers' comments – changes made to reduce word count are not reflected (but we could make these available as an additional tracked changes document if requested). We have now added supplementary material to deal with the reviewers' requests for additional data.

Reviewer #1:

1. It looks like there were ascitic fluid and pleural fluid tested were those the only non-sputum sources?

   Response:
   Mycobacterial blood cultures were sent in all participants, as mentioned in the methods section. We have also amended the sentence in the methods, which now reads: "Extrapulmonary samples (e.g. pleural fluid) were sent for mycobacterial culture when appropriate". A table detailing culture samples from different sources has been added as supplementary table 1, which gives the number of positive cultures from specimens from pulmonary and extrapulmonary sites.

2. What were the smear results in these I assume no gene expert on non-sputum samples.

   Response:
   Smear yield is already given for sputum in table 2. Smear is not done on mycobacterial blood cultures, which were done on all participants. We do not feel that smear yield of extrapulmonary samples sent (see supplementary table 1) will be informative because of the small numbers. Xpert MTB/RIF was not done on non-sputum samples.

3. What is break down Table 2 for induced/non-induced sputum?

   Response:
   Culture & Xpert MTB/RIF yield by induction were given in line 224 – 227, and we have now added smear yield by induction.

4. Were all cases at least pulmonary even if pull/extrapulmonary as well?

   Response:
   We have now added the proportion with extrapulmonary, pulmonary, and disseminated TB as supplementary data.

5. Break down of pulm/extrapulm only and or both should be specified.

   Response:

As above.

6. Were there any non-pulmonary only cases?

   Response:
   As above

Reviewer #2:

This manuscript by Griesel et al describes a fairly large study to evaluate prognostic indicators of tuberculosis amongst severely ill HIV-infected patients with cough. They assess the utility of the current WHO algorithm and derive a new clinical prediction score for tuberculosis in this setting. The utility of the Xpert MTB/RIF assay is demonstrated. Strengths are the prospective nature of the study and, for the most part, it utilises 'real world' clinical parameters (though see comments below). It represents a useful addition to the literature.

I would make the following points:

1. As always in tuberculosis studies, a limitation is that the 'gold standard' of positive culture (used here to define a positive case) is imperfect. The study design already means that results are not applicable to those not coughing (i.e. with solely extrapulmonary tuberculosis), and this should be explicitly stated. Similarly, the exclusion of those unable to expectorate further limits the generalizability of the results to 'real-world' settings.

   Response:
   We have added these limitations to the discussion. We did not exclude patients who could not expectorate – we did sputum induction on these participants. We only excluded participants if sputum induction failed, which resulted in screening failure for only 26 out of 338 induced, therefore we disagree that this seriously limits the generalisability of our findings.

2. Even within those able to expectorate, there is clearly a percentage of tuberculosis cases who are genuinely sputum culture negative, and these would be missed with the strict definition applied here. Did the authors apply their analysis and prediction score to 'probable' TB cases as defined by investigators, even if culture negative? For that analysis, they would presumably not need to exclude people who cannot expectorate.

   Response:
   We accept that culture does not have 100% sensitivity for diagnosing TB, but we felt it was essential to have an objective reference standard. We did two mycobacterial sputum cultures and one blood culture in all participants, as well as doing cultures from extrapulmonary sites where relevant. Therefore we feel that we have been rigorous in attempting to improve the sensitivity

of our reference standard. We have added a limitation to the discussion regarding the sensitivity of our reference standard. We do not think it is appropriate to add a post hoc probable TB category.

3. Although an even less perfect 'gold standard', the decision to treat for tuberculosis by attending physicians is nevertheless an important outcome. Can the authors report how decisions to treat for TB correlated with the assessed predictor variables? Again, this analysis would not need to exclude those who did not expectorate. It would also be interesting to know how decisions to treat correlated with eventual culture results.

   Response:
   We have now added the proportion of patients empirically treated (defined as participants with negative sputum smears and Xpert MTB/RIF assays), and the proportion of these who were culture positive to the results. See comment above about participants who could not expectorate (we enrolled them if sputum induction was successful, which was the case in >90%). We feel that additional analyses of empiric treatment decisions by predictor variables is inappropriate as there were only 56 participants treated empirically (due to the high yield of sputum smears and Xpert MTB/RIF assays), so we would lack power (in regression analyses at least 10 events per variable are recommended).

4. The authors report that missing data was imputed, which may well be reasonable, but it would be useful to know the percentage of missing data for the key variables.

   Response:
   Missing data were given as a footnote in Table 1.

5. Radiological predictors are based on the assessment by an expert radiologist. As the authors point out, this expertise may not always be available. The Methods section reports that attending clinicians made an interpretation of chest radiographs at presentation: can the authors say whether this assessment was a useful predictor for culture positive TB?

   Response:
   We have now added the following sentence to the results: “Medical officers’ categorisation of likely tuberculosis on chest radiograph had an odds ratio of 9.4 (95% CI 5.7 to 15.6) for culture-positive tuberculosis.”

6. High white cell count was a negative predictor for tuberculosis in the current study, but when leukocytosis (especially neutrophilia) is present in tuberculosis it is associated with poor outcome and death. Furthermore, as the authors point out, co-infection is common and thus patients may well have bacterial infection concomitantly with TB. I am slightly concerned that the message from this manuscript might be to delay tuberculosis treatment

in those with leukocytosis, and the caveats here should be made more explicitly.

Response:
We have amended the discussion to be explicit that no single feature of our clinical prediction rule should be used in decisions to start treatment.

7. It is reported that mycobacterial blood cultures were taken on all patients: were any of these positive?

   Response:
   This has been added in supplementary table 1 (see response to reviewer 1, point 1).

8. Can the authors clarify the time-frame from presentation for measuring physiological predictor variables (pulse, temperature etc)? Clearly some of these parameters, especially temperature, will not be constant and if monitored for 24 hours may reveal higher values (eg temperature spikes > 39 C).

   Response:
   Pulse, temperature, respiratory rate and BP were all measured on admission to the hospitals – this has been added to the methods.

9. Although the authors have performed bootstrapping, there is no independent validation cohort and this limitation should be stated.

   Reponses:
   We did have a statement to this effect in the concluding paragraph of the discussion (lines 370-372). We have amplified this limitation - see response to reviewer 3, point 26.

10. The Figures and probably Table 6 could potentially be moved to Supplementary.

    Response:
    We would prefer to retain these in the paper, but will abide by an editorial decision.

Reviewer #3:

Thanks for the opportunity to review this paper. Overall it is well written, and addresses an important issue regarding the diagnosis of TB in seriously ill HIV-infected inpatients in high HIV/TB burden settings. My specific feedback is detailed below.

Introduction:

1. TB in HIV+ patients who are seriously ill commonly disseminates beyond the lungs, and studies in hospitalised cohorts have found approximately 40% of HIV/TB patients report no cough (Lawn et al, BMC 2015 13:192). This should be mentioned as another limitation of the WHO 2007 algorithm

   Response:
   We have included this as a limitation in the discussion.

2. The authors state 'no study [has] explored the ability of the 2007 WHO algorithm to diagnose TB'. Why have the authors not made this an aim of the current study?

   Response:
   Our study aim was to improve the WHO algorithm – we have now made this explicit in the final paragraph of the introduction.

3. Can the authors clarify that they sought to determine the accuracy of Xpert 'in seriously ill patients' (as the diagnostic accuracy of Xpert in the general inpatient population is well defined).

   Response:
   We have now made this explicit in the final paragraph of the introduction.

Methods

4. Can the authors justify why they are submitting these data now when they were collected at least 3 years ago?

   Response:
   There were logistic reasons for this delay, related to training of the lead author, data management, and identifying appropriate experts to conduct the analyses.

5. The authors excluded patients without a cough - please acknowledge this as a limitation of the study given that it is not uncommon for seriously ill patients without a cough to have HIV/TB co-infection (see above).

   Response:
   We have added this as a limitation to the discussion.

6. Can the authors clarify if there were any other exclusion criteria, for example, how were patient with altered conscious levels dealt with?

   Response:

There were no other exclusion criteria. The following statement under "Ethics approval" addresses the procedure for patients with altered consciousness: "Confused participants were enrolled and given the option to continue with participation once orientated; their data were removed from the study if consent was declined."

7. Please can the authors present data on how many variables had missing values imputed

   Response:
   Missing data was given as a footnote in Table 1.

8. Can the authors give more detail on the regression approach to selecting predictive variables (eg was a backwards or forwards stepwise approach)? The reference cited is a textbook on modelling, therefore not very specific.

   Response:
   The Collett's approach to variables selection is a form of backward selection, which we have added to the methods.

9. Did the authors use 'significance' levels to decide on which parameters went into the model, and, if so, were these decided a priori?

   Response:
   As stated in the methods, we selected variables a priori, which has been shown to result in less bias. The 6 variables which went into the final model were selected as they were independently predictive of tuberculosis on multivariate analysis (we have added supplementary table 2 showing the multivariable analysis of the a priori selected variables).

10. The methods section states associations between CXR features and culture positive TB were evaluated. Can the authors add this to the aims of the study, as currently only cough/TB symptoms, Hb and WCC are mentioned in the aims.

    Response:
    We have added this as an aim.

11. Given that the authors have assessed specific CXR features and their association with culture confirmed TB, can the authors justify why they have not used existing (validated) radiological scoring tools to assess CXRs?

    Response:
    Most CXR scoring systems for tuberculosis were developed in HIV-uninfected populations. We have recently published a study from a different cohort with high HIV prevalence and showed that a well-validated CXR scoring system had very poor specificity (Open Forum Infect Dis. 2017 Jun 17;4(3):ofx123.

doi: 10.1093/ofid/ofx123). We are not aware of any scoring tools that have been validated in HIV-infected inpatients.

Results:

12. Can the authors present data on how many patients were diagnosed with culture negative TB, and the basis for the diagnosis?

    Response:
    This has been added to the results - see response to reviewer 2, point 3.

13. Can the authors present data on how many patients were initiated on Abx, and of those how many failed to respond and were therefore started on TB treatment? Furthermore, can the authors present data on the how many patients failing to respond to Abx were culture positive?

    Response:
    All patients were given antibiotics, usually started at the primary care clinic prior to referral to hospital, in keeping with WHO recommendations – we have added this to the results. Failure to respond to antibiotic therapy was used as one of the criteria to initiate empiric anti-TB therapy, but we did not wait for response to antibiotics before starting anti-TB therapy in the majority of patients as their sputum smear and/or Xpert MTB/RIF assay were available within 24 hours of admission, and both tests had high sensitivities in our cohort (57.0% and 86.3% respectively). Therefore we are unable to do this analysis.

14. Can the authors please present data on which specimens were positive in the 255 culture confirmed TB cases?

    Response:
    This has been added in supplementary table 1 (see response to reviewer 1).

15. Were there any patients with contaminated TB cultures or failed cultures for other reasons? How were these handled? Did any patients have no culture results?

    Response:
    All participants had two sputum cultures performed, to mitigate against the problem of contamination, and one mycobacterial blood culture. 38/1030 of sputum cultures were contaminated – this has been added as supplementary data.

16. Can the authors present data on the sensitivity and specificity of the 2007 WHO algorithm in this cohort?

    Response:

The WHO algorithm for seriously ill inpatients recommends TB therapy for sputum smear negative TB suspects if the chest radiograph is suggestive of TB. We have added the sensitivity and specificity of the WHO algorithm based on the medical officer's chest radiographic diagnosis in the subset of participants with negative sputum smears to the results.

17. Table 1: typo 'previous' misspelt

    Response:
    This has been corrected.

18. Table 2: please state what the reference standard is and how many patients were positive by the reference standard?

    Response:
    We have added this to the table legend.

19. Can the authors clarify in the text which variables went into the final predicative model?

    Response:
    The variables are listed in table 4, we do not want to include them in the text as well as we are already over the word count.

20. Did the authors do a sensitivity analysis on the performance of the predictor score including TB cases that were culture negative, to acknowledge that some TB may be 'missed' by their reference standard?

    Response:
    See response to reviewer 2, point 2.

Discussion:

21. The authors acknowledge the latest WHO TB algorithms for seriously ill patients, and state they were able to evaluate the updated algorithm too. However, the new algorithm defines 'suspicion of TB' as any ONE of current cough, fever, night sweats or weight loss. This study excluded patients without cough, therefore cannot fully evaluate the new algorithm. This should be acknowledged as a limitation

    Response:
    Thank you for pointing this out - we have added this limitation to the discussion.

22. The authors state the six variables in the prediction score are 'readily obtainable in resource-constrained settings'. I would disagree, as several settings outside South Africa have difficulty accessing WCC and radiography

reliably (e.g. Malawi). Please can the authors acknowledge this as limitation of their score

Response:
We have amended the phrase in the discussion, which now reads "…all of which are readily obtainable in **most** resource-constrained settings."

23. Line 289, page 12: the authors state the new WHO algorithm 'requires current cough'. I disagree- see above.

    Response:
    We have corrected this error.

24. Lines 324-332, page 14: please can the authors cite Kerkhoff et al JAIDS 2014 66:33-40 (ref 12) as it better supports the association between anaemia and TB in HIV+ populations, and the use of anaemia as a predictor

    Response:
    We have added a citation to this reference.

25. Can the authors state the high reliance on induction as a potential limitation, as induction is also not reliably available in settings outside South Africa

    Response:
    We have now added a call for greater access to induction in the discussion as we feel this is an affordable technology that resulted in improved diagnostic yields in our study.

26. Can the following be added to the limitations/discussion section:
    -limitations of internal validation using bootstrapping, and the need for external validation
    -potential for over fitting in the predictive model, linked to internal validation being optimistic
    -relatively low ART coverage in this cohort (only 35% of patients on ART)

    Response:
    We had called for external validation in the concluding paragraph of the discussion. We have now added the following statements to the limitations section of the discussion: "Finally, while bootstrap re-sampling has several advantages over other internal validation methods, it is not enough to demonstrate the external applicability of the derived prediction rule. Because internal validation in general is optimistic, a drop in the performance of the prediction rule could be observed when it is applied to different settings. While our measure of optimism suggests that such a drop would be marginal, external validation ideally conducted by different investigators, is needed to confirm the performance of our prediction rule before wide uptake in routine

practice”. We agree ART coverage is low, but due to lack of space we have decided not to comment on it.

Appendix II

Algorithm for HIV-TB Diagnosis: Baseline information

Study Number				Date of admission	
Patient Initial		Sex M/F		Date enrolled	
Eligiblity criteria*		Consent *		Date of Birth	

Cough*		T°>39°C*		HR>120*		RR>30*		Unable to walk*	

HIV					
HIV known*		New HIV diagnosis*		Date tested (if new Dx)	
Year of Diagnosis		Current WHO stage		CD4 & date	
No prior OI's or TB		Yes		No	

ART							
Currently on ART*		Defaulted ART	yes		no		
ART start date			Current Regimen				
Period(s) previously on ART							
Recent VL			VL date				

Past OI's & TB episodes	Month / Year

Antibitiotic	yes		no		Time & date started	
Cotrimoxazole prophylaxis		yes			no	

Current Medication	
1.	6.
2.	7.
3.	8.
4.	9.
5.	10.

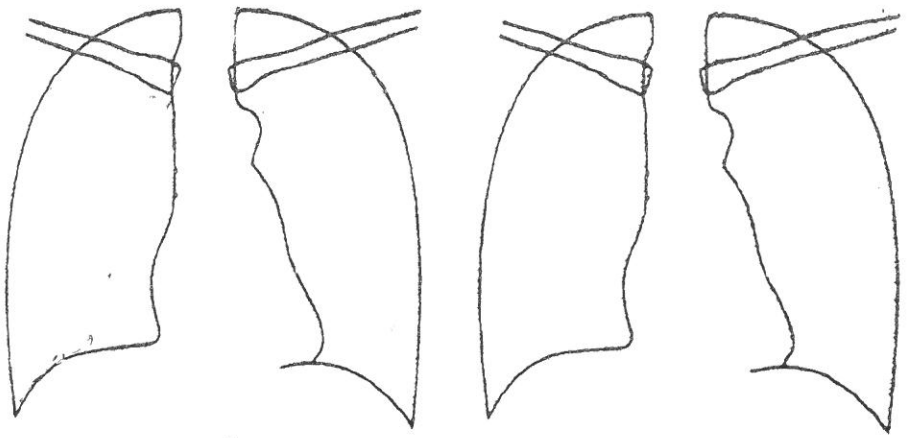
*y or n

Observations					
T°		HR		RR	
Wt		Height (m)		Sats on RA	
BP		Independent Y/N		CRB-65 score*	

*Confusion, RR≥30/min, BP: S ≤90 or D ≤65 , ≥65 yrs

Symptom enquiry (y or n)							
Cough		Duration		Fever		Duration	
Night sweats		Duration		Shortness of breath		Duration	
Chest pain		Duration		Weight loss		Amount	

History and Exam	
Local ART clinic	Happy Weight



CXR				Date	
				Effusion*	
				Nodes	
				Miliary	
				Cavitation	
				Bilateral Interstitial	
				Nodular (>2mm)	
				Reticulonodular	
Radiol Criteria for TB	yes	no	Criteria for PJP	yes	no

*Y/N

Assessment /Diagnoses

Management Plan		
	B-lactam*	
	Macrolide*	
	Bactrim*	
	Prednisone*	

*Y/N

Follow up dates:			
Day 28		Day 56	

HIV-TB Algorithm: Admission Outcome Form

General			
Study Number		Date of Admission	
		Date of Discharge	

Admission Detail					
Length of Stay		Duration of B-lactam		Duration Macrolide	
TB treatment started Y/N		TB treatment start date			

Outcome			
Diagnosis*			*
Complications			
Transfer to convalescent care		Name of facility	
Death Y/N		Date of Death	
Probable cause of death			
Absconded Y/N		Date	

*C= microbiologically confirmed; P= probable -on basis of clinical and radiological findings E= Empiric TB treatment –no response to Abs & no radiological or microbiology

Follow up date	
Day 28 (from admission)	
Day 56 (from admission)	

Appendix III

UNIVERSITY OF CAPE TOWN

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An evidence based algorithm for diagnosis of tuberculosis in HIV patients

INFORMED CONSENT and INFORMATION FORM

My name is _____

I wish to invite you to participate in a study that is trying to speed up the diagnosis of tuberculosis in patients with HIV who are ill and admitted to hospital. The Groote Schuur Hospital Department of Medicine is running this study. A/Prof Marc Mendelson and Prof Gary Maartens are the Principal Investigators.

Firstly, I wish to explain some of these terms to you:

Tuberculosis is a disease that is seen very commonly in Cape Town and in most of South Africa. People breathe in the germs that cause it. Tuberculosis can cause many symptoms, including cough, fevers and weight loss. Tuberculosis can be more severe in patients with HIV infection.

Early diagnosis and treatment of tuberculosis can save lives. The current ways of diagnosing tuberculosis can take up to two months and are not always successful in patients with HIV.

There are now new tests for tuberculosis, including the Xpert MTB/RIF test on sputum, which diagnoses more cases than the current microscopy-based tests and provides an answer within one day. We believe that by using this new test, plus some simple clinical observations and ultrasound tests of the abdomen, we can improve on the current World Health Organisation guidelines and thereby speed-up the start of tuberculosis treatment in those HIV-infected patients that have the disease.

We request your participation in this study, as tuberculosis is a possible cause for your illness. You have also undergone voluntary counselling and testing for HIV infection (VCT) as part of this process or are already known to have HIV from previous testing. Being a part of this study will not affect your treatment in any way and you will receive the same drugs for treating tuberculosis as all other patients. Similarly, if you require antiretroviral treatment for HIV and are not already receiving it, then we will refer you to the appropriate clinic to start treatment.

The decision to participate is entirely your own. IF YOU DECIDE NOT TO PARTICIPATE, YOUR TREATMENT WILL NOT BE DIASDVANTAGED IN ANY WAY. Also, you are free to withdraw at any point during the trial without telling us why.

If you do decide to take part, you will be asked to sign this consent form. We will then ask you to provide us with extra blood samples (total of 8 teaspoonful, which is regarded as safe) that can be taken at the same time as your normal blood tests. We will also ask you to provide us three sputum samples. Some of the sputum samples will be processed and stored and will not be used for genetic testing.

After discharge from the hospital, we will need to see you twice more in clinic, once after 28 days and lastly at 56 days. We will pay R150 per visit to cover your travel expenses and time whilst with us.

Although blood testing very rarely causes problems, if anything goes wrong the University provides insurance to cover this possibility. This study will also be monitored by the Research Ethics Committee of the University of Cape Town. Their job is to ensure your safety and protect you during the study.

Since this is a research study, the results of some of the tests will not be made available to you, but we hope the results will help other patients in the future. Throughout the trial your privacy will be maintained and nobody other than the doctors and nurses looking after you will know that you are participating. Samples will be labelled with code numbers and hence the laboratory staff will not know your identity. When the results of the study become available, names of the participating patients will not be included.

Do you have any questions? During the study you may contact either the **Research Ethics Committee** (021 406 6492) or **Prof Marc Mendelson** (021 404 5105) or **Dr Helen van der Plas** (082 4153041) if you have further questions.

Consent to participate in the study:

I have read the above / the above has been read to me and I have had the opportunity to discuss the study with Dr _____ and ask any questions. I consent to take part in this study:

Signature _____

Name _____

Date _____

Name of person performing the consent procedure _____

Signature _____

Date _____

Name of Witness _____

Signature _____

Date _____

Name of the Witness _____

Signature _____

Date _____

Appendix IV

Supplementary data

Supplementary Table 1 Different sites of specimens that were culture-positive for tuberculosis among 484 seriously ill HIV-infected participants presenting with a cough of any duration and one or more WHO danger sign. Note that rows and columns do not sum to the total given in bold for each site as multiple sites were commonly culture positive for individual participants, and that 38/1030 sputum cultures were contaminated.

	Sputum	**Blood**	**CSF[a]**	**Lymph node(s)**	**Ascitic fluid**	**Pleural fluid**	**Pus**	**Urine**
Sputum	221							
Blood	86	114						
CSF[a]	5	1	6					
Lymph node(s)	1	2	0	2				
Ascitic fluid	0	1	0	0	1			
Pleural fluid	12	7	1	0	0	19		
Pus	1	1	1	0	0	0	1	
Urine	8	8	1	1	0	1	0	11

[a] cerebrospinal fluid

Clinical sites of tuberculosis:

Pulmonary in 43·1% (110/255); extrapulmonary in 1·2% (3/255); and disseminated (defined as mycobacteraemic and/or culture-positive from ≥2 non-contiguous sites) in 49·8% (127/255).

Supplementary Table 2 Multivariable logistic regression model of variables used to establish a clinical prediction rule for the diagnosis of culture-positive tuberculosis among 484 seriously ill HIV-infected participants presenting with a cough of any duration and one or more WHO danger sign

Variables	**β-coeficient**	**95% CI**	**Wald's p-value**
Cough duration ≥14 days			
No	*Referent group*		
Yes	0·80	0·29 to 1·30	0·002
WHO danger signs			
Temperature >39^{o}C			
No	*Referent group*		
Yes	0·57	-0·08 to 1·22	0·084
Unable to walk unaided			
No	*Referent group*		
Yes	0·65	0·14 to 1·15	0·012
Radiographic assessment of tuberculosis			
Unlikely	*Referent group*		
Possible	1·15	0·26 to 2·04	0·011
Likely	2·63	1·69 to 3·58	<0·001
Laboratory Investigations			
Haemoglobin (3.3 - 8.3 g/dL)	*Referent group*		
Haemoglobin (8.4 - 10.6 g/dL)	0·73	0·14 to 1·31	0·016
Haemoglobin (10.7 - 20.6 g/dL)	1·39	0·76 to 2·02	<0·001
WCC (0.0 - 6.48 x10^{9}/L)	*Referent group*		
WCC (6.5 – 11.17 x10^{9}/L)	0·43	-0·17 to 1·02	0·161
WCC (11.2 - 40.35 x10^{9}/L)	-0·74	-1·35 to -0·13	0·017

Appendix V

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

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13 November 2017

HREC REF: 334/2011

Prof M Mendelson
Medicine
Infectious Diseases
G16.68
NGSH

Dear Prof Mendelson

PROJECT TITLE: AN EVIDENCE-BASED ALGORITHM FOR DIAGNOSIS OF TUBERCULOSIS IN HIV PATIENTS (MMed candidate Dr Rulan Griesel)

Thank you for submitting your study staff amendment form to the Faculty of Health Sciences Human Research Ethics Committee.

The HREC acknowledges and approves the MMed candidate, Dr Rulan Griesel, from the 01 August 2013 as part of the above-mentioned study undertaken for his MMed degree.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Appendix VI

Manuscript preparation instructions for the Journal of Clinical Infectious Diseases can be found at: https://academic.oup.com/cid/pages/General_Instructions