

Point-of-care C-reactive protein-based tuberculosis screening for people living with HIV: a diagnostic accuracy study



Christina Yoon, Fred C Semitala, Elly Atuhumuza, Jane Katende, Sandra Mwebe, Lucy Asege, Derek T Armstrong, Alfred O Andama, David W Dowdy, J Luke Davis, Laurence Huang, Moses Kamya, Adithya Cattamanchi

Summary

Background Symptom-based screening for tuberculosis is recommended for all people living with HIV. This recommendation results in unnecessary Xpert MTB/RIF testing in many individuals living in tuberculosis-endemic areas and thus poor implementation of intensified case finding and tuberculosis preventive therapy. Novel approaches to tuberculosis screening are needed to help achieve global targets for tuberculosis elimination. We assessed the performance of C-reactive protein (CRP) measured with a point-of-care assay as a screening tool for active pulmonary tuberculosis.

Methods For this prospective study, we enrolled adults (aged ≥ 18 years) living with HIV with CD4 cell count less than or equal to 350 cells per μL who were initiating antiretroviral therapy (ART) from two HIV/AIDS clinics in Uganda. CRP concentrations were measured at study entry with a point-of-care assay using whole blood obtained by fingerprick (concentration ≥ 10 mg/L defined as screen positive for tuberculosis). Sputum samples were collected for Xpert MTB/RIF testing and culture. We calculated the sensitivity and specificity of point-of-care CRP and WHO symptom-based screening in reference to culture results. We repeated the sensitivity analysis with Xpert MTB/RIF as the reference standard.

Findings Between July 8, 2013, and Dec 15, 2015, 1237 HIV-infected adults were enrolled and underwent point-of-care CRP testing. 60 (5%) patients with incomplete or contaminated cultures were excluded from the analysis. Of the remaining 1177 patients (median CD4 count 165 cells per μL [IQR 75–271]), 163 (14%) had culture-confirmed tuberculosis. Point-of-care CRP testing had 89% sensitivity (145 of 163, 95% CI 83–93) and 72% specificity (731 of 1014, 95% CI 69–75) for culture-confirmed tuberculosis. Compared with WHO symptom-based screening, point-of-care CRP testing had lower sensitivity (difference -7% , 95% CI -12 to -2 ; $p=0.002$) but substantially higher specificity (difference 58% , 95% CI 55 to 61; $p<0.0001$). When Xpert MTB/RIF results were used as the reference standard, sensitivity of point-of-care CRP and WHO symptom-based screening were similar (94% [79 of 84] vs 99% [83 of 84], respectively; difference -5% , 95% CI -12 to 2; $p=0.10$).

Interpretation The performance characteristics of CRP support its use as a tuberculosis screening test for people living with HIV with CD4 count less than or equal to 350 cells per μL who are initiating ART. HIV/AIDS programmes should consider point-of-care CRP-based tuberculosis screening to improve the efficiency of intensified case finding and increase uptake of tuberculosis preventive therapy.

Funding National Institutes of Health; President's Emergency Plan for AIDS Relief; University of California, San Francisco, Nina Ireland Program for Lung Health.

Introduction

Since 2000, the global incidence of tuberculosis has fallen by an average of 1.5% per year.¹ Yet tuberculosis remains the leading infectious cause of death, responsible for 1.5 million deaths overall and 400 000 HIV-related deaths (a third of all HIV-related deaths) in 2015 alone.¹ To reduce the burden of tuberculosis, WHO recommends systematic screening for the disease in all people living with HIV, irrespective of symptoms.²

The goals of screening are to detect active tuberculosis early to reduce the risk of poor disease outcomes and tuberculosis transmission, and to identify individuals eligible for preventive therapy to reduce incident tuberculosis.² A major barrier to implementing systematic screening of high-risk groups is the absence of an adequate tuberculosis screening test. The WHO target

product profile for such a test requires that sensitivity is at least 90% and specificity at least 70%.³ The high sensitivity requirement ensures that individuals who screen negative have a low probability of active tuberculosis and can therefore initiate preventive therapy safely. The moderately high specificity requirement limits the need for confirmatory diagnostic testing to a small subgroup of high-risk individuals. In addition to these technical requirements, the test should be simple, low cost, and available at the point of care so that tuberculosis screening can be done by front-line health-care workers.

For people living with HIV, no current test or algorithm satisfies the minimum criteria for a tuberculosis screening test. Although simple and highly sensitive ($>90\%$) for active tuberculosis, symptom-based tuberculosis screening, recommended by WHO, has insufficient specificity.^{4,5}

Lancet Infect Dis 2017

Published Online

August 25, 2017

[http://dx.doi.org/10.1016/S1473-3099\(17\)30488-7](http://dx.doi.org/10.1016/S1473-3099(17)30488-7)

[http://dx.doi.org/10.1016/S1473-3099\(17\)30472-3](http://dx.doi.org/10.1016/S1473-3099(17)30472-3)

See Online/Comment

[http://dx.doi.org/10.1016/S1473-3099\(17\)30472-3](http://dx.doi.org/10.1016/S1473-3099(17)30472-3)

Department of Medicine,

Division of Pulmonary and

Critical Care Medicine

(C Yoon MD, Prof L Huang MD,

A Cattamanchi MD), and

Department of Medicine,

HIV/AIDS, Infectious Diseases

and Global Medicine

(Prof L Huang), University of

California, San Francisco,

Zuckerberg San Francisco

General Hospital, San Francisco,

CA, USA; Department of

Medicine, Makerere University

College of Health Sciences,

Kampala, Uganda

(F C Semitala MMed,

A O Andama MSc,

Prof M Kamya MMed); Makerere

University Joint AIDS Program,

Kampala, Uganda (F C Semitala,

J Katende BSc, Prof M Kamya);

Makerere University—University

of California, San Francisco

Research Collaboration, Mulago

Hospital Complex, Kampala,

Uganda (E Atuhumuza MSc,

S Mwebe BSN, L Asege BBLT,

A O Andama, Prof L Huang);

Johns Hopkins University School

of Medicine, Baltimore, MD, USA

(D T Armstrong MHS);

Department of Epidemiology,

Division of Infectious Disease

Epidemiology, Johns Hopkins

Bloomberg School of Public

Health, Baltimore, MD, USA

(D W Dowdy MD); School of

Medicine, Department of

Medicine, Pulmonary, Critical

Care, and Sleep Medicine

Section, Yale University,

New Haven, CT, USA

(J L Davis MD); Department of

Epidemiology of Microbial

Diseases, Yale University, School

of Public Health, New Haven, CT,

USA (J L Davis); and Curry

International Tuberculosis

Center, University of California,

San Francisco, Oakland, CA, USA

(A Cattamanchi)

Correspondence to:
Dr Christina Yoon, Zuckerberg
San Francisco General Hospital,
San Francisco, CA 94110, USA
christina.yoon@ucsf.edu

Research in context

Evidence before this study

C-reactive protein (CRP) is an acute-phase reactant; serum and plasma concentrations of CRP rise in response to pyogenic infections such as active pulmonary tuberculosis, independently of HIV status. Although increases in CRP (≥ 10 mg/L) are not specific for active tuberculosis, this protein has consistently shown higher sensitivity for active tuberculosis than other inflammatory markers. Moreover, CRP can be measured with low-cost and simple point-of-care assays from blood obtained by fingerprick. To identify all studies that measured blood CRP concentrations in consecutive patients undergoing screening or assessment for active pulmonary tuberculosis, we searched PubMed with the terms "C-reactive protein" and "tuberculosis" for English-language studies published before June 26, 2017. We found that most studies investigated the diagnostic accuracy of CRP testing for active tuberculosis in the setting of passive case finding (ie, diagnosis-seeking patients with symptoms suggestive of tuberculosis). In these studies, CRP testing had high sensitivity but low specificity for active tuberculosis. We identified two studies that assessed the diagnostic accuracy of CRP testing for active tuberculosis in the setting of active case finding (ie, provider-initiated screening). By testing stored serum samples of HIV-infected patients initiating antiretroviral therapy (ART), both studies found that CRP testing had high sensitivity for active tuberculosis, similar to that of symptom-based screening (the current standard), and substantially higher specificity. However, no study has prospectively assessed CRP testing as a tuberculosis screening tool for people living with HIV and it is unknown whether CRP testing meets WHO's target product profile

(sensitivity $\geq 90\%$ and specificity $\geq 70\%$) for a tuberculosis screening test.

Added value of this study

To our knowledge, our study is the first to prospectively assess the diagnostic accuracy of CRP testing in the context of tuberculosis screening. Our findings showed that CRP, measured with a point-of-care assay, had 89% sensitivity and 72% specificity for culture-confirmed tuberculosis, whereas symptom-based screening had higher sensitivity (96%) but substantially lower specificity (14%). When Xpert MTB/RIF, the confirmatory test most commonly used in resource-limited settings, was used as the reference standard, point-of-care CRP testing detected 94% of all Xpert MTB/RIF-positive tuberculosis cases. Thus, point-of-care CRP testing is the first tuberculosis screening tool to meet the minimum accuracy targets established by WHO.

Implications of all the available evidence

Previously published data and our results support the use of CRP testing to systematically screen people living with HIV initiating ART for active tuberculosis. Point-of-care CRP-based tuberculosis screening could improve the efficiency of intensified case finding and increase the uptake of tuberculosis preventive therapy in people living with HIV. To support policy recommendations, studies and programmes doing systematic tuberculosis screening in people living with HIV should incorporate point-of-care CRP testing into their tuberculosis screening protocols and analyse costs and yield relative to current options.

Prospective studies from sub-Saharan Africa have shown that the specificity of symptom-based screening is low (range 5–33%).^{6–10} If done routinely, symptom-based screening would require nearly all people living with HIV to undergo confirmatory testing¹¹ before initiating life-saving tuberculosis preventive therapy. Therefore, to facilitate implementation of intensified case finding and preventive therapy, there is an urgent need for a screening strategy that has higher specificity for active tuberculosis than WHO symptom-based screening but retains high negative predictive value (NPV) and can be used at peripheral health centres in resource-limited settings.

C-reactive protein (CRP) is an acute-phase reactant; serum and plasma concentrations of this protein rise in response to inflammation induced by diseases such as active tuberculosis.^{12–19} CRP has consistently shown higher sensitivity for pulmonary tuberculosis compared with other non-specific markers of inflammation such as erythrocyte sedimentation rate, lactate dehydrogenase, and procalcitonin.^{12–14,20–23} Although increases in CRP (≥ 10 mg/L) are not specific for active tuberculosis, two studies that investigated CRP as a screening test in people living with HIV initiating antiretroviral therapy

(ART) found CRP testing of stored serum samples to have two to six times higher specificity (58% and 81%) than symptom-based tuberculosis screening.^{18,19} These findings suggest that CRP testing, which is already available as a simple, rapid, and low-cost point-of-care test (results in 3 min, <US\$2 per test), might be a promising approach to tuberculosis screening.

We report the first prospective study of point-of-care CRP-based tuberculosis screening in people living with HIV who present to prototypical HIV/AIDS clinics for ART initiation. Our objective was to determine whether point-of-care CRP testing meets the minimum sensitivity and specificity targets recommended by WHO for tuberculosis screening, thereby assessing whether point-of-care CRP testing might improve the effectiveness and efficiency of screening relative to the WHO-recommended symptom-based screening.

Methods

Study participants

From July 8, 2013, to Dec 15, 2015, we enrolled consecutive adults (aged ≥ 18 years) initiating ART from two HIV/AIDS clinics within the Mulago Hospital

Complex (Kampala, Uganda). Patients were included if they were ART-naïve and had a pre-ART CD4 cell count less than or equal to 350 cells per μL within 3 months of study enrolment. Patients with a known diagnosis of active tuberculosis or taking medication with antimycobacterial activity (antituberculosis or tuberculosis preventive therapy, fluoroquinolones) within 3 days of enrolment were excluded. All patients provided written informed consent and the study was approved by the institutional review boards at the University of California, San Francisco, Makerere University, and the Uganda National Council for Science and Technology. This study conforms to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) initiative guidelines.²⁴

Procedures

Trained study personnel collected demographic and clinical data and administered the WHO symptom screen at the time of enrolment. **CRP concentrations were measured at study entry using whole blood obtained by fingerprick with a US Food and Drug Administration (FDA)-approved standard sensitivity point-of-care assay (iCHROMA, Boditech, South Korea).**

Two spot sputum samples (the second induced with nebulised 3% hypertonic saline, if necessary) were collected at study entry. A minimum of 1 mL of sputum from the first sample was processed for Xpert MTB/RIF testing (Cepheid, USA). The remaining volume from the first sputum sample and the second sputum sample underwent decontamination with N-acetyl-L-cysteine and sodium hydroxide. Mycobacterial culture was done on the decontaminated sediments; sediments were cultured on liquid media using the BACTEC 960 Mycobacterial Growth Indicator Tube (MGIT) system (Becton Dickinson, Franklin Lakes, NJ, USA), with parallel solid (Löwenstein-Jensen) media added from June 10, 2014, to Dec 1, 2015. Laboratory technicians confirmed the identity of any growth by acid-fast bacilli smear microscopy and molecular speciation testing (Capilia TB, TAUNS, Japan, or MPT64 assay, Standard Diagnostics, South Korea). All staff doing Xpert MTB/RIF testing and culture were masked to clinical and demographic data, including symptom screen status and point-of-care CRP concentrations.

Definitions

We defined a priori a point-of-care CRP concentration of 10 mg/L or more (rounding to the nearest whole number) as screen positive for tuberculosis on the basis of large-scale epidemiological studies of other conditions.^{25,26} In accordance with WHO guidelines, we considered patients to be symptom screen positive if they reported any of four symptoms (current cough, fever, night sweats, weight loss) in the previous 30 days.²

We considered patients to have active tuberculosis if *Mycobacterium tuberculosis* was isolated from any sputum

culture. We considered patients not to have active tuberculosis if (1) *M tuberculosis* was not isolated from any sputum culture, and (2) at least two sputum cultures were negative. Patients with insufficient culture data (eg, because of contamination) were excluded from the analysis. Subsequently, to assess CRP performance in the context of routine intensified case finding, we also did a pre-planned analysis of tuberculosis status classified on the basis of Xpert MTB/RIF results.

Statistical analysis

The sample size for the study required at least 150 patients with tuberculosis to show that the sensitivity of point-of-care CRP screening was at least 90% plus or minus 5% (using a normal approximation to the binomial calculation). On the basis of an estimated tuberculosis prevalence of 15%, we therefore needed to enrol at least 1000 patients. We inflated this number by 20% to a target enrolment of 1200 patients to account for missing data and for the possibility of a lower tuberculosis prevalence than estimated.

We compared categorical and continuous variables with the Wilcoxon rank sum test, *t* test, or χ^2 test as appropriate. For the primary analysis, we calculated the point estimates and 95% CIs for the sensitivity, specificity, NPV, and positive predictive value (PPV) of point-of-care CRP and WHO symptom-based screening in reference to culture results. We compared differences in sensitivity and specificity between WHO symptom-based screening and the point-of-care CRP test with McNemar's test of paired proportions. To identify the optimal cutoff point for point-of-care CRP-based tuberculosis screening, we did a receiver operating characteristic analysis to explore the sensitivity, specificity, and predictive values of alternative thresholds. To explore the prognostic value of point-of-care CRP concentrations with mycobacterial load, we calculated the Pearson's correlation coefficient between point-of-care CRP concentrations and days-to-culture positivity. We repeated the analysis of sensitivity with Xpert MTB/RIF as the reference standard. We did all analyses in Stata version 13.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During the study period, 1246 consecutive people living with HIV were screened, of whom 1237 were eligible and underwent point-of-care CRP testing (figure 1). 60 (5%) patients with incomplete or contaminated cultures were excluded from the analysis. Table 1 shows the demographics and clinical characteristics of the remaining 1177 patients. Overall, 163 (14%) patients

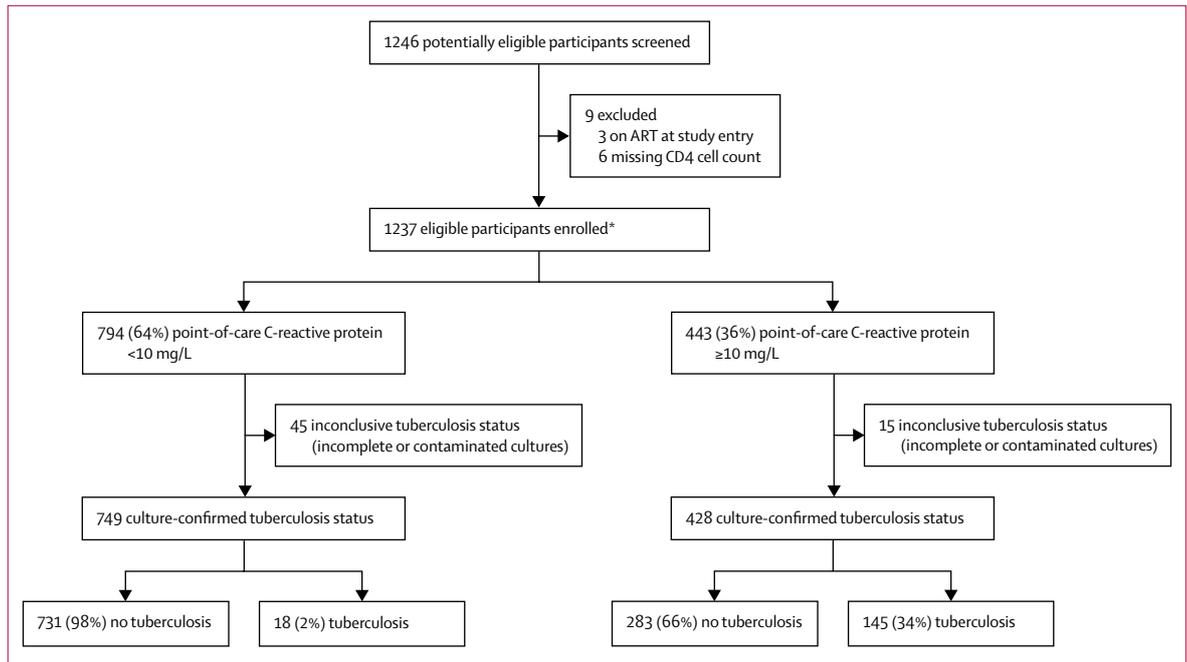


Figure 1: Patient flow diagram

ART=antiretroviral therapy. *All enrolled participants underwent point-of-care C-reactive protein testing and submitted two spot sputum samples for liquid culture. Tuberculosis defined as at least one sputum culture positive for *Mycobacterium tuberculosis*. No tuberculosis defined as any sputum cultures negative for *M tuberculosis*.

All patients (n=1177)	
Age (years)	33 (27–40)
Sex	
Women	626 (53%)
Men	551 (47%)
New to HIV care	742 (63%)
CD4 count (cells per μL)	165 (75–271)
<50	211 (18%)
50–99	169 (14%)
100–199	311 (26%)
≥ 200	486 (41%)
Body-mass index (kg/m^2)	21.2 (18.9–24.0)
Previous tuberculosis	42 (4%)
Tuberculosis contact	457 (39%)
WHO symptom screen	1025 (87%)
Current cough	568 (48%)
Fever	585 (50%)
Night sweats	410 (35%)
Weight loss	848 (72%)
Raised point-of-care CRP (≥ 10 mg/L)	428 (36%)
Point-of-care CRP (mg/L)	4.6 (2.5–24.5)
Data are median (IQR) or n (%). CRP=C-reactive protein.	

Table 1: Demographics and clinical characteristics

See Online for appendix

had culture-confirmed tuberculosis, with prevalence increasing as CD4 cell count decreased (>200 cells per μL , 33 [7%] of 486 patients; 100–200 cells per μL , 48 [15%] of

311; 50–99 cells per μL , 32 [19%] of 169; <50 cells per μL , 50 [24%] of 211; $p<0.0005$ for trend). Xpert MTB/RIF was positive in 84 of 163 patients (sensitivity 52%, 95% CI 44–59) with culture-confirmed tuberculosis and eight of 1014 patients (specificity 99%, 95% CI 99–100) with negative cultures.

Point-of-care CRP concentrations were raised (≥ 10 mg/L) in 428 (36%) of 1177 patients, including 145 of 163 patients with culture-confirmed tuberculosis (sensitivity 89%, 95% CI 83–93; table 2) and 79 of 84 patients with Xpert MTB/RIF-positive tuberculosis (sensitivity 94%, 95% CI 87–98). In patients with CD4 counts less than 200 cells per μL , sensitivity of CRP testing for culture-confirmed tuberculosis was 93% (121 of 130, 95% CI 87–97; appendix p 1) and did not vary by CD4 strata within this group ($p=0.65$ for trend; figure 2), but was lower (73% [24 of 33], 95% CI 55–87) in patients with CD4 counts more than or equal to 200 cells per μL (difference –20%, 95% CI –36 to –5; $p=0.0009$). NPV was high overall (98%, 95% CI 96–99) and for all CD4 strata (range 96–100%).

Point-of-care CRP concentrations were not raised (<10 mg/L) in 731 of 1014 patients without tuberculosis (specificity 72%, 95% CI 69–75; table 2). In patients with CD4 counts less than 200 cells per μL , specificity was 66% (367 of 561, 95% CI 61–69; appendix p 1) and was similar (range 62–67%) for all CD4 strata within this group ($p=0.73$ for trend; figure 3). Specificity was higher (80% [364 of 453], 95% CI 76–84) in patients with CD4 counts more than or equal to 200 cells per μL than in

	Point-of-care CRP	WHO symptom-based screening	Difference, % (95% CI)	p value for difference
Sensitivity				
True positive/culture positive (% , 95% CI)	145/163 (89%, 83 to 93)	156/163 (96%, 91 to 98)	-7% (-12 to -2)	0.002
Specificity				
True negative/culture negative (% , 95% CI)	731/1014 (72%, 69 to 75)	145/1014 (14%, 12 to 17)	58% (55 to 61)	<0.0001
NPV				
True negative/true negative+false negative (% , 95% CI)	731/749 (98%, 96 to 99)	145/152 (95%, 91 to 98)	2% (-1 to 6)	0.13
PPV				
True positive/true positive+false positive (% , 95% CI)	145/428 (34%, 29 to 39)	156/1025 (15%, 13 to 18)	19% (14 to 24)	<0.0001

CRP=C-reactive protein. NPV=negative predictive value. PPV=positive predictive value.

Table 2: Diagnostic accuracy of point-of-care CRP testing versus WHO symptom-based screening for active tuberculosis (in reference to culture)

those with CD4 counts less than 200 cells per μL (difference -14%, 95% CI -20 to -10; $p<0.0001$).

Median point-of-care CRP concentrations were higher in patients with culture-confirmed tuberculosis than in those with negative cultures (51.3 mg/L [IQR 21.9–112.8] vs 3.4 mg/L [2.5–11.6], respectively; $p<0.0001$), and higher in patients with Xpert MTB/RIF-positive tuberculosis than in those with Xpert MTB/RIF-negative tuberculosis (67.1 mg/L [IQR 30.7–141.2] vs 36.9 mg/L [13.1–88.7], respectively; $p=0.003$). Point-of-care CRP concentrations increased as days-to-culture positivity decreased, although correlation was modest ($r=0.28$, $p=0.0003$; appendix p 2).

By contrast with point-of-care CRP, most patients (1025 [87%] of 1177) screened positive by symptoms (difference 51%, 95% CI 48–54). Compared with point-of-care CRP, WHO symptom-based screening had higher sensitivity (89% vs 96%, respectively; difference 7%, 95% CI 2 to 12; $p=0.002$) but substantially lower specificity (72% vs 14%; difference -58%, 95% CI -61 to -55; $p<0.0001$; table 2). When Xpert MTB/RIF was used as the reference standard, sensitivity of point-of-care CRP and WHO symptom-based screening was similar (94% [79 of 84] vs 99% [83 of 84], respectively; difference 5% [95% CI -2 to 12]; $p=0.10$; appendix p 1). For all CD4 strata, specificity of WHO symptom-based screening (range 4–19%) was substantially lower than the specificity of point-of-care CRP testing (range 62–80%; appendix p 1).

The diagnostic accuracy of two combination tuberculosis screening strategies (any test positive [point-of-care CRP or WHO symptom-based screening] and both tests positive) in reference to culture is shown in the appendix (p 3). Neither combination approach to tuberculosis screening improved the diagnostic accuracy beyond that of an individual screening test.

Point-of-care CRP testing met the minimum tuberculosis screening test sensitivity ($\geq 90\%$) and specificity ($\geq 70\%$) targets when the cutoff point was lowered to 8 mg/L (area under the receiver-operating curve [AUROC] 0.80, 95% CI 0.77–0.83) or 9 mg/L (AUROC 0.81, 95% CI 0.78–0.83; table 3; figure 4).

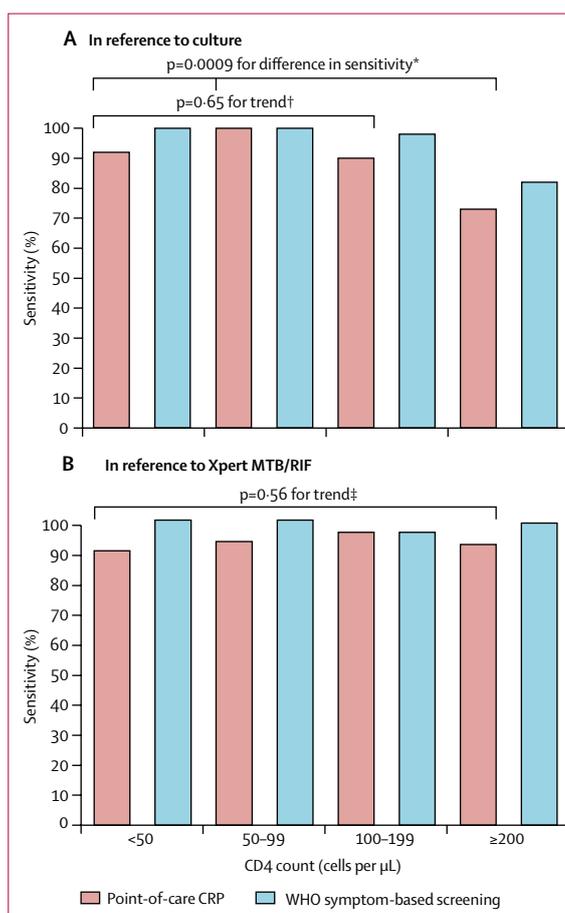


Figure 2: Sensitivity of screening tests for culture-confirmed tuberculosis and Xpert MTB/RIF-positive tuberculosis, stratified by CD4 cell count
CRP=C-reactive protein. *p value for difference in CRP sensitivity between patients with ≥ 200 CD4 cells per μL and those with <200 CD4 cells per μL . †p-value for trend in CRP sensitivity by CD4 strata, but only among patients with <200 CD4 cells per μL . ‡p value for trend in CRP sensitivity in reference to Xpert, by CD4 strata, for all patients.

Discussion

In this study, we screened 1177 HIV-infected adults who were initiating ART and had CD4 counts less than or equal to 350 cells per μL for active tuberculosis with a

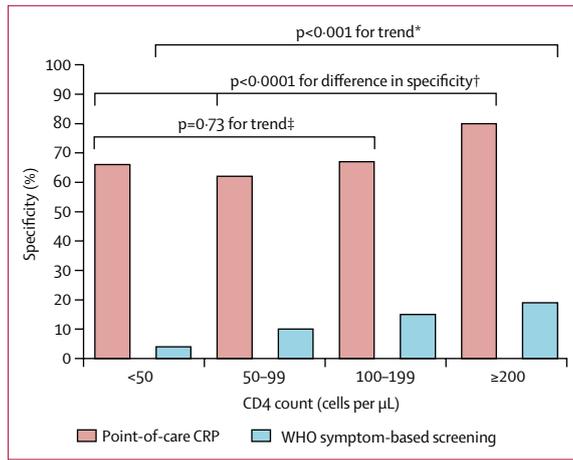


Figure 3: Specificity of screening tests for culture-positive tuberculosis, stratified by CD4 cell count

CRP=C-reactive protein. *p value for the trend in WHO symptom screen specificity by CD4 strata for all patients. †p value for the difference in CRP specificity between patients with ≥200 CD4 cells per µL and those with <200 CD4 cells per µL. ‡p value for the trend in CRP specificity by CD4 strata, but only among patients with <200 CD4 cells per µL.

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)
≥3 mg/L	93.9% (89.0–97.0)	46.5% (43.4–49.7)	22.0% (19.0–25.3)	97.9% (96.2–99.0)
≥4 mg/L	93.3% (88.2–96.6)	53.2% (50.0–56.3)	24.2% (20.9–27.8)	98.0% (96.4–99.0)
≥5 mg/L	92.6% (87.5–96.1)	59.7% (56.6–62.7)	27.0% (23.3–30.8)	98.1% (96.6–99.0)
≥6 mg/L	92.0% (86.7–95.7)	63.9% (60.9–66.9)	29.1% (25.2–33.2)	98.0% (96.7–98.9)
≥7 mg/L	91.4% (86.0–95.2)	67.6% (64.6–70.4)	31.2% (27.0–35.5)	98.0% (96.7–98.9)
≥8 mg/L	90.2% (84.5–94.3)	69.6% (66.7–72.4)	32.3% (28.0–36.8)	97.8% (96.4–98.7)
≥9 mg/L	89.6% (83.8–93.8)	71.5% (68.6–74.3)	33.6% (29.1–38.2)	97.7% (96.4–98.7)
≥10 mg/L	89.0% (83.1–93.3)	72.1% (69.2–74.8)	33.9% (29.4–38.6)	97.6% (96.2–98.6)
≥11 mg/L	87.1% (81.0–91.8)	74.6% (71.8–77.2)	35.5% (30.8–40.4)	97.3% (95.9–98.3)
≥12 mg/L	85.3% (78.9–90.3)	75.3% (72.6–78.0)	35.7% (31.0–40.7)	97.0% (95.5–98.0)

PPV=positive predictive value. NPV=negative predictive value.

Table 3: Effect of varying point-of-care C-reactive protein threshold on diagnostic accuracy

rapid and inexpensive point-of-care CRP assay using whole blood obtained by fingerprick. Point-of-care CRP-based screening detected 89% of all culture-confirmed and 94% of all Xpert MTB/RIF-positive tuberculosis cases. Furthermore, this screening method correctly identified 72% of all people living with HIV without active tuberculosis as immediately eligible for preventive therapy. These results identify point-of-care CRP as the first test to meet the minimum accuracy criteria established by WHO for a tuberculosis screening tool in people living with HIV, a key high-risk population targeted for systematic screening.

Our findings are consistent with many previous studies showing that raised CRP concentrations strongly predict the presence of active tuberculosis in people living with HIV.^{12–19} Studies assessing CRP in patients self-reporting tuberculosis symptoms (ie, passive case detection) have consistently shown CRP testing to have high sensitivity

(range 89–100%) for active tuberculosis.^{12–17} However, because patients self-reporting symptoms have a higher prevalence of pyogenic infections (eg, bacterial pneumonia), specificity of CRP testing has generally been low (range 0–43%) in this population.^{13–15,17} Two studies used stored serum samples to assess CRP in the context of tuberculosis screening in people living with HIV (ie, active case detection).^{18,19} CRP testing had similar sensitivity but two to six times higher specificity for active tuberculosis, relative to WHO symptom-based screening.^{18,19} Our findings from the first prospective assessment of point-of-care CRP testing as a tuberculosis screening tool validate these previous analyses, and strongly support that this approach could improve the efficiency of intensified case finding and increase the uptake of tuberculosis preventive therapy.

Despite WHO recommendations, only 7 million people living with HIV were screened for tuberculosis and fewer than 1 million received tuberculosis preventive therapy worldwide in 2014.²⁷ The high false-positive rate of WHO symptom-based screening, the currently recommended screening test for people living with HIV, has hampered efforts to scale up both intensified case finding and preventive therapy. Consistent with previous prospective studies,^{6–10} our data show that 86% of people living with HIV without active tuberculosis screened false-positive by symptoms. Although WHO symptom-based screening exceeded the minimum sensitivity threshold (≥90%) for a tuberculosis screening test, the poor specificity (14%) of this method precludes its usefulness in this population.

For a tuberculosis screening test to be considered by policy makers and clinicians alike, the test must prioritise sensitivity over specificity. The extent to which sensitivity of a tuberculosis screening test should be prioritised over specificity is described by WHO’s target product profile for a tuberculosis screening test, which recommends at least 90% sensitivity and at least 70% specificity.³ This trade-off between sensitivity and specificity takes into consideration the risks associated with a patient who screens false-negative (eg, generation of isoniazid-resistant tuberculosis) and the burden patients who screen false-positive would have on the health-care system (eg, costs and workload of unnecessary confirmatory testing). Our data suggest that if culture is used as the confirmatory tuberculosis test, WHO symptom-based screening would detect 7% more tuberculosis cases than point-of-care CRP testing but would require nearly all (87%) patients to undergo culture, which is likely to be cost-prohibitive even in the few high-burden countries where culture is more readily available. By contrast, point-of-care CRP testing would detect 89% of all culture-confirmed tuberculosis cases but would require only 36% of all patients to undergo culture, an absolute reduction of 51% relative to WHO symptom-based screening. When either an 8 mg/L or 9 mg/L cutoff point was used, point-of-care CRP testing met both the minimum sensitivity and specificity thresholds recommended by WHO. Furthermore, point-

of-care CRP testing would perform particularly well in patients with low CD4 cell counts. Additional studies are needed to confirm whether point-of-care CRP-based screening with a cutoff point of 8 mg/L or 9 mg/L would further improve the effectiveness of tuberculosis screening, and formal cost-effectiveness studies are needed to better estimate the costs and yield of this approach relative to current options.

In most settings that use Xpert MTB/RIF as the confirmatory test, our data show that point-of-care CRP testing would detect 94% of all Xpert MTB/RIF-positive patients but reduce by more than half (60% absolute reduction) the number of patients who would require Xpert MTB/RIF testing relative to symptom-based screening. Therefore, point-of-care CRP-based screening would identify nearly all Xpert MTB/RIF-positive tuberculosis cases (cases that pose the greatest infectious risk to the community), but substantially lower the cost of intensified case finding activities.

Our study has several strengths. First, our findings are likely to be generalisable to several other HIV-endemic settings because our study participants represent a prototypical population for whom tuberculosis screening is recommended. Second, all patients, irrespective of symptoms, were screened and then assessed for tuberculosis with a robust standard for tuberculosis diagnosis: two MGIT cultures. Third, CRP concentrations were measured with a commercially available, simple, and low-cost point-of-care assay. As such, point-of-care CRP testing is available for immediate scale-up for HIV/AIDS clinics wishing to implement this method of tuberculosis screening and further strengthen its evidence base. Future studies should also assess whether the diagnostic accuracy of point-of-care CRP-based tuberculosis screening could be further improved when used in combination with chest radiography or other promising biomarkers, particularly in patients with high CD4 cell counts or patients treated with ART.

Our study also has limitations. First, we chose to study ART-naive patients with advanced HIV-associated immunosuppression because tuberculosis risk is greatest in this population. Our findings might therefore be less applicable to other HIV subgroups. Future studies should assess the diagnostic accuracy of point-of-care CRP testing in ART-treated people living with HIV, other high-risk populations (eg, household contacts, miners, prisoners) for whom systematic tuberculosis screening is also recommended, and other high-burden settings.² Second, we classified tuberculosis status on the basis of culture results, the gold standard for tuberculosis diagnosis. Although tuberculosis classification based on clinical criteria might have resulted in additional cases identified for our analysis, empirical tuberculosis treatment was uncommon in our screening cohort (ie, people living with HIV undergoing active case finding rather than those seeking care for symptoms suggestive of tuberculosis). Third, we did not compare CRP

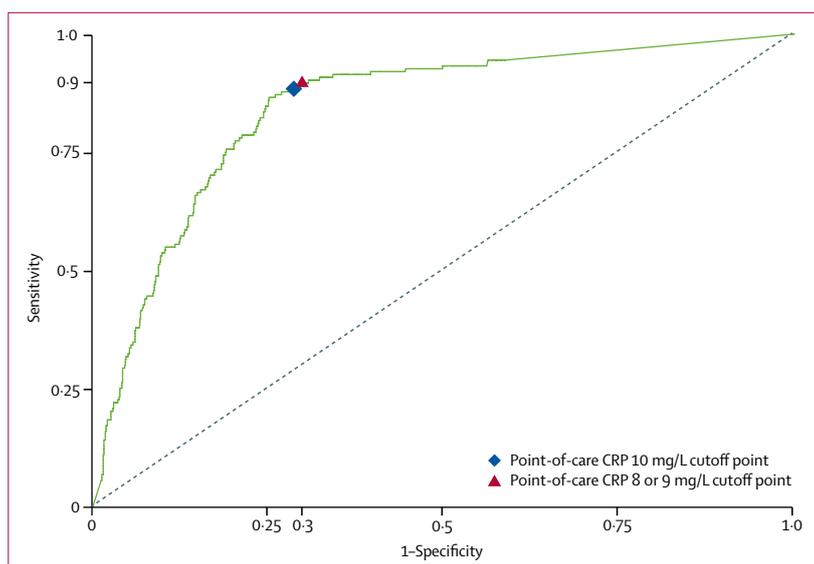


Figure 4: Receiver operating characteristic curves for the detection of culture-confirmed pulmonary tuberculosis by point-of-care CRP testing

Area under the receiver-operating curve for 10 mg/L (0.81, 95% CI 0.78–0.83), 9 mg/L (0.81, 95% CI 0.78–0.83), and 8 mg/L (0.80, 95% CI 0.77–0.83) cutoff points. CRP=C-reactive protein.

concentrations measured with a point-of-care assay versus a laboratory-based assay because previous studies, including an FDA-led investigation, **have demonstrated excellent correlation of the iCHROMA point-of-care CRP assay with the reference standard, and minimal variation with repeated testing of the same sample over a large range of CRP concentrations.**²⁸ Lastly, we did not assess patients for extrapulmonary tuberculosis or for non-tuberculosis disease. Future studies should investigate the diagnostic accuracy of point-of-care CRP for extrapulmonary tuberculosis and assess the causes and significance of a raised CRP concentration in people living with HIV with non-tuberculosis disease.

In conclusion, our findings have important implications for clinical care and programme implementation. Point-of-care CRP-based tuberculosis screening could substantially increase the number of people living with HIV initiating ART identified as eligible for tuberculosis preventive therapy and reduce the number of people requiring confirmatory tuberculosis testing. Thus, point-of-care CRP testing could increase uptake of tuberculosis preventive therapy and decrease costs of implementing intensified case finding beyond that of symptom-based screening. These results support the use of point-of-care CRP testing as a part of a public health strategy to reduce the global burden of tuberculosis in people living with HIV.

Contributors

CY and AC designed the study. FCS, EA, DTA, AOA, and MK oversaw the local collection of data. JK, SM, and LA collected the data. CY analysed the data and wrote the first draft of the manuscript. AC and DWD critically revised the manuscript. All authors read and approved the final manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

This study was supported by the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID; K23 AI114363 to CY); NIH and University of California, San Francisco-Gladstone Institute of Virology and Immunology (UCSF-GIVI) Center for AIDS Research (CFAR; P30 AI027763 to CY); the UCSF Nina Ireland Program for Lung Health (CY); NIH/NIAID-President's Emergency Plan for AIDS Relief (PEPFAR) CFAR Administrative Supplement (P30 AI20163 to AC); and the NIH/NHLBI (K24 HL087713 to LH). The funding organisations had no role in the design, collection, analysis, and interpretation of data, or in the writing of the manuscript. We thank Amy J Markowitz for her editorial contribution to the manuscript and Isabel E Allen for her feedback on the analysis.

References

- WHO. Global tuberculosis report. Geneva: World Health Organization, 2016. http://www.who.int/tb/publications/global_report/en/ (accessed July 31, 2017).
- WHO. Systematic screening for active tuberculosis: principles and recommendations. Geneva: World Health Organization, 2013. <http://www.who.int/tb/tbscreening/en/> (accessed July 31, 2017).
- WHO. High-priority target product profiles for new tuberculosis diagnostics: report of a consensus meeting. Geneva: World Health Organization, 2014. http://www.who.int/tb/publications/tpp_report/en/ (accessed July 31, 2017).
- WHO. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings. Geneva: World Health Organization, 2011. <http://www.who.int/hiv/pub/tb/9789241500708/en/> (accessed July 31, 2017).
- Getahun H, Kittikraisak W, Heilig CM, et al. Development of a standardized screening rule for tuberculosis in people living with HIV in resource-constrained settings: individual participant data meta-analysis of observational studies. *PLoS Med* 2011; **8**: e1000391.
- Lawn SD, Brooks SV, Kranzer K, et al. Screening for HIV-associated tuberculosis and rifampin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. *PLoS Med* 2011; **8**: e1001067.
- Kufa T, Mngomezulu V, Charalambous S, et al. Undiagnosed tuberculosis among HIV clinic attendees: association with antiretroviral therapy and implications for intensified case finding, isoniazid preventive therapy, and infection control. *J Acquir Immune Defic Syndr* 2012; **60**: e22–e28.
- Hanifa Y, Fielding KL, Charalambous S, et al. Tuberculosis among adults starting antiretroviral therapy in South Africa: the need for routine case finding. *Int J Tuberc Lung Dis* 2012; **16**: 1252–59.
- Ahmad Khan F, Verkuijl S, Parrish A, et al. Performance of symptom-based tuberculosis screening among people living with HIV: not as great as hoped. *AIDS* 2014; **28**: 1463–72.
- Swindells S, Komarow L, Tripathy S, et al. Screening for pulmonary tuberculosis in HIV-infected individuals: AIDS Clinical Trials Group Protocol A5253. *Int J Tuberc Lung Dis* 2013; **17**: 532–39.
- WHO. Policy update: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Geneva: World Health Organization, 2013. <http://www.who.int/tb/publications/xpert-mtb-rif-assay-diagnosis-policy-update/en/> (accessed July 31, 2017).
- Polzin A, Pletz M, Erbes R, et al. Procalcitonin as a diagnostic tool in lower respiratory tract infections and tuberculosis. *Eur Respir J* 2003; **21**: 939–43.
- Schleicher GK, Herbert V, Brink A, et al. Procalcitonin and C-reactive protein levels in HIV-positive subjects with tuberculosis and pneumonia. *Eur Respir J* 2005; **25**: 688–92.
- Sage EK, Noursadeghi M, Evans HE, et al. Prognostic value of C-reactive protein in HIV-infected patients with *Pneumocystis jirovecii* pneumonia. *Int J STD AIDS* 2010; **21**: 288–92.
- Wilson D, Nacheha J, Morroni C, Chaisson R, Maartens G. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. *Int J Tuberc Lung Dis* 2006; **10**: 31–38.
- Wilson D, Badri M, Maartens G. Performance of serum C-reactive protein as a screening test for smear-negative tuberculosis in an ambulatory high HIV prevalence population. *PLoS One* 2011; **6**: e15248.
- Drain P, Mayeza L, Bartman P, et al. Diagnostic accuracy and clinical role of rapid C-reactive protein testing in HIV-infected individuals with presumed tuberculosis in South Africa. *Int J Tuberc Lung Dis* 2014; **18**: 20–26.
- Yoon C, Davis JL, Huang L, et al. Point-of-care C-reactive protein testing to facilitate implementation of isoniazid preventive therapy for people living with HIV. *J Acquir Immune Defic Syndr* 2014; **65**: 551–56.
- Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic and prognostic value of serum C-reactive protein for screening for HIV-associated tuberculosis. *Int J Tuberc Lung Dis* 2013; **17**: 636–43.
- Lawn SD, Obeng J, Acheampong JW, Griffin GE. Resolution of the acute-phase response in West African patients receiving treatment for pulmonary tuberculosis. *Int J Tuberc Lung Dis* 2000; **4**: 340–44.
- Breen RA, Leonard O, Perrin FM, et al. How good are systemic symptoms and blood inflammatory markers at detecting individuals with tuberculosis? *Int J Tuberc Lung Dis* 2008; **12**: 44–49.
- Kang YA, Kwon SY, Yoon HI, Lee JH, Lee CT. Role of C-reactive protein and procalcitonin in differentiation of tuberculosis from bacterial community acquired pneumonia. *Korean J Intern Med* 2009; **24**: 337–42.
- Choi CM, Kang CI, Jeung WK, Kim DH, Lee CH, Yim JJ. Role of the C-reactive protein for the diagnosis of TB among military personnel in South Korea. *Int J Tuberc Lung Dis* 2007; **11**: 233–36.
- Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. *BMJ* 2015; **351**: h5527.
- Claus DR, Osmand AP, Gewurz H. Radioimmunoassay of human C-reactive protein and levels in normal sera. *J Lab Clin Med* 1976; **87**: 120–28.
- Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta* 1981; **117**: 13–23.
- WHO. Global Tuberculosis Report. Geneva: World Health Organization, 2015. http://www.who.int/tb/publications/global_report/gtbr15_main_text.pdf (accessed July 31, 2017).
- Pfafflin A, Schleicher E. Inflammation markers in point-of-care testing (POCT). *Anal Bioanal Chem* 2009; **393**: 1473–80.