

# C-reactive protein as a screening test for HIV-associated pulmonary tuberculosis prior to antiretroviral therapy in South Africa

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**Background:** There is an urgent need for more accurate screening tests for tuberculosis

(TB). We assessed the diagnostic accuracy of C-reactive protein (CRP) as a screening test for active TB in HIV-infected ambulatory adults.

**Methods:** CRP levels were measured in blood collected at the time of HIV testing.

Diagnostic accuracy of CRP for pulmonary TB was calculated (reference standard: TB culture), compared to the WHO 4-symptom screen, consisting of cough, fever, night sweats, and weight loss. Diagnostic accuracy was also calculated for CRP in a larger cohort of HIV-infected adults with a positive symptom screen (reference standard: clinical or microbiological TB).

**Results:** Among 425 HIV-infected outpatients systematically tested for pulmonary TB, TB culture was positive in 42 (10%), 279 (66%) had at least one TB-related symptom and 197 (46%) had a CRP more than 5 mg/l. The sensitivity of CRP and the TB symptom screen to detect TB was the same [90.5%; 95% confidence interval 77.4–97.3] but specificity of CRP was higher than for the TB symptom screen (58.5% vs. 37.1%,  $P < 0.001$ ). Of persons with no symptoms and normal CRP, 99 (98%) had no TB. In another cohort of 749 patients presenting with at least one TB-related symptom and clinically evaluated, CRP had a sensitivity of 98.7% and specificity of 48.3%.

**Conclusion:** In HIV-infected outpatients, CRP was as sensitive but substantially more specific than TB symptom screening. Use of CRP as a screening tool to exclude active TB could identify the same number of HIV-associated TB cases, but reduce the use of diagnostic sputum testing in TB-endemic regions.

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## Introduction

Tuberculosis (TB) is the leading cause of mortality in people living with HIV in sub-Saharan Africa [1]. Exclusion of TB is recommended prior to starting

antiretroviral therapy (ART), and is essential prior to and starting isoniazid preventive therapy (IPT) [2–4]. The World Health Organization (WHO) and South African guidelines recommend screening all HIV-infected persons for TB using a four-symptom screen, based on any

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current cough, fevers, weight loss, and night sweats. A positive TB symptom screen consisting of at least one of the four symptoms should prompt further diagnostic TB evaluations.

This symptom screen has numerous limitations. A low estimated pooled specificity of the symptom screen in HIV-infected persons results in the need for unnecessary, expensive, and time-consuming confirmatory sputum-based testing for many people without TB. Diagnostic tests for TB such as the gold standard TB culture or Xpert MTB/RIF are costly, time consuming, and typically limited to centralized laboratories [5] if available, which introduces multiple logistical complications of infectious specimen transportation, processing and turnaround [6,7]. These delays in turn can delay ART initiation and require patients to make additional clinic visits, incurring additional costs to the patient. Sputum smear microscopy for TB is less expensive than culture or Xpert and may be available on-site, but has unacceptably low sensitivity (<20%) in HIV-infected individuals [8,9]. There is an urgent need to identify a better screening test to identify or exclude TB that is rapid, inexpensive, and can be performed at the point of care with minimal training.

C-reactive protein (CRP) is a nonspecific inflammatory marker that has been found to be elevated in both HIV-infected and uninfected people with pyogenic infections including active tuberculosis [10]. CRP has been proposed as a potential biomarker for TB disease as well as a prognostic indicator of disease and treatment [11,12]. CRP testing is quick, inexpensive (~USD2 per test), and point-of-care (POC) testing can be performed easily by field and clinical staff. For these reasons, CRP is an attractive candidate for an initial TB screening test. Several studies have suggested that CRP may be able to improve clinician diagnosis of TB or exclude TB in persons with clinical signs of TB, but there is limited evaluation of direct comparisons of CRP to the WHO symptom screen, using a gold standard confirmatory test for TB [13,14]. We evaluated the diagnostic accuracy of CRP to screen for TB in HIV-infected adults being evaluated for ART initiation in Durban, South Africa.

## Methods

### Study setting and participants

We conducted two cross-sectional studies nested within a prospective clinical cohort in an urban HIV clinic in Umlazi township near Durban, South Africa. The first study evaluated CRP as a primary screening test to be performed on all persons with HIV regardless of symptoms. The second study assessed the use of CRP as a secondary screen, or triage test, to identify persons

requiring diagnostic testing, in persons reporting one or more TB symptoms. We enrolled adults older than 18 years attending the clinic at the time of HIV testing, between November 2013 and August 2016. The clinic provided HIV testing, treatment, and primary care, including ART and treatment of co-infections. ART was typically started for eligible persons within 7–14 days after diagnosis with HIV. Isoniazid preventive therapy (IPT) was available in the clinic during most of the study enrollment period, but in practice was rarely provided to patients.

### Screening study

Between February 2014 and February 2015, HIV-infected participants were asked to provide sputum specimens for research reference TB testing. To determine the diagnostic accuracy of CRP, we tested CRP in this cohort of participants ( $N=425$ ) who were systematically tested (regardless of symptoms) using TB sputum culture, the gold standard diagnostic test for TB. The TB case definition was at least one positive of two TB sputum cultures performed. We simultaneously evaluated the accuracy of the recommended WHO symptom screen to identify or exclude TB in this cohort.

### Triage study

To evaluate CRP as a triage test to determine persons requiring diagnostic sputum testing, we tested CRP in stored specimens from all HIV-infected persons enrolling between 2013 and 2016 who reported at least one symptom. Persons without symptoms are considered to be TB-negative and do not routinely undergo subsequent testing. The clinician decision to order microbiologic testing, diagnosis of TB, and decision to initiate anti-TB treatment was made without knowledge of CRP result. In clinical practice, TB is often diagnosed and treated in the absence of a positive gold-standard reference test [15]. Thus, for the triage analysis, we defined a clinically relevant reference standard of ‘probable TB’, which included empiric as well as microbiologic diagnoses of TB, and used only microbiologic testing available to the treating clinician. Positive results obtained within 3 months after enrollment were considered diagnostic for TB that was present at baseline. The majority of clinician-initiated TB microbiologic testing was sent at the baseline visit. In the triage study, ‘probable TB’ was defined as any one of the following: a positive clinician-ordered microbiologic test (Xpert, culture, or sputum smear) or documentation of initiation of antituberculosis therapy within 3 months of enrollment.

### Participant testing

A research staff member collected demographic, socioeconomic, health, and behavior information prior to participants receiving HIV test results. A study nurse screened all participants for symptoms of TB at the time of enrollment using the WHO four-symptom screen (current cough, fever, weight loss, night sweats). Serum

specimens were collected by a study nurse at enrollment, prior to ART initiation, and stored in a biorepository at  $-80^{\circ}\text{C}$ . After the enrollment encounter, participants had no additional research testing and proceeded to initiate routine clinical care with a nonstudy clinician, including baseline  $\text{CD4}^{+}$  cell count testing, screening for opportunistic infections and ART when indicated by South African national guidelines [3]. Clinicians could request additional testing, including sputum testing for TB (Xpert MTB/RIF, sputum microscopy, or culture) or referral for chest radiography. These investigations were not systematically performed and were requested based on clinician suspicion during routine clinical care. Clinical, pharmacy, and laboratory data from participant clinic charts were periodically abstracted into a study database over a 12-month follow-up period.

### Laboratory testing

We measured CRP levels in thawed specimens using the Roche Integra analyzer (Mannheim, Germany; reference range  $<5\text{ mg/l}$ , 18 years or older). CRP is stable in serum stored at  $-80^{\circ}\text{C}$  for up to 11 years [16]. Between February 2014 and February 2015, HIV-infected participants, regardless of symptoms, were asked to provide a sputum specimen for mycobacterial testing for research purposes. Participants unable to spontaneously expectorate sputum underwent sputum induction with nebulized hypertonic saline. Specimens were digested and decontaminated using NALC-NaOH. We performed Ziehl-Nielsen direct microscopy of AFB and liquid mycobacterial culture (BACTEC MGIT 960, Sparks, MD) on decontaminated, concentrated sputum specimens in a certified BSL-3 in central Durban. All laboratory testing requested by clinicians as part of routine clinical care was performed in the South African National Health Laboratory System. Personnel performing and reporting TB culture results were not aware of participant symptoms or CRP result. The CRP assay was performed without knowledge of symptoms or clinical TB diagnosis.

### Statistical analyses

For both the screening and the triage analyses, we constructed nonparametric receiver operating characteristic (ROC) curves to evaluate the accuracy of CRP to distinguish between presence or absence of TB disease, and calculated the area under the curve (AUC). Sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated with 95% confidence intervals. We assessed multiple CRP cut-points in an exploratory analysis. Cut-points of 5 and 10 mg/l were chosen of particular interest to be included, as 5 mg/l is the manufacturer-defined upper limit of normal and 10 mg/l has been used as a cut-point in other evaluations of CRP as a screening test for TB [13,17]. In the primary analysis using TB culture as a reference standard, we compared diagnostic accuracy of

CRP to the WHO-endorsed symptom screen as well as combinations of CRP and symptoms.

Participant characteristics were compared using the chi-squared test for binary variables and the Mann-Whitney *U* test for continuous variables. Sensitivity and specificity of the symptom screen and CRP were compared using McNemar's test of paired proportions. All statistical analyses were performed in Stata software (Stata Corp., College Station, Texas, USA).

### Ethics

The study was approved by the Biomedical Research Ethics Committee (BREC) of the University of KwaZulu-Natal and the University of Washington IRB. All participants gave written informed consent to participate in the study.

## Results

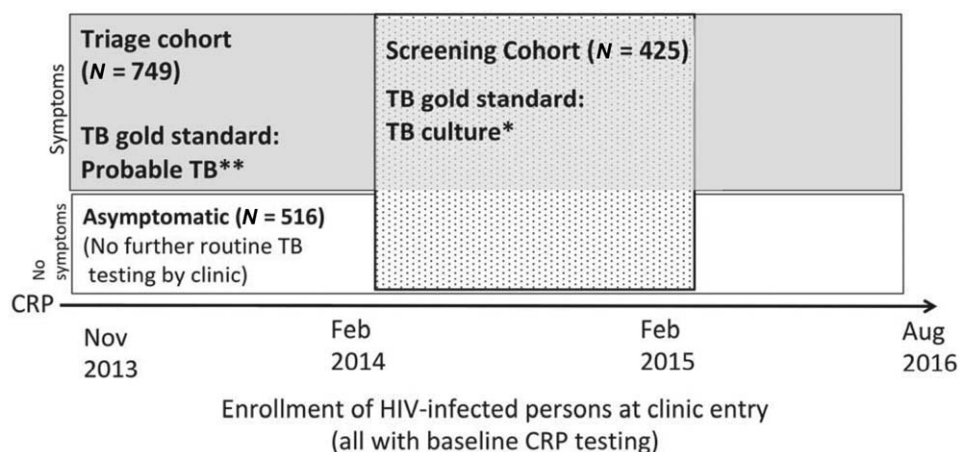
### Study population

The cohort enrolled 1390 untreated HIV-infected individuals between November 2013 and August 2016 (Fig. 1). Between February 2014 and February 2015, sputum specimens were systematically collected at enrollment. During this time period, 439 cohort participants provided a sputum specimen for culture, comprising the screening cohort (Fig. 2a). C-reactive protein levels were available for 425 (97%) individuals. Of the 425 participants who had both diagnostic CRP and TB testing performed, 42 (10%) had a positive TB culture.

Characteristics of the 425 participants included in the screening cohort are shown in Table 1 A. Over half of the population was female (58%), the median age was 32, and the median  $\text{CD4}^{+}$  cell count at the time of HIV testing and enrollment was 306 cells/ $\mu\text{l}$  (IQR 176–468). Persons with positive TB cultures were on average older, had lower BMIs, and had more advanced immunosuppression compared to persons without TB. Two-thirds of the study population reported at least one TB symptom at the time of enrollment, representing 63% of TB-negative participants and 91% of TB-positive participants. Median CRP level was significantly higher among persons with culture-positive TB (42.3 mg/l, IQR 16.2–102.8) compared to TB-negative participants (3.5 mg/l, IQR 1.2–11.8).

### Accuracy of C-reactive protein as a primary screening test

Using CRP to discriminate between presence of TB (positive culture) and absence of TB (negative culture) resulted in an area under the ROC curve of 0.80 (95% confidence interval (CI) 0.72–0.88) (Supplemental Figure 1A, <http://links.lww.com/QAD/B298>). We evaluated test performance using multiple potential CRP thresholds (Table 2A). Using a CRP threshold more than 5 mg/l resulted in a sensitivity of 90.5% (95%



**Fig. 1. Enrollment timeline of HIV-infected persons into clinic cohort, screening cohort (speckled) and triage cohort (shaded).**

\*TB culture: liquid mycobacterial culture; \*\*Probable TB: positive microbiologic test or TB treatment initiated within 3 months of enrollment. TB, tuberculosis.

CI 77.4–97.3) and specificity of 58.5% (95% CI 53.4–97.3). Using a CRP threshold more than 10 mg/l, sensitivity decreased to 78.6% (95% CI 63.2–89.7) and specificity increased to 72.3% (95% CI 67.6–76.7). Further increasing the CRP threshold resulted in decreased sensitivity and increased specificity (Table 2A).

We calculated the accuracy of the 4-symptom screen to discriminate presence vs. absence of TB. The presence of any one symptom (positive screen) had a sensitivity of 90.5% (95% CI 7.4–97.3), specificity of 37.1% (95% CI 32.2–42.1), positive predictive value of 13.6% (95% CI 9.82–18.2) and negative predictive value of 97.3% (95% CI 93.1–99.2) (Table 2A). Using a CRP threshold of more than 5 mg/l thus had equivalent sensitivity, but markedly improved specificity compared to the symptom screen as a diagnostic test ( $P < 0.001$ ).

A screening test requiring both a positive symptom screen and a CRP more than 5 mg/l had a sensitivity of 85.7% (95% CI 71.5–94.6) and specificity 69.7% (95% CI 64.8–74.3); a screening test requiring either a positive symptom screen or a CRP more than 5 mg/l had a marginally higher sensitivity 95.2% (95% CI 83.8–99.4) but decreased specificity 25.8% (95% CI 21.5–30.5).

Four persons with culture-positive TB had normal CRP levels (<5 mg/l). Of these, two also had negative symptom screens. Two had positive symptom screens with 1 symptom each.

We investigated whether the test properties of either the symptom screen or the CRP screen (threshold of 5 mg/l) were affected by CD4<sup>+</sup> T-cell count (Table 2A). In a stratified analysis, CRP sensitivity was highest (96.0%) in persons with CD4<sup>+</sup> cell counts of 200 cells/ $\mu$ l or less and decreased to 80.0% at higher CD4<sup>+</sup> cell counts, but

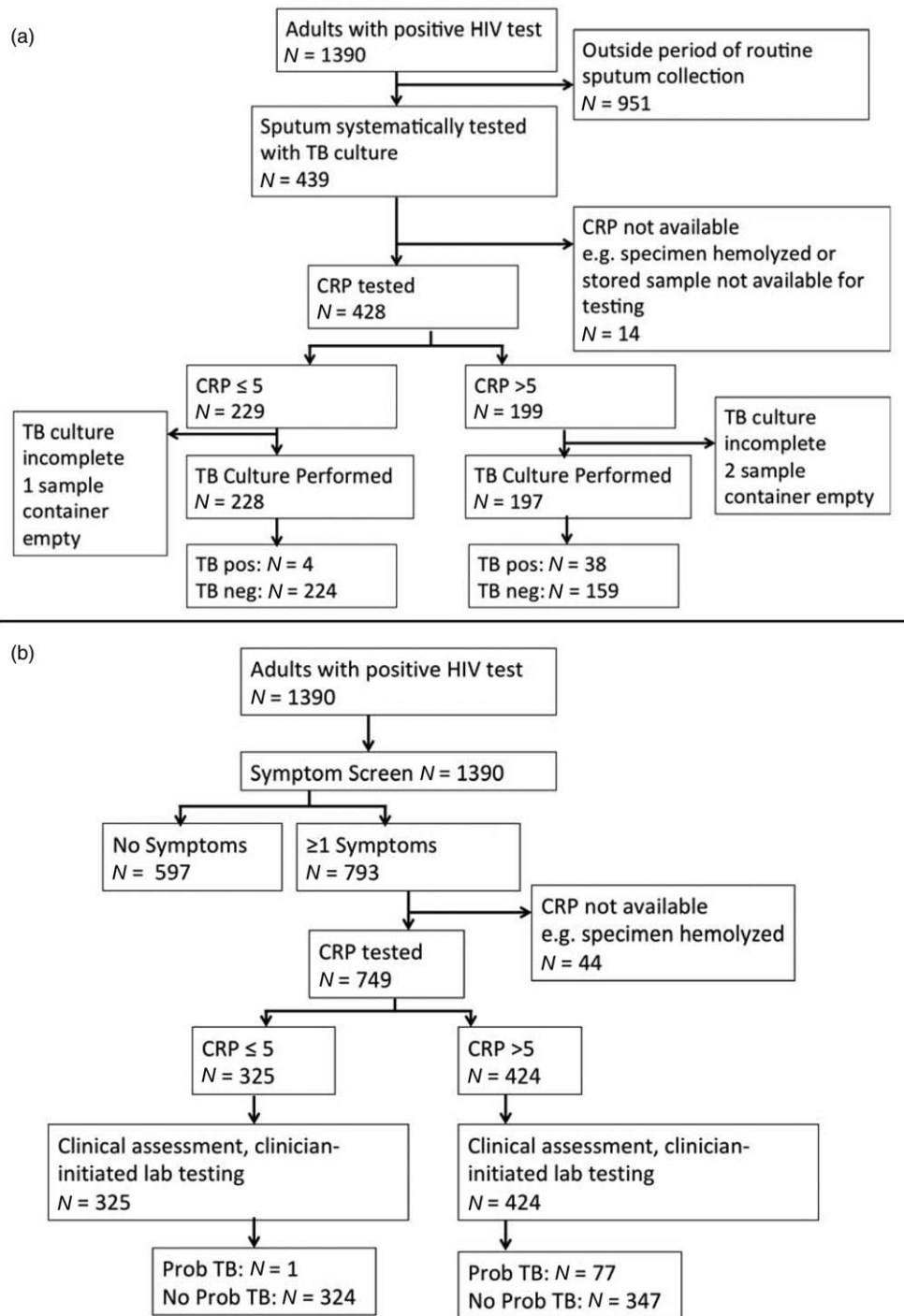
this difference was not significant. Specificity of CRP was lower (38.2%) in the low-CD4 group compared to 66.9% in the higher CD4 stratum. At all CD4 strata, sensitivity of CRP and the symptom screen were not significantly different, but specificity of CRP was significantly higher in those with higher CD4<sup>+</sup> cell counts (Fig. 3).

#### Accuracy of C-reactive protein as a triage test after symptom screen

Of 1390 enrolled HIV-infected individuals, 793 (57%) had a positive symptom screen at enrollment (Fig. 2B). CRP results were available for 749 (94%) persons with a positive symptom screen, constituting the triage cohort. All had a clinical evaluation and 262 (35%) had clinician-initiated microbiologic testing. Seventy-eight (10%) of the triage cohort were judged to have probable TB based on clinician-initiated microbiologic testing ( $N = 70$ , 90%) or on clinical criteria without microbiologic confirmation ( $N = 8$ , 10%).

Over half the triage cohort was female (54%), median age was 33.4 (IQR 27.0–39.5), and median CD4<sup>+</sup> cell count 268 cells/ $\mu$ l (Table 1B). Cough was the most common symptom, reported by 61% of all persons with symptoms and 89% of persons with TB. Male sex, lower CD4<sup>+</sup> cell count, lower BMI, and lower CRP were all significantly associated with probable TB in the triage cohort. Median CRP was 83 mg/l (IQR 37.7–135.3) in symptomatic persons with TB compared to 5.3 mg/l (IQR 1.5–24.4) in symptomatic persons without TB.

The area under the ROC curve using CRP to discriminate between presence and absence of probable TB in persons with symptoms was 0.87 (95% CI 0.84–0.90) (Supplemental Figure 1B, <http://links.lww.com/QAD/B298>). Using a threshold of CRP >5 mg/l, sensitivity and



**Fig. 2. Study subcohorts.** (a) Screening cohort: HIV-infected persons systematically screened with WHO symptom screen, TB culture, and CRP. (b) Triage cohort: HIV-infected persons with a positive WHO symptom screen. Pos, positive; neg, negative; Prob TB, probable TB (positive clinical diagnostic test or initiated anti-TB treatment within 3 months of enrollment). CRP, C-reactive protein; TB, tuberculosis.

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**Table 1. Clinical and demographic characteristics of participants.**

	Total	No pulmonary TB	Pulmonary TB	P
<b>(A) Screening cohort for analysis of CRP as a primary screen, using TB culture as the reference standard</b>				
Screening cohort, N (%)	425 (100)	383 (90)	42 (10)	
Sex, N (%)				
Male	177 (42)	154 (40)	23 (55)	
Female	248 (58)	229 (60)	19 (45)	0.069
Age, Median (IQR)	31.6 (26.6–39.3)	31.3 (26.2–38.7)	36.2 (31.6–41.6)	0.001
CD4 (cells/ $\mu$ l), median (IQR)	306 (176–468); N=411	340 (189–485); N=371	129 (70–311); N=40	<0.001
Prior TB, N (%)	19 (4)	17 (4)	2 (4)	0.92
BMI, Median (IQR)	24 (21–28)	25 (21–30)	24 (19–27)	0.008
TB symptoms, N (%)				
Current cough	191 (55)	159 (42)	32 (76)	<0.001
Fever	129 (30)	107 (28)	22 (52)	0.001
Weight loss	139 (33)	118 (31)	28 (67)	<0.001
Night sweats	146 (34)	114 (30)	25 (59)	<0.001
Number of symptoms, N (%)				
0	146 (34)	142 (37)	4 (9)	
1	100 (24)	94 (25)	6 (14)	
2	79 (19)	70 (18)	9 (21)	
3	53 (12)	44 (11)	9 (21)	
4	47 (11)	33 (8)	14 (33)	<0.001
CRP mg/l; median (IQR)	4.1 (1.3–17.4)	3.5 (1.2–11.8)	42.3 (16.2–102.8)	<0.001
	Total	No TB	TB	P
<b>(B) Triage cohort, persons with a positive symptom screen, using probable TB as the reference standard</b>				
Triage cohort, N (%)	749 (100)	671 (90)	78 (10)	
Sex, N (%)				
Male	341 (46)	290 (43)	51 (65)	
Female	408 (54)	381 (57)	27 (35)	<0.001
Age, Median (IQR)	33.4 (27.0–39.5)	33.1 (27.7–40.5)	34.4 (30.2–40.9)	0.11
CD4 (cells/ $\mu$ l) median (IQR)	268 (118–431); N=722	286 (141–449); N=650	98 (41–226); N=72	<0.001
Prior TB, N (%)	51 (6.8)	45 (6.7)	6 (7.7)	0.74
BMI, Median (IQR)	23.3 (20.2–27.0)	23.6 (20.5–27.1)	20.5 (18.9–23.9)	<0.001
TB symptoms, N (%)				
Current cough	458 (61)	389 (58)	69 (89)	<0.001
Fever	386 (52)	326 (49)	60 (77)	<0.001
Weight loss	386 (52)	325 (48)	61 (78)	<0.001
Night sweats	334 (45)	285 (42)	49 (63)	0.001
Number of symptoms, N (%)				
0	–	–	–	
1	292 (39)	287 (43)	5 (6)	
2	212 (28)	195 (29)	17 (22)	
3	132 (18)	118 (16)	24 (31)	
4	113 (15)	81 (12)	32 (41)	<0.001
CRP mg/l; median (IQR)	6.9 (1.7–34.6)	5.3 (1.5–24.4)	83 (37.7–135.3)	<0.001

CRP, C-reactive protein; IQR, interquartile range.

specificity were 98.7% (95% CI 93.1, 100) and 48.3% (95% CI 44.4, 52.1) (Table 2B). The negative predictive value was 99.7% (95% CI 98.3, 100) and negative likelihood ratio was 0.027 (95% CI 0.004, 0.19).

One person in the triage cohort had a normal CRP (2.2 mg/l) and a positive TB diagnosis made by clinician-initiated sputum GeneXpert MTB/RIF, representing a false negative CRP.

Among the initial 1,390 individuals enrolled, 561 persons with CRP levels available had a negative symptom screen (Fig. 1). Six (1%) had a probable TB diagnosis, all with all with microbiologic confirmation, of whom 4 had CRP > 50 mg/l (one had smear-negative, culture-positive TB, CRP = 2 mg/l, one had Xpert-positive TB, CRP = 2.2 mg/l).

## Discussion

In this large clinical cohort of South African adults newly testing positive for HIV, a TB screening test using CRP at the time of HIV testing had equivalent sensitivity (90.5%) to the currently recommended four-symptom screen and had substantially higher specificity. The lower negative likelihood ratio and higher negative predictive value of CRP compared to the symptom screen indicate that CRP is a better 'rule-out' test for TB. This result was consistent across CD4 levels. Both symptom screen and CRP misclassified 4 TB cases as negative in this cohort, but CRP correctly classified 82 persons as negative in addition to the 142 correctly classified by the symptom screen. This suggests that using CRP testing instead of symptom screening could reduce the number of persons required to have additional diagnostic testing for TB,

**Table 2. Diagnostic characteristics of screening tests for tuberculosis in HIV-infected adults.**

Gold standard: TB culture	Sens % (95% CI)	Spec % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR- (95% CI)
(A) Diagnostic test properties of TB screening tests at selected cut-points: C-reactive protein (CRP), WHO 4-symptom screen, and combinations (N = 425)						
CRP > 5	90.5 (77.4–97.3)	58.5 (53.4–63.5)	19.3 (14–25.5)	98.2 (95.6–99.5)	2.18 (1.87–2.54)	0.16 (0.064–0.42)
CD4 <sup>+</sup> ≤ 200 (N = 127)	96.0 (79.6–99.9%)	38.2 (28.8–48.4)	27.6 (18.5–38.2)	97.5 (86.8–99.9)	1.55 (1.31–1.85)	0.11 (0.015–0.725)
CD4 <sup>+</sup> > 200 (N = 284)	80.0 (51.9–95.7)	66.9 (60.9–72.5)	11.9 (6.29–19.8)	98.4 (95.3–99.7)	2.42 (1.78–3.28)	0.30 (0.11–0.83)
CRP > 10	78.6 (63.2–89.7)	72.3 (67.6–76.7)	23.7 (16.9–31.7)	96.9 (94.1–98.6)	2.84 (2.26–3.56)	0.30 (0.17–0.53)
CRP > 50	47.6 (32.0–63.6)	89.3 (85.8–92.2)	32.8 (21.3–46)	94.0 (91–96.2)	4.45 (2.9–6.8)	0.59 (0.44–0.78)
Any WHO symptom	90.5 (77.4–97.3)	37.1 (32.2–42.1)	13.6 (9.82–18.2)	97.3 (93.1–99.2)	1.44 (1.27–1.63)	0.26 (0.1–0.66)
CD4 <sup>+</sup> ≤ 200 (N = 127)	92.0 (74.0–99.0)	22.5 (14.9–31.9)	22.5 (14.9–31.9)	92.0 (74.0–99.0)	1.19 (1.02–1.39)	0.36 (0.09–1.41)
CD4 <sup>+</sup> > 200 (N = 284)	86.7 (59.5–98.3)	43.5 (37.5–49.6)	7.88 (4.26–13.1)	98.3 (94.1–99.8)	1.53 (1.23–1.92)	0.31 (0.08–1.12)
Gold standard: probable TB						
	Sens % (95% CI)	Spec % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	LR+ (95% CI)	LR- (95% CI)
(B) Triage cohort: test properties of CRP in symptomatic persons (N = 749)						
CRP > 5	98.7% (93.1–100)	48.3% (44.4–52.1)	18.2% (14.6–22.0)	99.7% (98.3–100)	1.91 (1.77–2.06)	0.027 (0.004–0.19)
CRP > 10	94.9% (87.4–98.6)	62.3% (58.5–66)	22.6% (18.2–27.6)	99.1% (97.6–99.7)	2.52 (2.25–2.81)	0.082 (0.031–0.21)

CI, confidence interval; LR-, negative likelihood ratio; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity. Symptom, at least one of current cough, fever, weight loss, night sweats. CRP units, mg/l.

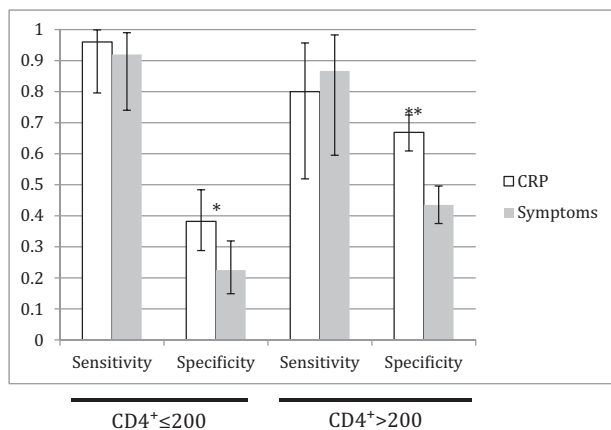
saving time and costs, and who thus could more rapidly proceed to therapies requiring TB exclusion such as ART and IPT. No additional TB cases were missed by CRP compared to symptom screen in the gold-standard screening evaluation.

In this study, as in many high-burden settings, symptoms from the WHO screen are highly prevalent among persons with newly diagnosed HIV being evaluated for ART. The symptoms comprising the screen are nonspecific for TB – many common co-infections as well as HIV itself can cause nonspecific TB-related symptoms. When the symptom screen is used to indicate further diagnostic testing, the symptom screen can result in substantial over-testing using tests that are expensive (prices vary by region, but Xpert and TB culture are approximately USD 25 per test) [18,19] and are frequently not available on-site where the person is being screened, introducing delays. The symptom screen,

with theoretical benefits of speed, ease of use, and no laboratory cost or delays, has limitations beyond its poor specificity and positive predictive value [9,20]. It may be inadvertently omitted during a brief visit in a high-volume clinic [21]. Symptom questions are subjective; patients may answer differently depending on how the questions are asked, and clinicians may not act on a positive screen due in part to subjectivity and lack of specificity [22,23]. In contrast, an objective test like CRP provides an objective value that could be a more concrete indicator to pursue additional testing. CRP testing using a threshold of 5 mg/l is an attractive alternative to exclude active TB and reduce the need for further testing. Replacing the symptom screen with CRP, or adding CRP testing for persons with a positive symptom screen, are both approaches that could decrease the need for diagnostic testing.

The current diagnostic algorithm in South African guidelines recommends that all HIV-infected persons with at least one TB symptom be investigated with a sputum test (Xpert MTB/RIF). Under these recommendations, 279 (66%) persons in the screening cohort should receive a diagnostic test. However, if CRP were used as the primary screen instead of symptoms, only 197 (46%) persons would require diagnostic testing, resulting in a 56% increase, or 82 additional persons, who could be immediately initiated on ART and TB preventive therapy at the time of screening.

In the triage cohort, all 749 individuals had a guideline indication for a diagnostic TB test, due to a positive symptom screen. Application of the CRP screen to this cohort would have reduced the number needing a diagnostic test to 424, a reduction of 42%. The negative predictive value of 99.7% and negative likelihood ratio of 0.032 indicate excellent performance of CRP as a ‘rule-out’ test to exclude TB in persons with symptoms. The CRP screen would have misclassified one person (1.5% of all TB cases) as ‘no TB’ who was categorized as having TB by the reference definition.



**Fig. 3. Sensitivity and specificity of CRP vs. symptom screen in the screening cohort (gold standard: TB sputum culture), stratified by CD4<sup>+</sup> cell count. (\*P = 0.004, \*\*P < 0.001).** CRP, C-reactive protein.

Several previous studies have examined CRP as a screening test for TB in different populations of HIV-infected individuals. Among 201 persons initiating ART in Uganda, POC CRP testing (threshold >10 mg/l) had a sensitivity of 80% and specificity of 87% to detect TB, and if used in place of symptoms would have resulted in a 66% increase of patients in whom TB was excluded and could initiate IPT; in this study the reference standard used for TB was a clinical diagnosis with or without microbiologic tests [17]. A subsequent study of CRP in 1177 HIV-infected Ugandans all with CD4<sup>+</sup> cell count less than 350 cells/ $\mu$ l, compared to a diagnostic gold standard of TB culture, found POC CRP had a sensitivity of 89% and specificity of 72%. CRP sensitivity was lower than symptom screen in this population, but specificity was significantly higher [14]. In South Africa, 496 HIV-infected persons were screened with CRP and TB culture prior to ART and found sensitivity and specificity of 90.1% and 43.9% respectively, with a negative predictive value of 95.8% for a CRP threshold of 5 mg/l [24]. Our findings were similar overall and performance in our setting was optimized at a threshold of 5 mg/l. In the screening cohort, raising the CRP threshold from 5 to 10 mg/l decreased the sensitivity substantially (90.5% to 79%). Using a cutoff of 10 mg/l would have missed an additional five of 42 (11%) of TB cases found to have only mildly elevated CRP levels. In the triage cohort (i.e. among persons who had at least one symptom), raising the threshold from 5 to 10 mg/l did not change the sensitivity significantly (99% to 95%) (Table 2B). This suggests that using the lower CRP threshold may be necessary to maintain sensitivity if CRP is used as a single test, but a higher threshold could be used in combination with other tests as part of a screening algorithm. The ideal threshold value may vary by CRP testing platform and should be re-evaluated for different assays. Regardless of the specific threshold, this study supports the increasing body of evidence that CRP could improve the ability to exclude active TB in persons with HIV in high-burden settings.

As HIV programs adopt WHO recommendations to provide ART regardless of CD4 T-cell count [25], TB screening prior to ART will increasingly take place in persons with higher CD4<sup>+</sup> cell counts (e.g. >200 cells/ $\mu$ l). This study is the first to evaluate CRP across a wide range of CD4<sup>+</sup> cell counts; prior studies were primarily limited to persons with CD4<sup>+</sup> cell counts less than 250 cells/ $\mu$ l. We found CRP consistently had higher specificity, higher negative predictive value, and lower negative likelihood ratio than symptom screen at relatively high CD4<sup>+</sup> cell counts (>200 cells/ $\mu$ l). CRP was consistently better than the symptom screen in persons with lower CD4<sup>+</sup> cell counts at highest risk for TB.

The WHO has defined a target product profile for a POC TB triage test to be used by first-contact healthcare providers [26]. The goal of such a test is to distinguish

those who do not have TB from those who require additional investigations. Desired characteristics include sensitivity >90%, specificity >70%, and equipment features that provide rapid results (<30 min), minimal maintenance requirements, and cost per test less than USD 2. If comparable results from our study were obtained with existing POC CRP assays, CRP would be close to meeting these minimum criteria. CRP exceeds minimum sensitivity requirements at multiple thresholds and either alone or in combination with a positive symptom screen. The one limitation preventing CRP from fulfilling all WHO target criteria is that the specificity of CRP is lower than the desired target specificity of 70%. The observed specificity of CRP at a threshold of 5 mg/l was 58% with the most rigorous reference standard for TB. CRP used in an algorithm combined with a positive symptom screen achieved a specificity of 62% at a threshold of 10 mg/l, compared to a clinical reference standard, both close but slightly less than the 70% minimum target specified by the WHO. Though not a complete fit with the target criteria, POC CRP is available for clinic-based implementation now, in contrast to other candidate assays that remain in early stages of laboratory or commercial development [27]. There is potential to realize clinical and diagnostic benefits by using CRP to exclude TB until more accurate tests are available.

Our study has several strengths and limitations. We screened a large population of outpatients at a South African HIV clinic in an area with high TB prevalence, which is typical of many outpatient HIV care settings. Previous evaluations of CRP for TB screening were conducted in persons with CD4<sup>+</sup> cell counts less than 250 cells/ $\mu$ l; our cohort included nearly 44% of participants with CD4 >350 cells/ $\mu$ l and is thus more generalizable to patients currently initiating ART. In our primary analysis, we used a rigorous gold standard of TB culture collected in all participants regardless of symptoms, and thus were able to directly compare symptoms to CRP. This analysis was complemented by a second analysis in a larger population, assessing how CRP testing could be used and interpreted in conjunction with the symptom screen. Among limitations, we used a lab-based CRP assay, though implementation of CRP screening would be most feasible and potentially cost-effective with POC testing. However, prior studies have shown that lab-based and POC CRP values are comparable [28]. CRP data was missing from a small percentage of patients, which could introduce bias, but we found no evidence of systematic difference in participants with and without CRP available. Our conclusions are limited to ambulatory outpatients and cannot be generalized to acutely ill, hospitalized patients, who may have very different acute inflammatory conditions contributing to their hospitalization that would lower the specificity of CRP. We were also limited in our diagnostic capacity for a TB reference standard: although chest X-ray has been used to increase



sensitivity and specificity of TB screening algorithms, X-ray was not part of the screening protocol at the clinical site and was not routinely available on-site. Additionally, our setting had limited diagnostic capacity to definitively identify extrapulmonary TB. There may have been some extrapulmonary TB present in the cohort that was missed by the gold standard of a positive sputum TB culture. It is possible that some participants had extrapulmonary TB with an elevated CRP and/or symptoms; in this case we would expect that the true specificity of CRP and symptoms would be higher than what we observed. Further studies including more extensive diagnostic sampling and chest X-ray may enable improved screening algorithms.

In conclusion, CRP has promise as a test to rule out active TB in HIV-infected outpatients preparing to initiate ART, with or without a concomitant symptom screen, and reduce the need for costly diagnostic testing. Further studies should be done to determine the effect of using of CRP on clinically and operationally meaningful outcomes [29] such as effect on TB diagnosis, rate of IPT initiation, time to ART initiation, and cost-effectiveness.

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## Conflicts of interest

There are no conflicts of interest.

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