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C-Reactive Protein Testing for Active Tuberculosis among Inpatients without HIV in Uganda: a Diagnostic Accuracy Study

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ABSTRACT The objective of this prospective cross-sectional study, conducted at a national referral hospital in Kampala, Uganda, was to determine diagnostic performance of serum C-reactive protein (CRP) as a triage test for tuberculosis (TB) among HIV-seronegative inpatients. We calculated the sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values to determine the diagnostic performance of a CRP enzyme-linked immunosorbent assay (ELISA) (Eurolyser) in comparison to that of a reference standard of *Mycobacterium tuberculosis* culture on two sputum samples. We constructed receiver operating curves and reported performance in reference to the manufacturer's cutoff and also to a threshold chosen to achieve sensitivity of >90%, in accordance with the WHO's target-product profile for a triage test. Among 119 HIV-seronegative inpatients, 46 (39%) had culture-positive pulmonary TB. In reference to *M. tuberculosis* culture, CRP had a sensitivity of 78% (95% confidence interval [CI], 64 to 89%) and a specificity of 52% (95% CI, 40 to 64%) at the manufacturer's threshold of 10 mg/liter. At a threshold of 1.5 mg/liter, the sensitivity was 91% (95% CI, 79 to 98%) but the specificity was only 21% (95% CI, 12 to 32%). Performance did not differ when stratified by illness severity at either threshold. In conclusion, among HIV-seronegative inpatients, CRP testing performed substantially below targets for a TB triage test. Additional studies among HIV-seronegative individuals in clinics and community settings are needed to assess the utility of CRP for TB screening.

KEYWORDS CRP, Africa, diagnosis

In routine settings, not all presumed tuberculosis (TB) patients are willing or able to produce sputum for evaluation (1). When patients can produce sputum, TB diagnoses may be missed because sputum microbiologic testing is insufficiently sensitive (2–4), especially in settings where mycobacterial culture is not routinely available or affordable. Another challenge is that sputum collection generates cough aerosols that increase the risk of TB transmission; this risk may also give rise to fears of acquiring

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TB, thereby limiting engagement of both patients and health workers in sputum collection and reducing their success in obtaining sputum (1, 5). The availability of a sensitive and easily detected, blood-based biomarker test for active TB would circumvent these operational challenges and potentially lower costs and enhance the yield of TB diagnostic evaluation. Indeed, among the World Health Organization's (WHO) highest priorities for new TB diagnostics is the need for novel nonsputum tests and for novel, rapid, point-of-care "triage" tests with high sensitivity and moderate to high specificity (6).

One non-sputum-based test that could fit this profile is serum C-reactive protein (CRP). CRP is an acute-phase reactant released during the interleukin-6-mediated innate immune response to infection. CRP levels reported in large cross-sectional studies of patients (7) presenting with active TB disease are substantially higher than those reported in large cross-sectional studies of patients with community-acquired pneumonia (8). Moreover, a variety of simple, inexpensive, point-of-care enzyme-linked immunosorbent assay (ELISA)-based assays are commercially available at low cost ($2 to $4 [U.S. dollars] per test) and can provide results within minutes. A recent systematic review (9) found CRP levels of $\geq$10 mg/liter among persons undergoing TB screening to have a pooled sensitivity of 93% (95% confidence interval [CI], 88 to 98) and pooled specificity of 60% (95% CI, 44 to 75) for culture-positive pulmonary TB, approximating the WHO's minimum requirements for sensitivity of $>$90% and specificity of $>$70% for a triage test for active TB (6). CRP levels have also outperformed the standard triage instrument, the WHO symptom screen (10–14). Only a few, relatively small studies of CRP diagnostic performance have enrolled individuals without HIV or inpatients (15). Therefore, we examined the diagnostic performance of CRP compared with that of sputum mycobacterial culture for active TB diagnosis among hospitalized adults living without HIV in an area where TB is endemic.

MATERIALS AND METHODS

Study design and population. We carried out a prospectively designed, nested cross-sectional study to determine the sensitivity, specificity, positive and negative likelihood ratios, and positive and negative predictive values of serum CRP for active pulmonary TB among HIV-seronegative inpatients in Kampala, Uganda. From August 2012 through July 2013, study staff enrolled consecutive adult patients admitted to Mulago National Referral Hospital with cough of less than 6 months and referred for TB evaluation through the Mulago Inpatient Non-invasive Diagnosis of Pneumonia-International HIV-associated Opportunistic Pneumonia study (henceforth referred to as the parent study), as previously described (16–18). For this substudy, we included all participants who tested HIV seronegative, excluding those whose TB status was indeterminate in reference to a gold standard consisting of one or more positive or at least two negative mycobacterial cultures, as well as those for whom serum was unavailable.

Procedures. After obtaining written informed consent, research assistants obtained demographic and clinical information, including WHO patient performance status (19, 20), using standardized case record forms. Starting at enrollment, they collected sputum samples on two consecutive days for acid-fast bacillus (AFB) smear microscopy, GeneXpert MTB/RIF testing, and *Mycobacterium tuberculosis* culture on Lowenstein-Jensen solid medium, as previously described (16–18). We performed GeneXpert testing on the first sample and cultured two different sputum samples from two different days to reduce misclassification arising from day-to-day and sample-to-sample variation in sputum quality or mycobacterial load. We defined a positive culture result as growth of one or more CFU of *Mycobacterium tuberculosis* from at least one sputum sample and a negative culture result as a lack of growth from both samples. Those examining mycobacterial cultures were blinded to all clinical characteristics and to the results of the index test. All participants underwent routine frontal chest radiography, with standardized interpretation by a chest physician. On the day of enrollment, trained laboratory assistants collected whole blood by phlebotomy, transported it to an on-site laboratory at −4°C, separated out the serum component, and, within 24 h of collection, placed it in long-term, temperature-regulated storage at −20°C. In August 2017, trained laboratory technologists blinded to the clinical characteristics and TB status of the participants measured CRP levels in a routine clinical laboratory located in a primary health clinic in Kampala, Uganda. Testing was performed according to the manufacturer's instructions using photometric ELISA on the Eurolyser Cube S platform (Eurolyser Diagnostica, Salzburg, Austria), a CE-marked point-of-care diagnostic instrument with a high level of repeatability (coefficient of variation, ±3.5%) and correlation with reference instruments ($r = 0.993$ with Roche Cobas 6000) (21). We selected the Eurolyser Cube S as an appropriate technology for the triage testing application in this setting because it provides results rapidly (≤5 min) using a small volume of blood (5 μl) at a low unit cost ($4). In addition, its small form factor (16 by 13 by 14.5 cm) made it an appropriate choice for a small primary health clinic laboratory. We stored testing reagents at 4°C and used them before their 3-month expiration

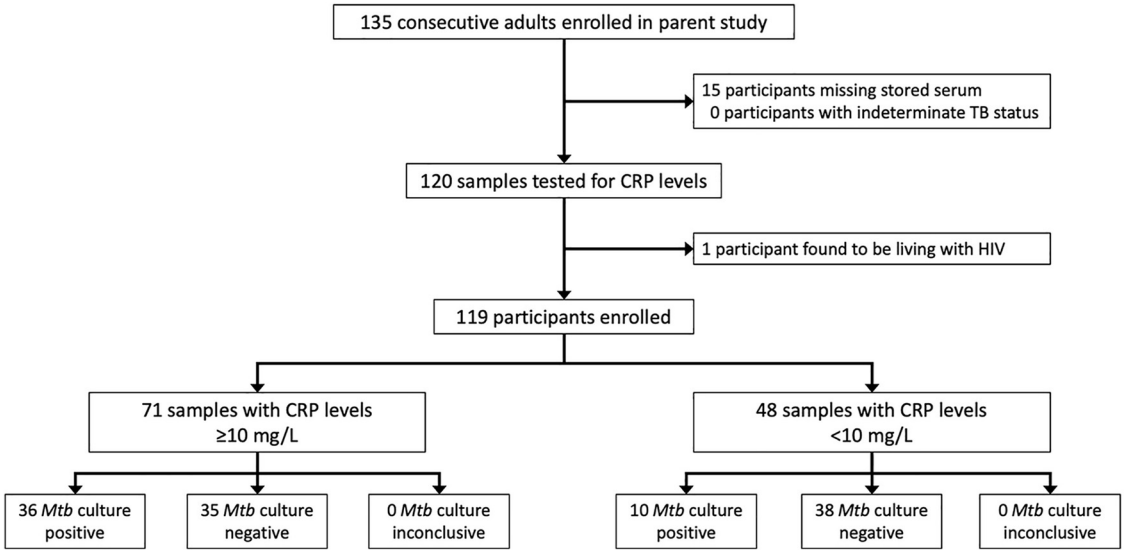

FIG 1 Flow diagram showing enrollment of participants. Abbreviations: CRP, C-reactive protein; *Mtb*, *Mycobacterium tuberculosis*.

date. Technicians recorded CRP results in a CommCare application (Dimagi, Cambridge, MA) using double data entry.

Statistical analysis. We performed bivariate analyses of participant characteristics stratified by CRP levels. We compared dichotomous variables using χ^2 tests and continuous variables using Wilcoxon rank-sum tests. We calculated sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios for CRP at specific thresholds, all in reference to a gold standard of mycobacterial culture on two sputum samples. We also calculated the same diagnostic indicators in reference to several alternative gold standards. These included GeneXpert MTB/RIF testing performed directly on a single expectorated sputum sample and clinical definitions that incorporated either (i) culture results and empirical TB treatment or (ii) culture results and standardized interpretation of chest radiography. We planned to exclude from analyses those with missing, indeterminate, or inconclusive results of the index or reference test. However, individuals who had a positive test result on AFB smear microscopy but did not have a subsequent GeneXpert test were assumed to be GeneXpert test positive.

We first dichotomized CRP levels using the manufacturer's suggested threshold, which considers those with CRP levels of ≥10 mg/liter test positive. We also explored performance at the CRP thresholds that would provide sensitivity >90% and then specificity >70%, as specified by WHO's target product profile for a triage test for TB (6). We plotted the receiver operating characteristic (ROC) and reported the area under the curve (AUC). We calculated exact binomial 95% confidence intervals (95% CI) for all parameters. We stratified all diagnostic results by WHO performance status as an exploratory analysis. We estimated that a sample size of 120 patients would provide 95% confidence at a TB prevalence of 33% to estimate the sensitivity of CRP at 90% ± 10% and the specificity of CRP at 70% ± 10%.

Ethical considerations. This study was approved by the Makerere School of Medicine Research Ethics Committee, the Mulago Hospital Research and Ethics Committee, the Uganda National Council for Science and Technology, the Committee on Human Research at the University of California San Francisco, and the Yale University Human Investigation Committee. All study participants provided written informed consent.

RESULTS

Population characteristics. Of 135 enrolled adults without HIV referred for TB evaluation, 15 patients did not have stored serum available to measure CRP levels (Fig. 1). Those without available serum were slightly older than those with serum (median age, 47 versus 35 years [$P = 0.04$]), but all other characteristics were similar. One individual was found to be living with HIV upon further testing, leaving 119 individuals for analysis (Table 1). The median age was 35 years (interquartile range, 26 to 52). Forty-one (34%) individuals were female. Thirteen (11%) had a history of TB. Thirty-seven (31%) were smokers. Overall, 113 (95%) reported one or more TB symptoms at study enrollment, including 99 (83%) reporting cough of ≥2 weeks, 87 (73%) reporting fever, chills, or night sweats within the previous 7 days, and 89 (75%) reporting weight loss. WHO performance status was unaffected in 34 (29%), mildly affected in 64 (54%), moderately affected in 15 (13%), and severely affected in 6 (5%). Seventy-one patients

TABLE 1 Characteristics of the study population

Characteristic	Value for: All patients ($n = 119$)	Patients with CRP level of ≥10 mg/liter ($n = 71$)	Patients with CRP level of <10 mg/liter ($n = 48$)	*P* value[a]
Median age (IQR[c]), yrs	35 (26–52)	34 (26–52)	40 (27–55)	0.33[b]
Female sex (%)	41 (34)	24 (34)	17 (35)	0.86
History of previous TB (%)	13 (11)	6 (8)	7 (15)	0.29
Smoke tobacco[d] (%)	37 (31)	24 (34)	13 (27)	0.44
Cough for ≥2 wks (%)	99 (83)	61 (86)	38 (79)	0.33
Fever, night sweats, or chills (%)	87 (73)	54 (76)	33 (69)	0.38
Weight loss of ≥3 kg (%)	89 (75)	57 (80)	32 (67)	0.09
Severity score				
Not affected (%)	34 (29)	16 (23)	18 (38)	0.002
Mild, ambulatory (%)	64 (54)	35 (49)	29 (60)	
Significant (%)[e]	15 (13)	15 (21)	0 (0)	
Severe (%)[f]	6 (5)	5 (7)	1 (2)	
Xpert MTB/RIF positive (%)	43 (36)	34 (48)	9 (19)	0.001
M. tuberculosis culture positive (%)	46 (39)	36 (51)	10 (21)	0.001

[a]Pearson χ^2 *P* value utilized unless otherwise noted.
[b]Wilcoxon rank-sum test utilized for age *P* value.
[c]IQR, interquartile range.
[d]Defined as those who have smoked more than 99 cigarettes in their lifetime.
[e]Defined as ≤50% of time spent in bed.
[f]Defined as >50% of time spent in bed.

(60%) had CRP levels of ≥10 mg/liter. CRP levels were strongly associated with performance status, with 20 (95%) of those in the moderately or severely impaired category having elevated CRP, compared with 51 (52%) of those in the unaffected or mildly impaired category (risk ratio, 13.5; 95% CI, 1.9 to 97.4; $P < 0.001$). There were no adverse events from performing the index test or reference standard.

Diagnostic performance of CRP using a reference standard of sputum *Mycobacterium tuberculosis* culture. Of 119 subjects, 46 (39%) had positive *M. tuberculosis* cultures, while 73 (61%) had negative *M. tuberculosis* cultures. Among those who were *M. tuberculosis* culture positive, 36 had CRP levels of ≥10 mg/liter, providing a sensitivity of 78% (95% CI, 64 to 89%). Among those with *M. tuberculosis*-negative cultures, 38 had CRP levels of <10 mg/liter, giving a specificity of 52% (95% CI, 40 to 64%). Other indicators of diagnostic performance are found in Table 2.

We also explored diagnostic performance at a CRP threshold of ≥1.5 mg/liter, selected empirically to provide >90% sensitivity. At this cutoff, 100 individuals had a positive CRP result, providing a sensitivity of 91% (95% CI, 79 to 98%) and a specificity of 21% (95% CI, 12 to 32%). We also explored diagnostic performance at a CRP threshold of ≥13 mg/liter, selected to provide >70% specificity. This threshold yielded a sensitivity of 61% (95% CI, 45 to 75%) and a specificity of 71% (95% CI, 59 to 81%).

TABLE 2 Diagnostic performance of CRP among study participants[a]

Parameter ($n = 119$)	*M. tuberculosis* culture reference standard[b] (95% CI)	GeneXpert MTB/RIF reference standard[c] (95% CI)
Sensitivity, %	78 (64–89)	79 (64–90)
Specificity, %	52 (40–64)	51 (40–63)
AUC	0.65 (0.57–0.74)	0.65 (0.57–0.74)
LR^+	1.63 (1.23–2.17)	1.62 (1.23–2.14)
LR^-	0.42 (0.23–0.75)	0.41 (0.22–0.76)
PPV, %	51 (39–63)	48 (36–60)
NPV, %	79 (65–90)	81 (67–91)

[a]Using standard manufacturer-recommended cutoff of 10 mg/liter. Abbreviations: CI, confidence interval; AUC, area under the curve for the receiver operating characteristic; LR^+, positive likelihood ratio; LR^-, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.
[b]Performed on two sputum samples collected over two consecutive days.
[c]Performed on one sputum sample collected on the day of enrollment.

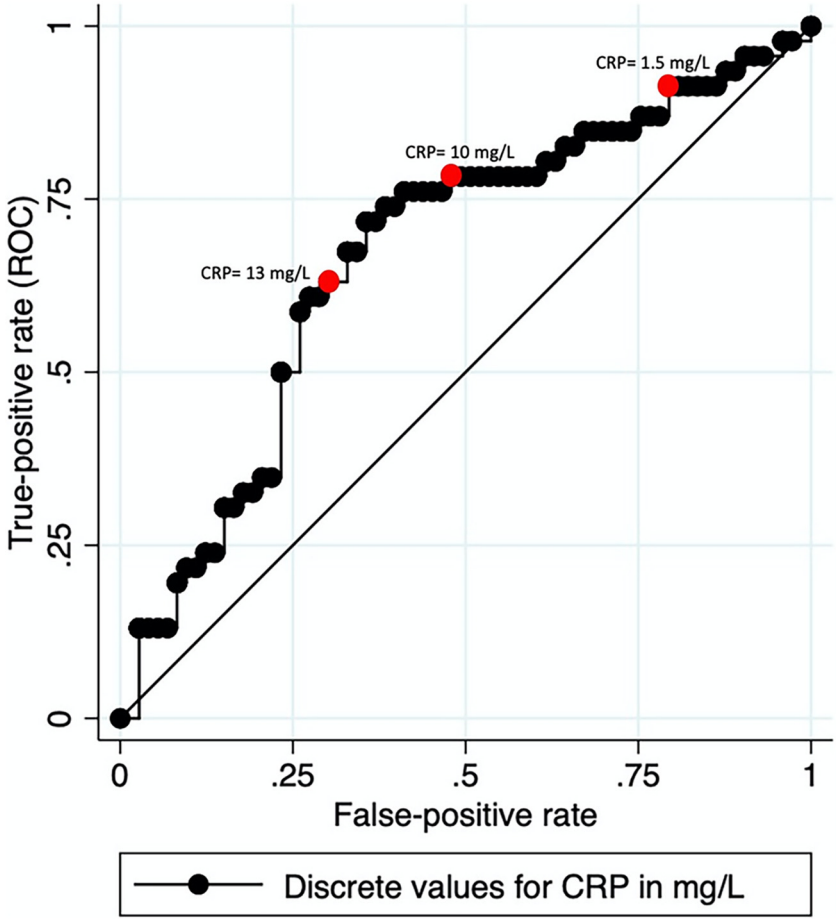

FIG 2 Receiver operating characteristic (ROC) plot for C-reactive protein in reference to a gold standard of *Mycobacterium tuberculosis* culture. Red points represent different CRP cutoffs of importance. A CRP level of 10 mg/liter is the recommended manufacturer cutoff. A CRP level of 1.5 mg/liter provides a sensitivity of >90%, and a CRP level of 13 mg/liter provides a specificity of >70%. These performance targets were chosen based on the WHO's minimum requirements for a triage test.

The ROC curve is plotted in Fig. 2. Similar results for diagnostic accuracy were obtained among patients with unaffected or mildly impaired performance status (sensitivity, 72% [95% CI, 53 to 86%]; specificity, 58% [95% CI, 45 to 70%]) and among patients with moderate or severely impaired performance status (sensitivity, 93% [95% CI 66 to 100%]; specificity, 0% [95% CI, 0 to 41%]).

Of the 35 individuals with false-positive results according to comparison of CRP levels with a threshold of ≥10 mg/liter to the reference standard of *M. tuberculosis* culture, 31 (89%) had chest X rays completed. All of these were read as abnormal, with the patterns interpreted as consistent with probable TB in 12 (39%), possible TB in 3 (10%), community-acquired pneumonia in 10 (32%), or another pulmonary process in 6 (19%). One (0.8%) additional individual with a CRP level of ≥10 mg/liter received empirical TB treatment within 2 months of initial diagnostic testing. There were no important changes in the diagnostic accuracy of CRP at the manufacturer's cutoff when calculated in reference to a variety of alternative reference standards, including reclassification of culture-negative patients as clinical TB patients based on empirical treatment or as radiographic TB patients based on probable or possible TB (data not shown).

Diagnostic performance using GeneXpert MTB/RIF as the gold standard. Of 119 subjects, 43 had TB-positive GeneXpert results, while 76 had TB-negative GeneXpert results. Among those with TB-positive GeneXpert results, 34 had CRP levels of ≥10 mg/liter. Among those with TB-negative GeneXpert results, 39 had CRP levels of <10 mg/

liter. This resulted in a sensitivity of 79% (95% CI, 64 to 90) and a specificity of 51% (95% CI, 40 to 63). Other indicators of diagnostic performance can be found in Table 2.

DISCUSSION

There are many barriers to adequately evaluating individuals for active TB disease, as symptom screening often misses those with subclinical disease (22, 23), obtaining sputum that is needed for highly sensitive diagnostic techniques may be difficult or dangerous, and testing them may simply be too costly (1). Therefore, identifying a blood-based biomarker that can be easily assayed in all patients could greatly facilitate TB evaluation. Although CRP has previously been shown to perform well as a screening test among outpatient adults living with HIV, CRP had substantially lower sensitivity and specificity in this study of adults without HIV in a hospital setting and did not appear to add significant diagnostic value to standard symptom screening and sputum-based diagnostic testing with GeneXpert MTB/RIF.

Current diagnostic recommendations involve using the WHO symptom-based screening algorithm, which often leads to large numbers of false-positive results not only among people living with HIV (PLWH) (24, 25) but also among those without HIV (25–27). This results in a sensitivity as low as 38% and a specificity as low as 32% in some populations (22, 26). The WHO recommends that new diagnostic tests being considered for use in triage or screening should have a sensitivity of >90% and a specificity of >70% (28). The primary advantage to integrating a triage test into diagnostic algorithms for TB would be to reduce the number of confirmatory assays that need to be performed, because reference standard tests are costly both to health systems and to patients who have to travel to access them. An additional objective would be to improve the sensitivity of testing among those who have difficulty producing sputum and among those with extrapulmonary TB whose tests are negative for concurrent pulmonary disease. In this study of HIV-seronegative inpatients, CRP testing missed those benchmarks for accuracy, regardless of the reference standard used. When thresholds ensuring high sensitivity were selected, specificity fell substantially to levels that would likely eliminate any cost benefits from reduced testing using the reference standard. This is consistent with findings of a recent systematic review in which no study using CRP as a diagnostic test for TB in HIV-seronegative participants or in inpatient settings met these thresholds (15). Only one study, carried out in a community setting with a vast majority of participants living with HIV, showed that CRP met the WHO guidelines for a triage test, including 21 persons without HIV (29). Similarly, a recent study also conducted in Uganda showed that CRP levels can be utilized as an acceptably accurate screening tool among PLWH, in whom CRP testing had a sensitivity of 90% and a specificity of 69% (24). Thus, there is a need for additional studies of CRP among outpatients without HIV, who are much less likely than inpatients to have alternative inflammatory conditions that may reduce specificity. Although we could not adequately compare the sensitivity and specificity of CRP to symptoms in this study because only patients exhibiting cough were enrolled, future studies that include HIV-negative outpatients with and without symptoms and that employ a reference standard that also incorporates evaluation and testing for clinically diagnosed and extrapulmonary TB could provide these valuable comparisons.

This study had a few limitations. First, we enrolled an inpatient rather than an outpatient population. Hospitalized patients are more likely to have active TB disease, which may reduce the relative benefits of triage tests, whose value lies primarily in helping to rule out active TB. Inpatients are also more likely to have non-TB-related infectious and inflammatory conditions, further reducing the value of CRP as a triage test because of reduced specificity. We hypothesize that in a community setting where TB is less prevalent and alternative inflammatory conditions are less common, the impact and specificity of CRP could be higher. Second, the alternative diagnoses that we provided for patients without TB are based on chest radiography rather than clinical or microbiologic confirmation. Understanding these alternative diagnoses—specifically whether they represent TB that is microbiologically negative or another diagnosis—is

particularly important given the above-mentioned concerns about the nonspecificity of CRP. However, in previous studies, we have found that extensive microbiologic testing yields very few alternative diagnoses and that chest radiography provides a very reasonable substitute for assigning clinical diagnoses (17, 30). Third, we did not include clinically diagnosed or extrapulmonary TB in our reference standard, which may have led us to overestimate sensitivity and underestimate specificity. Fourth, because sensitivity and specificity were lower than hypothesized, the confidence intervals surrounding our estimates were wider than expected, although this did not influence our conclusions because the upper bounds of these intervals excluded WHO's sensitivity and specificity targets. Finally, the study was carried out using banked specimens that were 4 to 5 years old at the time of the CRP measures. However, prior studies show that CRP levels remain stable in properly stored frozen serum when measured using laboratory immunoassays (31–33). Although data on the stability of CRP when measured by point-of-care immunoassays are lacking, any decay in CRP levels would likely have affected all samples similarly. Storage is therefore unlikely to have impacted our estimates of sensitivity and specificity, since these parameters are calculated based on relative rank rather than absolute level and since we explored a range of test positivity thresholds to optimize performance.

This study also had several strengths. First, we are among the first to examine the diagnostic accuracy of CRP as a triage test in HIV-negative inpatients. Second, we conducted this study in a high-TB-burden setting using a commercially available point-of-care assay, which demonstrates that measuring CRP under routine laboratory conditions is feasible using this technology. Third, we employed rigorous methods for diagnostic test evaluation as outlined in reporting guidelines, a previously identified need in the field (34–36). For example, we demonstrated that test performance did not surpass the minimum requirements across a range of positivity thresholds for the index test or with various definitions of the reference standard.

Overall, our study excludes any utility of CRP as a triage test among inpatients without HIV. CRP still holds great potential for TB evaluation for PLWH. In addition, more studies are needed to assess the diagnostic accuracy of CRP among HIV-negative individuals in community settings. If sufficiently accurate in this setting, point-of-care CRP testing could reduce the overall turnaround time for diagnostic evaluation and conserve patient and health system resources for GeneXpert or other time-intensive testing procedures, such as sputum induction. In addition, in the future, researchers should explore the potential added value of CRP in identifying individuals with forms of TB that are difficult to diagnose by sputum examination, including incipient TB and extrapulmonary TB, in studies that include more comprehensive clinical reference standards.

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