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ABSTRACT

Objectives To evaluate the performance of point-of-care C-reactive protein (CRP) as a screening tool for tuberculosis (TB) using a threshold of 10 mg/L in both people living with HIV (PLHIV) and HIV-negative individuals and compare it to symptom screening using a composite reference for bacteriological confirmation of TB.

Methods Prospective cross-sectional study.

Setting A primary healthcare facility in Lusaka, Zambia.

Participants Consecutive adults (≥18 years) presenting for routine outpatient healthcare were enrolled. Of the 816 individuals approached to participate in the study, 804 eligible consenting adults were enrolled into the study, of which 783 were included in the analysis.

Primary outcome measures Sensitivity, specificity, positive predictive value and negative predictive value (NPV) of CRP and symptom screening.

Results Overall, sensitivity of WHO-recommended four-symptom screen (W4SS) and CRP were 87.2% (80.0–92.5) and 86.6% (79.6–91.8) while specificity was 30.3% (26.7–34.1) and 34.8% (31.2–38.6), respectively. Among PLHIV, sensitivity of W4SS and CRP was 92.2% (81.1–97.8) and 94.8% (85.6–98.9) while specificity was 37.0% (31.3–43.0) and 27.5% (22.4–33.1), respectively. Among those with CD4 ≥350, the NPV for CRP was 100% (92.9–100). In the HIV negative, sensitivity of W4SS and CRP was 83.8% (73.4–91.3) and 80.3% (69.5–88.5) while specificity was 30.3% (26.7–34.1) and 34.8% (31.2–38.6), respectively. Parallel use of CRP and W4SS yielded a sensitivity and NPV of 100% (93.8–100) and 100% (91.6–100) among PLHIV and 93.3% (85.1–97.8) and 90.0% (78.2–96.7) among the HIV negatives, respectively.

Conclusion Sensitivity and specificity of CRP were similar to symptom screening in HIV-positive outpatients. Independent use of CRP offered limited additional benefit in the HIV negative. CRP can independently accurately rule out TB in PLHIV with CD4 ≥350. Parallel use of CRP and W4SS improves sensitivity irrespective of HIV status and can accurately rule out TB in PLHIV, irrespective of CD4 count.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Our study included HIV-negative individuals, so it adds to the limited evidence on the utility of C-reactive protein (CRP) in the HIV-negative population.
⇒ We used a composite of culture or Xpert MTB/RIF Ultra as the reference standard, providing an accurate reference against which presence or absence of pulmonary tuberculosis (TB) could be defined in routine practice.
⇒ The sample size limits the ability to detect differences in the subgroup analysis.
⇒ The study population had a high burden of TB, so it is not representative of all populations.
⇒ We did not compare the performance of the CRP threshold of 10 mg/L to that of 5 mg/L used by WHO.

BACKGROUND

C-reactive protein (CRP) is one of the biomarkers of tuberculosis (TB) irrespective of HIV status4–10; it is also a biomarker of several other diseases associated with acute or chronic inflammation.11–16 Among people living with HIV (PLHIV), CRP has a high sensitivity but the specificity is variable depending on the CD4 count, Anti-Retroviral Therapy (ART) status and whether it is used in an inpatient or outpatient setting.8 17 CRP has a higher specificity among outpatients in comparison to inpatients2 and among patients with higher CD4 count.8 Additionally, while very low CRP values exclude active TB, high CRP values are a predictor of mortality.7 18 There is paucity of literature on utility of CRP for TB screening among HIV-negative individuals and it shows variable sensitivity and specificity of the test.5 9 10
In contrast to other TB screening and diagnostic tools like chest X-ray and molecular diagnostics which might not be readily deployed at lower levels of care due to cost and cost-effectiveness constraints, CRP can be readily deployed at all levels of care including the primary healthcare level where most individuals with TB first present as they seek care for their symptoms.\textsuperscript{19,20} There is need for more tools that can be deployed at primary healthcare level.

WHO recently updated its guidelines on systematic screening for TB disease where it recommended CRP as a screening tool for TB among adults and adolescents living with HIV.\textsuperscript{15} Using a cut-off of 5 mg/L, WHO analysis showed that CRP has a similar sensitivity and similar or higher specificity than WHO-recommended four-symptom screen (W4SS) (which comprises any of current cough, fever, night sweats or weight loss),\textsuperscript{17} among all the subpopulations of adults and adolescents living with HIV except pregnant women living with HIV. Additional research to evaluate accuracy and predictive value of measuring CRP above any cut-off higher than 5 mg/L for TB screening is recommended by WHO.\textsuperscript{17} There is no WHO recommendation on use of CRP among HIV-negative individuals.

We evaluated the performance of Actim CRP rapid test as a screening tool for TB using a threshold of 10 mg/L in both PLHIV and HIV-negative individuals and compared it to symptom screening using a composite reference for bacteriological confirmation of TB.

**METHODS**

**Study setting and design**

This was a prospective cross-sectional study conducted at a primary healthcare facility servicing a periurban population setting in Lusaka, Zambia. It was conducted between June 2018 and December 2018 as a nested study within a larger TB REACH wave 5 project.\textsuperscript{21} Consecutive adults (≥18 years) presenting at the healthcare facility to seek routine outpatient healthcare were enrolled on giving written informed consent, until the substudy sample size was reached. The study sample size was 784 which was determined based on the following assumptions: a prevalence of 0.2 in the population, a difference in sensitivity of 0.18 between W4SS and CRP and a 25% loss due to contamination of culture samples.

**Study procedures**

Each participant underwent a full-routine clinical evaluation by a qualified medical practitioner, including TB symptom screening, determination of HIV status, a fingerstick CRP test and collection of two spot sputum specimens. HIV status was determined by both self-report and testing; those who were known HIV positive and on antiretroviral therapy were not retested but simply documented as such, while those who were HIV negative or with unknown HIV status were tested. Participants diagnosed with HIV were initiated on ART, irrespective of CD4 count.\textsuperscript{22} Participants with comorbidities associated with an increase in CRP were not excluded from the study.

**TB symptom screening**

In addition to the W4SS, history on chest pain and shortness of breath was collected. The two additional symptoms are recommended for TB screening by the Zambia National TB guidelines.\textsuperscript{23} A ‘yes’ response to any one or more of the six symptoms (six-symptom screen) was considered a positive result.

**Point-of-care CRP**

The point-of-care (POC) CRP test was performed using blood collected by fingerstick from all participants regardless of HIV status. Ten microlitres of blood was collected using a capillary tube provided with the kit and testing was performed as per the standard operating procedure for Actim CRP test (Oy Medix Biochemica Ab, Finland) (product insert). The test measured CRP levels banded at <10, 10–40, 40–80 and >80 mg/L. A CRP level <10 mg/L signified a negative CRP test result with no band appearing in the patient sample area. CRP levels at 10–40, 40–80 and >80 mg/L signified positive CRP test results with one, two and three bands appearing, respectively, in the patient sample area.

**Bacteriological testing**

For each participant, two spot sputum samples were collected regardless of symptoms. The sputum samples were sent to the central laboratory for culture, smear microscopy and Xpert MTB/RIF Ultra testing. Samples were transported to the CIDRZ Central Laboratory on the same day of collection and were processed within 24 hours. At the Central Laboratory, each sample was decontaminated using the NALC/NaOH method and was resuspended in 2 mL normal saline. Each decontaminated and resuspended pellet was then split to set up one MGIT culture (BACTEC MGIT 960 System), one Lowenstein-Jensen solid culture, one concentrated smear and an Xpert MTB/RIF Ultra assay. The primary smears were examined using fluorescence microscopy. Acid Fast Bacilli (AFB) positive cultures were confirmed as *Mycobacterium tuberculosis* (MTB) using the BD MGIT TBc Identification test. We used a composite reference of any MTB-positive culture or MTB detected on Xpert Ultra. A trace call result was considered positive among PLHIV and those without history of TB in the past 5 years.

**Data collection and management**

Data were collected using a paper-based case reporting form, capturing information on demographics, TB symptoms, physical examination findings, specimen collection and results and linkage to treatment for those diagnosed with TB. Data were entered into a custom web application with a Microsoft SQL Server database backend. Weekly and bimonthly reports were generated using Transact-SQL queries. Error reports were used to identify data inconsistencies that needed to be addressed and maintain data integrity. Data were backed up daily.
Data analysis
A descriptive analysis was done using STATA V.14 (Stata Corp Statistics/Data Analysis 14.2 Copyright 1985–2015 StataCorp). Missing data were excluded from the analysis. Performance of symptom screening was disaggregated by W4SS and six-symptom screen as defined in the Zambian setting. The overall performance of POC CRP was evaluated against a composite reference derived as described above. The sensitivity of using W4SS in parallel to CRP was also determined. Additional performance analysis was conducted disaggregated by HIV status and among PLHIV, further analysis was done disaggregated by CD4 absolute count <350 and ≥350 cells/mL. The CD4 threshold of ≥350 was used in the analysis to determine if there is any differential performance of CRP especially among the subgroup with CD4<350 where the risk of TB is higher.24 25

Patient and public involvement
Patients were only involved in the study as participants. They did not contribute to the study questions, study design and dissemination of study findings. However, representatives of the neighborhood health committee, which is responsible for supporting community health activities, were invited to the dissemination meeting at facility level.

RESULTS
Study population
A total of 816 consecutive individuals were approached to participate in the study of whom 804 (98.5%) were eligible and provided written informed consent (figure 1). We included 783 participants, with complete evaluable data, in the analysis of whom 452 (57.7%) were male, the median age was 38 years (IQR 29–47), 338 (43.2%) were HIV positive, 622 (79.4%) were six-symptom screen positive, 546 (69.7%) were W4SS positive, 539 (68.8%) had a positive CRP and 134 (17.1%) were diagnosed with TB (table 1). Among PLHIV, 80 (23.7%) had a negative CRP result while 258 (76.3%) had a positive CRP result

![Figure 1: Patient flow diagram. CRP, C-reactive protein; TB, tuberculosis.](image)
### Table 2  Comparison of CRP and symptom screening

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>n/N</th>
<th>Specificity (95% CI)</th>
<th>n/N</th>
<th>PPV (95% CI)</th>
<th>n/N</th>
<th>NPV (95% CI)</th>
<th>n/N</th>
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<tbody>
<tr>
<td><strong>W4SS</strong></td>
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<td></td>
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<tr>
<td>Overall, N=753</td>
<td>109/125</td>
<td>87.2% (80.0–92.5)</td>
<td>190/627</td>
<td>30.3% (26.7–34.1)</td>
<td>109/546</td>
<td>20.0% (16.7–23.6)</td>
<td>190/206</td>
<td>92.2% (87.7–95.5)</td>
</tr>
<tr>
<td>HIV positive, n=324</td>
<td>47/51</td>
<td>92.2% (81.1–97.8)</td>
<td>101/273</td>
<td>37.0% (31.3–43.0)</td>
<td>47/219</td>
<td>21.5% (16.2–27.5)</td>
<td>4/105</td>
<td>96.2% (90.5–99.0)</td>
</tr>
<tr>
<td>HIV negative, n=429</td>
<td>62/74</td>
<td>83.8% (73.4–91.3)</td>
<td>90/355</td>
<td>25.4% (20.9–30.2)</td>
<td>62/327</td>
<td>19.0 (14.9–23.6)</td>
<td>12/102</td>
<td>88.1% (80.4–93.8)</td>
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<td><strong>Six-symptom screen</strong></td>
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<td></td>
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<tr>
<td>Overall, N=753</td>
<td>117/125</td>
<td>93.6% (87.8–97.2)</td>
<td>123/628</td>
<td>19.6% (16.6–22.9)</td>
<td>117/622</td>
<td>18.8% (15.8–22.1)</td>
<td>123/131</td>
<td>93.9% (88.3–97.3)</td>
</tr>
<tr>
<td>HIV positive, n=324</td>
<td>49/51</td>
<td>96.1% (96.5–99.5)</td>
<td>75/273</td>
<td>27.5% (22.3–33.2)</td>
<td>49/247</td>
<td>19.8% (15.1–25.4)</td>
<td>75/77</td>
<td>97.4% (90.9–99.7)</td>
</tr>
<tr>
<td>HIV negative, n=429</td>
<td>68/74</td>
<td>91.9% (83.2–97.0)</td>
<td>48/355</td>
<td>13.5% (10.1–17.5)</td>
<td>68/375</td>
<td>18.1% (14.4–22.4)</td>
<td>48/54</td>
<td>88.9% (77.4–95.8)</td>
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<td><strong>POC CRP</strong></td>
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<tr>
<td>Overall</td>
<td>116/134</td>
<td>86.6% (79.6–91.8)</td>
<td>226/649</td>
<td>34.8% (31.2–38.6)</td>
<td>116/539</td>
<td>21.5% (18.1–25.2)</td>
<td>226/244</td>
<td>92.6% (88.6–95.6)</td>
</tr>
<tr>
<td>HIV positive</td>
<td>55/58</td>
<td>94.8% (85.6–98.9)</td>
<td>77/280</td>
<td>27.5% (22.4–33.1)</td>
<td>55/258</td>
<td>21.3% (16.5–26.8)</td>
<td>77/80</td>
<td>96.3% (89.4–99.2)</td>
</tr>
<tr>
<td>HIV negative</td>
<td>61/76</td>
<td>80.3% (69.5–88.5)</td>
<td>149/369</td>
<td>40.5% (35.3–45.6)</td>
<td>61/281</td>
<td>21.7% (17.0–27.0)</td>
<td>149/164</td>
<td>90.9% (85.4–94.8)</td>
</tr>
<tr>
<td><strong>W4SS and POC CRP used in parallel</strong></td>
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<tr>
<td>Overall</td>
<td>128/133</td>
<td>96.2% (91.4–98.8)</td>
<td>87/642</td>
<td>13.5% (11.0–16.4)</td>
<td>128/684</td>
<td>18.7% (15.9 to –21.8)</td>
<td>87/92</td>
<td>94.6% (87.8–98.2)</td>
</tr>
<tr>
<td>HIV positive</td>
<td>58/58</td>
<td>100% (93.8–100)</td>
<td>235/277</td>
<td>15.2% (11.2–19.9)</td>
<td>58/293</td>
<td>19.8% (15.4–24.8)</td>
<td>42/42</td>
<td>100% (91.6–100)</td>
</tr>
<tr>
<td>HIV negative</td>
<td>70/75</td>
<td>93.3% (85.1–97.8)</td>
<td>321/366</td>
<td>12.3% (9.1–16.1)</td>
<td>70/391</td>
<td>17.9% (14.3–22.1)</td>
<td>45/50</td>
<td>90.0% (78.2–96.7)</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; NPV, negative predictive value; POC, point of care; PPV, positive predictive value; W4SS, WHO-recommended four-symptom screen.
of whom 3 (3.7%) and 55 (21.3%) were diagnosed with TB, respectively. Among HIV-negative individuals, 164 (36.9%) had a negative CRP result while 281 (63.2%) had a positive CRP result of whom 15 (9.2%) and 61 (21.8%) were diagnosed with TB, respectively.

**Comparative performance of tests**

The overall sensitivity and specificity of W4SS were 87.2% (80.0–92.5) and 30.3% (26.7–34.1), respectively (table 2). While the point estimates for sensitivity, negative predictive value (NPV) and positive predictive value (PPV) were higher among PLHIV, the CIs were overlapping. The specificity of W4SS was significantly higher among PLHIV than among the HIV-negative individuals. The overall sensitivity, PPV and NPV of six-symptom screening were comparable to W4SS but six-symptom screening had a reduced specificity (19.6% vs 30.3%). We observed similar differences among the HIV positives and the HIV negatives.

The overall sensitivity and specificity, PPV and NPV of CRP were comparable to that of W4SS. While the point estimates for sensitivity and NPV were higher among the HIV positives, the point estimates for PPV were higher among the HIV negatives, and differences were not significant. The specificity of CRP was significantly higher among the HIV negatives than PLHIV at 40.5% (35.3–45.6) and 27.5% (22.4–33.1), respectively.

When W4SS and CRP were used in parallel, overall, the performance was observed for sensitivity, NPV and PPV of six-symptom screening relative to W4SS was statistically significant. While use of six-symptom screening was more sensitive than W4SS, the difference in sensitivity was not statistically significant. However, the loss in specificity when using six-symptom screening relative to W4SS was statistically significant. Parallel use of CRP and W4SS resulted into improved sensitivity with a reduction in specificity, with respective gains and losses being more marked in the HIV-negative population. Importantly, neither the W4SS nor the CRP independently or in combination reached WHO target product profile for TB screening due to low specificities.

**DISCUSSION**

At a cut-off of 10 mg/L, the sensitivity, specificity, PPV and NPV of CRP were similar to those of W4SS among HIV-positive outpatients, irrespective of CD4 count. However, among HIV-negative individuals, while sensitivity, PPV and NPV were similar, CRP had higher specificity than W4SS. CRP also had a comparable sensitivity but higher specificity than six-symptom screen irrespective of HIV status.

The sensitivity, specificity and NPV for W4SS among individuals with CD4<350 and those with CD4≥350 were comparable with overlapping CIs (table 3). The PPV of symptom screening was higher among individuals with CD4<350 compared with those with CD4≥350. A similar performance was observed for sensitivity, NPV and PPV when using CRP as the screening tool; however, specificity of CRP was higher among individuals with CD4≥350 compared with those with CD4<350.

**Table 3** Comparison of CRP and symptom screening among PLHIV by CD4 threshold

<table>
<thead>
<tr>
<th>CD4 threshold</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4&lt;350</td>
<td>95.1% (83.5–99.4)</td>
<td>30.1% (22.7–38.3)</td>
<td>28.1% (20.8–36.3)</td>
<td>95.6% (84.9–99.5)</td>
</tr>
<tr>
<td>CD4≥350</td>
<td>70% (34.8–93.3)</td>
<td>43.4% (34.5–52.7)</td>
<td>9.21% (3.78–18.1)</td>
<td>94.6% (85.1–98.9)</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; NPV, negative predictive value; PLHIV, people living with HIV; POC, point of care; PPV, positive predictive value; W4SS, WHO-recommended four-symptom screen.
healthcare facility-based active case finding programme. To the best of our knowledge, this is the only study that has evaluated the role of CRP in TB screening among HIV-negative outpatients. Studies that have evaluated the role of CRP in TB screening and diagnosis among HIV-negative individuals were conducted in inpatient settings.\textsuperscript{3}\textsuperscript{9}\textsuperscript{10}

Compared with a study done in Uganda among HIV-negative inpatients using a similar cut-off of 10 mg/dL, the specificity reported by our study was lower.\textsuperscript{9} This is unexpected, as generally inpatients are more likely to have other conditions associated with a raised CRP, and likely lowering specificity. However, it could be because in the Ugandan study CRP was evaluated in the context of TB diagnosis including only symptomatic individuals, while in our setting CRP was evaluated in the context of TB screening. Similarly, among PLHIV, the specificity reported in our study was lower than that reported in other outpatient settings.\textsuperscript{2} However, both the sensitivity and specificity by our study were comparable to those reported by a recent meta-analysis among HIV-positive inpatients.\textsuperscript{26} Our study reported a slightly higher specificity than that reported by the meta-analysis when W4SS and CRP were used in parallel. The overall sensitivity and specificity of W4SS and six-symptom screening were similar to those by a previous study conducted in the Zambian setting\textsuperscript{27} and by WHO.\textsuperscript{17}

Our findings suggest that the overall performance of CRP is not superior to W4SS. However, CRP offers higher specificity compared with the six-symptom screen. Additionally, the findings suggest that among individuals with CD4 count $\geq 350$, CRP can rule out active TB since it has an NPV of 100%. Use of CRP for TB screening among PLHIV would thus facilitate scale-up of TB preventative treatment (TPT) as concerns that a negative symptom screen cannot fully rule out active TB are part of the barriers to TPT.\textsuperscript{28}\textsuperscript{29} This is important because with implementation of test and treat in many settings, including Zambia, an increasing proportion of PLHIV start ART with CD4 count $\geq 350$.\textsuperscript{30}\textsuperscript{31} However, even in settings where the median CD4 count at initiation of treatment is still $<350$,\textsuperscript{32}\textsuperscript{36}\textsuperscript{35} due to late presentation for HIV testing, the role of CRP in ruling out active TB can be optimised by using it in combination with symptom screening. By dichotomising the participants by CD4 counts $\geq 350$ and $<350$, we clarify the role of CRP for TB screening in both PLHIV with a lower and higher incidence of TB, respectively.\textsuperscript{24}\textsuperscript{36}\textsuperscript{37} Among the HIV negatives, CRP showed a 5% increase in specificity but comparable sensitivity, PPV and NPV to symptom screening suggesting additional benefit, although limited. Use of CRP as a stand-alone screening tool in HIV-negative individuals should be explored further to determine whether there are any subpopulations within which its performance is optimised.

There are several strengths of this study: (1) we evaluated the performance of CRP for facility-based systematic screening and included HIV-negative individuals; (2) we used a composite of culture or Xpert Ultra as the reference standard, providing an accurate reference against which presence or absence of pulmonary TB can be defined in routine practice; and (3) we compared the performance of CRP to the current standard of care in Zambia (six-symptom screen) to better understand the additional benefit of use of CRP. The limitations of this study include: (1) we did not exclude participants with other comorbidities that are associated with a raised CRP; (2) the sample size limits the ability to detect differences in the subpopulation analysis; (3) the study population had a high burden of TB which limits the generalisability of our findings to high burden settings; and (4) we did not compare the performance of the CRP threshold of 10 mg/L to that of 5 mg/L used by WHO.

In conclusion, in PLHIV with a CD4 count $\geq 350$, our study suggests that CRP can be used as a stand-alone screening test to promptly rule out active TB and identify patients who are eligible for TPT. Parallel use of W4SS and CRP can also rule out active TB in PLHIV irrespective of CD4 count. This could increase uptake of TPT especially that the tool can be easily used in other settings even at lower levels of care. In HIV-negative individuals, specificity of CRP is slightly higher than symptom screening so use of CRP as an adjunct screening tool in HIV-negative individuals should be explored further to determine subpopulations in which its performance is optimised.

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**Contributors** Conceived and designed the study: MM. Database design and data curation: PS, WM, MM. Implemented the study: WM, MK, LC, MM. Data analysis: PS, MM. Wrote the manuscript: WM, PS, MK, MM. Critically reviewed the manuscript: JC. Approved the final version to be published: WM, PS, MK, LC, JC, MM. MM accepts full responsibility for the finished work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by the University of Zambia Biomedical Research Ethics Committee (No 012-05-17). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** All data related to the study has been uploaded as supplementary information.

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