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Impact of hematocrit on point-of-care C-reactive protein-based tuberculosis screening among people living with HIV initiating antiretroviral therapy in Uganda

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Abstract

Point-of-care C-reactive protein (POC CRP) testing is a potential tuberculosis (TB) screening tool for people living with HIV (PLHIV). Unlike lab-based assays, POC assays do not routinely adjust CRP levels for hematocrit, potentially resulting in TB screening status misclassification. We compared the diagnostic accuracy of unadjusted and hematocrit-adjusted POC CRP for culture-confirmed TB among PLHIV with CD4 cell-count ≤350 cells/uL initiating antiretroviral therapy (ART) in Uganda. We prospectively enrolled consecutive adults, measured POC CRP (Boditech; normal <8 mg/L), collected two spot sputum specimens for comprehensive TB testing, and extracted pre-ART hematocrit from clinic records. Of the 605 PLHIV included, hematocrit-adjusted POC CRP had similar sensitivity (80% vs. 81%, difference +1% [95% CI −3 to +5], p=0.56) and specificity (71% vs. 71%, difference 0% [95% CI −1 to +1], p=0.56) for culture-

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6.0 AUTHOR'S CONTRIBUTIONS

SZM, CY, AC and FCS designed the study. AA and FCS oversaw the local collection of data. JK, SZM, MN and LA collected the data. CY analyzed the data and SZM wrote the manuscript with input from all authors. FCS, CY and CY critically revised the manuscript. All authors read and approved the final manuscript.

[*]First author

4.1 Declarations

4.1.1 Ethics approval and consent to participate.

All participants completed an informed consent, and the parent study was approved by the University of California San Francisco Committee on Human Research, the Makerere University School of Medicine Research Ethics Committee, and the Uganda National Council for Science and Technology.

4.1.2 Work publication.

This work has not been published anywhere and is not under consideration for publication anywhere else.

4.1.3 Declarations of interest: None

confirmed TB, relative to unadjusted POC CRP. When used for TB screening, POC CRP may not require adjustment for hematocrit. However, larger studies may be required if differences close to the clinically meaningful threshold are to be detected.

Keywords

tuberculosis; screening; C-reactive protein; Haematocrit; PLHIV; point of care

1.0 INTRODUCTION:

1.1 Background

Tuberculosis (TB) is a leading cause of death among people living with HIV (PLHIV)[1, 2]. To reduce TB burden, the World Health Organization (WHO) recommends that all PLHIV be screened for TB at each clinical encounter using a four-part symptom screen [3, 4]. Recent prospective studies have shown that the symptom screen has poor specificity[5–10]. As a consequence, the vast majority of PLHIV screen positive and require further confirmatory TB testing, which strains clinic resources and limits uptake of TB preventive therapy. Better screening methods are needed urgently in order to reduce TB burden among PLHIV.

C-reactive protein (CRP) is an acute phase reactant whose levels increase in response to interleukin-6 (IL-6)-mediated inflammation, including pyogenic infections such as active TB [11–14]. CRP levels can be measured using simple, inexpensive and point-of-care (POC) tests and POC CRP testing was recently shown to be the first and only test to date to meet the WHO-recommended accuracy thresholds for a TB screening test [14, 15]. In a subsequent study [10], POC CRP-based TB screening was demonstrated to improve the efficiency of intensified TB case finding among PLHIV, relative to symptom-based TB screening, without compromising diagnostic yield. Furthermore, elevated POC CRP levels have been associated with an increased risk of early mortality for both patients with and without prevalent TB, suggesting that POC CRP testing may have a dual use: both to screen PLHIV for active TB and to identify patients at high risk of poor outcomes, irrespective of prevalent TB status[16].

POC CRP assays use the immunodetection method wherein the antibody in buffer binds to antigen in the sample, forming antigen-antibody complexes that are captured on the test strip[17–21]. Unlike lab-based CRP assays, POC CRP assays do not typically mathematically adjust for hematocrit (Hct), which is performed to improve prevalence estimates of iron-deficiency anemia, particularly useful in low-income settings where both iron-deficiency anemia and elevations in CRP due to systemic inflammation caused by infection are common. If used to screen PLHIV for active TB, failure to adjust POC CRP for Hct has the potential to 1) overestimate CRP concentrations when Hct is elevated, increasing the proportion of patient without active TB referred for unnecessary confirmatory testing (reduced POC CRP specificity) and 2) underestimate CRP concentrations among patients with anemia due to chronic disease, such as anemia caused by active TB, increasing the proportion of TB cases missed by screening (reduced POC CRP sensitivity). Although

manufacturers recommend mathematically adjusting measured POC CRP levels for Hct [19], if Hct is unknown or not available at the point-of-contact, adjusting POC CRP levels for Hct may not be feasible.

To determine whether Hct adjustments impact the accuracy of POC CRP-based TB screening, we compared the diagnostic accuracy of Hct-adjusted and -unadjusted POC CRP levels for active TB among a cohort of PLHIV undergoing systematic TB screening.

2.0 METHODS:

2.1 Study design and population

This cross-sectional study was part of a larger prospective study that compared the WHO four-part symptom screen to POC CRP-based TB screening for HIV-infected adults initiating antiretroviral therapy (ART) at two large HIV clinics in Kampala, Uganda. Findings of the parent study have been published previously[14]. Briefly, the parent study included HIV-infected ART-naïve adults (age ≥18 years) with a pre-ART CD4 T-cell count ≤350 cells/uL taken within three months of study enrollment. For this study, we restricted analysis to participants for whom baseline Hct levels were available (Hct ordered as part of routine pre-ART care). The parent study was approved by the University of California San Francisco Committee on Human Research, the Makerere University School of Medicine Research Ethics Committee, and the Uganda National Council for Science and Technology.

2.2 Study Procedures

2.2.1 Data collection.—As part of the parent study, all participants were administered a survey to collect demographic and clinical data. For all participants, we measured CRP levels from capillary blood using a standard POC assay (iCHROMA POC CRP Reader, BodiTech Med Inc., South Korea) and obtained two spot sputum specimens collected by patient expectoration or sputum induction for Xpert MTB/RIF testing and liquid mycobacterial culture as described [14]. We considered participants with POC CRP ≥8 mg/L to have elevated CRP levels, based on previously reported receiver operating characteristics [14]. We considered participants to have prevalent TB if at least one sputum culture was positive for *Mycobacterium tuberculosis*. We considered participants with two negative sputum cultures to not have active TB. For this analysis, pre-ART Hct levels measured on the same day as POC CRP testing were extracted from clinic records.

2.2.2 Adjusting POC CRP levels for Hct: POC CRP levels were adjusted for Hct in accordance with the manufacturer's instructions: we corrected the measured CRP level by multiplying by the correction factor provided in the manufacturer's manual[18]. We calculated sensitivity and specificity of HCT-unadjusted and -adjusted POC CRP in reference to culture results. We compared the sensitivity and specificity of HCT-unadjusted and -adjusted POC CRP for culture-confirmed TB (cut-point ≥8 mg/L) using culture as the reference standard. We compared sensitivity and specificity differences using McNemar's test of paired proportions. All analyses were performed using Stata 13 (College Station, TX).

2.3 RESULTS:

2.3.1 Demographic and clinical characteristics.—We prospectively enrolled 1314 consecutive adults initiating ART from October 2014 to December 2016 and excluded 709 patients for the following reasons: previously received ART (6), unknown ART status (11), missing CD4 cell-count (14), currently receiving treatment for active TB (3), unknown baseline TB status (217; due to incomplete or contaminated cultures), missing POC CRP results (2), and missing Hct levels (456) (Figure 1). Of the 605 participants included in this analysis, 308 (51%) were female, median age was 34 years (IQR 27–40), median CD4 cell-count 140 cells/uL (IQR 56–233) and median Hct was 40% (IQR 35–44) (Table 1). Overall, 229 (38%) had elevated (≥8 mg/L) unadjusted POC CRP levels, median unadjusted POC CRP levels was 3.0 mg/L (IQR 2.5–20.3) and 90 (16.5%) had culture-positive TB (Table 1). Compared to patients with known Hct levels, patients with unknown Hct levels had higher median CD4 cell counts (168 cells/ul, IQR 80–264; p=0.001), were more likely to have elevated unadjusted POC CRP levels (203/456, 45%; p=0.03) and had higher median unadjusted POC CRP levels (4.9 mg/L, IQR 2.5–37.3; p=0.0005); all other demographic and clinical characteristics, including TB prevalence, were similar.

2.3.2 Diagnostic accuracy of Hct-unadjusted and -adjusted POC CRP levels.—Unadjusted POC CRP levels were elevated in 82/103 patients with active TB (sensitivity 81, 95% CI: 72–88) and 146/502 patients without TB (specificity 71%, 95% CI 67–75) (Figure 2). Adjusting for Hct reclassified 3 patients with Hct ≤36% who screened positive for TB by unadjusted POC CRP as screen negative, including 2 patients with active TB. Adjusting for Hct reclassified 3 patients with Hct ≥43% who screened negative for TB by unadjusted POC CRP levels as screen positive, including 1 patient with active TB. Compared to unadjusted POC CRP, Hct-adjusted POC CRP had similar sensitivity (81% [95% CI 72–88] vs. 80% [95% CI: 71–87], difference −1% [95% CI −5 to −3], p=0.56) and similar specificity (71% [95% CI: 67–75] vs. 71% [95% CI: 67–75], difference 0% [95% CI −1 to +1], p=0.56) for culture-confirmed TB.

3.0 DISCUSSION:

In this study we assessed whether Hct adjustment modifies outcomes of POC CRP-based TB screening among PLHIV - a high-risk population for whom systematic TB screening is recommended[19, 23]. Overall, few PLHIV were re-classified as either screen-positive or screen-negative and there was no difference in diagnostic accuracy of TB screening when using adjusted vs. unadjusted POC CRP values. These data support use of POC CRP without the need for measurement of HCT during routine TB screening.

No prior studies have assessed the impact of Hct adjustment on the outcomes of TB screening using POC CRP. However, for TB screening, our data support that Hct adjustment is not only unnecessary but also may be harmful. Anemia is common in populations at risk for TB, including PLHIV [24, 25]. Adjusting for Hct in anemic patients lowers CRP values, potentially resulting in screen-positive patients being re-classified as being screen-negative as occurred in 3 patients in our study (including 2 with TB). Thus, using unadjusted POC CRP values is less likely to lead to missed TB cases in those with severe anemia. However,

in populations where elevated Hct is common (i.e., children), using unadjusted POC CRP levels to screen for active TB has the potential to miss TB cases.

This study had several strengths. First, we performed POC CRP testing as part of routine TB screening of PLHIV initiating ART – a population in whom TB screening is of particular importance. We show that POC CRP testing is practical as a screening tool for TB in HIV clinics in resource limited settings. Second, we compared accuracy in reference to sputum culture results, the gold standard for TB testing.

The limitations of our study included that a significant proportion of patients did not have a Hct value measured as part of routine pre-ART care. Although non-random selection of patients for Hct testing has the potential to bias results, baseline characteristics including TB prevalence and proportion of patients with elevated unadjusted POC CRP levels were similar among patients with and without Hct values. Furthermore, because patients referred for Hct testing can be expected to have additional risk factors for anemia (relative to patients not referred for Hct testing), sensitivity and specificity estimates of Hct-adjusted POC CRP are likely to be higher (more similar to unadjusted POC CRP estimates) if Hct results were available for all patients. Second, the study population was from a single high TB/HIV burden country and the prevalence of anemia may be higher in other settings. However, the likely impact as we show here, would be lower sensitivity of POC CRP with Hct-adjustment as prevalence of anemia increases. Third, we did not assess patients for other conditions that may affect POC CRP levels, such as adipose tissue. Fourth, enrollment was limited to HIV-infected adults and did not include children, a population in whom elevated Hct is more prevalent. Studies evaluating POC CRP-based TB screening in pediatric patients are needed to determine whether Hct-adjusted POC CRP improves the sensitivity of TB screening. Lastly, larger studies may improve detection of clinically meaningful difference in sensitivity.

To our knowledge, this is the first study evaluating the impact of Hct adjustment on outcomes of POC CRP-based TB screening. We show that in an ambulatory population of PLHIV, Hct adjustment results in small changes in POC CRP values and the direction of change does not support Hct adjustment in the context of TB screening.

In conclusion, although sensitivity was similar, adjusting for Hct has the potential to miss TB in HIV infected patients with anemia. When used for TB screening, results of POC CRP assays may not require adjustment for Hct and unknown Hct levels should not delay POC CRP-based TB screening.

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List of Abbreviations:

ART	Antiretroviral therapy
CRP	C-reactive protein
CI	Confidence Interval
HIV	Human Immunodeficiency Virus
Hct	Heamatocrit
IQR	Interquartile Range
PLHIV	People living with HIV
POC	Point of care
TB	Tuberculosis
WHO	World Health Organization

5.0 REFERENCES

1. World, Health, Organisation. Global Tuberculosis Report. Geneva, Switzerland: WHO; 2017.
2. World, Health, Organisation. Global Tuberculosis Report Geneva, Switzerland: WHO; 2018.
3. 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 medicine. 2011;8(1):e1000391.doi:10.1371/ [PubMed: 21267059]
4. World, Health, Organisation. Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resourceconstrained settings. Geneva, Switzerland; 2011.
5. 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(10):1463–72.doi:10.1097/QAD.0000000000000278 [PubMed: 24681417]
6. Floridia M, Ciccacci F, Andreotti M, et al. Tuberculosis Case Finding With Combined Rapid Point-of-Care Assays (Xpert MTB/RIF and Determine TB LAM) in HIV-Positive Individuals Starting Antiretroviral Therapy in Mozambique. Clin Infect Dis. 2017;65(11):1878–83.doi:10.1093/cid/cix641 [PubMed: 29020319]
7. Henostroza G, Harris JB, Chitambi R, et al. High prevalence of tuberculosis in newly enrolled HIV patients in Zambia: need for enhanced screening approach. Int J Tuberc Lung Dis. 2016;20(8):1033–9.doi:10.5588/ijtld.15.0651 [PubMed: 27393536]
8. Van't Hoog AH, Langendam MW, Mitchell E, et al. A systematic review of the sensitivity and specificity of symptom- and chest-radiography screening for active pulmonary tuberculosis in HIV-negative persons and persons with unknown HIV status. Report. 2013. Contract No.: version March, 2013.
9. World, Health, Organisation. Systematic screening for active tuberculosis: Principles and recommendations. Geneva Switzerland; 2013.
10. Yoon C, Dowdy DW, Esmail H, MacPherson P, Schumacher SG. Screening for tuberculosis: time to move beyond symptoms. The Lancet Respiratory Medicine. 2019;7(3):202–4.doi:10.1016/s2213-2600(19)30039-6 [PubMed: 30823972]
11. 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(5):636–43.doi:10.5588/ijtld.12.0811 [PubMed: 23575330]

12. Pfäfflin A, Schleicher E. Inflammation markers in point-of-care testing (POCT). Analytical and Bioanalytical Chemistry. 2008;393(5):1473.doi:10.1007/s00216-008-2561-3 [PubMed: 19104782]
13. 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(6):939–43.doi:10.1183/09031936.03.00055103 [PubMed: 12797485]
14. Yoon C, Chaisson LH, Patel SM. Diagnostic accuracy of C-reactive protein for active pulmonary tuberculosis: a meta-analysis Int J Tuberc Lung Dis. 2017;21(9):1013–9.doi:10.5588/ijtld.17.0078
15. Yoon C, Semitala FC, Asege L, et al. Yield and efficiency of novel intensified tuberculosis case-finding algorithms for people living with HIV. AJRCCM. 2018.10.1164/rccm.201803-0490OC
16. Chaisson LH, Semitala FC, Asege L, et al. Point-of-care C-reactive protein and risk of early mortality among adults initiating antiretroviral therapy. AIDS. 2019;33(5):895–902.doi:10.1097/QAD.0000000000002130 [PubMed: 30649057]
17. Suman Bains, Chidi Anyaeche, Angus Wyatt, Olu Coker, John B. Evaluation of Point of Care Test (POCT), i-CHROMA™ Serum C-Reactive Protein (CRP) Assay and Microalbumin Urine (MAU) Methods. Ann Clin Lab Res. 2017;05(03).doi:10.21767/2386-5180.1000192
18. Boditech, Med, Incorporated. ichroma™ CRP: INS-CR-EN-CRP-Rev.17_150701.pdf. Google scholar 2016
19. Bolodeoku J, Bains S, Pinkney S, et al. Comparison of the point of care test (POCT), i-CHROMA™ human chorionic gonadotrophin (HCG), leutinzing hormone (LH) and follicle stimulating hormone (FSH) methods with the other laboratory methods in the Randox International Quality Assessment Scheme (RIQAS). COGRM. 2017;3.doi:10.15761/COGRM.1000187
20. Bolodeoku J BS, Pinkney S, Coker O, Kim TK and Anyaeche C. An evaluation of the Boditech i-CHROMA Thyroid-Stimulating Hormone (TSH) method: Precision and Accuracy. Annals of Clinical and Laboratory Research. 2019;7 (2:302).doi:10.21767/2386-5180.100302
21. Beltran Luisa, Sureshni de Fonseka Emily Leach, Bolodeoku John, Chinegwundoh F. An Evaluation of the Novel i-CHROMA™ Point-of-Care Testing (POCT) Method for the Analysis of Prostate-Specific Antigen (PSA) in Serum. BJSTR. 2018;9(4):2–4.doi:10.26717/bjstr.2018.09.001822
22. Drain PK, Mayeza L, Bartman P, et al. Diagnostic Accuracy and Clinical Role of Rapid C-Reactive Protein Testing in HIV-infected TB Suspects in South Africa. Int J Tuberc Lung Dis. 2014;18(1):20–6.10.5588/ijtld.13.0519 [PMC free article] [PubMed] [Google Scholar]
23. World, Health, Organistaion. Global Tuberculosis Report. Geneva; 2019.
24. Minchella PA, Donkor S, Owolabi O, Sutherland JS, McDermid JM. Complex anemia in tuberculosis: the need to consider causes and timing when designing interventions. Clin Infect Dis. 2015;60(5):764–72.doi:10.1093/cid/ciu945 [PubMed: 25428413]
25. Rohini K, Surekha Bhat M, Srikumar PS, Mahesh Kumar A. Assessment of Hematological Parameters in Pulmonary Tuberculosis Patients. Indian J Clin Biochem. 2016;31(3):332–5.doi:10.1007/s12291-015-0535-8 [PubMed: 27382206]

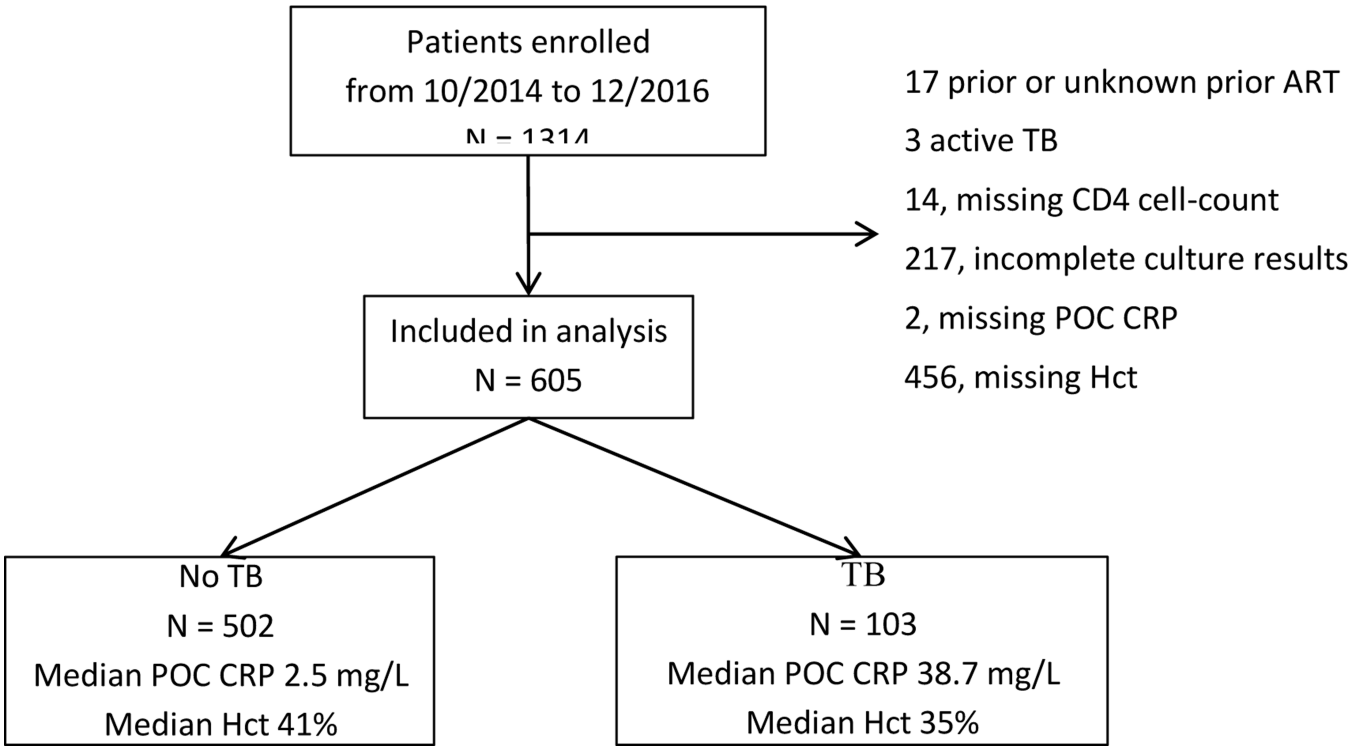

Figure 1.
PATIENT FLOW DIAGRAM Abbreviations: ART (antiretroviral therapy); TB (tuberculosis); POC CRP (point-of-care C-reactive protein); Hct (hematocrit).

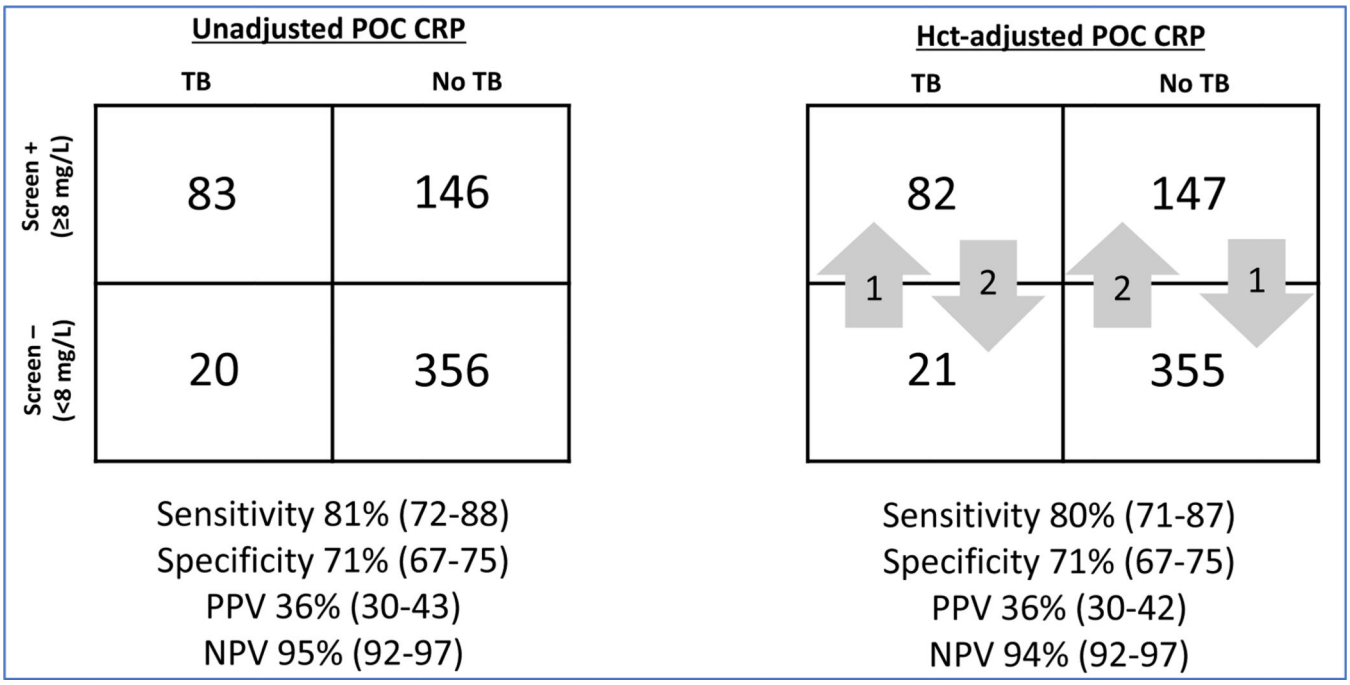

Figure 2.

Diagnostic accuracy of hematocrit-unadjusted and -adjusted POC CRP for culture-confirmed TB. Abbreviations: POC CRP (point-of-care C-reactive protein); TB (tuberculosis); PPV (Positive predictive value); NPV (negative predictive value).

Table 1

Demographics and clinical characteristics.

Characteristic, N (%)	Median (IQR)/Number (%) (Total N=605)
Age (years)	34 (27–40)
Female	308 (51%)
CD4+ T-cell count (cells/μL)	140 (56–233)
Hemoglobin (g/dL)	12.7 (11.0–14.3)
Hematocrit (%)	39.8 (35.1–43.8)
BMI (kg/m^2)	20.8 (18.8–23.8)
Elevated unadjusted POC CRP (≥8 mg/L)	229 (38%)
Unadjusted POC CRP (mg/L)	3.0 (2.5–20.3)
Elevated adjusted POC CRP (≥8 mg/L)	229 (38%)
Hematocrit-adjusted POC CRP (mg/L)	3.3 (2.5–21.1)

Abbreviations: IQR (interquartile range); BMI (body mass index); POC CRP (point-of-care C-reactive protein).