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Chiweka, Evarist

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C-Reactive Protein as a Triage Test in Guiding Who Should Get a Confirmatory Test for Pulmonary Tuberculosis Diagnosis among Adults: A Case-Control Proof-of-Concept Study from Urban Tanzania

Evarist Chiweka[1,2], Thomas Maroa[1], Hosiana Temba[1], Joseph Ponera[1], Sharifa Athumani[1], Lujeko Kamwela[1], Mohamed Sasamalo[1], Rastard Naftari[1], Mirambi Tito[1], Francis Mhimbira[1], Jerry Hella[1*]

[1]Ifakara Health Institute, Dar es Salaam, Tanzania
[2]Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
Email: *jhella@ihi.or.tz

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Abstract

Background: The current screening tools for tuberculosis (TB) are inadequate resulting in insufficient TB case detection and continued community transmission of TB. As the world is geared into finding missing TB cases, new strategies are called for to aid in rapid identification of TB cases. This study aimed to evaluate the role C-reactive protein (CRP) in triaging patients to get a definitive test for active pulmonary TB diagnosis in urban Tanzania. Methods: A case-control study was conducted among pulmonary TB (PTB) patients and contacts without active PTB. The diagnosis of PTB was performed using GeneXpert MTB/RIF assay and culture. Blood was collected from cases and controls for measuring CRP levels during recruitment. We compared socio-demographic characteristics, clinical and laboratory parameters obtained during recruitment and performed diagnostic accuracy analyses for CRP. Results: Out of all 193 study participants who were involved in final analysis, 147 (76.2%) were males. Pulmonary TB cases had significantly lower median BMI than controls (median 17.4 kg/m^2 [IQR: 15.8 - 19.2 kg/m^2] vs., 24.9 kg/m^2 [IQR: 22.1 - 28.5 kg/m^2), $p < 0.001$). There was no statistical difference in prevalence of HIV between PTB cases and controls *i.e.*, 13.33% vs., 11.7%, $p = 0.48$. CRP was significantly higher in PTB cases vs., controls (median 67.8 mg/L, [IQR: 36.5 - 116.9 mg/L] vs., 1.55 mg/L, [IQR: 0.59 - 6.0 mg/L], $p = 0.003$). Furthermore, CRP at cut-off ≥10 mg/L was associated with best combination of sensitivity, specificity and area under the curve of 89.9%, 95% CI:

82.2 - 95.0, 80.9%, CI: 71.4 - 88.2 and 0.85, 95% CI: 0.80 - 0.90 respectively. A multivariate logistic regression model adjusted for fever, night sweats and body mass index showed that CRP above 10 mg/L was significantly associated with PTB, aOR 5.2, 95% CI 1.2 - 22.8. Conclusions: CRP at cut-off ≥10 mg/L can be used to screen pulmonary TB. These findings can be used to improve TB screening algorithm by incorporating CRP in combination with TB symptoms to identify patients who need further confirmatory TB tests. However, additional prospective studies are required to support our findings and contribute into policy recommendations on use of CRP in a screening algorithm for pulmonary TB.

Keywords

C-Reactive Protein, Pulmonary Tuberculosis, Screening, Temeke, Tanzania

1. Introduction

Tuberculosis remains one of the world's deadliest communicable diseases. It was estimated that 10 million people fell ill with tuberculosis (TB) and 1.5 million died of the disease globally in 2018 [1]. Prompt case detection and subsequent start of effective treatment especially in TB high burden countries are a major strategy for its control [2] [3] [4] [5]. Systematic screening of individuals presenting to health facilities seeking health care and certain risk groups with high TB prevalence is an important approach to improve early detection of TB.

Symptoms and chest radiography are the available methods to screen for active TB in most resources limited settings while sputum smear microscopy, mycobacterial culture and currently endorsed molecular Xpert MTB/RIF assay (Cepheid, Sunnyvale, Califormia) are confirmation tests [6] [7] [8]. However, current screening algorithm relies mostly on TB symptoms questionnaire followed by chest radiography—a strategy that is inadequate in TB case detection [9]. Furthermore, the rapid and widely used smear microscopy has varied and limited sensitivity while culture is often not available in resource limited setting for routine use [10] [11] [12]. Therefore, majority of TB patients remain undetected and there is a need for alternative TB diagnostic algorithm especially in TB high burden countries with limited resources.

Inflammatory markers are emerging as tools of choice for TB screening with potentials of being rapid and point of care utility [13]. C-reactive protein (CRP) is an acute phase molecule found in plasma whose levels rise significantly in response to tissue damage and infections such as active TB independently of HIV status [14] [15] [16] [17]. Several observational studies have shown that CRP has association and high sensitivity for the presence of TB [16] [18] [19] [20]. However, despite the evidence shown in the previous observations on the role of CRP as a biomarker for TB, performance of screening using CRP has varied across different settings. It is therefore important to determine performance of CRP for screening pulmonary TB in our setting.

We aimed to establish the role of CRP as a screening tool for active pulmonary TB in an observational study of patients with PTB and controls without PTB in urban Tanzania. To address this need, we specifically 1) compared baseline characteristic and distribution of CRP in pulmonary TB cases and controls 2) determined the discriminatory accuracy of CRP and TB symptoms for pulmonary TB among adults.

2. Methods

2.1. Study Setting

We included participants from an ongoing prospective cohort of bacteriological confirmed pulmonary TB patients in Tanzania (TB-DAR) initiated in October 2013. The study site is in Temeke district of Dar es Salaam, the economic capital of Tanzania. Dar es Salaam has approximately 4.4 million people and notifying 22% of all TB cases in Tanzania [21]. Participants included were sputum smear-positive adult TB patients (TB cases) and household contacts (controls) that did not have active disease (≥18 years).

2.2. Selection of Study Participants

Among 359 TB patients recruited between 2014 and 2015, we randomly selected 103 TB cases and 103 people with documented contact to a TB cases but without active disease (controls). The final analysis was restricted to 193 participants *i.e.*, 99 (51.3%) TB cases and 94 controls with serum CRP and sputum samples examination results [22].

2.3. Study Procedures and Data Collection

All participants recruited were clinically evaluated during recruitment as either TB cases or controls after ruling out presence of active TB among controls. Socio-demographic data, clinical data and biological samples were collected at the time of recruitment. Briefly, data were captured using the OpenDataKit application (www.opendatakit.org) on Android tablets, and data quality was monitored in real-time using the *odk_planner* tool [23]. Serum samples were taken at the time of TB diagnosis before starting TB treatment (pulmonary TB cases) or at the time of recruitment (controls) and stored at −80˚C. GeneXpert MTB/RIF assay was used to rule out TB in controls. During recruitment, we collected urine and stool for diagnosis of helminths infections as well as nasopharyngeal swabs (Copan, USA) to detect respiratory viruses and bacteria.

2.4. Laboratory Procedures

We used extensive laboratory techniques to evaluate the presence of various soil transmitted helminths from urine and stool samples. Kato-Katz method (in triplicates), Baermann technique (in duplicates), urine filtration (in duplicates), and circulating cathodic rapid antigen test (POC-CCA; Rapid Medical Diagnostics, South Africa) were used to diagnose helminths (*Strongyloides stercoralis*, *Tri-*

churis trichiura, *Schistosoma mansoni*, *Schistosoma haematobium*, *Ascaris lumbricoides*).

Sputum samples from TB cases and controls were collected and stored at 4˚ Celsius and transported in temperature-controlled cool boxes to a biosafety level 2+ laboratory at Bagamoyo Research and Training Center, IHI. Specimens were homogenized and decontaminated using *N-acetyl L-cysteine Sodium Hydroxide* and then incubated according to standard procedures on Löwenstein-Jensen medium and read once each week until there is *Mycobacterium tuberculosis* (Mtb) growth otherwise they were declared as negative after 8 weeks. In case of Mtb growth, the isolate was subjected to a Capilia TB/MPT64 antigen test to confirm the presence of *Mycobacterium tuberculosis* complex species.

Nasopharyngeal swabs were analysed to detect presence of respiratory pathogens using a multiplex real-time PCR with a broad panel of 16 viral (Anyplex II RV16) and seven bacterial (Allplex panel 4) respiratory pathogens (Table 1) according to the manufacturer's instructions (Seegene, Seoul, South Korea).

Haematological tests were performed immediately after sample collection when possible while those tests which needed specialized laboratory and not available within the country were stored at −80˚C until shipping them abroad. HIV screening was done using Alere Determine HIV rapid test, and the Uni-gold HIV (Trinity Biotech, USA) rapid test served as a confirmatory test in case of a positive screening test. Full blood counts were done with a MS4 Vet haematology analyser (Diamond Diagnostics, Massachusetts, USA) at the Temeke Regional Referral Hospital laboratory. CRP analysis was performed at the Labor Risch, Bern (Switzerland) using the Siemens Nephelometer BN II (soluble transferrin receptor) and the Cobas 6000, Roche diagnostics, Switzerland. Lastly, we confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Table 1. Detection of respiratory viral and bacterial pathogens using a multiplex real-time PCR in nasopharyngeal swabs.

Viral species- Anyplex II RV16 (panels A and B)	**Bacterial species** - Allplex respiratory panel 4
Adenovirus	*Mycoplasma pneumonia*
Influenza A/B	*Chlamydophila pneumonia*
Rhinovirus A/B/C	*Legionella pneumophila*
Respiratory syncytial virus A/B	*Haemophilus influenza*
Parainfluenza virus 1/2/3/4	*Streptococcus pneumoniae*
Bocavirus 1/2/3/4	*Bordetella pertussis*
Metapneumovirus	*Bordetella parapertussis*
Coronavirus 229	
Coronavirus OC4	
Coronavirus NL63	
Enterovirus	

2.5. Definitions of Terms

We used at CRP concentration cut-off ≥10 mg/L to indicate presence of active TB vs., <10 mg/L indicated absence of active TB [24]. Helminths infection was defined as infection with any helminth species, and respiratory infection as detection of any respiratory viral or bacterial pathogen. We classified TB symptoms as the presence of fever, weight loss, night sweats and fever. We used BMI ≤ 18.5 kg/m^2 as underweight.

2.6. Statistical Analysis

Socio-demographic characteristics, clinical and laboratory parameters of pulmonary TB cases and controls were compared for data obtained during recruitment. Wilcoxon rank-sum test or Student's *t*-tests were used for continuous variables while chi-square or Fisher's exact tests for comparison of categorical variables. CRP concentrations for pulmonary TB cases discrimination were explored using sensitivity, specificity and receiver operating characteristic curves. We used odds ratios and 95% confidence interval associated with CRP cut-offs concentration and TB symptoms to determine their role in predicting pulmonary TB cases using univariate and multivariable logistic regression analyses. All statistical tests were two-sided, and we set threshold of a statistically significant difference at an alpha level of 0.05. We performed all analyses using Stata version 15.1 (Stata corporation, Texas, USA).

2.7. Ethical Considerations

The study was approved by the institutional review board of Ifakara Health Institute (IHI, reference no. IHI/IRB/04-2015), the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (NIMR, reference no. NIMR/HQ/R.8c/Vol. I/357), and the Ethics Committee of the Canton of Basel (EKNZ, reference no. UBE-15/42). All participants gave written informed consent before enrolment.

3. Results

3.1. Socio-Demographic and Clinical Characteristics

The median age was 32.7 years (interquartile range [IQR] 26.5 - 40.0); and 147 (76.2%) were males out of all 193 study participants who were involved in final analysis. Overall, the median body mass index (BMI) at the time of recruitment was 20.1 kg/m^2, IQR 17.3 - 25.1 kg/m^2. Pulmonary TB cases had significantly lower median BMI than recruited controls (median 17.4 kg/m^2 [IQR: 15.8 - 19.2 kg/m^2] vs., 24.9 kg/m^2 [IQR: 22.1 - 28.5 kg/m^2], $p < 0.001$) (**Table 2**).

The prevalence of HIV infection among study participants was 12.44%, with no statistical difference in prevalence of HIV infection between pulmonary TB cases and controls *i.e.*, 13.33% vs., 11.7%, $p = 0.48$. We found a higher prevalence of helminths infections among pulmonary TB cases than controls *i.e.*, 32.2% vs., 21.28%, however this difference in prevalence between the two study

Table 2. Baseline socio-demographic and clinical characteristics of adult pulmonary TB cases and controls at Temeke, Dar es Salaam.

Characteristics	All, n = 193 (100%)	Controls, n = 94 (48.7%)	TB cases, n = 99 (51.3%)	*P*-value
Age, years, median (IQR)	32.7 (26.5 - 40)	32.7 (26.5 - 39.3)	33 (26 - 40)	0.98††
Sex, n (%)				0.59$
Female	46 (23.8)	24 (25.5)	22 (22.2)	
Male	147 (76.2)	70 (74.5)	77 (77.8)	
BMI, kg/m^2, median (IQR)	20.1 (17.3 - 25.1)	24.9 (22.1 - 28.5)	17.4 (15.8 - 19.2)	<0.001††
BMI category, n (%)				<0.001$$
Underweight	73 (37.8)	6 (6.38)	67 (67.68)	
Normal weight	71 (36.8)	41 (43.62)	30 (30.30)	
Overweight	34 (17.6)	32 (34.04)	2 (2.02)	
Obese	15 (7.8)	15 (15.96)	-	
Education, n (%)				0.403$$
No formal education	36 (18.65)	15 (15.96)	21 (21.21)	
Primary	117 (60.62)	62 (65.96)	55 (55.56)	
Secondary	31 (16.06)	12 (12.77)	19 (19.19)	
University	9 (4.66)	5 (5.32)	4 (4.04)	
Occupation, n (%)				0.031$$
Housewife	14 (7.29)	5 (5.38)	9 (9.09)	
Unskilled labor	17 (8.85)	7 (7.53)	10 (10.10)	
Semiskilled manual	63 (32.81)	36 (38.71)	27 (27.27)	
Semiskilled non-manual	76 (39.58)	32 (34.41)	44 (44.44)	
Student	4 (2.08)	-	4 (4.04)	
Unemployed	18 (9.38)	13(13.98)	5 (5.05)	
Income ($), mean ± SD	85.99 ± 73.53	91.18 ± 96.58	81.07 ± 41.12	0.341†
Cigarette smoking, n (%)	39 (20.21)	14 (14.89)	25 (25.25)	0.073$
HIV infection, n (%)	24 (12.44)	11 (11.70)	13 (13.13)	0.48$
Helminthiasis, n (%)	52 (26.94)	20 (21.28)	32 (32.32)	0.084$
Strongyloidiasis	31 (16.06)	9 (9.57)	22 (22.22)	0.019$$
Schistosomiasis	11 (5.70)	5 (5.32)	6 (6.06)	1.0
URTI, n (%)				
Bacterial, (n = 124)	58 (46.77)	18 (62.07)	40 (42.11)	0.059$
Viral	42 (21.76)	18 (19.15)	24 (24.24)	0.391$
TB Symptoms, n (%)				
Night sweats	108 (55.96)	12 (12.77)	96 (96.97)	<0.001$$
Fever	111 (57.51)	17 (18.09)	94 (94.95)	<0.001$$
Cough	145 (75.13)	46 (48.94)	99 (100)	<0.001$$
Weight loss	114 (59.07)	15 (15.96)	99 (100)	<0.001$$

n, number; SD, standard deviation; IQR, Interquartile range; †Student *t* test †† Wilcoxon rank sum test; $ Pearson Chi-squared test; $$Fisher's exact test; USD, United States Dollars (1 USD = 2171 Tanzanian Shillings, June 2016).

groups were not statistically significant different. *Strongyloides stercoralis* contributed more into the burden of soil transmitted helminths among pulmonary TB cases than controls *i.e.*, 22.22% vs., 9.57%, $p = 0.019$. Pulmonary TB cases recruited in our study reported classical TB symptoms more frequently than controls with cough and weight loss experienced by all cases, $p < 0.001$ (Table 2).

3.2. Haematological Parameters and Acute Phase Proteins

The overall median CRP concentration was 18.7 mg/L (IQR: 1.4 - 80 mg/L) where this biomarker was significantly higher in pulmonary TB cases compared to controls (median 67.8 mg/L, [IQR: 36.5 - 116.9 mg/L] vs., 1.55 mg/L, [IQR: 0.59 - 6.0 mg/L], $p = 0.003$). CRP when used at a cut-off of ≥10 mg/L could statistically differentiate active TB vs., no active TB disease among adults recruited (Table 3). Similarly, the average haemoglobin (Hb) level was 12.3 mg/dL ± 2.18 mg/dL and we found that pulmonary TB cases were more likely to have lower average Hb levels than controls (mean 11.8 mg/mL ± 2.1 mg/dL vs., 12.8 mg/mL ± 2.1 $p = 0.001$). Serum ferritin levels were higher in TB cases than in controls (median 355.5 µg/L vs., 103.5 µg/L, $p < 0.001$).

Furthermore, serum albumin levels at the time of pulmonary TB diagnosis and recruitment of controls was significantly lower among TB cases than among controls (mean 28.58 ± 6.14 g/L vs., 39.25 ± 5.54 g/L, $p < 0.001$, see Table 3).

3.3. Receiver Operator Characteristic (ROC) Curve and Logistic Regression Analyses

We evaluated the utility of different CRP cut-offs in discriminating cases of pulmonary TB using gradual increasing of concentration from 5 mg/L to 25 mg/L. CRP at cut-off ≥5 mg/L were associated with sensitivity, specificity, and AUC of 93.9%, 95% CI: (87.3 - 97.7), 69.1%, 95% CI: (58.8 - 78.3) and 0.82, 95% CI: (0.76 - 0.87) respectively. Increasing CRP cut-off ≥10 mg/L were associated with sensitivity, specificity and AUC of 89.9%, 95% CI: (82.2 - 95.0), 80.9%, CI: (71.4 - 88.2) and 0.85, 95% CI: (0.80 - 0.90) respectively. CRP cut-offs values of ≥15 mg/L, 20 mg/L and 25 mg/L were associated with increasing specificity and AUC. However, they gradually compromise their sensitivity (Table 4 and Figure 1). In univariate logistic regression analysis underweight, night sweats, fever and CRP ≥ 10 mg/L were significantly associated with pulmonary TB cases (OR for underweight 15.3, 95% CI: 5.9 - 39.8; OR for night sweats 218.7, 95% CI: 59.7 - 801.6; OR for fever 85.2, 95% CI: 30.0 - 241.3; OR for CRP ≥ 10 mg/L 37.6, 95% CI: 16.4 - 86.3). In multivariable logistic regression all symptoms remained independently significantly associated with pulmonary TB cases (aOR for underweight 9.2, 95% CI: 1.4 - 61.5; aOR for night sweats 9.7, 95% CI: 1.9 - 49.9; aOR for fever 85.2, 95% CI: 30.0 - 241.3; aOR for CRP ≥ 10 mg/L5.2, 95% CI: 1.2 - 22.8) (see Figure 2 & Figure 3).

Table 3. Distribution of haematological acute and chronic inflammatory markers and disease status among TB cases and controls at Temeke, Dar es Salaam.

Serum parameters	No. included* Controls/Cases	Controls, n = 94 (48.7%)	TB cases, n = 99 (51.3%)	*P*-value
Albumin, mean ± SD	94/99	39.25 ± 5.54	28.58 ± 6.14	<0.001†
Ferritin, µg/L, median (IQR)	89/99	103.5 (59.5 - 159.5)	355.3 (162.2 - 642.7)	<0.001††
sTfR, mg/L, mean ± SD	75/89	1.54 ± 0.54	1.92 ± 0.74	0.0003†
Transferrin, mean ± SD	94/99	2.52 ± 0.48	1.7 ± 0.49	<0.001†
CRP, mg/L, median (IQR)	94/99	1.55 (0.59 - 6)	67.8 (36.5 - 116.9)	<0.001††
Neutrophil, median (IQR)	47/53	2.29 (1.47 - 3.23)	5.07 (3.85 - 6.86)	<0.001††
Monocytes, median (IQR)	47/53	0.47 (0.31 - 0.66)	0.94 (0.67 - 1.26)	<0.001††
Haemoglobin, g/dL, mean ± SD	94/99	12.8 ± 2.1	11.8 ± 2.1	0.001†
Thrombocytes, mean ± SD	94/99	247.4 ± 87.4	363.6 ± 124.2	<0.001†
Anaemia, n (%)	94/99			0.001§§
No anaemia		52 (55.32)	28 (28.28)	
Mild anaemia		26 (27.66)	38 (38.38)	
Moderate anaemia		15 (15.96)	29 (29.29	
Severe anaemia		1 (1.06)	4 (4.04)	
TB disease by CRP, n (%)	94/99			<0.001§§
No active disease		76 (80.85)	10 (10.10)	
Active disease		18 (19.15)	89 (89.90)	
TB symptom scoring, n (%)	94/99			
No TB		94 (100.0)	-	
Mild TB symptoms		-	57 (57.58)	
Severe TB symptoms		-	42 (42.42)	

n, number; SD, standard deviation; IQR, Interquartile range; †Student *t* test, ††Wilcoxon rank sum test; § Pearson Chi-squared test; §§Fisher's exact test; sTfR, soluble transferrin receptor; CRP, C-reactive protein. * Participants with available inflammatory serum parameters.

Table 4. Performance characteristics of different CRP cut-offs for pulmonary TB discrimination among adults at Temeke, Dar es Salaam.

Cut point mg/dl	Sensitivity, (95% CI)	Specificity, (95% CI)	PPV, (95% CI)	NPV, (95% CI)	AUC, (95% CI)
5 mg/L	93.9%, (87.3 - 97.7)	69.1%, (58.8 - 78.3)	76.2%, (67.7 - 83.5)	91.5%, (82.5 - 96.8)	0.815, (0.763 - 0.868)
10 mg/L	89.9%, (82.2 - 95.0)	80.9%, (71.4 - 88.2)	83.2%, (74.7 - 89.7)	88.4%, (79.7 - 94.3)	0.854, (0.804 - 0.904)
15 mg/L	86.9%, (78.6 - 92.8)	85.1%, (76.3 - 91.6)	86.0%, (77.6 - 92.1)	86.0%, (77.3 - 92.3)	0.859, (0.811 - 0.909)
20 mg/L	85.9%, (77.4 - 92.0)	89.4%, (81.3 - 94.8)	89.5%, (81.5 - 94.8)	85.7%, (77.2 - 92)	0.876, (0.829 - 0.923)
25 mg/L	84.8%, (76.2 - 91.3)	89.4%, (81.3 - 94.8)	89.4%, (81.3 - 94.8)	84.8%, (76.2 - 91.3)	0.871, (0.824 - 0.918)

CI, Confidence interval; PPV, Positive predictive value; NPV, Negative predictive value; AUC, Area under the curve.

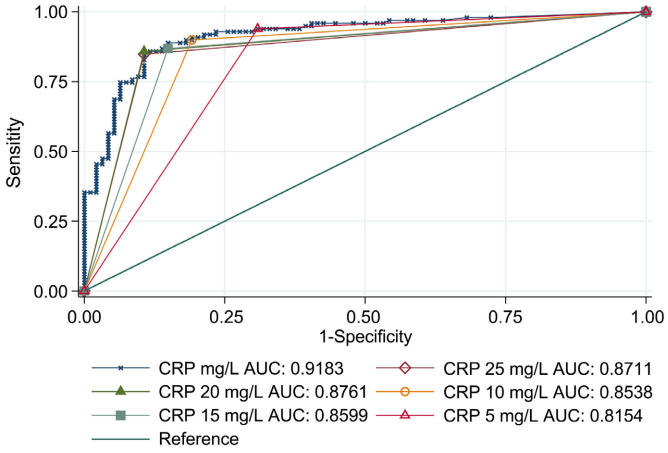

Figure 1. Receiver operator characteristic (ROC) curves for different CRP cut-offs for TB diagnosis among adults in Temeke, Dar es Salaam.

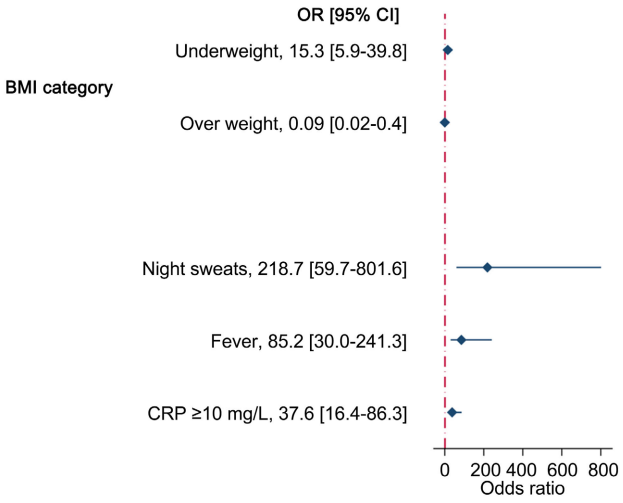

Figure 2. Univariate logistic regression analyses for CRP cut-off ≥10 mg/L and pulmonary TB symptoms.

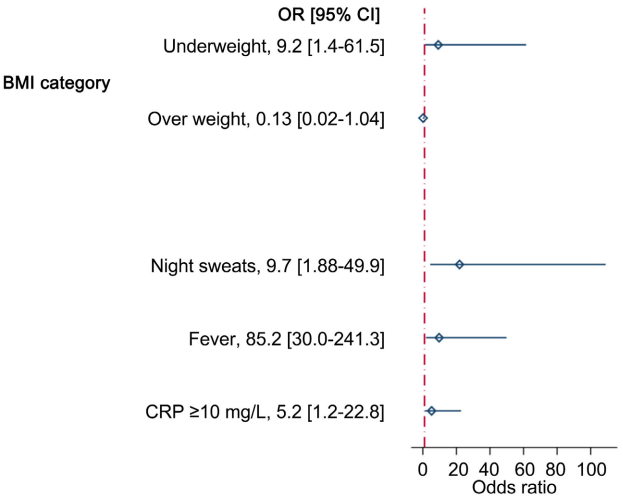

Figure 3. Multivariate logistic regression analyses for CRP cut-off ≥10 mg/L and TB symptoms.

4. Discussion

In this observational study of adult pulmonary TB cases and controls in urban Tanzania, we determined screening performance of CRP for pulmonary TB. We found that CRP at concentration cut-off ≥10 mg/L was associated with best combination of sensitivity, specificity and AUC for discriminating pulmonary TB cases. We also demonstrated that classical TB symptoms and CRP concentration cut-off ≥10 mg/L can accurately predict pulmonary TB cases. These findings are of clinical relevant because CRP can be easily measured and utilized at point of care [25]. Furthermore, night sweats, fever and nutritional status are routinely sought by clinicians when evaluating patients.

The findings on performance of CRP on identifying pulmonary TB cases are in agreements with other findings elsewhere. Lawn and his colleagues in South Africa demonstrated a CRP of 10 mg/L and above have a relatively high sensitivity of 85.2% and reliably rule out active PTB [26]. However, their finding applies only to half of their study population. Another study in the same country revealed any elevated CRP with confirmed TB can be useful for ruling out TB at NPV of 0.72 - 0.96 [27]. However, raised CRP might have been caused by other diseases processes, notably intestinal parasites and respiratory infection [28] [29]. Thus, other diseases should be considered and looked for in a patient with significantly raised CRP levels. In the present study we addressed the impact of helminthiasis and respiratory infections on serum levels of CRP. We found no statistically significant differences in frequencies of their distribution in the two groups. These findings were in contrary to observations from other studies. Studies in Tanzania and Ethiopia demonstrated high co-existence of pulmonary TB and helminthiasis [30] [31]. It was known that both diseases have impact on cellular mediated immunity and influence the natural course of one another [31] [32] [33]. However, similar prevalence in present study could be partially explained by several factors. First, our study setting is urban which is characterized by low exposure to worms and high literacy associated with frequent practice of de-worming. Second, our study participants were recruited from same environment. Last, our two groups have equal distribution of socio-demographic characteristics, which are important determinants of helminthiasis [34] [35]. A major difference in our study was a similar proportion of HIV infection in cases and controls, *i.e.*, 13.13% vs 11.7%. This is in disagreement with the fact that the two diseases are closely related. Previous studies in pulmonary TB have reported higher rates of HIV infection and patterns of co-existence were described in many places. Studies in Tanzania observed high prevalence of TB-HIV co-infection [36] [37]. Similar observations of high prevalence were demonstrated in India [38] [39]. Meta-analysis of studies done in Sub-Saharan Africa demonstrated a very high prevalence (34.4%) of HIV infection among pulmonary TB patients [40]. However, overall prevalence of HIV in our study population is higher than Tanzanian general population [41]. The similar distribution in HIV prevalence between pulmonary TB cases and controls can be partly explained by overly selection of study participants of a case-control study design.

Importantly, data from our study demonstrated that using CRP at cut-off ≥10 mg/L were associated with excellent combination of sensitivity, specificity and AUC for discriminating pulmonary TB cases, while higher cut-off values are associated with increasing specificity and AUC in expenses of sensitivity thus, compromising ability to discriminate pulmonary TB cases. Thus, CRP cut-off value ≥10 mg/L will reliably discriminate pulmonary TB from a vast majority of TB suspects in high TB burden settings. The demonstrated relatively high sensitivity and specificity of 89.9% and 80.9%respectively at CRP concentration of ≥10 mg/L is superior to varied low sensitivity of sputum smear microscopy ranging 20% - 80% [42]. Again, data from our study demonstrated that classical pulmonary TB symptoms and CRP at cut-off ≥10 mg/L were significantly more associated with pulmonary TB cases than controls. The observed association between pulmonary TB and classical TB symptoms has been described by other groups. In Kenya, 75%, 100% and 83% of pulmonary TB cases reported fever, night sweats and weight loss respectively, while in South Africa 78%, 78% and 100% reported the symptoms respectively [43]. One group in TB high burden country, China reported that TB symptoms were found in 75.8% of cases [44]. However, symptoms alone have been shown to be less reliable for pulmonary TB especially in areas with high prevalence of TB-HIV co-infection. The relationship between pulmonary TB and nutritional status is known for years. Underweight is a well-recognized risk factor for pulmonary TB and pulmonary TB can lead to underweight. Night sweats and fever are non-specific symptom of TB and are constitutional symptoms of many disease processes. Therefore, data from our study are informing CRP at cut-off ≥10 mg /L, underweight, night sweats and fever scan be used for screening pulmonary TB in high burden settings.

In spite of the findings of this study, there are limitations which need to be addressed. First, we could not exclude all possible disease processes that might be responsible for rise in CRP. Second, we are not able to perform stratified analysis base on disease severity. Third, the study was done in urban setting where the patient population might differ from other rural primary care settings. Finally, the case-control study design associated with overly selection of participants can explain the limitations observed on role of CRP and classical TB symptoms to screen pulmonary TB case.

5. Conclusion

In conclusion, this study has demonstrated that CRP is a potential tool for discriminating pulmonary TB in adults. Furthermore, CRP at cut-off ≥10 mg/l and classical pulmonary TB symptoms can be used systematically to screen pulmonary TB among suspects. The findings can therefore be used to improve TB screening algorithm by incorporating CRP concentration of ≥10 mg/L in combination with classical TB symptoms to identify patients who need further TB work out or empirical treatment. This approach can significantly contribute in reduction of TB burden. However, additional well-designed large-scale studies

are required in setting of intended use to estimates the role of CRP and TB symptoms for pulmonary TB screening to support policy recommendations. Furthermore, studies are required to determine appropriate strategy of screening algorithm for pulmonary TB using CRP and TB symptoms.

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Declarations

Ethics Approval and Consent to Participate

The study was approved by the institutional review board of the Ifakara Health Institute (IHI; reference no. IHI/IRB/04-2015) and the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania (NIMR; reference no. NIMR/HQ/R.8c/Vol. I/357). All study participants gave written informed consent before enrolment into the study.

Availability of Data and Material

The datasets that were used for analysis and preparation of this manuscript are not publicly available due the national policy on data sharing. The datasets will be available from the corresponding author upon reasonable request where concerned parties will sign a data transfer agreement approved by the Medical Research Coordinating Committee.

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Authors' Contribution

Conceived and designed the study: EC, LK, MS, TM, HT, FM and JH. EC, MS, FM, and JH analyzed the data. TM, HT, JP, SA, RN and MT contributed clinical data. MS, LK, RJ, JP and MT contributed laboratory data. EC, FM and JH prepared the first draft of the manuscript. All authors contributed in final manuscript revisions and approved the final version.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

aOR	Adjusted odds ratio
AUC	Area under the curve
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
Hb	Haemoglobin
HIV	Human immune deficiency virus
IHI	Ifakara Health Institute
IQR	Inter quartile range
IRB	Institutional Review Body
MTB	*Mycobacterium tuberculosis*
NIMR	National Institute for Medical Research
NPV	Negative predictive value
OR	Odds ratio
POC CCA	Point of care-Circulating Cathodic Antigen
PPV	Positive predictive value
RIF	Rifampicin
ROC	Receiver operating characteristic
TB	Tuberculosis
USA	United States of America