Predictive value of C-reactive protein for tuberculosis, bloodstream infection or death among HIV-infected individuals with chronic, non-specific symptoms and negative sputum smear microscopy

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Abstract

Background: To determine whether C-reactive protein (CRP) is an inflammatory biomarker that may identify patients at risk of infections or death. Mortality among HIV-infected persons commencing antiretroviral therapy (ART) is often attributed to tuberculosis (TB) or bloodstream infections (BSI).

Methods: In two district hospitals in southern Malawi we recruited HIV-infected adults with one or more unexplained symptoms present for at least one month (weight loss, fever, or diarrhea), and negative expectorated sputum microscopy for TB. CRP determination for 452/469 (96 %) participants at study enrolment was analyzed for associations with TB, BSI or death to 120 days post-enrolment.

Results: Baseline CRP was significantly elevated among patients with confirmed and probable TB (52), BSI (50) or death (60) compared to those with no identified infection who survived at least 120 days (269). A CRP value of >10 mg/L was associated with confirmed and probable TB (adjusted odds ratio 5.7; 95% CI 2.6, 14.3; 87% sensitivity) or death by 30 days (adjusted odds ratio 9.2; 95% CI 2.2, 55.1; 88% sensitivity). CRP was independently associated with TB, BSI or Death but prediction of these endpoints was enhanced by including hemoglobin (all outcomes), CD4 count (BSI, death), and

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whether ART was started (death) in logistic regression models.

Conclusion: High CRP at the time of ART initiation is associated with TB, BSI and early mortality, and so has potential utility for stratifying patients for intensified clinical and laboratory investigation and follow-up. They may also be considered for empirical treatment of opportunistic infections including TB.

Keywords: C-reactive protein, HIV, tuberculosis, biomarker, bloodstream infection, risk of death

Introduction

Early mortality among HIV-infected persons initiating ART in sub-Saharan African settings is significantly higher than that observed in treatment cohorts in well-resourced settings.[1] Several studies of febrile hospitalized patients, as well as autopsy studies, have shown tuberculosis (TB) to be a major cause of morbidity and mortality.[2-5] Bloodstream infections (BSI) are also well-documented in this population.[6, 7] Given the limited capacity for clinical assessment to detect or rule out TB, BSI or risk of death, there is considerable interest in the evaluation of host biomarkers which could predict the presence of latent or active TB, and risk of death,[8-11 12, 13]including the non-specific biomarker C-reactive protein (CRP).

CRP elevation has been documented in relation to a wide range of inflammatory conditions, both infectious and non-infectious. It is known to be elevated in active TB, bacterial and fungal infections, and to fall with treatment of TB and other infections; [14] elevated CRP (>10 mg/L) predicts disease-specific and all-cause mortality risk among HIV-infected persons. [10, 15] Although CRP elevation is relatively nonspecific, a low or normal CRP (<5 or <8 mg/L, depending on the assay) has been evaluated as a test to rule out active TB, with negative predictive values for bacteriologically-confirmed TB of 96% reported for a cohort of HIV-positive outpatients being investigated for TB disease after self-presenting with symptoms. [16] In Gugulethu Township near Cape Town, South Africa, however, CRP lacked diagnostic utility in a cohort of patients due to start ART, with all but a few participants having moderately raised CRP irrespective of final TB diagnosis.[17]

We have previously reported results from a cohort of patients recruited from two HIV clinics in Malawi in 2010 who were starting ART as outpatients with smear-negative WHO-stage 3/4 disease, for whom we identified a high risk of BSI (mainly non-typhoidal Salmonella), TB and death.[18] The main aim of the current study from this same cohort was to describe the utility of CRP, and to investigate positive and negative predictive values of CRP relating to TB, BSI and death.

Methods

Study design

As part of a prospective observational cohort study[18] investigating the prevalence of TB and BSI among expectorated sputum smear-negative, HIV-infected persons qualifying for ART on the basis of chronic non-specific symptoms (detailed below), venous blood was collected at the time of study enrolment for later CRP determination (post-study). Infections (case definitions are provided below) and deaths retrospectively diagnosed up to 120 days post-enrolment were used for the analysis presented here.

Ethics

This study received ethics approval from the National Health Sciences Research Committee, Lilongwe, Malawi, and from the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease, Paris, France (IUATLD).

Study population

HIV-infected adults (≥ 15 years of age is considered adult in the Malawi health system) undergoing assessment for initiation of ART, or seeking health care in outpatient clinics, were consecutively recruited at Zomba Central Hospital and Thyolo District Hospital, in southern Malawi between February and November 2010. Patients were eligible for study enrolment if they had three negative expectorated sputum smears (or were unable to expectorate sputum) *and* fulfilled at least one of the following criteria: history of unexplained weight loss >10% of baseline body weight (estimated or measured), or unexplained fever (intermittent or continuous) for >1 month duration, or unexplained diarrhea >1 month in duration, or weight loss <10% of baseline body weight but with CD4 count <250 cells/microliter. Anti-TB therapy in the past month or pregnancy were exclusion criteria. The study population for this analysis consisted of 452 participants with an enrolment CRP value available.

Enrolment and follow-up procedures

Eligible patients provided written (or witnessed thumbprint if illiterate) informed consent before enrolment, or assent with written informed consent from the guardian for the (one) participant under 18 years of age.

A baseline interview included questions on demographics, clinical history and current symptoms. Weight and height were recorded. Blood was taken for full blood count, culture into Myco/F Lytic™ (supportive of mycobacterial as well as standard bacterial growth), cryptococcal antigen (latex

agglutination test), CD4 count, and CRP assay. A single induced sputum specimen was collected for high quality microscopy after specimen concentration, and mycobacterial solid culture.

A chest radiograph was obtained if not already available. Participants were then managed under the routine care system at each hospital, but with the benefit of results provided by the study team, except for CRP (which was measured only post-study). Xpert MTB/Rif was not available at the time of this study. A comprehensive description of the parent study and its procedures is available. [18]

Clinical endpoints

Participants were enrolled in the study only if they had initial negative routine expectorated sputum smears or if they were unable to expectorate. Additional study diagnostics included sputum induction and concentration of induced sputum for acid-fast bacilli staining and mycobacterial culture.

Confirmed TB: ≥1 positive *M. tuberculosis* culture (sputum and/or blood); *probable* TB: 1 positive (induced, concentrated) sputum smear, but negative or contaminated culture results; *possible* TB: decision to start anti-TB therapy despite negative bacteriological results, or without culture. For this analysis, *possible TB* cases were included if treatment was started within 60 days of enrolment and no other BSI was identified on the enrolment blood culture.

Bloodstream infection (BSI): pathogenic species isolated from blood culture taken at the time of study enrolment, or a serum cryptococcal antigen titre of ≥1:10 (considered to be evidence of disseminated cryptococcal infection).

Death was determined by report of participants' relatives or guardian and/or tracing of participants lost to follow-up. [19]

Laboratory methods

These have been described in detail in an earlier paper as follows. [18] TB microscopy and cultures were performed at the TB laboratory of the College of Medicine/Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW), Blantyre. This laboratory provides TB diagnostic support to clinical trials funded by the National Institutes of Health, Division of AIDS (NIH/DAIDS)/AIDS Clinical Trials Group (ACTG) and as such employs the quality management system recommended by the NIH, including an annual audit. The laboratory takes part in the External Quality Assurance programs of both the NIH and the UK National External Quality Assurance Scheme (NEQAS).

After same-day transport to the laboratory, induced sputum (IS) was decontaminated with an equal volume of 4% NaOH for 15 minutes and concentrated with centrifugation before culture onto Lowenstein-Jensen (LJ) media for up to eight weeks. Smears made from both direct and concentrated sputum were examined under fluorescent microscopy (Auramine O), with any positive re-

sults confirmed by Ziehl-Neelsen staining (ZN). Mycobacterial isolates were further speciated as *Mycobacterium tuberculosis* (MTB) or nontuberculous mycobacteria (NTM) using microscopic cording and MBP-64 lateral flow assays (Capilia®; TAUNS Laboratories, Inc., Numazu, Japan) and, if either test was negative, growth on media with p-nitrobenzoic acid (PNB) at room temperature and/or 45 deg C.

Blood for culture of mycobacteria and other bacterial (or fungal) pathogens was collected using a sterile technique and transferred the same day to the Microbiology Laboratory at Queen Elizabeth Hospital, Blantyre (supported by the Malawi-Liverpool-Wellcome Trust Clinical Research Programme). For bacterial culture, venous blood (5–7.5 mL) was taken for aerobic culture in 50 mL of broth and incubated for 7 days (BacT/ALERT 3D®; bioMérieux SA, Marcy l'Etoile, France). Isolates were then identified using standard diagnostic techniques. For the purpose of this study, all organisms which are found as normal skin or oral flora were considered to be contaminants, including coagulase-negative Staphylococci, alpha-hemolytic Streptococci other than *S. pneumoniae*, and diphtheroids. Antibiotic susceptibility was determined by disc testing (Oxoid, Cambridge, UK).

For mycobacterial culture, venous blood (5 mL) was inoculated into 50 mL broth (BACTEC Myco/F Lytic®; Becton Dickinson Microbiology Systems, Sparks, MD, USA) and incubated at 37 ^{o}C. Bottles were inspected daily for the first 14 days and then once every 2 days using a handheld UV Woods lamp. Contents of bottles were concentrated by centrifugation (3000 g for 15 minutes) either within 48 hours after first detection of fluorescence, or at the end of 6 weeks' incubation (whichever occurred sooner). The concentrate was examined with ZN and Gram's staining to exclude bacterial contaminants, and subcultured onto LJ media. ZN positive subcultures were then speciated as above.

Cryptococcal antigen tests were conducted on a single 1∶10 dilution of serum using a *Cryptococcus* rapid latex agglutination test (Oxoid, Cambridge, UK).

CRP measurements were performed using CRP latex (Beckman Coulter, Brea, USA) after study completion on thawed plasma (collected at the time of study enrolment and frozen at -20˚C) by personnel without knowledge of microbiological test results, to exclude any influence on clinical decision-making. The assay provided CRP values in the range of 3.0 to 480 mg/L.

Statistical analysis

Median/IQR was used to summarize continuous variables. Wilcoxon rank sum tests were used to test the equality of the distribution of two continuous random variables. Binomial logistic regression was used to evaluate the relative contribution of CRP and other variables (with known biological associations to the outcomes) to the prediction of each of TB, BSI or death, stated as odds ratios for a

10-unit increase in CRP, with 95% CI's and P-values for each. Final multivariable logistic regression models were constructed based on the results of the bivariate binomial logistic regression modelling. Sample size was chosen for the parent study of TB prevalence. In accordance with STARD (Standards for Reporting Diagnostic Accuracy) guidelines, [20] sensitivity and specificity of CRP as a classifier of each of TB, BSI and death, respectively, were estimated. We present sensitivity and specificity when the CRP cut-off point is set to 10 mg/L (the justification for this cut-off point is provided in Results). Binomial confidence intervals are calculated for sensitivity and specificity. We also estimated ROC (receiver operating characteristic) curves plotting estimated sensitivities and specificities at various CRP cut-off points. We estimated AUC (area under the curve) for the corresponding ROC curves (see Figures, Supplemental Digital Content 1, 2, 3 and 4, showing ROC curves for CRP in relation to TB (confirmed or probable), BSI, Death by 30 days, and Death by 120 days, respectively). Levels of significance were set at 5%.

Results

Of 469 participants (279 female) enrolled in the parent study, 452 had a CRP value obtained at the time of study enrolment for this analysis (Figure 1). The median CD4 count was 128 cells /microliter (IQR 48-214). Study participants reported chronic fever (62.4%), weight loss >10% of baseline body weight (53%), and/or chronic diarrhea (35%); 68% of participants reported >1 of these inclusion criteria. There were 48 confirmed TB cases, 4 probable TB cases, 48 possible TB cases and 50 bloodstream infections (29 non-typhoidal Salmonella, 5 E. coli, 4 other pathogenic bacteria, 8 C. neoformans, and 4 non-tuberculous mycobacteria). Participants with confirmed or probable TB (but not possible TB), or BSI, or who died within 120 days of study enrolment had significantly higher baseline CRP values than participants with no infection who survived >120 days post-enrolment (all paired comparisons $p < 0.01$, except for 'possible TB' compared to 'no death or infection' which was non-significant, p 0.7,) (Figure 2).

Binary logistic regression with CRP as a continuous variable confirmed that CRP level was significantly associated with confirmed & probable TB (but not possible TB), BSI and death by 30, 60, 90 and 120 days from enrolment (not shown).

Various specific threshold values of CRP were evaluated for their potential predictive value as a part of baseline screening at the time of assessment for ART enrolment. A cut off value of CRP >10 mg/L demonstrated both strong associations with each of the outcomes of interest, with minimal compromise of sensitivity (although low specificity of 50.9%) (Table 1). This cut off point is consistent with that used in previous studies of CRP[8, 10] and with the usual definition of elevated CRP in clinical medicine.[21, 22]

To determine the relative predictive contribution of CRP, binary logistic regression was used to evaluate several other predictor variables with a plausible biological association with each outcome of interest. Final logistic regression models for confirmed & probable TB, and BSI included CRP, CD4 category (<50, 50-200, with reference category >200 cells/microL), and severe anemia (Hemoglobin <8 g/dL); logistic regression models for death by 30, 60, 90 and 120 days included CRP, CD4 category, severe anemia, and use of ART (Table 2). Results for death by 60 and 90 days are not shown. We are not attempting to build a rigorous predictive model (for any of the 3 outcomes) but rather seeking to illustrate the relative contributions of other major risk factors in combination with elevated CRP.

To explore the use of CRP in combination with other commonly available baseline predictors, the composite probability of TB, BSI and Death was estimated using multivariable logistic regression with various combinations of dichotomized predictor variables (Table 3).

Discussion

This study demonstrates that baseline CRP elevation is independently associated with increased risk of confirmed and probable TB, BSI or death among chronically ill, HIV-infected participants.

It has been shown previously that when examining various threshold values of CRP for their utility in predicting TB, sensitivity is lost and specificity improved at successively higher CRP values.[17] We evaluated various threshold values and found associations for CRP >10 mg/L with confirmed and probable TB (OR = 5.7; 95% CI 2.6, 14.3) that retained moderately high sensitivity (87%), although with low specificity (51%), despite negative routine sputum smear microscopy for TB. When CRP exceeded this threshold and severe anemia was also present the probability of confirmed or probable TB was as high as 37% in this setting of high TB prevalence, compared to <5% if neither risk factor was present. The association of TB with baseline CRP > 10 mg/L and anemia among ART initiators was observed by Shivakoti et al.[13] As previously observed by Lawn et al,[8] CRP was not associated with CD4 count in TB patients.

CRP elevation was not associated with possible TB, which were cases defined on clinical grounds (with or without radiological evidence) but without laboratory evidence for the diagnosis. The lack of association may reflect misdiagnosis of non-TB cases, which is inevitable (but difficult to quantify) in a context where the sensitivity of the TB diagnostic methods was limited (this study was conducted before the introduction of Xpert MTB/Rif), and where smear-negative pulmonary TB is commonplace. CRP values are thought to correlate with bacillary burden. [14, 16] It is possible that true TB cases with a very low bacillary burden in sputum (such that they were smear and culture negative on single sputum culture) may not demonstrate the inflammatory response typically seen to *M. tu-*

berculosis, and that CRP values were consequently lower in these cases. We are unable to resolve this conundrum in this analysis.

The more modest, although significant, association between CRP rise and BSI may have resulted because some BSI cases present in a clinically subtle manner, with few signs of systemic inflammation typically associated with CRP elevation. This may be explained by impairment of the inflammatory response in the context of HIV infection; previous analysis of this cohort showed no association of fever with non-typhoidal Salmonella (NTS) BSI. [18]The majority (29/50, 58%) of the pathogenic (non-TB) isolates in this study were NTS; 8 others were *Cryptococcus neoformans* infections. These WHO clinical stage 4 infections are associated with low CD4 counts so it is not surprising that CD4 counts were associated with BSI in this population, as previously demonstrated. As with TB, the coexistence of severe anemia markedly increased the probability of BSI.

The strongest performance by CRP was in the prediction of risk of death. As a clinical endpoint death has the advantage of capturing all of the participants with diagnosed or undiagnosed but ultimately fatal infections. The association of baseline CRP elevation with all-cause risk of death is well known [23, 24] and our analysis illustrates that this association is durably present at 30, 60, 90 and 120 days after enrolment. Nonetheless, the strength of the association is notably higher for death by 30 days, where participants with CRP >10 mg/L had a 9-fold risk of death compared to those with lower CRP values. Higher CRP thresholds offered no advantage in terms of the strength of association with death, or sensitivity which was 88% for the threshold CRP value of >10 mg/L.

Participants who failed to start ART had the strongest probability of death at any interval, among the predictor variables we studied. Among such patients the presence of the 3 other risk factors (CRP >10, severe anemia, CD4 <200) yielded a 96% probability of death by 30 days. If ART was started the probability of death by 30 days fell to 13.4% despite the presence of the other 3 poor prognostic factors at baseline, underscoring the importance of urgent ART initiation – even for patients with CD4 >200 among whom the probability of 30 day mortality was 72% if CRP was >10 mg/L and severe anemia was present. Unlike the baseline risk factors, ART initiation is clearly within programmatic control. All patients in this study were ART eligible even according to the 2010 WHO ART guidelines but the urgency of starting ART may not have been recognized.

Baseline CRP could potentially be used in clinical screening of HIV-infected patients with negative routine sputum smear microscopy in various ways. Although we have shown moderately high sensitivity for the prediction of death or TB, it would be unreasonable to expect high specificity from a non-specific inflammatory biomarker. The greatest utility of CRP will therefore be to use its sensitivity for risk stratification in combination with other risk factors, rather than in making a specific diagnosis. Since a CRP value above 10 mg/L identifies patients with a comparatively high risk of

death by 30 days (PPV 0.15; 95% CI 0.10-0.21)(NPV 0.98; 95% CI 0.94-0.99), and of confirmed & probable TB (PPV 0.25; 95% CI 0.19-0.33)(NPV 0.95; 95% CI 0.90-0.98) and BSI (PPV 0.21; 95% CI 0.15-0.28)(NPV 0.90 95% CI 0.84-0.94), it could be used to identify patients who deserve to be more carefully and frequently evaluated both clinically and with higher sensitivity TB diagnostic tools (Xpert MTB/Rif) and blood cultures, and to ensure there are no unintended delays in ART initiation, particularly if hemoglobin and CD4 count are also available for risk stratification. Empirical anti-TB treatment or BSI treatment could be considered case-by-case, but would ideally be evaluated in clinical trials. Inpatient versus outpatient management could be evaluated.

The potential for baseline CRP to be used as a 'rule out' test to exclude (or at least predict very low risk for) TB, BSI or risk of death was evaluated using a CRP threshold of ≤3 mg/L. A baseline CRP value at or below this level would have correctly predicted the absence of confirmed & probable TB in 94% of cases, but would have missed 6% of cases. The problem with this threshold value is that the median CRP was 9.6 mg/L for participants who did not die or have TB by 120 days – so only a minority of patients had CRP <3 mg/L. This shortcoming, also noted by Lawn et al [17], limits the practical use of CRP for ruling out TB.

Limitations

We were able to perform blood and induced sputum cultures only once, at the time of study enrolment, so it was not possible to microbiologically verify any infections arising after enrolment. Bacteremia may have been missed by a single culture and we were unable to exclude use of antibiotics (or other agents that could modulate the inflammatory response) prior to study enrolment. We employed a single solid media TB culture of sputum, which can be expected to detect roughly half of all potentially culture positive TB that would be detected with 3 liquid media TB cultures.[25] Many cases that were not culture positive are likely to have been diagnosed on clinical (and possibly radiological) grounds, but we have no way of ascertaining what proportion of *possible* TB cases identified in this study would have been sputum culture positive, or positive by molecular testing, in an ideal setting. This may have resulted in some unintentional misclassification of TB cases and non-cases that would tend to diminish differences between these groups. Our choice of a comparator group of patients without identified infection and surviving to at least 120 days was intended to ensure that the comparator group was very unlikely to contain undiagnosed TB cases. It is possible that there were undiagnosed TB cases in the group that died by 120 days but this would not alter our interpretation of the results related to risk of death.

Conclusion

Among chronically ill, WHO Stage 3 & 4 HIV-infected patients with negative routine sputum smear microscopy, baseline CRP > 10 mg/L may offer a simple means of identifying individuals at higher risk of death and of TB, and the need for intensified investigation and clinical follow-up. Further research could evaluate the utility of CRP screening among all HIV-infected persons (regardless of symptoms), and the effectiveness of empiric treatments based on CRP elevations.

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Figure Legend

Figure 1: Participant flow diagram* (figures shown for confirmed or probable TB)

The reference standard for confirmed or probable TB is described above in Methods. Diagnosis of confirmed or probable TB was categorized as *Inconclusive* if any of the following conditions were met: possible TB diagnosis (no laboratory evidence), death at <120 days from enrolment with no infection or with a non-TB bloodstream infection diagnosed. Confirmed or probable TB was categorized as *Absent* if no infection was diagnosed and the subject survived to at least 120 days post-enrolment.

*Format as stipulated in STARD 2015[20]

List of Supplemental Digital Content:

SDC 1.tiff

SDC 2.tiff

SDC 3.tiff

SDC 4.tiff

Tables

Table 1: Sensitivity and specificity, and positive and negative predictive values of various CRP cut-off values for TB, BSI and Death by 30 days

CRP (mg/L)		CRP>3	CRP>5	CRP>10	CRP>20	CRP>50
Confirmed or Proba-ble TB	**Sens**	0.88 (0.77, 0.96)	0.88 (0.77, 0.96)	0.87 (0.74, 0.94)	0.79 (0.65, 0.89)	0.52 (0.37, 0.66)
	Spec	0.33 (0.28, 0.40)	0.40 (0.34, 0.46)	0.51 (0.45, 0.57)	0.62 (0.56, 0.68)	0.79 (0.73, 0.84)
	PPV	0.21 (0.15, 0.26)	0.22 (0.17, 0.29)	0.25 (0.19, 0.33)	0.28 (0.21, 0.37)	0.32 (0.22, 0.43)
	NPV	0.94 (0.87, 0.98)	0.95 (0.89, 0.98)	0.95 (0.90, 0.98)	0.94 (0.89, 0.97)	0.89 (0.85, 0.93)
BSI	**Sens**	0.82 (0.69, 0.91)	0.80 (0.66, 0.90)	0.70 (0.55, 0.82)	0.60 (0.45, 0.74)	0.32 (0.20, 0.47)
	Spec	0.33 (0.28, 0.40)	0.40 (0.34, 0.46)	0.51 (0.45, 0.57)	0.62 (0.56, 0.68)	0.79 (0.73, 0.84)
	PPV	0.19 (0.14, 0.25)	0.20 (0.15, 0.26)	0.21 (0.15, 0.28)	0.23 (0.16, 0.31)	0.22 (0.13, 0.33)
	NPV	0.91 (0.84, 0.96)	0.92 (0.85, 0.96)	0.90 (0.84, 0.94)	0.89 (0.84, 0.93)	0.86 (0.81, 0.90)
Death by 30 days	**Sens**	0.88 (0.70, 0.98)	0.88 (0.70, 0.98)	0.88 (0.70, 0.98)	0.88 (0.70, 0.98)	0.77 (0.56, 0.91)
	Spec	0.34 (0.28, 0.40)	0.40 (0.34, 0.46)	0.51 (0.45, 0.57)	0.62 (0.56, 0.68)	0.79 (0.73, 0.84)
	PPV	0.11 (0.07,0.17)	0.13 (0.08, 0.18)	0.15 (0.10, 0.21)	0.18 (0.12, 0.26)	0.26 (0.17, 0.37)
	NPV	0.97 (0.91, 0.99)	0.97 (0.89, 0.99)	0.98 (0.94, 0.99)	0.98 (0.95, 0.99)	0.97 (0.94, 0.99)

Figures in brackets are 95% confidence intervals for proportion above

Sens = Sensitivity; Spec = Specificity; PPV = Positive Predictive Value; NPV = Negative Predictive Value

Table 2: Odds Ratios (adjusted) for clinical endpoints with multivariable logistic regression

Confirmed & Probable TB	**Adjusted OR (95% CI)**	**p-value**
CRP>10	5.7 (2.6-14.3)	<0.001
Severe Anemia	2.3 (1.2-4.6)	0.016
CD4 <50	1.2 (0.5-2.8)	0.729
CD4 50-200	0.9 (0.4-2.0)	0.847
BSI	**Adjusted OR (95% CI)**	**p-value**
CRP>10	2.1 (1.1-4.2)	0.037
Severe Anemia	2.3 (1.1-4.7)	0.020
CD4 <50	5.1 (2.0-14.5)	0.001
CD4 50-200	1.8 (0.7-5.2)	0.212
Death by 30 days	**Adjusted OR (95% CI)**	**p-value**
CRP>10	9.2 (2.2-55.1)	0.006
Severe Anemia	11.6 (3.4-50.6)	<0.001
CD4 <50	6.8 (1.0-53.8)	0.057
CD4 50-200	9.6 (2.1-54.9)	0.006
ART not taken	142.9 (31.2-970.9)	<0.001
Death by 120 days	**Adjusted OR (95% CI)**	**p-value**
CRP>10	2.9 (1.4-6.4)	0.005
Severe Anemia	3.6 (1.8-7.4)	<0.001
CD4 <50	5.2(1.83-15.9)	0.003
CD4 50-200	4.8 (1.9-13.2)	0.002
ART not taken	18.0 (8.0-44.1)	<0.001

Table 3: Probability of clinical endpoint with selected combinations of dichotomized risk factors

CRP >10 mg/L	**Severe Anemia**			**Confirmed & Probable TB (95% CI)***
Yes	Yes	-	-	37% (21-57)
Yes	No	-	-	21 % (12-33)
No	No	-	-	4% (2-10)
CRP >10 mg/L	**Severe Anemia**	**CD4 <200**		**BSI (95% CI)****
Yes	Yes	Yes	-	35% (23-50)
No	Yes	Yes	-	21% (11-37)
Yes	No	Yes	-	20% (14-29)
Yes	Yes	No	-	16% (7-35)
No	No	No	-	4% (2-10)
CRP >10 mg/L	**Severe Anemia**	**CD4 <200**	**ART not started**	**Death by 30 days (95% CI)*****
Yes	Yes	Yes	Yes	96% (81-99)
Yes	Yes	No	Yes	72% (42-90)
No	Yes	Yes	Yes	70% (27-93)
Yes	No	Yes	Yes	65% (35-87)
Yes	Yes	Yes	No	13% (5-29)
No	No	No	No	<<1% (0-<1)

*Cohort prevalence of confirmed or probable TB 52/371 (16.2%); (censored: possible TB cases, deaths to 120 days where confirmed/probable TB or BSI was not diagnosed)

**Cohort prevalence of BSI 50/371 (15.7%); (censored: possible TB cases, deaths to 120 days where confirmed/probable TB or BSI was not diagnosed)

***Overall 30-day mortality risk was 26/418 (6.2%); (censored: deaths >30 days but ≤120 days)