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MAJOR ARTICLE

Accuracy of C-reactive Protein and Procalcitonin for Diagnosing Bacterial Infections Among Subjects With Persistent Fever in the Tropics

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Background. In low-resource settings, inflammatory biomarkers can help identify patients with acute febrile illness who do not require antibiotics. Their use has not been studied in persistent fever (defined as fever lasting for ≥7 days at presentation).

Methods. C-reactive protein (CRP) and procalcitonin (PCT) levels were measured in stored serum samples of patients with persistent fever prospectively enrolled in Cambodia, the Democratic Republic of Congo, Nepal, and Sudan. Diagnostic accuracy was assessed for identifying all bacterial infections and the subcategory of severe infections judged to require immediate antibiotics.

Results. Among 1838 participants, CRP and PCT levels were determined in 1777 (96.7%) and 1711 (93.1%) samples, respectively, while white blood cell (WBC) count was available for 1762 (95.9%). Areas under the receiver operating characteristic curve for bacterial infections were higher for CRP (0.669) and WBC count (0.651) as compared with PCT (0.600; P <.001). Sensitivity for overall and severe bacterial infections was 76.3% (469/615) and 88.2% (194/220) for CRP >10 mg/L, 62.4% (380/609) and 76.8% (169/220) for PCT >0.1 µg/L, and 30.5% (184/604) and 43.7% (94/215) for WBC >11 000/µL, respectively. Initial CRP level was <10 mg/L in 45% of the participants who received antibiotics at first presentation.

Conclusions. In patients with persistent fever, CRP and PCT showed higher sensitivity for bacterial infections than WBC count, applying commonly used cutoffs for normal values. A normal CRP value excluded the vast majority of severe infections and could therefore assist in deciding whether to withhold empiric antibiotics after cautious clinical assessment.

Keywords. C-reactive protein; antibiotic; persistent fever; procalcitonin; resource-limited.

The approach to febrile illness in tropical countries has dramatically changed since the introduction of malaria rapid diagnostic tests. When malaria can be excluded, clinicians now often choose to "preventively" cover bacterial infections due to fear of adverse outcomes, even though evidence from different tropical fields has demonstrated that acute febrile illness (AFI) in primary care is caused mainly by viral infections [1, 2]. Consequently, while overconsumption of antimalarials has declined, first-line antibiotic use has increased [3]. This contributes to antimicrobial resistance, which poses a larger threat to low- and middle-income countries (LMICs) than to high-income countries [4]. On the other hand, previous studies among hospitalized patients in LMICs have found that those with the highest exposure to empiric antimicrobials are the most likely to be on inappropriate therapy and are at the greatest risk of death [5]. Therefore, there is a significant need for diagnostic tests that provide accurate identification of those cases for whom antibiotic treatment is really needed vs those for whom antibiotic treatment could be safely withheld.

In high-resource settings, it has been demonstrated that inflammatory biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) can help exclude bacterial etiologies of AFI and reduce antibiotic use without increasing mortality or treatment failure [6, 7], although controversy remains [8, 9]. Point-of-care (semi-)quantitative or qualitative lateral-flow rapid diagnostic tests (RDTs) for both biomarkers are commercially available and relatively affordable, raising their potential use in resource-limited settings (LRS). A few studies conducted in Southeast Asia and Sub-Saharan Africa have shown acceptable diagnostic accuracy in patients with AFI [10]. Similar to high-resource settings, trials in LRS assessing CRP levels as a clinical decision point have shown a reduction in antibiotic use without an increase in adverse outcomes [11, 12]. However, uncertainty remains about ideal cutoffs, and results

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have varied according to study populations, settings, and methods [10, 13, 14]. In practice, the incremental diagnostic added value of CRP and PCT is insufficient for use as a standalone test, and their optimal use is generally as a part of clinical decision tools or algorithms that take into account various clinical and laboratory arguments [15, 16].

So far research on febrile illnesses in the tropics has focused almost exclusively on acute fever, while etiologies of persistent fever (defined pragmatically as fever lasting for ≥7 days at medical evaluation) remain underexplored [17]. Recently, the Neglected Infectious Diseases DIAGnosis (NIDIAG; www.nidiag.eu) consortium published the results of a large prospective study aimed at identifying the causes of persistent fever in 4 tropical countries, focusing on a set of priority (ie, severe and treatable) diseases [18]. The study showed that no clinical or basic laboratory features reliably distinguish between specific bacterial diseases [19].

In the present study, we sought to characterize the ability of additional biomarkers to distinguish between causes of persistent fever that would benefit from empiric antimicrobial treatment and conditions in which antimicrobials may be safely delayed or avoided. Therefore, we measured CRP and PCT levels on stored serum samples obtained during the NIDIAG persistent fever study. The primary objectives were to describe the diagnostic accuracy of CRP and PCT to discriminate bacterial infections (as a group as well as for specific bacterial infections of interest) from other causes of persistent fever and to rule out bacterial infections in case of low biomarker levels. Secondary objectives were to compare the accuracy of the biomarkers with that of white blood cell (WBC) count and to estimate the potential impact of immediate biomarker results on antibiotic use.

METHODS

Study Population and Diagnostic Ascertainment

Detailed methodology, setting, and main findings of the NIDIAG persistent fever study are described elsewhere [18, 19]. Concisely, between January 2013 and October 2014, patients presenting with fever of ≥7 days' duration were enrolled from 6 study sites in 4 countries: Cambodia, the Democratic Republic of Congo (DRC), Nepal, and Sudan. Study sites were a rural outpatient clinic (DRC), rural (district) hospitals (Sudan, DRC, Nepal), and higher-level urban hospitals (Nepal and Cambodia). Exclusion criteria were age <5 years (in Cambodia <18 years because no children were attended in the study site), clinical instability with need for intensive care, or a known diagnosis at presentation. Twelve priority conditions (enteric fever, leptospirosis, rickettsiosis, relapsing fever, brucellosis, melioidosis, visceral leishmaniasis, human African trypanosomiasis, amebic liver abscess, malaria, tuberculosis, and HIV) were preselected as they are (locally) epidemiologically relevant, severe, and treatable (in LRS). For these conditions, reference diagnostic tests were systematically performed, whereas for viral infections diagnostics such as molecular assays and antigen tests were not available. Expert clinicians reviewed case files with the results of all reference and diagnostic tests (including WBC count) and assigned 1 or more final diagnoses based on preestablished case definitions for probable and confirmed priority conditions, or for the other, nonpriority diagnoses, clinical judgment and available test results (diagnostic criteria and reference tests performed are shown in Supplementary Table 1).

Table 1. Distribution of Main Diagnoses According to the Relevant Categories and Subcategories in the Overall Study Population (n = 1838)

Total	n = 1838
Bacterial infections	628 (34.2)
Severe bacterial infections	227 (11.8)
Enteric fever[a]	26 (1.4)
Melioidosis[a]	15 (0.8)
Other bloodstream infections[b]	18 (1.0)
Clinical sepsis[c]	8 (0.4)
Bacterial meningitis (confirmed or presumed)	4 (0.2)
Pneumonia	127 (6.9)
Acute abdominal infections	36 (2.0)
Other bacterial infections	411 (22.3)
Relapsing fever[a]	9 (0.5)
Brucellosis[a]	28 (1.5)
Leptospirosis[a]	64 (3.5)
Rickettsiosis[a]	38 (2.1)
Tuberculosis[a]	120 (6.5)
Urinary tract infection	116 (6.3)
Skin/soft tissue infection	23 (1.3)
Pelvic inflammatory disease	4 (0.2)
Presumed bacterial dysentery	5 (0.3)
Other	4 (0.2)
Nonbacterial infections	431 (23.4)
Suspected viral infection (respiratory/other)	165 (8.9)
New HIV diagnosis/opportunistic infection (other than tuberculosis)[a]	12 (0.7)
Malaria[a]	131 (7.1)
Visceral leishmaniasis[a]	104 (5.7)
Amoebic liver abscess[a]	10 (0.5)
Other infections (parasitic, fungal)	9 (0.5)
Noninfectious causes	34 (1.8)
Unknown/unspecified cause	745 (40.5)

All results are presented as No. (%).

[a]The target priority conditions in the source study, for which disease-specific diagnostic tests were performed.

[b]All subjects with positive blood cultures, excluding contaminants and *Salmonella, Brucella,* and *Burkholderia pseudomallei* species; of these, 17 cases presented with a clinical focus for bloodstream infection and were included as one of the "other bacterial infections" (10 cases) or another "severe bacterial infection" (7 cases).

[c]Includes patients with clinical presentation of sepsis or septic shock. One of these was diagnosed with infected endocarditis and 3 with strongyloidiasis hyperinfection syndrome (all in Cambodia).

Disease Categories

For the present study, we retained both probable and confirmed cases for all diagnoses in the NIDIAG persistent fever study. We grouped final diagnoses into relevant disease

categories and subcategories (Table 1). Among all bacterial infections, we defined the subcategory "severe bacterial infections," including enteric fever, melioidosis, other bloodstream infections (BSIs), clinical sepsis, bacterial meningitis, pneumonia, and acute abdominal infections. For these infections, broad-spectrum antibiotic treatment cannot be delayed, and a good excluding power of the biomarkers is therefore paramount. The diagnosis "other BSI" consisted of all subjects with positive blood cultures, after excluding contaminants and *Salmonella, Brucella,* and *Burkholderia pseudomallei* species, the latter 3 being analyzed separately as they were "target priority conditions" in the NIDIAG study.

Diagnostic Procedures

WBC count was measured as part of the on-site evaluation in the NIDIAG study, using available methods at the routine laboratories of the study sites, in line with NIDIAG good clinical laboratory practice standards [18]. For the present study, we measured CRP and PCT levels on archived serum samples from the NIDIAG study. After on-site processing, we divided each serum sample into 2 aliquots, stored them in liquid nitrogen, and shipped them on dry ice to the Institute of Tropical Medicine, Antwerp, Belgium, to be stored in the biobank at −80°C. Biomarker measurement took place in April–May 2018 using Vitros Chemistry Products CRP Slides (Ortho-Clinical Diagnostics, Rochester, NY, USA) on the Vitros 5600 Integrated System (Ortho-Clinical Diagnostics) and BRAHMS PCT-sensitive Kryptor (Thermo Fisher Scientific, BRAHMS GmbH, Hennigsdorf, Germany) on BRAHMS Kryptor Compact PLUS (Thermo Fisher Scientific, BRAHMS GmbH). These assays had a limit of detection of 5 mg/L for CRP and of 0.075 µg/L for PCT. CRP levels <10 mg/L and PCT levels <0.1 µg/L are considered the reference values by the manufacturers and will henceforward be referred to as "normal values." For WBC count, the cutoff for normal values was set at 11 000/µL [20].

Data Analysis

We pragmatically excluded cases with multiple diagnoses when it was not clear which was the true cause of fever or how each diagnosis could have influenced biomarker production. However, we retained cases if 1 of multiple diagnoses likely explained the other (eg, in cases of leptospirosis with a secondary diagnosis of pneumonia, only leptospirosis was assigned). In some cases in hyperendemic settings (DRC), we considered low malaria parasitemia to be an accidental bystander to a more likely alternative diagnosis and retained only the latter [21].

We summarized biomarker levels using median and interquartile range (IQR) and visualized them using box plots. For CRP and PCT values below the limit of detection, we artificially set values for calculation at 2.5 mg/L and 0.01 µg/L, respectively. We compared biomarker levels using the Wilcoxon rank-sum test for 2-group comparison and the Kruskal-Wallis test for multigroup comparison.

We generated receiving operating characteristic (ROC) curves, compared the area under the ROC curve (AUROC) between CRP, PCT, and WBC count using the approach of Delong et al. [22], and identified optimal cutoffs using the Youden index [23]. We calculated sensitivity, specificity, and positive and negative likelihood ratios (LRs) at these optimal cutoffs, as well as at the commonly used predefined cutoffs that are provided on commercially available semiquantitative biomarker RDTs [10].

SAS 9.4 was used for statistical analyses. We used the 2015 Standards for the Reporting of Diagnostic Accuracy Studies (STARD) (for details, see checklist in Supplementary Table 2) [24].

Patient Consent

The source NIDIAG study was conducted under a specifically designed ethical charter [25] and was registered at clinicaltrials.gov under the identifier NCT01766830. Ethics approval was provided by the Institutional Review Board (IRB) of the Institute of Tropical Medicine (ITM) in Antwerp, the ethical committee of the University Hospital of Antwerp, and at least 1 accredited ethical committee or IRB in the 4 study countries. All study participants gave written informed consent. It was stated that clinical data could be used for additional study purposes and that the samples would be stored for 10 years for possibly conducting additional investigations. Therefore, no additional ethical committee clearance was required for this post hoc biomarker study, apart from a notification to the ITM IRB.

RESULTS

Study Population

Baseline clinical and demographic characteristics have been described in detail previously. Around 20% of subjects were aged <18 years [19]. Serum samples were available for a total of 1919 subjects in the source study. Eighty-one subjects were excluded because their fever possibly had multiple causes, leaving 1838 subjects for biomarker evaluation. Due to an insufficient amount of sample or technical problems, biomarker levels could not be determined in 61 samples for CRP and 127 samples for PCT, leaving 1777 (96.7%) and 1711 (93.1%) samples with available CRP and PCT levels, respectively. WBC count was available for 1762 subjects (95.9%).

Diagnoses as identified in the source study are shown in Table 1, ordered by disease category. A single disease-specific diagnosis was identified in 1093 subjects (59.5%), while for 745 subjects (40.5%) the diagnosis remained unknown. Bacterial infections represented 34.2% of the cohort (628 subjects), and the predefined subcategory of severe bacterial

infections represented 11.8% (227 subjects). Viral infections were suspected in <10%. Malaria was the most frequent single diagnosis (7.1%), followed by pneumonia (6.9%) and tuberculosis (6.5%).

Biomarker Levels

Figure 1 shows box plots describing CRP and PCT levels across disease categories. Box plots for selected individual diagnoses are shown in Supplementary Figure 1. CRP and WBC count levels were significantly higher in subjects with bacterial infections compared with nonbacterial infections and unknown/unspecified causes of fever ($P < .001$), but not compared with noninfectious causes ($P = .57$ for CRP; $P = .60$ for WBC). PCT levels were significantly higher in bacterial infections compared only with unknown/unspecified causes ($P < .001$), and not with nonbacterial infections ($P = .79$) or noninfectious causes ($P = .54$). A longer duration of fever was inversely correlated to both CRP (Spearman correlation = −0.067; $P = .006$) and PCT levels (Spearman correlation = −0.086; $P < .001$).

Diagnostic Accuracy for All and Severe Bacterial Infections

The AUROC (left panels of Figure 2) associated with all bacterial infections was higher for CRP (0.669) and WBC count (0.624) compared with PCT (0.600; $P < .001$), and not significantly different comparing CRP with WBC count ($P = .57$). For identifying the subcategory of severe bacterial infections, AUROCs were generally higher than for all bacterial infections, but were not significantly different comparing the 3 tests (0.726 for CRP; 0.689 for PCT; 0.686 for WBC).

Sensitivities, specificities, and LRs associated with disease categories are shown in the right panel of Figure 2. For comparison, the results of an analogous analysis that included only subjects who met the case definitions for confirmed cases are shown in Supplementary Table 3. For all bacterial infections, sensitivity was <80% for both CRP and PCT even at the cutoff for normal values; it was higher for CRP than for PCT (76.3% for CRP >10 mg/L and 62.4% for PCT >0.1 µg/L; $P < .001$). WBC count at the commonly used cutoff of 11 000/µL showed a sensitivity of 30.5%. Only a CRP >80 mg/L and WBC count >11 000/µL reached a positive LR >2.

For identifying the subcategory of severe bacterial infections, sensitivity was highest (88.2%) for CRP >10 mg/L, while for PCT >0.1 µg/L it was 76.8%. The lowest negative LR (0.27) was obtained with a normal CRP value. Positive LR exceeded 2.5 for CRP >80 mg/L, PCT >2 µg/L, and WBC count >11 000/µL.

Diagnostic Accuracy for Selected Bacterial Infections

Table 2 shows sensitivities, specificities, and LRs associated with selected specific infections. As the main interest for practical use is to exclude these infections, values are shown only for the 2 lowest studied cutoffs for CRP and PCT.

Among the 25 cases of blood culture–proven enteric fever, none showed a CRP below normal values, and only 1 showed a CRP ≤20 mg/L (sensitivity, 96% for CRP >20 mg/L). Two cases, however, showed normal PCT values (sensitivity, 92%).

For pneumonia (n = 122), sensitivity was <90% for both biomarkers, even at the cutoffs for normal values. Of note, results were similar for radiologically confirmed cases compared with those without x-ray confirmation.

For tuberculosis (n = 120 for CRP, n = 119 for PCT), sensitivity was 95% for CRP and 72.3% for PCT at the cutoff for normal values.

For brucellosis and leptospirosis, the sensitivity of both biomarkers at values above the normal cutoff was ∼60% at best. For rickettsiosis, CRP performed somewhat better (sensitivity, 83.3%), but PCT did not (sensitivity, 67.6%).

Potential Impact of Biomarker Results on Antibiotic Use

As detailed elsewhere, about 22% of the participants had been exposed to antibiotics before study inclusion [26]. At study inclusion (and within 1 day after inclusion), antibiotics were prescribed to 68.9% of all subjects (1267/1838). Among those, 44.5% (546/1226) and 54.6% (645/1182) in retrospect appeared to show normal CRP (<10 mg/L) and PCT (<0.1 µg/L) values, respectively (data not shown).

Among the 465 subjects with a nonbacterial cause of fever (the categories "nonbacterial infection" and "noninfectious cause" combined), 261 (56%) received antibiotics at inclusion. Of these unnecessary treatments, 39.6% (99/250) and 51.5% (122/237) were given to patients with normal CRP and PCT values, respectively, and could have therefore been avoided using biomarker-guided treatment. However, in comparison, among subjects with bacterial infections 76.8% (482/628) received antibiotics, and of these necessary treatments, 25.4% (120/473) and 39.0% (183/469) were given to subjects with normal CRP and PCT values, respectively, and could have therefore been erroneously withheld if biomarker-guided treatment was applied.

DISCUSSION

Few data inform the approach to persistent fever in tropical settings, and few clinical and basic laboratory features have some discriminative value in this clinical scenario [19]. Diagnostic uncertainty often leads to antibiotic escalation, which was also reflected in this study, as almost 70% of participants were given antibiotics at initial evaluation. Here, both inflammatory biomarkers CRP and PCT performed unsatisfactorily to exclude all bacterial infections, whereas for the subcategory of severe bacterial infections normal levels of CRP, but not of PCT and WBCs, had moderate to good performance. For confirmation of bacterial infections, both CRP and PCT underperformed, with weak positive LRs even at high serum levels.

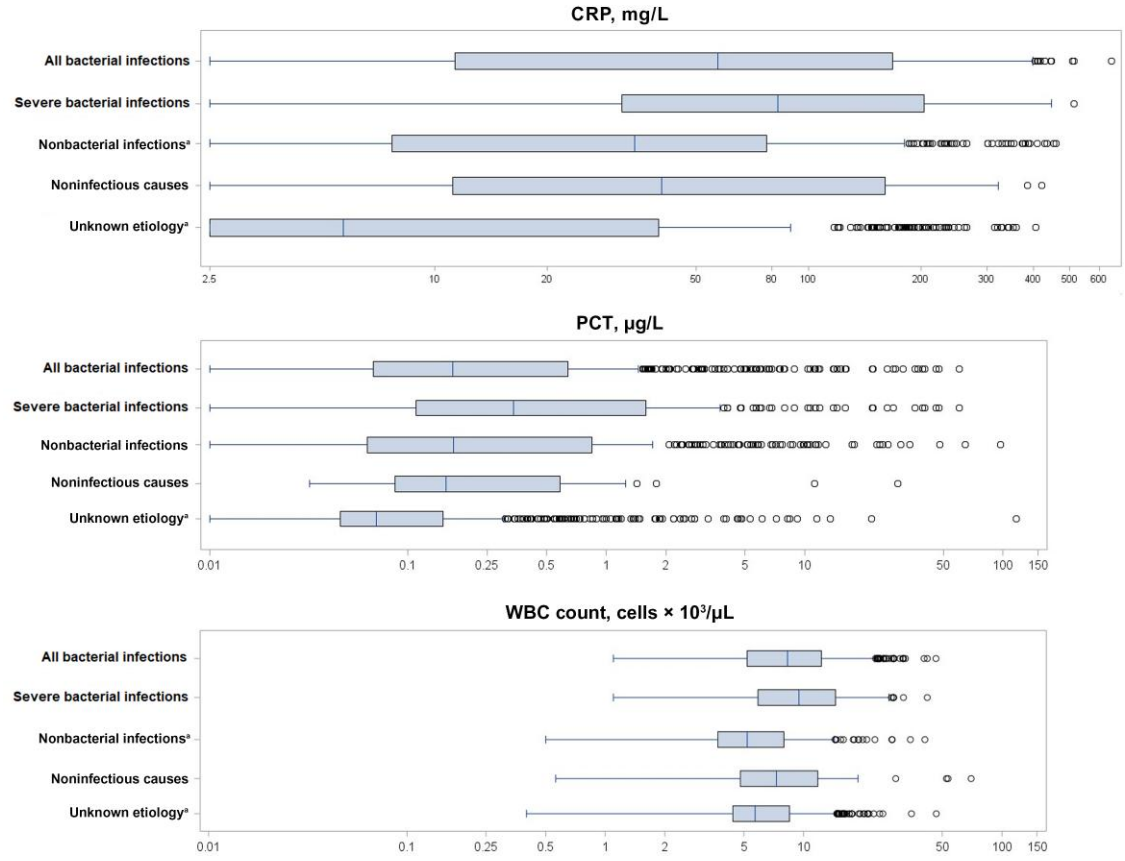
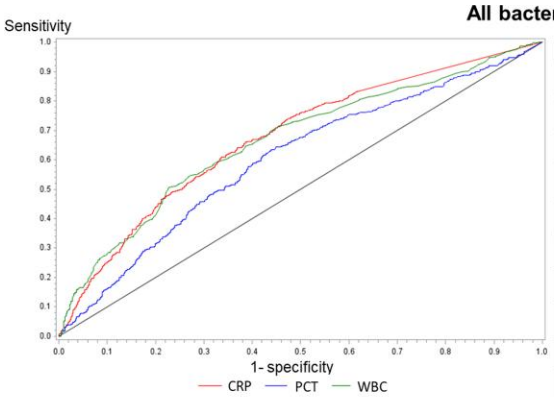


Figure 1. Box plots (median and interquartile range) for CRP, PCT, and WBC values in etiological categories. [a]Disease categories with significantly lower biomarkers levels as compared with the category bacterial etiology (Wilcoxon rank-sum test, $P < .001$). No comparison was made between bacterial etiology and severe bacterial infections as these categories are not mutually exclusive. Abbreviations: CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood cell.

Among subjects who were prescribed antibiotics, a large proportion (at least 40%) appeared to show normal CRP levels, which denotes the potential of this biomarker to reduce antibiotic use also in persistent fever.

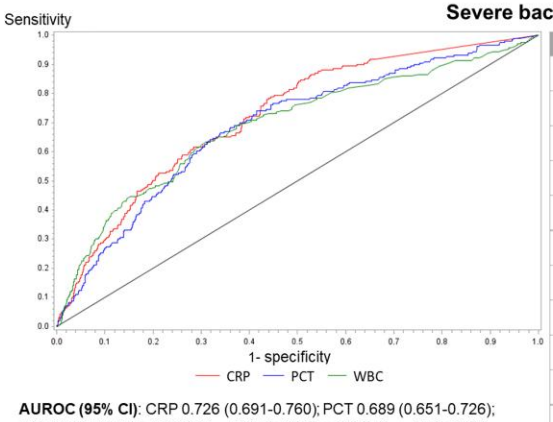
In this study, we managed to evaluate the diagnostic accuracy of CRP and PCT in large prospective cohorts in different tropical settings, and the extensive workup using disease-specific reference tests allowed us to study their diagnostic performance for identifying the preselected priority conditions. However, there were several methodological limitations, some of which were related to the source NIDIAG study itself; these have been discussed in detail previously [19]. Most importantly, the diagnostic tools focused mainly on the target priority conditions, leaving some diagnostic uncertainty for other diseases, sometimes applying merely clinical criteria. No diagnostic tests for viral pathogens were performed, although we assumed that the inclusion criteria of fever ≥7 days would have reasonably excluded most cases of acute respiratory or undifferentiated viral illnesses such as dengue, chikungunya, and influenza. Although strict diagnostic criteria were applied for most bacterial infections, the results of WBC count (as opposed to CRP and PCT, which were analyzed later) were not blinded during diagnostic ascertainment, which could have introduced bias to the diagnostic accuracy analysis [27]. In addition to the limitations related to the source study, one must acknowledge that the current study has been retrospectively implemented, using additional analysis in combination with available data from the source study, which was not specifically designed for the research questions addressed in this paper. Also, patients infected with multiple pathogens were excluded from this secondary analysis, aiming to describe diagnostic performance for each infection individually. However, doing so we accepted that this does not entirely reflect real-life situations, as co-infections occur and furtherly increase diagnostic uncertainty [28]. Other limitations are more technical, such as the delay (3–4 years) between clinical sampling and laboratory processing, although previous work showed little decay of both biomarkers if stored in appropriate conditions [29, 30]. Also, CRP and PCT levels were determined using reference assays on stored serum, rather than RDTs performed in the field. Despite good correlation observed between both methods in field studies, accuracy is usually a bit lower using RDTs [31]. Lastly, only 1 blood sample at



All bacterial infections

	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	LR+	LR-
CRP >10 mg/L	469/615 (76.3; 72.7-79.5)	556/1162 (47.8; 45.0-50.7)	1.46	0.50
CRP >20 mg/L	422/615 (68.6; 64.8-72.2)	649/1162 (55.9; 53.0-58.7)	1.55	0.56
CRP >38 mg/L	374/615 (60.8; 56.9-64.6)	765/1162 (65.8; 63.1-68.5)	1.78	0.60
CRP >80 mg/L	232/615 (37.7; 34.0-41.6)	965/1162 (83.0; 80.8-85.1)	2.23	0.75
CRP >100 mg/L	207/615 (33.7; 30.0-37.5)	993/1162 (85.5; 83.3-87.4)	2.31	0.78
PCT >0.09 µg/L	398/609 (65.4; 61.5-69.0)	575/1102 (52.2; 49.2-55.1)	1.37	0.66
PCT >0.1 µg/L	380/609 (62.4; 58.5-66.2)	621/1102 (56.4; 53.4-59.3)	1.43	0.67
PCT >0.5 µg/L	183/609 (30.0; 26.5-33.8)	900/1102 (81.7; 79.3-83.8)	1.64	0.86
PCT >2 µg/L	79/609 (13.0; 10.5-15.9)	1008/1102 (91.5; 89.7-93.0)	1.52	0.95
PCT >10 µg/L	26/609 (4.3; 2.9-6.2)	1078/1102 (97.8; 96.8-98.5)	1.96	0.98
WBC >8000/µL	311/604 (51.5; 47.5-55.5)	851/1158 (73.5; 70.9-76.0)	1.94	0.66
WBC >11 000/µL	184/604 (30.5; 26.9-34.2)	1007/1158 (87.0; 84.9-88.8)	2.34	0.80

AUROC (95% CI): CRP 0.669 (0.643-0.695); PCT 0.600 (0.572-0.629); WBC 0.651 (0.624-0.679). $P < .001$ for CRP vs PCT and WBC count vs PCT; $P = .57$ for CRP vs WBC count.
Optimal cutoff: CRP 38 mg/L; PCT 0.09 µg/L; WBC 8000/µL



Severe bacterial infections

	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	LR+	LR-
CRP >10 mg/L	194/220 (88.2; 83.2-91.8)	676/1557 (43.4; 41.0-45.9)	1.56	0.27
CRP >20 mg/L	179/220 (81.4; 75.7-86.0)	801/1557 (51.4; 49.0-53.9)	1.68	0.36
CRP >26 mg/L	174/220 (79.1; 73.2-83.9)	859/1557 (55.2; 52.7-57.6)	1.76	0.38
CRP >80 mg/L	113/220 (51.4; 44.8-57.9)	1241/1557 (79.7; 77.6-81.6)	2.53	0.61
CRP >100 mg/L	105/220 (47.7; 41.2-54.3)	1286/1557 (82.6; 80.6-84.4)	2.74	0.63
PCT >0.1 µg/L	169/220 (76.8; 70.8-81.9)	799/1491 (53.6; 51.1-56.1)	1.66	0.43
PCT >0.17 µg/L	147/220 (66.8; 60.4-72.7)	975/1491 (65.4; 62.9-67.8)	1.93	0.51
PCT >0.5 µg/L	95/220 (43.2; 36.8-49.8)	1201/1491 (80.5; 78.5-82.5)	2.22	0.71
PCT >2 µg/L	48/220 (21.8; 16.9-27.7)	1366/1491 (91.6; 90.1-92.9)	2.60	0.85
PCT >10 µg/L	18/220 (8.2; 5.2-12.6)	1459/1491 (97.9; 97.0-98.5)	3.81	0.94
WBC >8000/µL	133/215 (61.9; 55.2-68.1)	1062/1547 (68.6; 66.3-70.9)	1.97	0.56
WBC >11 000/µL	94/215 (43.7; 37.3-50.4)	1306/1547 (84.4; 82.5-86.1)	2.81	0.67

AUROC (95% CI): CRP 0.726 (0.691-0.760); PCT 0.689 (0.651-0.726); WBC 0.686 (0.645-0.726). $P = .049$ for CRP vs PCT; $P = .15$ for CRP versus WBC count; $P = .94$ for WBC count vs PCT
Optimal cutoff (Youden): CRP 26 mg/L; PCT 0.17 µg/L; WBC 8000/µL

Figure 2. Left panels: ROC curves associated with all bacterial infections and severe bacterial infections in the total patient population, with optimal cutoffs according to the Youden index, and comparison between the AUROCs. Right panels: diagnostic performance of the biomarkers at selected cutoffs (including optimal cutoff) for all bacterial infections and severe bacterial infections. Sensitivity and specificity are reported as n/n (%; 95% CI). Abbreviations: AUROC, area under the ROC curve; CRP, C-reactive protein; LR+, positive likelihood ratio; LR-, negative likelihood ratio; PCT procalcitonin; ROC, receiver operating characteristics.

presentation was evaluated for biomarker levels, not allowing evaluation of serial measurements in time.

This study is the first to explore the diagnostic value of inflammatory biomarkers in persistent fever syndrome in the tropics, so sound comparisons with other published experience is difficult. While in studies on AFI, sensitivity of around or above 90% is often reached for CRP [10, 13], here it was <80% for both CRP and PCT, even at lower cutoff values, which is considered insufficient to safely exclude all bacterial infections [32]. For many diseases, the inflammatory profile likely spontaneously varies (and decreases) over time. Also, many patients (>20%) were (partially) treated with antibiotics before study evaluation, with a possible effect on biomarker levels. For the subcategory of severe bacterial infections, however, CRP >10 mg/L did approach a sensitivity of 90% (with a negative LR of 0.27). The distinction of this category is not trivial, as these infections usually require immediate antibiotic treatment, and negative CRP levels could therefore allow delay of specific treatment while awaiting the results of additional targeted testing for less severe infections. However, disease-specific testing for tropical infections is very difficult as RDTs generally do not exist, or they perform poorly, as we also have recently shown [19], while both serological and molecular tests still have important limitations, even in experienced laboratories [33]. Also, cautious use with respect to the clinical status of the patient remains absolutely necessary, as a few life-threatening diseases (mostly pneumonia) remained undetected at the lower cutoff for CRP. Additionally, the value of biomarkers is less clear in some vulnerable groups such as malnourished children and immunosuppressed patients [34].

As CRP and PCT levels above the normal cutoffs showed higher sensitivity than WBC count above the most commonly used cutoff of 11 000 cells/µL, both studied biomarkers seem more appropriate to decide for which patients antibiotics can

Table 2. Diagnostic Performance of the Biomarkers at Selected Cutoffs for Selected Diagnoses in the Study Cohort

	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	LR+	LR–
Enteric fever				
CRP >10 mg/L	25/25 (100.0; 86.7–100.0)	702/1752 (40.1; 37.8–42.4)	1.67	0.00
CRP >20 mg/L	24/25 (96.0; 80.5–99.3)	841/1752 (48.0; 45.7–50.3)	1.85	0.08
PCT >0.1 µg/L	23/25 (92.0; 75.0–97.8)	848/1686 (50.3; 47.9–52.7)	1.85	0.16
PCT >0.5 µg/L	10/25 (40.0; 23.4–59.3)	1311/1686 (77.8; 75.7–79.7)	1.80	0.77
WBC >11 000/µL	0/23 (0.0; 0.0–14.3)	1404/1739 (80.7; 78.8–82.5)	0.00	1.24
Melioidosis				
CRP >10 mg/L	14/15 (93.3; 70.2–98.8)	701/1762 (39.8; 37.5–42.1)	1.55	0.17
CRP >20 mg/L	14/15 (93.3; 70.2–98.8)	841/1762 (47.7; 45.4–50.1)	1.79	0.14
PCT >0.1 µg/L	15/15 (100.0; 79.6–100.0)	850/1696 (50.1; 47.7–52.5)	2.00	0.00
PCT >0.5 µg/L	10/15 (66.7; 41.7–84.8)	1321/1696 (77.9; 75.9–79.8)	3.02	0.43
WBC >11 000/µL	12/15 (80.0; 54.8–93.0)	1424/1747 (81.5; 79.6–83.3)	4.33	0.25
Other bloodstream infections				
CRP >10 mg/L	18/18 (100.0; 82.4–100.0)	702/1759 (39.9; 37.6–42.2)	1.66	0.00
CRP >20 mg/L	18/18 (100.0; 82.4–100.0)	842/1759 (47.9; 45.5–50.2)	1.92	0.00
PCT >0.1 µg/L	17/18 (94.4; 74.2–99.0)	849/1693 (50.1; 47.8–52.5)	1.89	0.11
PCT >0.5 µg/L	13/18 (72.2; 49.1–87.5)	1321/1693 (78.0; 76.0–79.9)	3.29	0.36
WBC >11 000/µL	10/15 (66.7; 41.7–84.8)	1422/1747 (81.4; 79.5–83.2)	3.58	0.41
Probable pneumonia[a]				
CRP >10 mg/L	102/122 (83.6; 76.0–89.1)	682/1655 (41.2; 38.9–43.6)	1.42	0.40
CRP >20 mg/L	95/122 (77.9; 69.7–84.3)	815/1655 (49.2; 46.8–51.7)	1.53	0.45
PCT >0.1 µg/L	80/122 (65.6; 56.8–73.4)	808/1589 (50.8; 48.4–53.3)	1.33	0.68
PCT >0.5 µg/L	46/122 (37.7; 29.6–46.6)	1250/1589 (78.7; 76.6–80.6)	1.77	0.79
WBC >11 000/µL	54/124 (43.5; 35.1–52.3)	1357/1638 (82.8; 80.9–84.6)	2.54	0.68
Confirmed pneumonia[a]				
CRP >10 mg/L	84/98 (85.7; 77.4–91.3)	688/1679 (41.0; 38.6–43.3)	1.45	0.35
CRP >20 mg/L	80/98 (81.6; 72.8–88.1)	824/1679 (49.1; 46.7–51.5)	1.60	0.37
PCT >0.1 µg/L	69/98 (70.4; 60.7–78.5)	821/1613 (50.9; 48.5–53.3)	1.43	0.58
PCT >0.5 µg/L	42/98 (42.9; 33.5–52.7)	1270/1613 (78.7; 76.7–80.7)	2.02	0.73
WBC >11 000/µL	46/97 (47.4; 37.8–57.3)	1376/1665 (82.6; 80.7–84.4)	2.73	0.64
Probable tuberculosis[b]				
CRP >10 mg/L	114/120 (95.0; 89.5–97.7)	696/1657 (42.0; 39.6–44.4)	1.64	0.12
CRP >20 mg/L	110/120 (91.7; 85.3–95.4)	832/1657 (50.2; 47.8–52.6)	1.84	0.17
PCT >0.1 µg/L	86/119 (72.3; 63.6–79.5)	817/1592 (51.3; 48.9–53.8)	1.48	0.54
PCT >0.5 µg/L	35/119 (29.4; 22.0–38.1)	1242/1592 (78.0; 75.9–80.0)	1.34	0.90
WBC >11 000/µL	39/117 (33.3; 25.4–42.3)	1349/1645 (82.0; 80.1–83.8)	1.85	0.81
Confirmed tuberculosis[b]				
CRP >10 mg/L	80/81 (98.8; 93.3–99.8)	701/1696 (41.3; 39.0–43.7)	1.68	0.03
CRP >20 mg/L	78/81 (96.3; 89.7–98.7)	839/1696 (49.5; 47.1–51.8)	1.91	0.07
PCT >0.1 µg/L	64/80 (80.0; 70.0–87.3)	834/1631 (51.1; 48.7–53.6)	1.64	0.39
PCT >0.5 µg/L	28/80 (35.0; 25.5–45.9)	1274/1631 (78.1; 76.0–80.1)	1.60	0.83
WBC >11 000/µL	29/78 (37.2; 27.3–48.3)	1378/1684 (81.8; 79.9–83.6)	2.05	0.77
Brucellosis				
CRP >10 mg/L	15/26 (57.7; 38.9–74.5)	691/1751 (39.5; 37.2–41.8)	0.95	1.07
CRP >20 mg/L	12/26 (46.2; 28.8–64.5)	828/1751 (47.3; 45.0–49.6)	0.88	1.14
PCT >0.1 µg/L	12/26 (46.2; 28.8–64.5)	836/1685 (49.6; 47.2–52.0)	0.92	1.09
PCT >0.5 µg/L	4/26 (15.4; 6.2–33.5)	1304/1685 (77.4; 75.3–79.3)	0.68	1.09
WBC >11 000/µL	0/28 (0.0; 0.0–12.1)	1399/1734 (80.7; 78.8–82.5)	0.00	1.24
Leptospirosis				
CRP >10 mg/L	38/63 (60.3; 48.0–71.5)	677/1714 (39.5; 37.2–41.8)	1.00	1.00
CRP >20 mg/L	32/63 (50.8; 38.8–62.7)	811/1714 (47.3; 45.0–49.7)	0.96	1.04
PCT >0.1 µg/L	27/60 (45.0; 33.1–57.5)	817/1651 (49.5; 47.1–51.9)	0.89	1.11
PCT >0.5 µg/L	10/60 (16.7; 9.3–28.0)	1276/1651 (77.3; 75.2–79.2)	0.73	1.08
WBC >11 000/µL	17/63 (27.0; 17.6–39.0)	1381/1699 (81.3; 79.4–83.1)	1.44	0.90
Rickettsiosis				
CRP >10 mg/L	30/36 (83.3; 68.1–92.1)	696/1741 (40.0; 37.7–42.3)	1.39	0.42
CRP >20 mg/L	27/36 (75.0; 58.9–86.2)	833/1741 (47.8; 45.5–50.2)	1.44	0.52

Table 2. Continued

	Sensitivity (%; 95% CI)	Specificity (%; 95% CI)	LR+	LR–
PCT >0.1 µg/L	25/37 (67.6; 51.5–80.4)	838/1674 (50.1; 47.7–52.5)	1.35	0.65
PCT >0.5 µg/L	16/37 (43.2; 28.7–59.1)	1305/1674 (78.0; 75.9–79.9)	1.96	0.73
WBC >11 000/µL	6/36 (16.7; 7.9–31.9)	1397/1726 (80.9; 79.0–82.7)	0.87	1.03

Sensitivity and specificity are reported as n/n (%; 95% CI).

Abbreviations: CRP, C-reactive protein; LR+, positive likelihood ratio; LR-, negative likelihood ratio; PCR, polymerase chain reaction; PCT, procalcitonin.

[a]Probable pneumonia: clinical diagnosis based on fever, cough, presence of crackles on lung auscultation. Confirmed pneumonia required clinical suspicion and lung infiltrate on x-ray.

[b]Tuberculosis could be confirmed by microscopy, culture, or PCR, or classified as probable based on clinical criteria (see Supplementary Table 1 for case definition).

be omitted. Similarly, CRP more reliably excluded (severe) bacterial infections than PCT, which has also been seen in AFI studied in the tropics [35–37]. For practical use in LRS, CRP would probably be preferred over PCT, also since more CRP-based RDTs are available and often at a lower cost [10, 31].

Using the biomarkers for ruling in bacterial infections appeared difficult, as positive LRs remained <2.5 even at higher biomarker levels. This is likely explained by the larger heterogeneity of etiologies in persistent fever syndrome, while AFI is predominantly caused by viral illnesses in the tropics [1]. On the other hand, as the prevalence (pretest probability) of severe bacterial infections in the cohort was ∼10% and CRP levels >80 mg/L showed a positive LR of ∼3, the resulting post-test probability of ∼20%–25% can be considered above the threshold for prescribing lifesaving antibiotic treatment.

The overall size of the cohort allowed us to study diagnostic accuracy for some pooled individual diagnoses. No cases of (blood culture–positive) enteric fever showed CRP levels <10 mg/L. With some reservations due to the rather low number of cases (n = 25), this would mean that normal CRP values allow for exclusion of enteric fever with more certainty than any typhoid RDT [38]. Also of interest was the good excluding power for tuberculosis at lower cutoffs for CRP. Previously, similar results were observed, and it has been proposed to use CRP in LRS as a screening tool to identify patients for whom additional testing targeting tuberculosis could be omitted [39]. Conversely, for pneumonia excluding power was somewhat lower, with CRP again appearing more useful than PCT. Finally, among the zoonotic infections, sensitivity for brucellosis and leptospirosis was disappointing, and it was slightly better for rickettsiosis. A study on AFI found better results (sensitivity of 87% for leptospirosis, rickettsiosis, *Coxiella*, and brucellosis combined) [40], suggesting more inflammation in the early stages of these diseases. As generic biomarkers could not help identify these conditions, the need for improved disease-specific field diagnostics is even more pressing [19].

In conclusion, the results of our study suggest that normal CRP values can help exclude severe and some selected bacterial infections in patients with persistent fever in the tropics. Confirming power is more limited, although high biomarker levels may support the prescription of antibiotics. If used with careful clinical consideration or integrated in validated diagnostic aid tools [41], inflammatory biomarkers might be of value to limit immediate antibiotic prescription in a rather large group of patients presenting with persistent fever, who are currently almost systematically treated with antibiotics in LRS. Whether such a strategy could reduce antibiotic use in a safe way in this particular clinical syndrome remains to be studied in large cluster-randomized or observational prospective studies.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. L.V., E.B., C.P.Y., M.B., K.V., and F.C. performed the literature search, elaborated the study design, supervised the field study, interpreted the data, and wrote the different drafts of the manuscript. J.B. and A.T. contributed to the data analyses and interpretation. B.B., J.J., M.V.E., and K.R. performed the clinical study in the different fields and/or conducted the laboratory analyses either on site or in reference laboratories and participated in the data collection. All authors contributed to the writing and read and approved the final manuscript.

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