Review Article

Usefulness of C-Reactive Protein and Other Host BioMarker Point-of-Care Tests in the Assessment of Non-Malarial Acute Febrile Illnesses: A Systematic Review with Meta-Analysis

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Abstract. In low- and middle-income countries, in resource-limited settings, the implementation of diagnostic tools discriminating bacterial from nonbacterial fever is a matter of primary concern. The introduction of malaria rapid diagnostic tests highlighted the need for point-of-care tests (POCTs) supporting clinical decision-making for non-malarial febrile illnesses. The purpose of this work was to review the use of host biomarker POCTs for the assessment of acute non-malarial fever in resource-constraint settings. Specific objectives were as follows: 1) to estimate the accuracy of such tests in differentiating fever of bacterial from nonbacterial origin and 2) to assess the impact of host biomarkers on antibiotic prescription and clinical outcome. We conducted a systematic review searching PubMed, Embase, the Cochrane Library, and Bireme. The protocol was registered with PROSPERO (n CRD42019141735). Data on the accuracy of C-reactive protein (CRP) for the detection of bacterial infections were meta-analyzed using the hierarchical summary receiver operating characteristic model, obtaining a summary ROC (SROC). We identified 2,192 articles, eight of which were included in the review. Among the different biomarkers evaluated, CRP was the one most frequently studied. The SROC presented an area under the curve = 0.77 (CI: 0.73–0.81), which indicates good accuracy to distinguish bacterial from nonbacterial infections. However, the optimal cutoff of CRP could not be assessed, and we found insufficient evidence about its impact on antibiotic prescription and clinical outcome. The role of CRP and other host biomarker POCTs for the assessment of acute non-malarial febrile illnesses in resource-constraint settings deserves further studies.

INTRODUCTION

The management of acute febrile illnesses is a challenge worldwide, especially in low- and middle-income countries (LMICs). Resource-constraint settings have limited access to diagnostics, so the causative agent of fever most commonly remains unknown. Hence, algorithms based on clinical assessment have been implemented, with the aim of improving the management of febrile illnesses. However, this approach has limitations. As an example, the Integrated Management of Childhood Illness (IMCI), an algorithm for the management of childhood infections based on clinical evaluation, does not give proper indications when dealing with febrile children with no localizing symptoms.¹ Therefore, in recent years, attempts to integrate the IMCI with results of diagnostic tests have been made.¹

Since 2010, the deployment of malaria rapid diagnostic tests (RDTs) in endemic countries has made it possible to implement the WHO-recommended test-and-treat policy and more accurately target antimalarial treatment.^{2–4} However, at the same time, this has also created a treatment void for non-malaria fevers, which, because of lack of diagnostic tests, also unintentionally fueled irrational antibiotic prescriptions.^{5,6} Having tests that can assist case management decisions for fever not caused by malaria is therefore of paramount importance both in malaria-endemic and non-endemic areas. A test that could be deployed along the malaria RDT to help

screening out non-malaria, nonbacterial infections would reduce antibiotic overprescribing and improve the management of febrile illnesses. The example of malaria RDTs highlights the importance of point-of-care tests (POCTs). These are, in general, automated, bench-top, or hand-held tests that could be used by staff with limited laboratory skills at the primary healthcare level.⁷ A test that could provide results quickly to the healthcare providers, without involving any specific laboratory equipment, would facilitate appropriate, prompt case management decisions.⁷

In particular, the ideal test to be deployed in LMICs should follow the ASSURED criteria⁸: it should be affordable, sensitive, specific, user friendly, rapid and robust, equipment free, and deliverable to end users. In high-income countries (HICs), C-reactive protein (CRP), procalcitonin (PCT), and white blood cells (WBCs) (total and differential counts) are variably used⁹ to inform rapid clinical assessment of patients with fever presenting to emergency departments. Although these markers can be altered in the presence of several noninfectious conditions (such as inflammation), they are deemed useful as first-line tests in the evaluation of febrile patients.

The impact on antibiotic prescription of CRP and other biomarkers assessed with POCTs, thoroughly reviewed mainly for acute respiratory tract infections,¹⁰ still remains uncertain. Moreover, there is scant evidence on the diagnostic cutoff to differentiate between bacterial and nonbacterial infections,¹¹ and regulatory standards providing guidance for the interpretation of the results in clinical practice still lack.⁴

This work aimed at reviewing biomarker POCTs for acute non-malarial febrile illness in LMICs. Specific objectives were as follows: 1) to estimate the accuracy of such tests in differentiating fevers of bacterial from nonbacterial origin and 2) to

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describe both the impact of these tests on antibiotic prescription and clinical outcomes.

METHODS

A systematic review of the literature with meta-analysis was conducted. The protocol was registered with the PROSPERO international prospective registry of systematic reviews (registration n CRD42019141735). On February 10, 2020, the following databases were searched for relevant studies: PubMed, Embase, the Cochrane Library, and Bireme. The electronic search strategy was as follows: Biomarkers (Biomarker*, Diagnostic Tests, Routine, Biologic* Marker, Host biomarker*, Laboratory Marker*, Serum Marker*, Surrogate Endpoint*, Clinical Marker*, Viral Marker*, Biochemical Marker*, Immune Marker*, Immunologic Marker*, Surrogate Marker*, CPR, c reactive protein, diagnostic test, point of care) AND Fever (Fever*, Pyrexia*, Hyperthermia*, malarial febrile illness) AND LMICs (Poverty [MeSH Terms], Poverty Areas, low income, resource limited setting, middle income, low income countr*, middle income countr*, Africa [MeSH Terms], Latin America [MeSH Terms], Asia [MeSH Terms], Pacific Islands [MeSH Terms]). No restrictions were applied in relation to the language and the date of publication. We also screened the reference lists of all included studies for other potentially relevant studies and authors' personal collections (gray literature).

Two authors, G. B. and N. R., independently screened the list of articles generated from the electronic search, using the EndNote program, version 6, 2012 (Thomson Reuters, Philadelphia, PA). G. B. and N. R. extracted the data on the basis of the inclusion/exclusion criteria (reported in the following text) and entered all information in a database created with MS Office Excel 2013 (Microsoft Corporation, Redmond, WA). In case of discrepancies in the process of inclusion of articles/data extraction, a consensus was reached through the involvement of a third author (D. B.).

The inclusion criteria included prospective or retrospective studies on host biomarkers POCTs for acute non-malarial fever in LMICs; POCTs were defined as bench-top/handheld devices providing results within 2 hours.⁷ The exclusion criteria included literature reviews, studies conducted in HICs, case reports or case series, overviews on the acceptability (social impact) of POCTs, POCTs for a specific condition (e.g., malnutrition and typhoid fever), or apparatus (e.g., respiratory infections). For the meta-analysis, when an article reported cases of malaria, data were extracted but excluded from the analysis. Unspecified fevers were also excluded.

Statistical analysis and data synthesis. To characterize the study populations, demographic and clinical data were summarized with descriptive statistics using SAS software version 9.4 (SAS Inst., Cary, NC). When sensitivities and specificities of the biomarkers were not reported in the published article, we either contacted the authors or, when possible, extrapolated the data. We used RevMan version 5.3 (Review Manager, Copenhagen, Denmark) to produce coupled forest plots for these parameters.

C-reactive protein data were meta-analyzed using the hierarchical summary receiver operating characteristic (HSROC) model^{12,13} because CRP thresholds varied among studies—a variable cutoff was added to the model as covariate. To aid in data visualization, we present the coupled forest plot and the summary ROC (SROC) estimated by the HSROC model. We do not report summary estimates of sensitivity and specificity because studies

used different CRP cutoffs (as per Rutter and Gatsonis,¹² the SROC is drawn restricted to the range of specificities and sensitivities of the included studies to avoid extrapolation beyond the data). Meta-analysis was performed in SAS software version 9.4 using the METADAS v1.3 macro obtained from Cochrane website 13 and Stata software version 14 (StataCorp, College Station, TX). Parameters estimations are reported with calculated 95% CIs. Small sample size and high heterogeneity precluded fitting a HSROC model for PCT and meta-analyzing WBCs.

Quality assessment. For the qualitative assessment of the articles included in the meta-analysis, we entered data in RevMan version 5.3 and used the QUADAS-2 tool (University of Bristol, Bristol, UK).

RESULTS

The electronic search identified 2,192 articles; the study flow from electronic search to inclusion of articles is described in the flowchart (Figure 1). Eventually, eight articles were included in the review.

All studies were published from 2015 onward. Seven studies were prospective, two of which were randomized controlled trials; one study was retrospective. Table 1 reports the main characteristics of the included articles.

Five studies were conducted in Southeast Asia (SEA: Thailand, Laos, Myanmar, and Cambodia), and three in Africa (Tanzania).

FIGURE 1. Study flowchart.

TABLE 1
Main characteristics of the included articles

Reference	Country	Study design	Studied population	Sample size	Host biomarker	Setting
Althaus et al. ¹⁴	Thailand and Myanmar	Multicentric open RCT*	Adult and children > 1 year	2,410	CRP: NycoCard Reader II (Axis Shield, Oslo, Norway)†	OPD
Wangrangsimakul et al. ¹⁵	Thailand	Monocentric prospective observational study	Adults	200	CRP: NycoCard Reader II (Axis Shield)† PCT: ELISA-based VIDAS PCT (BioMeArieux, Marcy-L'Etoile, France)† WBC* and ANC*	IPD
Mahende et al. ¹⁶	Tanzania	Monocentric retrospective	Children (2–59 months)	867	CRP: Cobas c111 biochemistry analyzer (Roche diagnostics, Indianapolis, IN)† WBC and ANC Melet schloesing MS9-5 (Diamond Diagnostics, Holliston, MA)†	OPD
Keitel et al. ¹	Tanzania	Multicentric RCT*	Children (2–59 months)	3,192	CRP: semi-quantitative assay (bioNexia CRPplus, BiomeArieux)†† PCT: ELISA-based VIDAS PCT (BioMeArieux)†	OPD
Hildenwall et al. ¹⁷	Tanzania	Monocentric prospective	Children (3 months–5 years)	428	CRP: Afinion AS100 Analyzer™ (Axis-Shield)† WBC (HemoCue, Angelholm, Sweden)†	OPD
Phommasone et al. ¹⁸	Laos	Multicentric prospective	All population	837	CRP: DTS233 (Creative Diagnostics, New York, NY)†† CRP: WD-23 (Assure Tech Co., Ltd., Hangzhou, China)†† CRP: semi-quantitative assay (bioNexia CRPplus, BiomeArieux)††	OPD
Lubell et al. ¹⁹	Laos	Multicentric prospective	5–49 years	1,083	CRP: NycoCard Reader II (Axis Shield)†	OPD
Lubell et al. ²⁰	Cambodia Laos Thailand	Multicentric prospective	All population	1,372	CRP: NycoCard Reader II (Axis Shield)† PCT: ELISA-based VIDAS PCT (BioMeArieux)†	OPD

ANC = absolute neutrophil count; CRP = C-reactive protein; IPD = inpatient department; OPD = outpatient department; PCT = procalcitonin; RCT = randomized controlled trial; WBC = white blood cell.

* The article did not report the type of assay.

† POCT = point-of-care test.

‡ RDT = rapid diagnostic test.

The three studies from Africa enrolled children aged from 1 to 59 months; of the five studies from Southeast Asia, four included patients of all ages and one adult only. Seven studies included data from outpatients. The sample size varied from a minimum of 200 to a maximum of 3,192 individuals. Six studies had a sample size larger than 500 patients.

Main biomarkers studied. As shown in Table 1, only three studies evaluated a single host biomarker (namely, CRP), whereas most articles analyzed different host biomarkers. Overall, CRP was investigated in all studies, PCT in three, WBCs in three, and absolute neutrophil counts (ANCs) in two.

C-reactive protein. Different CRP POCTs were evaluated in the reviewed studies, the most frequent (4/8 studies) being NycoCard Reader II (Axis Shield, Oslo, Norway). Only two studies evaluated an RDT (Phommasone et al.¹⁸ and Keitel et al.¹). The main aim of Phommasone et al.¹⁸ was to assess the accuracy of RDTs based on CRP in LMICs. They found that three commercially available CRP-based RDTs (one qualitative and two semi-quantitative tests) provided reliable results when compared with quantitative tests such as the NycoCard Reader II. This study did not analyze the accuracy of the RDT in relation to the etiology of the disease (nonbacterial/bacterial infection).

Only four studies^{15–17,20} reported data on the accuracy of the POCTs in discriminating bacterial from nonbacterial

fevers, but only three of them, based on CRP, were included in the meta-analysis; Mahende et al.'s¹⁶ study was excluded because it did not provide etiological diagnosis. The quality analysis of the three studies included in the meta-analysis is shown in Figure 2; the risk of bias was acceptable (low/unclear) for all considered domains; two studies (Lubell et al.²⁰ and Wangrangsimakul et al.¹⁵) had only one unclear domain

FIGURE 2. Risk of bias and applicability concerns summary. This figure appears in color at www.ajtmh.org.

FIGURE 3. Forest plot for studies on the accuracy of C-reactive protein. This figure appears in color at www.ajtmh.org.

each, whereas Hildenwall et al.¹⁷ had four unclear domains because of incomplete information reported in the article.

The meta-analysis of the accuracy of CRP for the identification of bacterial infections was carried out on six datasets, retrieved from the three included studies (Figure 3). Five different CRP cutoff values were included in the analysis. In general, the higher the cutoff, the lower the sensitivity and the higher the specificity for the values tested within the same study (Lubell et al.²⁰ and Wangrangsimakul et al.¹⁵), but only in the latter, CIs between the 10 and 20 mg/L cutoff did not overlap.

The SROC obtained from the HSROC model, with covariate cutoff, is shown in Figure 4. The SROC represents a prediction of the accuracy of CRP obtained by including in the model the results of different studies, with different CRP cutoff values, which is why the accuracy reported by each study does not lie on the summary curve. Each circle represents one dataset, and their areas are proportional to the sample size of each study. Estimations from Hildenwall et al.¹⁷ with a sensitivity of 44% and Wangrangsimakul et al.¹⁵ (cutoff = 36) fall outside the 95% CI, whereas the only studies with estimation entirely within the CI are Lubell et al.²⁰ (cutoff = 20) and Wangrangsimakul et al.¹⁵ (cutoff = 20). The area under the summary curve (AUC) is 0.77 (CI: 0.73–0.81), which indicates good accuracy of the test, although the best cutoff to obtain this performance could not be assessed.

Procalcitonin. Two studies^{1,20} reported data on the diagnostic accuracy of PCT-based POCTs for the identification of bacterial infections. For three studies,^{1,15,20} different PCT cutoff values (0.1, 0.25, and 0.5 ng/mL) were retrieved from four datasets. Sensitivities and specificities were extrapolated from data reported in each article, and presented in the forest plot (Figure 5). The cutoff 0.5 ng/mL led to different accuracy of PCT, with a higher specificity and sensitivity in Wangrangsimakul et al.¹⁵ Both Lubell et al.²⁰ and Wangrangsimakul et al.¹⁵ conducted their works in Southeast Asia, but the former²⁰ involved people of any age, and the latter only adults.

White blood cell (WBC). The studies evaluating the association between WBC total counts and bacterial versus non-bacterial infections had heterogeneous results. Hildenwall et al.¹⁷ found no difference in WBC counts between children with and without signs of bacterial infections; Mahende et al.¹⁶ found only a weak association between WBC counts $> 15 \times 10^3/\text{mm}^3$ and a positive blood culture; and Wangrangsimakul et al.¹⁵ found that WBC count $< 7.9 \times 10^3/\text{mm}^3$ was predictor for viral infection.

Only two articles analyzed ANCs,^{15,16} and none of them found conclusive results.

Antibiotic prescriptions and clinical outcomes following the introduction of POCTs. Three studies^{1,14,19} examined the effect of POCTs on antibiotic prescription. One modeling study¹⁹ estimated 80% correct antibiotic prescriptions with CRP-based POCTs compared with 52% in routine practice.

The other two studies reported the overall impact on antibiotic prescription by testing strategy. Althaus et al.¹⁴ found that the use of POCTs resulted in a significant decrease in antibiotic prescription for low CRP values, and increased targeted prescription for high CRP values. Keitel et al.¹ compared antibiotic prescription in three different arms: routine care; the ALMANACH (an electronic algorithm derived from the IMCI, including urine dipstick test and a clinical predictor or rapid test for typhoid fever); and ePOCT (an innovative electronic algorithm including CRP and PCT-based POCTs). They observed that the proportion of children who were prescribed an antibiotic was 95%, 29.7%, and 11.5% in the routine care, in the ALMANACH, and in the ePOCT arms, respectively.

Althaus et al.¹⁴ attempted to address the potential impact of POCT–CRP testing on clinical outcome. Despite the improved use of antibiotics, the reported proportion of patients who recovered at days 5 and 14 of follow-up, comparing two CRP testing groups, with thresholds set at 20 mg/L (group A) or 40 mg/L (group B), with a control group (routine practice), was similar across the study groups.

On the other hand, Keitel et al.¹ observed significantly fewer clinical failures by day 7 (49%) when the experimental electronic algorithm was used.

FIGURE 4. Summary receiver operating characteristic displaying the accuracy of C-reactive protein. This figure appears in color at www.ajtmh.org.

FIGURE 5. Forest plot for studies on the accuracy of procalcitonin. This figure appears in color at www.ajtmh.org.

DISCUSSION

In LMICs, the evaluation and deployment of POCTs aimed to improve the accuracy of diagnosis and management of acute fevers are challenging. Little is known about the causes of fever locally, and excluding malaria RDTs, there are few effective pathogen-specific POCTs, which leaves healthcare providers with just their clinical judgment where malaria is not endemic or after excluding malaria. This sustains “just-in-case” antibiotic prescriptions, which is one of the root causes of antimicrobial resistance and inappropriate fever case management.^{5,21} In the absence of pathogen-specific POCTs other than malaria RDTs, the main question is how healthcare providers can be assisted in deciding whether antibiotic treatment is warranted or not. One possibility is using biomarkers that can orient toward a bacterial or nonbacterial cause of infection.⁶

However, there is a series of caveats to consider when interpreting findings and projecting the use of POCTs in clinical practice, including how age and patterns of prevailing infections differ in studies conducted in different geographical areas. Many infections are seasonal, and studies should cover a period long enough to capture these fluctuations; we know for malaria that the positive and the negative predictive values of RDTs differ in high- and low-transmission seasons.²² Furthermore, concomitant conditions like HIV infection and malnutrition could influence the expression of biomarkers, for example, lowering CRP concentrations, thus potentially causing bacterial infections being underdiagnosed and undertreated.²³ Conversely, some biomarkers, CRP in particular, can be elevated in case of malaria,²⁴ which makes it impossible to rule out or rule in other infections in patients with concomitant malaria infection.

We found a limited number of studies to support the use of POCT biomarkers, and we could reasonably draw conclusions only for CRP. These studies show, based on few good-quality studies, that CRP is reasonably accurate in distinguishing bacterial from nonbacterial infections (HSROC AUC 0.77). Although it was not possible to identify the best cutoff, 20 mg/L was the only cutoff lying within the 0.73–0.81 CIs of the AUC. Of note, using the HSROC model with covariate cutoff allowed to include test accuracy data obtained with different thresholds, although this meant not having a summary point for sensitivity and specificity. Limitations mainly relate the heterogeneity of the included studies, which could have influenced the final interpretation. Indeed, the studies were carried out in different geographical/epidemiological contexts, where different degree of coinfections (including malaria) might affect the results of the tests. Moreover, the methodologies, including study design, age-groups of studied populations, and case definition, for bacterial versus nonbacterial infections differed between studies, partially affecting the comparison.

The question then was whether applying different cutoff values for CRP plasma levels impacted antibiotic prescription and clinical outcome. Only three studies addressed these

questions: two^{1,19} for antibiotic prescription and one¹⁴ for clinical outcomes. Results were not entirely consistent, possibly because different populations were studied (adults in Southeast Asia and children in Africa) and different clinical decision algorithms were applied. Because available studies did not address simultaneously the effects of different CRP cutoffs on diagnostic accuracy, antibiotic prescriptions, and clinical outcomes, it is difficult to draw conclusions as to whether using CRP (or possibly other markers) translates into better case management decisions, reducing unnecessary antibiotic prescription while not denying patients’ antibiotics when needed. When the decision to prescribe or not an antibiotic is based on CRP alone (using cutoffs at 20 or 40 mg/L),¹⁴ clinical outcome was not affected as compared with a management decision made on clinical ground. The best prospects in providing better care are likely when biomarkers (CRP and PCT) are used in the context of improved electronic algorithms, but so far this evidence comes from only one non-inferiority study.¹

Another question that still remains unanswered is if the deployment of algorithms combining clinical signs (e.g., fever, rash, and cough) associated with host biomarkers and locally relevant pathogen-specific tests (e.g., malaria, arbovirology, HIV, influenza, and typhus) have a clinical impact on the management of fever of unknown origin. Although some electronic algorithms have shown a positive influence on antibiotic prescription, it is not clear if they can also have an impact on clinical outcome. Moreover, the deployment of an electronic device to run such algorithms might not be feasible in remote settings, where technologies requiring minimal skills and equipment are needed.

Indeed, only two of the POCTs studied were RDTs; all the others required readers and personnel training which may not be available at peripheral health centers in LMICs. Semi-quantitative CRP-based RDTs could be a reasonable compromise, although reading and interpretation might not be immediately obvious.¹⁸

Overall, the available data on CRP performance are enough to justify further studies to validate the best threshold for bacterial versus nonbacterial fever diagnostic triage. In parallel, research should go on to identify a battery of markers and pathogen-specific diagnostic tests, and develop diagnostic algorithms adapted to different levels of the health system.

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