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Evaluation of C-reactive protein and myxovirus resistance protein A to guide the rational use of antibiotics among acute febrile adult patients in Northwest Ethiopia

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ABSTRACT

Objectives: In low-resource settings, treatment is often given empirically without knowledge of the aetiology due to a lack of diagnostics. In the search for reliable rapid tests to guide treatment work-up, this study was performed to determine whether two biomarkers could differentiate bacterial from non-bacterial infections in acute febrile patients.

Methods: Adults with acute fever were recruited at a referral hospital in Ethiopia. The QuikRead Go test was used to quantify C-reactive protein (qCRP) and the FebriDx test was used for combined qualitative detection of the bacterial CRP marker with myxovirus resistance protein A (MxA), a viral biomarker.

Results: Of the 200 patients included in this study, most presented with 2–3 days of fever, headache, and joint pain. Antibiotics were prescribed for 83.5% and antimalarials for 36.5%, while a bacterial infection was only confirmed in 5% and malaria in 11%. The median qCRP level for confirmed bacterial infections was 128 mg/l. The FebriDx and QuikRead Go test had an overall agreement of 72.0%.

Conclusions: An over-prescription of antibiotics for febrile patients was observed, even for those with low CRP levels and without a confirmed bacterial infection. The added value of the FebriDx was limited, while the combined use of rapid tests for qCRP and malaria should be considered for the management of acute febrile illness and antibiotic stewardship.

Introduction

The management of acute undifferentiated febrile illness (AFI) and the identification of patients who would benefit from antibacterial treatment is challenging, particularly in low-resource settings where diagnostic laboratory services are often lacking (Althaus et al., 2020). Hence, clinicians have to select an empirical antibiotic prescription that is rarely based on a definitive diagnosis and with clinical findings that often provide insufficient information. Even when laboratory capacity is available in these settings, it is mostly focused on malaria diagnosis (Bhargava et al., 2018; D'Acremont et al., 2014), still leading to the overuse of antibiotics for non-malarial AFI (Kapasi et al., 2016). Thus, effective, rapid, low-cost diagnostic tools that can easily be integrated into clinical algorithms are needed to guide optimal antibiotic use (WHO, 2020; Acestor et al., 2012).

Assays that detect host inflammatory biomarkers can provide an assessment and prediction of infection to decide on the best therapeutic approach and patient referral (Dupuy et al., 2013). C-reactive protein (CRP), an acute-phase protein of hepatic origin, is one of the most commonly used biomarkers and has been shown to be highly sensitive for predicting bacterial infections (Althaus et al., 2020; Kapasi et al., 2016). However, the performance of CRP as a marker for bacterial infections varies considerably among study populations, settings, and the cut-off values applied, and can

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be influenced by co-morbidities such as malaria, HIV, and malnutrition (Dittrich et al., 2016). The latter is especially of significance in low-resource settings, although it is not well-studied there (Kapasi et al., 2016). Besides CRP, several other biomarkers have been assessed, and new multiplex assays for the combined detection of different biomarkers have been developed in the last few years (Escadafal et al., 2017). They are rarely made commercially available in user-friendly test formats and their uptake and implementation in healthcare systems remain low.

This study was performed to evaluate two biomarker point-of-care tests in adult febrile patients in Gondar, Ethiopia. The first quantifies CRP in peripheral blood with a user-friendly benchtop device. The second is a handheld rapid test (FebriDx) on fingerprick blood for the qualitative detection of CRP and myxovirus resistance protein A (MxA), a protein induced by type 1 interferons during active viral infection (Nakabayashi et al., 2006). While the latter rapid test is relatively novel and promising because of the combined detection of CRP and MxA to differentiate bacterial and viral causes in acute respiratory infections (Davidson, 2017; Self et al., 2017; Shapiro et al., 2018), its use for undifferentiated AFI in low-resource settings is not yet known.

Materials and methods

Study site and participants

A cross-sectional study was conducted in the emergency ward of the University of Gondar (UoG) Hospital, Ethiopia, among patients ≥15 years old, presenting with acute fever (an axillary temperature of ≥37.5 °C) of ≤7-day duration. Pregnant women, children below 15 years of age, and patients with a suspected urinary tract infection or upper/lower acute respiratory infection were excluded in order to focus on AFI alone.

Patients received standard clinical care. Treatment was prescribed by the treating physician, who was unaware of the study results, and was mainly based on clinical judgement as the availability of routine tests was limited (malaria microscopy, blood culture, tuberculosis testing). Routine microscopic examination of blood smears for malaria diagnosis was done for 35 patients (17.5%) in this study on the request of the treating physician and was performed according to the national guidelines.

Study-related tests were done after routine work-up. Clinical study data were collected at the bedside for each patient after informed consent and from the medical records. Data were entered offline into a case report form by the study physician using the mobile phone KoBoToolbox app (www.kobotoolbox.org). Data were uploaded online when internet access was available.

Study sample collection and processing

A finger prick of blood was collected and immediately tested with the FebriDx test (Rapid Pathogen Screening) in the emergency ward. For all other study-related laboratory testing, venous blood was collected in (1) one 4-ml ethylenediaminetetraacetic acid (EDTA) tube for quantitative CRP testing (qCRP; QuikRead Go), to test for malaria (by rapid diagnostic test (RDT)), and to test for relapsing fever Borrelia and typhus group rickettsiosis (by PCR); (2) one 4-ml serum separator tube (SST) serum tube to test for dengue (by RDT, ELISA, and RT-PCR) and chikungunya and yellow fever (by RT-PCR). Samples were transported immediately to the Immunology and Molecular Biology Laboratory of the Biomedical Department (UoG) for aliquoting, storage, and testing.

In addition, 8 ml of blood was collected in a haemo-aerobic culture bottle according to routine procedures and transferred to the Microbiology Laboratory (UoG Hospital) for blood culture.

FebriDx test

The FebriDx test (Rapid Pathogen Screening, Sarasota, FL, USA) is a lateral flow immunoassay that detects raised levels of CRP (a marker for bacterial infections; >20 mg/l) and of MxA (a marker for viral infections; >40 ng/ml) from a fingerstick blood sample. This test was performed according to the manufacturer's instructions. The test strip, which is held inside an all-in-one plastic housing with a built-in retractable lancet, a blood collection and transfer tube, and a button release buffer activation mechanism, has one control line and two result lines (MxA and CRP). Briefly, capillary blood (5 μl) was applied via the collection tube into the blood transfer well and delivered to the test strip by pressing the release button. Results were read after 10 min and interpreted according to the manufacturer's instructions for valid test results (presence of a control line), with a viral infection defined by a positive result line for MxA only or both MxA and CRP, and with a bacterial infection defined by a positive result for a CRP line only.

Quantitative CRP test

EDTA whole blood was tested for quantitative detection of CRP using the immunoturbidimetric QuikRead Go CRP test on the QuikRead Go instrument (Orion Diagnostica, Espoo, Finland) following the manufacturer's instructions. Briefly, 20 μl of sample was collected with a capillary and added to the buffer in prefilled cuvettes using the capillary plunger. The cuvette was capped and inserted in the QuikRead Go instrument. CRP concentrations are measured by changes in the turbidity and are automatically corrected for the haematocrit level. For whole blood samples, the measurement range is 5–200 mg/l at the normal haematocrit level of 40%. No result is displayed when the haematocrit is above 75%; in such cases, the test was then repeated on serum.

For the analysis of qCRP levels, results that were displayed as <5, >170, >190, >200, >210, and >240 mg/l, were converted to 4, 171, 191, 201, 211, and 241 mg/l, respectively.

Confirmatory testing and case definitions

The biomarker levels were compared to clinical and laboratory data. For qCRP, cut-off levels of <10 mg/l and <20 mg/l to rule out the need for antibiotics and cut-off levels ≥100 mg/l to rule in the need for antibiotics were used, as described previously (Lubell et al., 2015; Do et al., 2016). Laboratory-confirmed infections were investigated with a panel of selected diagnostic tests. Bacterial infections were confirmed by either blood culture or PCR (typhus group rickettsiosis), malaria cases were identified by the study RDT and/or routine microscopy, and viral infections (confirmed acute dengue virus cases) were identified by RT-PCR, NS1 Ag RDT, or IgM ELISA, as described elsewhere (manuscript in preparation). For 158 patients, the aetiology remained unknown. Samples were also tested for *Borrelia* spp by PCR on whole blood and by RT-PCR for chikungunya virus and yellow fever virus, but no additional infections could be identified.

Laboratory data collection and data analysis

All laboratory data were entered into the KoBoToolbox database on a laptop by the investigator and validated by a senior researcher. After export to Excel, the data were analysed using GraphPad Prism v5.03 software.

The quick Sepsis-related Organ Failure Assessment (qSOFA) scores were calculated using the online qSOFA calculator (http://qsofa.org/#calc) based on three parameters: altered mentation, respiratory rate, and systolic blood pressure. A qSOFA score of 0, 1,

2, and 3 indicates that the patient with suspected infection has a respective 1%, 3%, 6%, and 23% risk of a bad outcome, with sepsis considered as unlikely, possible, likely, and very likely.

Regarding the patient characteristics, a descriptive analysis was used to summarize the data as frequencies and percentages. Continuous data were recorded as the median with interquartile range (IQR) and categorical data as the number and frequency. The statistical significance of differences between two groups was determined with the non-parametric Mann–Whitney *U*-test for continuous variables, with significance set at a p-value of <0.01. The sensitivity of qCRP for confirmed bacterial infection was calculated at different levels. Similar to a previous study (Althaus et al., 2020), specificity was not assessed because of the lack of diagnostic testing to confirm non-bacterial aetiologies. For the FebriDx test, the sensitivity for viral infections was calculated for MxA only, CRP + MxA, and total MxA, and for bacterial infections by CRP only, CRP + MxA, and total CRP. Confidence intervals (CIs) for sensitivity were constructed using the Clopper–Pearson formula. Dot plots were created with GraphPad Prism.

Ethics statement

Ethical approval was obtained from the School of Biomedical and Laboratory Science, College of Medicine and Health Sciences, UoG and the Institutional Review Board of the Institute of Tropical Medicine (ITM), Antwerp, Belgium. A support letter from the School of Biomedical and Laboratory Science and written permission from the hospital medical director and Diagnostic Centre was obtained prior to patient recruitment and data collection. Written informed consent was obtained from each participant. The confidentiality of all participants was maintained throughout the study. The study was registered at ClinicalTrials.gov (NCT04268732).

Results

Sociodemographic, clinical, and laboratory characteristics

During the study period, 234 acute undifferentiated febrile patients who met the inclusion criteria presented to the emergency department of UoG Hospital. Two hundred of these patients were enrolled; 34 were not eligible, as they either refused to give consent (n = 11) or refused to give a blood sample for the study (n = 23).

Of the 200 febrile participants (Table 1, Supplementary Material Table S1), 43.5% were female and 47.0% were under 25 years of age. Most were living in urban areas (80.0%) and were students (26.5%) or government employees (22.5%). The patients presented with a median axillary temperature of 37.9 °C (IQR 37.7–38.2 °C), mostly with fever for 2 days (26.5%) or 3 days (20.0%), often accompanied by headache (56.0%) and joint pain (44.5%), followed by vomiting (38.5%) and diarrhoea (37.5%). The aetiology could be identified in 42 patients: malaria in 22, acute dengue in 15, and bacterial infections in 10 (of which one was a bacterial and malaria mixed infection and four were viral and malaria mixed infections). The patients had a median respiratory rate of 20 breaths/min (IQR 18–22 breaths/min), with a median systolic blood pressure of 110 mmHg (IQR 100–120 mmHg), resulting in a qSOFA score of 0 for 67.5%, 1 for 29.0%, and 2 for 3.5% of the patients. Almost all patients were ambulant and only 3% were hospitalized. As self-reported by the patients, antibiotics had been taken by 3.5% of the participants within the 7 days before enrolment in the study.

Antibiotic prescription and qCRP levels

Antibiotics were prescribed for 83.5% of the patients and antimalarials for 36.5%, while a bacterial infection was confirmed in only 5% of the patients and a malarial infection in only 11%. Of those with confirmed bacterial and malarial infections, 90% received antibiotics and 63.6% received antimalarials (Table 1). Antibiotic prescription practice did not vary substantially according to the qSOFA score or biomarker levels (qCRP, CRP, MxA). Hence, antibiotics were prescribed for all seven patients with a high qSOFA score of 2, of whom none had a laboratory-confirmed bacterial infection, and for 113 patients with a qSOFA score of 0, of whom only four had a laboratory-confirmed bacterial infection. Of note, five of the 10 patients with confirmed bacterial infections had a qSOFA score of 1, while the other five had a qSOFA score of 0. Importantly, 30.0% and 44.0% of the patients had qCRP levels below the 10 mg/l and 20 mg/l, cut-off values proposed to rule out the need for antibiotics, and antibiotics were prescribed for 80.0% and 85.7% of these cases, respectively. In addition, 17.5% of the patients had qCRP levels of 100 mg/l or above, a cut-off value that has been proposed to rule in the need for antibiotics, and 91.4% of these patients were prescribed antibiotics.

The median qCRP level was highest for bacterial infections (128 mg/l, IQR 45–215 mg/l) and significantly lower for unknown infections (23 mg/l, IQR 4–59 mg/l; p = 0.0012). The median qCRP level was 47 mg/l (IQR 14–89 mg/l) for malaria and 60.0 mg/l (IQR 13–94 mg/l) for viral infections (Figure 1A). Of note, qCRP was assessed in whole blood for 181 participants, while retesting on serum was needed for 19 patients, due to haematocrit levels that were too high.

All 10 confirmed bacterial infections had a qCRP above 10 mg/l, and all but one *Rickettsia* infection had a qCRP level ≥20 mg/l (Table 2). For the detection of bacterial infections, a qCRP cut-off of 10 mg/l had a sensitivity of 100% (95% CI 69.2–100%), while a cut-off of 20 mg/l had a sensitivity of 90% (95% CI 55.5–99.8%). All but two malaria cases had increased qCRP levels, which were ≥100 mg/l in four cases. Amongst the 15 dengue cases, qCRP levels were ≥10 mg/l in 12 cases and ≥20 mg/l in 10 cases (Table 2).

FebriDx rapid diagnostic test

All FebriDx tests were valid. A negative result was seen for 60.5% of participants, and 24.5% had a positive test line for CRP only (indicating a bacterial infection). In addition, 13.5% tested positive for both CRP and MxA and 1.5% for MxA only, both indicative of a viral infection according to the FebriDx manual, bringing the total indicative viral infections to 15% (Table 2). If participants who had a FebriDx test with both CRP and MxA test lines positive (considering that a bacterial infection cannot be excluded in such a case) were also included in the CRP-positive group, this would bring the total CRP positives to 38% indicative of bacterial infections (Table 2).

Amongst the 10 bacterial infections, five were positive for CRP only and four for both CRP and MxA, while one rickettsial infection tested negative. Three out of 22 malaria cases had both CRP and MxA test lines, nine had a CRP test line only, and one had only an MxA positive result. Of the 15 dengue infections, four were CRP and MxA positive, four were CRP only positive, and four were negative (Table 2). For the detection of bacterial infections, the FebriDx CRP marker had a sensitivity of 50.0% (95% CI 18.7–81.3%) for CRP only or 90% (95% CI 55.5–99.8%) for all CRP positives. For the detection of viral infections, the MxA marker had a sensitivity of 26.6% (95% CI 10.9–69.2%).

The qCRP levels per qualitative FebriDx test result are illustrated in Figure 1B. The median qCRP was highest for FebriDx CRP only positive test lines (100 mg/l, IQR 44–158 mg/l) and lowest for CRP negative FebriDx results (11 mg/l, IQR 4–34 mg/l), followed by MxA only (20 mg/l, IQR 16–22) and CRP + MxA positive test lines (36 mg/l, IQR 25–75 mg/l). The qCRP levels differed significantly between FebriDx CRP only positives and CRP negative test results

Table 1
Proportions of individuals prescribed antibiotics and antimalarials according to patient and laboratory characteristics.

	Total, *n* (%)	Antibiotics prescribed *n* (row %)	Antimalarials prescribed *n* (row %)
Total	200	167 (83.5)	73 (36.5)
Sex			
Female	87 (43.5)	76 (87.4)	27 (31.3)
Male	113 (56.5)	91 (80.5)	46 (40.7)
Age (years)			
<25	94 (47.0)	79 (84.0)	31 (33.0)
25–35	55 (27.5)	45 (82.0)	23 (41.8)
35–45	22 (11.0)	18 (82.0)	10 (45.5)
≥45	29 (14.5)	25 (86.0)	9 (31.0)
Aetiology[a]			
Unknown	158 (79.0)	131 (82.9)	52 (32.9)
Malaria[b]	22 (11.0)	14 (63.6)	12 (54.5)
Viral infection (DENV)	15 (7.5)	14 (93.3)	7 (46.7)
Bacterial infection	10 (5.0)	9 (90.0)	3 (30.0)
Bacteraemia	8 (4.0)	8 (80.0)	2 (20.0)
Rickettsia	2 (1.0)	1 (10.0)	1 (10.0)
qSOFA			
Score 0	135 (67.5)	113 (83.7)	37 (27.4)
Score 1	58 (29.0)	47 (81.0)	31 (53.4)
Score 2	7 (3.5)	7 (100.0)	5 (71.4)
Score 3	0 (0.0)		
qCRP			
<10 mg/l	60 (30.0)	48 (80.0)	17 (28.3)
≥10 mg/l	140 (70.0)	119 (85.0)	56 (40.0)
≥20 mg/l	112 (56.0)	96 (85.7)	47 (42.0)
≥100 mg/l	35 (17.5)	32 (91.4)	13 (37.1)
FebriDx			
Negative	121 (60.5)	102 (84.3)	34 (28.1)
CRP only (>20 mg/l)	49 (24.5)	42 (85.7)	25 (51.0)
CRP + MxA	27 (13.5)	21 (77.8)	12 (44.4)
MxA only (>40 ng/mL)	3 (1.5)	3 (66.7)	2 (66.7)

DENV, dengue virus; qCRP, quantitative C-reactive protein levels (by QuikRead Go); CRP, qualitative C-reactive protein levels (by FebriDx); MxA, qualitative detection of myxovirus resistance protein A (by FebriDx); qSOFA, quick Sepsis-related Organ Failure Assessment; *n*, number of patients.
[a] Including five mixed infections (one bacterial and malarial mixed infection, four viral and malarial mixed infections).
[b] Malaria was confirmed in 21 study participants by rapid diagnostic test, of whom 10 were microscopy-positive and 11 were not tested by microscopy. For one study participant, malaria was confirmed by routine microscopy only.

($p < 0.0001$) and between CRP+MxA and CRP negative test results ($p < 0.0001$) (Figure 1B).

To evaluate the agreement between quantitative and qualitative CRP, the positivity threshold of ≥20 mg/l was used for the qCRP test, which is at the detection threshold of the FebriDx test (Figure 1B, Supplementary Material Table S2). In total, 56% of patients were positive for qCRP and 38% for the FebriDx CRP. Both methods were positive for 33% and negative for 39% of samples, resulting in an overall agreement of 72%. False-positive qualitative CRP results were seen in 5%, while false-negative qualitative CRP results were seen in 23% of cases with a median qCRP value of 43.5 mg/l (IQR 30.0–71.3 mg/l).

Discussion

Over-prescription of antibiotics and antimalarials

More than 80% of the febrile participants were prescribed antibiotics, while bacterial infections were only confirmed in 5% of them. Almost all patients with a confirmed bacterial infection (90%), but also 82.9% of patients with an unknown infection, 63.6% with malaria, and 93.3% with a viral infection, received an antibiotic prescription. Most patients had a low risk of sepsis based on the qSOFA score, so even for relatively mild AFIs, clinicians tended to prescribe empirical antibiotic treatment. The overuse of antibiotics might be due to the underuse of microbiology laboratories, which are often poorly linked to clinical practice in hospitals in sub-Saharan Africa (Jacobs et al., 2019). Future research should try to understand the underlying reasons, including patient expectations, lack of standardized treatment guidelines, or a gap in knowledge on the aetiology. Also, antimalarials were too frequently prescribed. While national guidelines recommend not prescribing antimalarials for patients with a negative blood film, routine microscopic examination was not requested for more than 80% of the febrile patients, revealing a gap for malaria testing. Altogether, this urges the need for the use of easy and rapid tools that can guide clinicians and speed up treatment decisions for patients presenting with AFI in these challenging settings (Bhargava et al., 2018; Schneider et al., 2020).

Evaluation of two host biomarker rapid tests

The use of host biomarkers to identify bacterial causes of AFI is increasing, with CRP being the most recognized one, however this is still rare in low-resource settings (Kapasi et al., 2016). The test selected for CRP quantification in the present study is a user-friendly, small benchtop device with individually packed test cuvettes. It was easily implemented and applied on a small volume of blood with results available within minutes, although some cases (9.5%) needed repeat testing on serum due to a

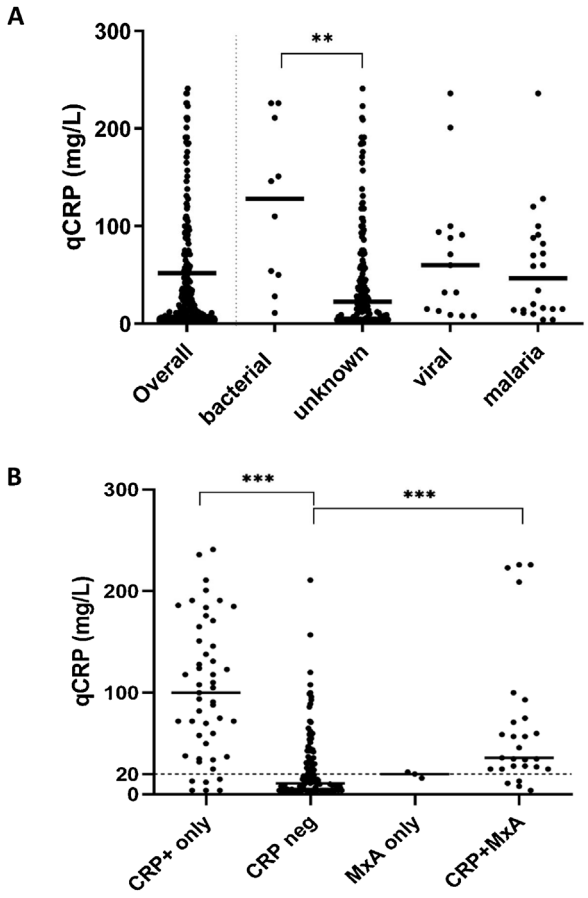

Figure 1. Dot plots of qCRP levels (mg/l), per aetiology (A) and per qualitative CRP/MxA detection (FebriDx test) (B).
Legend: Overall, qCRP ranged between detectable levels of 5 mg/l and >240 mg/l, with a median qCRP value of 42 (IQR 18–93) mg/l. Levels below the reference range limit (<5 mg/l), as seen for 23.5% of the acute febrile patients, were set at 4 mg/l. For bacterial, unknown, viral, and malarial infections, the median qCRP values were 128 (IQR 45–215) mg/l, 23 (IQR 4–59) mg/l, 60 (IQR 13–94) mg/l, and 47 (IQR 14–89) mg/l, respectively (A). In comparison to the qualitative FebriDx test results that were only CRP-positive (CRP + only; $n = 49$; >20 mg/l), negative for CRP (CRP neg; $n = 121$; <20 mg/l), only positive for MxA (MxA only; $n = 3$; >40 ng/mL), and CRP and MxA positive (CRP + MxA, $n = 27$), the median qCRP levels were 100 (IQR 44–158) mg/l, 11 (IQR 4–34) mg/l, 20 (IQR 16–22) mg/l, and 36 (IQR 25–75) mg/l, respectively (B). Horizontal line: median value. $^{**}p$-value between 0.001 and 0.01; $^{***}p$-value <0.001.

haematocrit level in whole blood that was too high/low. The test was used for adults with an acute undifferentiated febrile illness, attending a primary care hospital in a stable endemic malaria context.

In this setting, the qCRP test showed a sensitivity of 100% for the detection of bacterial infections with a threshold of greater than 10 mg/l and a sensitivity of 90% for a cut-off of 20 mg/l. The findings are in line with those of studies conducted among hospitalized children in rural Mozambique (Díez-Padrisa et al., 2010; Diez-Padrisa et al., 2012), children and adults in Southeast Asia (Lubell et al., 2015), and adults (>15 years old) in Thailand (Wangrangsimakul et al., 2018), which reported sensitivity of 86–95% at a cut-off of 10–20 mg/l. However, lower sensitivities of 44.6% (cut-off of 19 mg/l) and 80.0% (cut-off of 44.6 mg/l) have also been reported previously in malaria-negative paediatric fever in rural and urban Tanzania (Mahende et al., 2017; Hildenwall et al., 2016; Erdman et al., 2015) and other countries in Africa and Southeast Asia (Higdon et al., 2017). The performance of CRP can thus vary between settings; therefore the patient population targeted, epidemiological context, healthcare level, and urban or rural area setting should be well studied before clinical implementation.

The second biomarker test evaluated was the qualitative FebriDx immunoassay, which detects CRP and MxA simultaneously in a handheld cassette with a fingerprick blood transfer system. It appears that no other study on the use of this test in a low-resource setting has yet been published. Although the performance of the FebriDx test has been reported to be excellent for acute respiratory tract infections (Davidson, 2017; Self et al., 2017; Shapiro et al., 2018; Joseph and Godofsky, 2018; Sambursky and Shapiro, 2015), the present study showed rather low sensitivities of 50% (CRP only) and 26.6% (MxA) for predicting bacterial and viral infections, respectively, in those with AFI. Differences with previous studies might be related to the study design, which in the present study focused on AFI and the nature of febrile illnesses with only a few confirmed infections and without using procalcitonin and the white blood cell count as references in the test algorithm, as done by others (Self et al., 2017). Of note, we believe that in the case of AFI, a positive test line for both CRP and MxA is not only indicative of a viral infection, as stated by the manufacturer, but that a bacterial infection cannot be excluded, as shown here with the higher sensitivity of 90% when taking all CRP positives into account. In comparison to the qCRP test, the performance of the FebriDx CRP was fair, with an overall agreement of 72.0%, and this could be a potential tool to rule out the need for antibiotics with a CRP threshold below 20 mg/l; the rule-in threshold of 100 mg/l cannot be used with the FebriDx test.

Biomarkers to guide patient management

Immediate access to CRP results could be valuable to identify patients who require antibiotic treatment and those for whom antibiotics can be safely withheld (van Griensven et al., 2020).

When using a qCRP threshold of 10 mg/l and 20 mg/l to rule out the need for antibiotics, 30% and 44% of patients, respectively, would not have been prescribed antibiotics, while all patients with bacteraemia would have been treated. This could therefore have resulted in a remarkable reduction of antibiotic use, similar to that demonstrated previously in a randomized controlled trial in Tanzania (Keitel et al., 2017).

When applying a threshold of 100 mg/l to rule in the need for antibiotics, at least 17.5% of patients would have been prescribed antibiotics, including eight of 10 with confirmed bacterial infections. To decide on treatment for patients who have qCRP levels between these rule-in and rule-out thresholds, the clinical picture and additional laboratory testing could give guidance. In the present study, this could translate to additional laboratory testing being useful for 38.5–52.5% of the patients, depending on the threshold used. This would dramatically reduce the cost and resources needed for confirmatory testing.

Therefore, we strongly believe that CRP rapid tests could be supportive for clinical decision-making regarding antibiotic prescription in settings where laboratory facilities are not easily accessible. In malaria-endemic regions, malaria microscopy or RDTs should be performed first to rapidly exclude malaria and to prevent the overuse of antimalarials while avoiding antibiotic treatment for malaria cases. Blood culture or other diagnostic RDTs could be restricted to the subset of patients for whom the need for antibiotic treatment cannot be excluded or ruled in.

This study had some limitations. The number of confirmed infections was small, and this could have had an impact on the calculated sensitivity of the biomarkers, which should therefore be interpreted with caution, especially in the case of MxA. Additional testing, although hardly ever comprehensive enough, can be done on samples that remain unidentified. Furthermore, while there could have been clinical signs of severity in some patients that were not captured in our study and that would justify antibiotics, we think this would not have been common given the low qSOFA scores and low rates of hospitalization. Finally, we described the

Table 2
Performance of the CRP and MxA biomarkers to detect bacterial or viral infections in febrile patients.

qCRP level (mg/l)	Number	Bacterial infection (n = 10)	Non-bacterial infection/unknown (n = 158)	Viral infection (DENV) (n = 15)	Malarial infection (n = 22)
<10	60	0	60	3	2
≥10	140	10	130	12	20
≥20	112	9	103	10	13
≥100	35	6	29	3	4

FebriDx	Number	Bacterial infection (n = 10)	Non-bacterial infection/unknown (n = 158)	Viral infection (DENV) (n = 15)	Malarial infection (n = 22)
Total CRP+	76	9	67	11	12
CRP + only	49	5	44	7	9
CRP + MxA	27	4	23	4	3
MxA only+	3	0	3	0	1
Total MxA+	30	4	26	4	4
Negative	121	1	120	4	9

CRP, C-reactive protein; MxA, myxovirus resistance protein A; qCRP, quantitative C-reactive protein levels (by QuikRead Go); DENV, dengue virus.

added value of CRP testing to guide antibiotic prescription without taking into account patient-related factors such as the uptake of prescribed antibiotics and acceptance of a non-antibiotic management strategy by the patient when the clinician would have altered their clinical practice based on the CRP result. Not all clinical variables that may affect CRP, such as malnutrition, anaemia, diabetes, or endemic diseases (e.g. HIV, leishmaniasis, etc.), were included in the evaluation.

Conclusions

A high prescription of antibiotics and antimalarials was observed in acute febrile patients that greatly exceeded the number of laboratory-confirmed infections, including those with low CRP levels. The qualitative detection of CRP and MxA had limited value in the patient population and setting studied. Quantitative CRP was sensitive in predicting bacterial infections and could be considered for the management of AFI in adults in low-resource settings in order to reduce the diagnostic uncertainty of whether to treat with or withhold antibiotics, and to guide laboratory testing. To enhance antibiotic stewardship efforts, more research will be needed for the implementation of quantitative CRP rapid tests in clinical practice including the training of clinicians to assist in patient management, a cost–benefit analysis and its impact on clinical outcomes, antibiotic use, and antimicrobial resistance.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.09.1444.

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